Wastewater Treatment Laboratory Week 1

Course #2212

March 21-25, 2022

Fleming Training Center
Your Partner in Clean Water

TN Department of Environment & Conservation

Wastewater Treatment Laboratory -- Week 1
Course #2212
March 21-25, 2022

Monday:

8:30 Welcome and Class Introduction
8:45 Laboratory Safety
10:00 Laboratory Equipment
11:30 Lunch
12:30 Dissolved Oxygen (DO) Presentation
1:30 Biochemical Oxygen Demand (BOD) Presentation
Chemical Oxygen Demand (COD) Presentation

Tuesday:

8:30 BOD Analysis — Lab set up and Analysis
11:00 Lunch
12:00 Solids Presentation
1:00 Filter Prep for Solids Analysis
2:00 pH Presentation, Analysis and Calibration

Wednesday:

8:30 Solids Analysis
10:30 Bacteriological Presentation
12:00 Lunch
1:00 Bacterial Analysis (E-coli, Fecal coliforms)

Thursday:

8:30 QA/QC Presentation
11:00 Lunch
12:00 Sampling Presentation
1:00 Read Bacterial Analysis Results and Calculations

Friday:

8:30 Review
10:00 Exam—Lab Practical

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Fleming Training Center
Your Partner in Clean Water

TN Department of Environment & Conservation

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Section 1
Lab Basics

Wastewater Laboratory Class

Laboratory Safety

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Safety

- Operators work around many different kinds of hazards on a daily basis
  - Electrical
  - Bacteriological/Viral
  - Confined space
  - Mechanical
  - Traffic

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Safety

- Occupational Safety and Health Act (OSHA)
  - Demands that proper safety procedures be exercised in the lab at all times
  - "each employer has the general duty to furnish all employees with employment free from recognized hazards causing, or likely to cause, death or serious physical harm"
- TOSHA
  - Contact local office for specific questions

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Laboratory Hazards

- Infectious Materials
- Poisons
- Explosions
- Cuts and Bruises
- Electric Shock
- Toxic Fumes
- Fire
- Burns

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Be Aware

- Learn the lay-out of the lab
  - Emergency exits
  - Emergency routes
  - Emergency ventilation system
  - Fire-fighting equipment locations/ know how to use it
  - Eye wash station/Emergency shower
  - First Aid equipment
  - Emergency phone numbers

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Infectious Materials

- Wastewater and sludge contain millions of microorganisms
- Some are infectious and can cause disease
  - Tetanus
  - Typhoid
  - Dysentery
  - Hepatitis
  - Parasitic worms

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Infectious Materials

- Change out of work clothes before leaving
  - Prevent spread of infectious material into your home
- Inoculations from doctor/health dept.
  - Tetanus, polio, hepatitis A and B
  - Diseases contracted through breaks in skin, cuts, puncture wounds
  - Wastewater risk: breathing contaminated air

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Infectious Materials

- Always wash hands with soap and water, especially before handling food or smoking
  - Hand sanitizer is not sufficient
- Never pipet by mouth
  - Could lead to serious illness or death
  - Use mechanical or rubber bulbs

- Never drink from a beaker or other lab glassware

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Corrosive Chemicals

- Acids: a chemical substance that neutralizes alkalis, dissolves some metals; turns litmus paper red; typically a corrosive liquid
- Extremely corrosive to human tissue, metals, clothing, wood, cement, stone, concrete

- Sulfuric acid (H_2SO_4)
- Hydrochloric or muriatic (HCl)
- Nitric (NHO_3)
- Glacial acetic ($H_4C_2O_2$)

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Corrosive Chemicals

- Bases: turn litmus paper blue, pH greater than 7
- Extremely corrosive to skin, clothing, and leather

- Sodium hydroxide, aka "caustic soda" or "lye" (NaOH)
- Potassium hydroxide (KOH)
- Chlorine (and other oxidants)

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Corrosive Chemicals

- Commercially available chemical spill clean-up materials should be kept on hand
- Baking soda (bicarbonate) effectively neutralizes acids
- A jug of ordinary vinegar can be kept on hand to neutralize bases

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Toxic Materials

- Solids:
  - Cyanide, chromium, heavy metals
- Liquids:
  - Chlorine, nitric acid, ammonium hydroxide, chloroform, organic solvents
- Gases:
  - Chlorine, ammonia, hydrogen sulfide, sulfur dioxide, and chlorine dioxide

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Explosive or Flammable Materials

- Liquids:
  - Acetone, ethers, gasoline, benzene
- Gases:
  - Propane, hydrogen, acetylene

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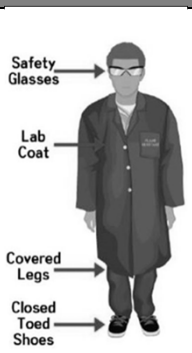
Personal Protective Equipment

- Known as **PPE**
- Safety Glasses
- Face Shield
- Lab Coat
- Lab Apron
- Gloves
  - Rubber, heat resistant
- Closed Toed Shoes
  - Steel-toed boots



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Personal Safety and Hygiene

1. Never work alone in the lab
   - In case of accident or fire
   - If necessary, have someone check on your regularly
2. Wear protective goggles or safety glasses at all times
   - Fumes can seep between contact lens and eyeball

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Personal Safety and Hygiene

3. Wear a face shield if there is danger of hot liquid erupting or flying glassware due to explosion

4. Wear protective or insulated gloves when handling hot or cold objects, or when handling liquids or solids that are skin irritants

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Personal Safety and Hygiene

5. Always wear a lab coat or apron
6. Never pipet by mouth
7. Never eat or smoke in the lab
8. Do not keep food in a refrigerator that is used for chemical or sample storage

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Personal Safety and Hygiene

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Personal Safety and Hygiene

9. Use ventilated lab fume hoods when handling toxic chemicals

- Average "face" velocity of 100 fpm with a min of 70 fpm at any point
- If carcinogens are handled, a face velocity of 150 fpm required
- Verification that air flow is active at each hood

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Personal Safety and Hygiene

9. Maintain clear access to emergency eye wash stations/showers
   - Flush weekly (OSHA)
   - Flush monthly (SAC Vol II)
   - Short flexible tube to wash chemicals off skin
10. Practice good housekeeping to prevent accidents

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Personal Safety and Hygiene

11. Never look into the open end of a container during a reaction or when heating a container
12. Always check labels on bottles to make sure you selected the proper chemical
    - Unlabeled containers only allowed if the are dispensed immediately after use
13. Never handle chemicals with bare hands
    - Use spoon or spatula

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Personal Safety and Hygiene

14. Unsafe glassware is the largest single cause of accidents in the lab
    - Fire polish chips – slowly heat chipped area until it reaches a temp at which glass will begin to melt, remove from heat, allow to cool
    - Never hold glassware (or equipment) with bare hands while heating
      - Use gloves and/or suitable tool
15. Special receptacle for broken glass

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Manufacturer Label Requirements

1. Product Identifier: The name used for a hazardous chemical on the label and in the SDS
2. Pictogram
3. Signal Word: Used to indicate the relative level of severity of hazard and alert the reader to a potential hazard
   - Danger—more severe hazard
   - Warning—less severe hazard
4. Hazard Statement: describes the nature of the hazard
5. Precautionary Statement: describes recommended measures that should be taken to minimize or prevent adverse effects resulting from exposure or improper storage or handling

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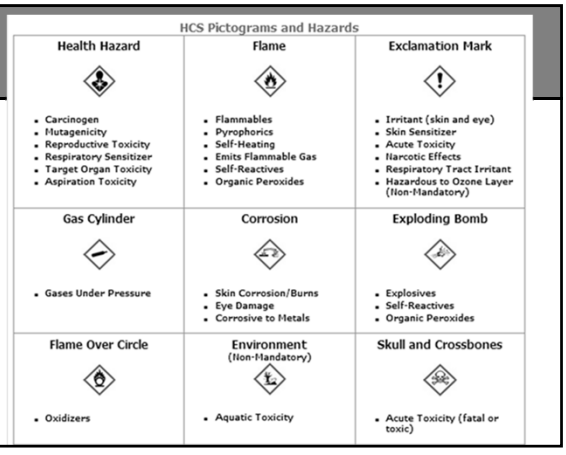

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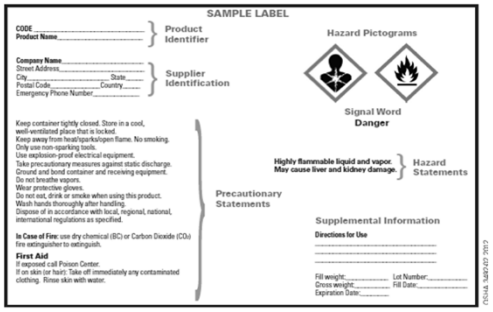
Manufacturer Container Label



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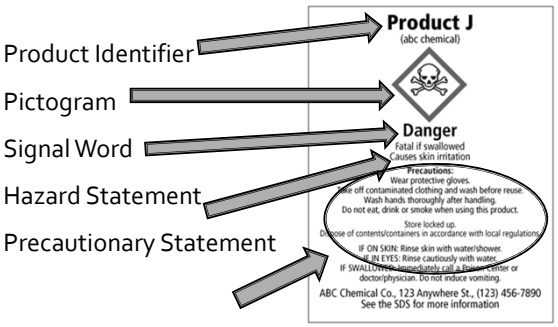
Manufacturer Container Label Container Label Requirement



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Safety Data Sheets (SDS)

- Includes all information on chemical label and specific info pertaining to that chemical
- 16 sections
  - ID, Hazards, Ingredients, First Aid, Fire-Fighting, Accidental Release, Handling/Storage, PPE, Physical and Chemical Properties, Stability/Reactivity, Toxicological Info, Ecological Info, Disposal, Transport Info, Regulatory Info

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SDS

- All chemicals in the facility currently:
  - In a labeled notebook or binder
  - Specific location near the entrance
  - Must be yellow or safety orange
- Must keep on file for all chemicals purchased
  - On file for at least 30 years

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Chemical Storage

- Properly ventilated
- Well lit
- Laid out to segregate incompatible chemicals
  - Not in alphabetical order
- Order and cleanliness must be maintained
- Clearly label and date all chemicals and bottles of reagents
  - Chemicals transferred to different containers MUST be labeled

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Chemical Storage

- Store heavy items on or as near to floor as possible
- Volatile liquids that may escape as a gas, such as ether, must be kept away from heat sources, sunlight, and electric switches
  - Flammable cabinet
- Cap and secure cylinders of gas in storage to prevent rolling or tipping

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Chemical Storage

- Store acids and bases in separate storage cabinets
- If incompatible chemicals are inadvertently mixed a fire, explosion, or toxic release can easily occur
- For especially dangerous materials, use a secondary container (e.g. plastic tub) large enough to contain a spill of the largest container

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Chemical Storage

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Moving chemicals

- Use cradles or tilters for carboys or other large chemical vessels
- Use a trussed hand truck for transporting cylinders of compressed gas
- Never roll a cylinder by its valve
- Clamp securely into place to prevent shifting or toppling
- Carry flammable liquids in safety cans
  - Gloves, safety shoes, rubber apron

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Proper lab technique

- Acids and other corrosives
  - Flush outside of acid bottles with water before opening
  - Do not lay stopper/lid on counter where person may rest arm or hand
  - Keep all acids tightly stoppered when not in use
  - Immediately clean up spills

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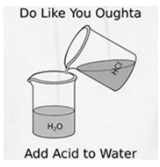
Proper lab technique

- Always add acid to water
  - If you pour water into acid, violent splashing may occur



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Mercury

- Even a small amount of spilled mercury can poison the atmosphere in a room
- To clean up a small spill (amount in a thermometer):
  - Put on rubber, nitrile, or latex gloves
  - Use squeegee or cardboard to sweep mercury beads together
  - Use disposable dropper/pipet to suck up beads
  - Slowly squeeze mercury onto damp paper towel
  - Place in labeled zip lock bag for proper disposal

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Electric shock

- Follow the usual "do's" and "do not's"
- Ground all apparatus with 3 prong plugs
- Do not continue to run a motor after liquid has spilled on it
  - Turn off immediately, clean and dry inside thoroughly before use
- Electrical units operated in an area exposed to flammable vapors should be explosion proof

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Cuts

- Some lab glassware must be inserted through rubber stoppers
  - Glass tubing, thermometers, funnels
  - Ends should be flame polished and either wetted or lubricated
    - Never use oil or grease
  - Wear gloves
  - Hold tubing as close to end being inserted as possible to prevent bending/breaking
- Never force rubber tubing or stoppers from glassware

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Cuts

- Examine all glassware before use
  - Discard any broken pieces in the appropriate sharps container
- Never store broken glassware in cabinets
  - Damaged glassware should either be sent for repair or disposed of properly
- Use gloves when sweeping up broken glass, do not use bare hands
  - Pick up fine glass particles with wet paper towel

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Burns

- Immediately wash off spatterings of acids, caustics, and strong oxidizers with large amounts of water
- Every worker should have access to a sink and emergency deluge shower
- Keep vinegar and baking soda handy to neutralize bases and acids
  - Vinegar neutralizes bases
  - Baking soda neutralizes acids

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Burns

- Heat resistant gloves
- Safety tongs to handle hot glassware
  - Do not juggle from hand to hand
- Most harmful and painful chemical burn = Eyes
  - Immediately flood eyes with water or special eyewash solution
  - Rinse within 1 minute of the burn
  - Flush at least 20 minutes
  - Consult doctor
- Alkali powder (such as lime) should be brushed off before adding water

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First Aid

- First Aid box should:
  - Be easy to access
  - Be easy to identify
  - Be adequately stocked (re-stocked quickly after use)
  - Contain a copy of basic first aid instructions
- Call 911 for major accidents
- Notify manager ASAP

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Toxic Fumes

- Use ventilated fume hood
  - Work at least 6 inches inside the hood
  - Annual maintenance
- Do not store chemicals in fume hood
  - Can impede proper air flow
  - Do not block rear exhaust slot
- When working with chlorine and other toxic substances, always wear a self-contained breathing apparatus

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Waste Disposal

- Corrosive materials should never be poured down the sink
  - Corrode the drain pipe or trap
- Corrosive acids should be neutralized and poured down corrosive-resistant sinks
  - Use large amounts of water to dilute and flush
- Broken glassware goes into designated sharps container

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Waste Disposal

- Do not place incompatible chemicals in same trash bag
- Store hazardous waste containers in a secondary container to prevent uncontrolled leaks
- Containers should be DOT approved for ultimate transport off site
- Keep hazardous waste container closed
  - Do not open, handle, or store in a manner that may cause it to rupture or leak

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Waste Disposal

- At least weekly, inspect areas where hazardous waste containers are stored
  - Look for evidence of leaks and deterioration
  - Take corrective action as required
- Label containers with hazardous waste, label describing the waste contained and date that accumulation started

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Chemical Hygiene Plan

- All treatment plants that have a laboratory should have a chemical hygiene plan
  - SOPs for using/handling hazardous chemicals
  - Criteria to reduce employee exposure, PPE, etc.
  - Operation of fume hoods to comply with regulatory requirements
  - Employee training – hazard communication
  - Provisions for medical consultation and exams
  - Assignment of chemical hygiene officer
  - Provisions for employee protection when working with carcinogens and reproductive toxins

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Fire

- Lab should be equipped with a fire blanket
  - Smother clothing fires
- Small fires in evaporating dish or beaker can be extinguished with
  - Glass plate
  - Wet towel
  - Wet blanket
- Do not use fire extinguisher on small beaker fire

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Fire

- You must use the proper fire extinguisher for each class of fire
- Ex: Never pour water onto grease fires, electrical fires, or metal fires
  - Increase the hazard – splattering the fire or electric shock
- Fires are classified according to the materials being consumed
  - A,B,C, or D

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Fire

- **Class A** = Ordinary combustibles
  - Wood
  - Paper
  - Cloth
  - Rubber
  - Many plastics
  - Grass, hay

- Use foam, water, soda-acid, carbon dioxide gas, or almost any type of extinguisher

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Fire

- **Class B** = Flammable and combustible liquids
  - Gasoline
  - Oil
  - Grease
  - Tar
  - Oil-based paint
  - Solvents
  - Flammable gases

- Use foam, carbon dioxide, or dry chemical extinguishers

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Fire

- **Class C** = Energized electrical equipment
  - Starters
  - Breakers
  - Motors

- Use carbon dioxide or dry chemical extinguishers to smother the fire
  - Both types are nonconductors of electricity

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Fire

- **Class D** = Combustible metals
  - Magnesium
  - Sodium
  - Zinc
  - Potassium

- Use a Class D extinguisher or use fine dry soda ash, sand, or graphite to smother the fire
- Operators rarely encounter this type of fire

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Fire Extinguishers

- A multipurpose ABC carbon dioxide extinguisher will handle most laboratory fires
  - Visual inspection – monthly
  - Maintenance check – annually
- Consult with your local fire dept. about best methods to use for specific hazards that exist at your facility

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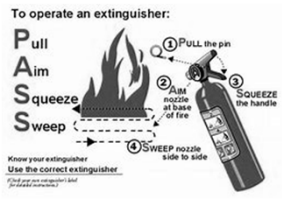
Fire Extinguishers

1. **P**ull the pin out
2. **A**im the nozzle at the base of the fire
3. **S**queeze the handle
4. **S**weep the nozzle/spray from side to side
   - To contain the fire



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Fire Prevention Practices

1. Maintain a neat and clean work area
2. Putting oil and paint-soaked rags in covered metal containers and regularly disposing of them in a safe manner
3. Observe all "no smoking" signs
4. Keep fire doors, exits, stairs, fire lanes, and firefighting equipment clear of obstructions
5. Keeping all combustible materials away from furnaces or other sources of ignition

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Fire Prevention Reminders

- Prevent fires by good housekeeping and proper handling of flammables
- Action in the first few seconds of ignition usually means the difference between destruction and control
- Know the proper fire extinguisher to use
- Learn how to operate the extinguishers *before* an emergency
- In necessary, evacuate immediately

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Water Supplies

- Inspect for cross connections
  - Water seals on pumps
  - Feed water to boilers
  - Hose bibs below grade where they could be subject to flooding with wastewater or sludges
- Install Air Gap device
  - Best backflow prevention method
- Never drink from outside water connections such as faucets and hoses
  - The hose could have been used to carry effluent or untreated wastewater
- Post signs that water is not potable where applicable

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Pregnancy

- Pregnant women should avoid teratogens
  - Teratogen = reproductive toxins that may cause damage to the fetus
- Ask supervisors to alter schedules/work assignments if the potential for exposure exists
- THM Plus method (Trihalomethanes) by Hach uses Chloroform, a teratogen

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Key Points to Remember

- Be aware of the hazards around you
- Educate yourself
- Review safety procedures on a regular basis
- Take charge of your own safety
- Don't get complacent

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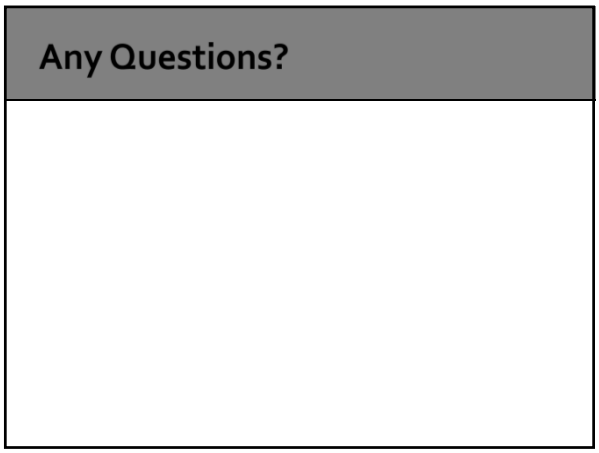

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Laboratory Equipment

Identification, Handling, and Cleaning

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Objectives

- Identify equipment commonly used in water treatment and wastewater laboratory
- Discuss accuracy and use of glassware
- Discuss how to use and maintain analytical equipment

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Beakers

Used for:

- Mixing
- Measuring approximate volumes
- ~10% accuracy

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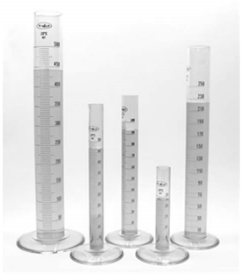
Graduated Cylinders

- Accurate to ~1%
- Measures liquid volumes more accurately than beakers, but still not the most accurate
- Measure quicker



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Volumetric Flasks

- Most accurate way to measure volume
- Disadvantage:
  - Only can measure one volume
  - Not used for storing or heating solutions

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What Are Pipets?

- Pipets are glass or plastic tubes, usually open at both ends, which are used to transfer specific amounts of liquid from one container to another
- They are usually used for volumes between 1 and 100 milliliters

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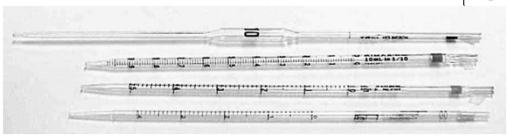
Types of Pipets



1. Volumetric
2. Measuring
   - Mohr
   - Serological

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Volumetric Pipets

- Used to deliver a single specific volume of liquid, usually between 1 and 100 ml
- Shaped like rolling pins with a large belly, one blunt end, the neck, and one tapering end, the tip

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Volumetric Pipets

- Used for accurate measurements, since it is designed to deliver only one volume and is calibrated at that volume
- Should be used when accuracy and reproducibility are crucial, because these can achieve accuracy to four significant figures

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Specifications on a Volumetric Pipet

- When emptying a volumetric pipet, the liquid is allowed to drain out
  - It is NOT forced out
- After it is emptied, the small amount of liquid which remains in the tip should not be blown out
- Volumetric pipets are NOT blow-out pipets

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Measuring Pipets

- They are straight glass or plastic tubes with one tapering end
- Calibrated into small divisions so that various amounts of liquid can be measured with the same pipet
- Usually used to measure any amount between 0.1ml and 25.0ml
- They are not as accurate due to the fact that any imperfection in their internal diameter will have a greater effect on the volume delivered

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Mohr and Serological Pipets

- Measuring pipets are divided into:
  - Mohr Pipets 
    - Graduations on these always end before the tip
  - Serological Pipets 
    - Graduation marks continue to the tip

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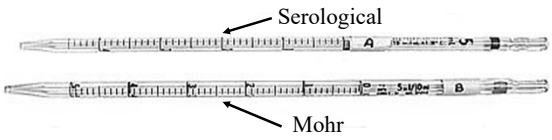
Examine pipets A and B
Which is the serological and which is the Mohr?



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Specifications on a Measuring Pipet

- Maximum volume of liquid that can be transferred
- Size of the divisions on the pipet
- Temperature at which calibrations were made
- If the pipet is a “to deliver”(TD) or “to contain”(TC) pipet

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5 in 1/10 ml
TD 20°C

- Specifications on a pipet as shown above indicate that the pipet is calibrated in 1/10ml divisions and will deliver up to 5.0 ml within published tolerance levels at 20°C

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1 ml in1/100
TD 20°C

- These specifications indicate that the pipet is calibrated in 1/100 ml divisions and it will deliver up to 1.00 ml within published tolerance levels at 20°C

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Using Pipets

- Select a clean, dry pipet of appropriate size
  - If clean/dry is not available, rinse several times with the solution to be used
- Avoid contaminating or diluting the solution by dipping a dirty or wet pipet into original solution
  - Pour some solution into a clean beaker, dip pipet into that beaker
  - Discard what remains in the beaker
  - Never put excess solution back into original reagent bottle

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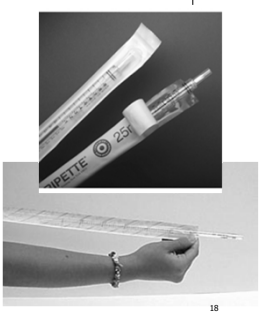
Handling Sterile Pipets

- When using sterile pipets, be sure to use proper sanitary techniques
- If you have a sterile package of disposable pipets, tear only a small corner of the package open and push one pipet out of this opening, then immediately close the package to prevent contamination



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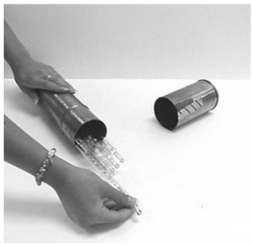
Handling Sterile Pipets

- If you are using sterile pipets in a pipet canister, place the canister on its side, slide off the cover, pull out one pipet and replace the cover immediately



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Handling and Disposing of Pipets

- Dispose dirty pipets by placing in soapy water solution in a tray or pipet washer
- Place disposable pipets in a cardboard holder
- Do not leave pipets on counters or sinks

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Transferring a Precise Volume of Liquid

- A pipet bulb is used to draw liquid up into the pipet
- There are many types of pipet bulbs

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Transferring a Precise Volume of Liquid

- You should observe the meniscus at eye-level
- Touch the tip of the pipet to the inside of the container when the meniscus is at the desired level

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Transferring a Precise Volume of Liquid

- Squeeze bulb and touch it to the mouth of the pipet
- Place other end of the pipet in liquid to be transferred and slowly release pressure on bulb
- Draw liquid up past desired level, quickly replacing bulb with index finger

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Transferring a Precise Volume of Liquid

- Let liquid drain until bottom of meniscus lines up with desired level on pipet
- Touch tip of pipet to inside of beaker to remove any adhering drops
- Transfer liquid to second beaker and touch tip to inside of beaker and let liquid drain out of pipet

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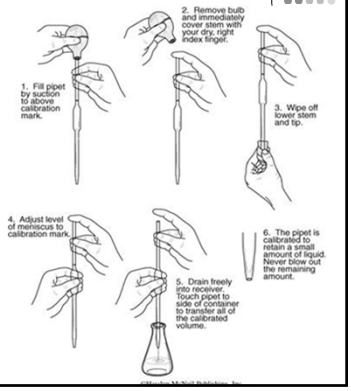
Handling and Disposing of Pipets

- Chipped and cracked pipets should be replaced as they are unsafe and may affect the accuracy of measurements.
- NEVER mouth pipet
- Hold the pipet by the upper third of the tube and keep the tip from touching anything



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Other Pipet Bulbs

- Other pipet bulbs that are often used include the Vadosa pipet filler, seen on the left, and the pipet Pumper, on the right

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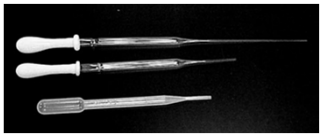
Other Pipet Types

- Transfer of uncalibrated volumes up to 2.5 ml can be accomplished using glass “transfer” or “Pasteur” pipets shown below. These may be sterilized before use
- Roughly calibrated volumes of 1 and 2 ml can be transferred with the one piece plastic transfer pipets which may be purchased as sterile or non-sterile units



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Burettes and Titrations

Burettes

- Used for titrations
- Treat like a Mohr pipet, do not let liquid completely drain out
- Also, make sure to remove air bubble in tip before titrating
  - Gently tap side with your finger to dislodge any bubbles clinging to walls

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Flasks

- Distilling Flask

- Florence (Flat Bottom) Flask

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Flasks

- Erlenmeyer Flasks

- Filter Flask

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How Scientific Glassware is made

- Glass blowing video
- Machine made video

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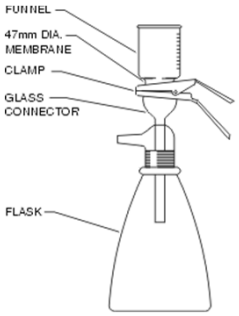
Filter Apparatus



- Vacuum Pump

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Bottles

- Dilution Bottles

- Sample Bottles

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Bottles

- Reagent Bottles

- Weighing Bottles

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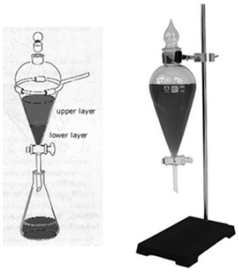
Funnels

- Separatory



- Buchner

- General

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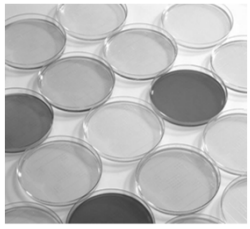
Petri Dish/Dessicator

- Petri Dish
  - Culturing container



- Desiccators
  - Dust and moisture free

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Evaporating Dish/Crucible

- Evaporating Dish
- Crucible

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Centrifuge

- Used to separate materials of different density
- Weight must be evenly distributed
  - Position test tubes across from each other to balance

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Autoclave

- Pressure cooker used to sterilize glassware, bottles, membrane filter equip, culture media and contaminated material to be discarded
- Standard temperature is set at 121°C and 15 PSI

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Refrigerators

Walk-in cooler

- Sample storage should maintain between 1-6 °C
- Never store samples and chemicals together

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Incubators

- Artificially heated container used for growing bacteria cultures
- Dry-Heat types hold temperatures to ± 0.5°C
- For E. coli and Total coliform = 35 ± 0.5°C
- Water Bath for fecal = 44.5 ± 0.2°C

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Incubators

- For BOD incubation at 20 ± 1 °C
- Do not store chemical solutions and samples in same refrigerator

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UV Sterilizer

- Use in Bacterial Lab to sterilize test equipment
- 3 minutes time

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Drying Oven

- Used more often in wastewater labs
- For solids testing set oven at 103-105°C

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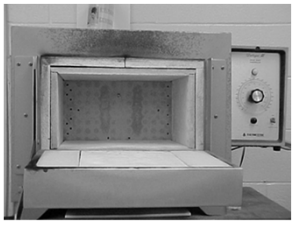
Muffle Furnace



- High temp oven used to ignite or burn solids
- Usually operate at temps of 550°C
- More often used in Wastewater lab work
  - MLVSS

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Fume Hood

- Can prevent serious accidents
- Use whenever heat is used in a test procedure
- Fumes vented out of lab
- Use when harmful smoke, gas, vapors, splashes or fumes are possible

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Water Still

- Produces distilled water for lab tests and rinsing washed glassware
- Removes dissolved minerals, organic and inorganic nonvolatile compounds
- Does not sterilize

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Heating and Stirring Samples

- Combo Heat/Stir Plate
  - Can be used to stir or heat and stir samples
  - Safer than heating with an open flame

- Gas Burner
  - Bunsen burner
  - Uses natural

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Balances

- Top Loading
  - Weighs to the nearest 0.01 g
- Analytical
  - Precise to 0.0001 g

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pH Meter

- Use buffer solutions to calibrate
- Store electrodes properly
- Calibrate daily
- Maintain records on daily calibrations

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Spectrophotometer

- HACH DR 6000
  - Factory pre-set programs for lab chemical analysis
- Very versatile

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Colorimeters

- Determine the concentration of many chemicals
- Most commonly used is chlorine type colorimeter
- Portable and battery powered

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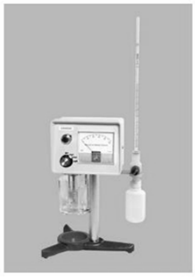
Amperometric Titrator

- Chlorine analysis
- Accurate and unaffected by sample color or turbidity
- Takes greater skill to use than DPD method with colorimetric devices



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Turbidimeter

- Desk top and continuous on-line monitoring
- Position away from direct sunlight and have extra light bulb on hand
- Ensure sample bottles maintained; no scratches; acid clean if necessary

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Chemical Storage

- Do not store volatile chemicals together
- Have separate storage cabinets for acids and bases/caustics

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Flammable Cabinet

- Flammable chemicals should be kept in a flammable cabinet

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Safety Equipment

PPE (Personal Protective Equipment):

- Goggles
- Gloves
- Aprons
- Wear safety clothing

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Eye Wash and Shower

- Should be checked weekly

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Cleaning Glassware

- Just because it looks clean does not mean residues are not left behind
- Results need to be accurate to use data for process control and/or reporting to the State
- Detergents, such as Alconox, may be sufficient
  - Should be phosphate-free

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Cleaning Glassware

- Residues of minerals and other substances can build up on glassware, causing erroneous test results
- If the water beads up on the inside of glassware *after* it has been cleaned, there is residue present

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Steps for Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse at least three times with distilled water
- Let air dry

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Steps for Acid-Washing

- Clean glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse with 1:1 hydrochloric acid or nitric acid
  - 1:1 means equal parts distilled water and acid
- Rinse well with distilled water
- Let air dry

Note: always use gloves and goggles when handling acids

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Laboratory Grade Water

- High quality lab grade water is essential
- Must be as free of contaminants as possible
  - Blanks, Standards, Etc.
- Follow manufacturer's instructions
  - Change filter cartridges regularly
  - Inspect distillation unit for scale build-up
- Water quality tested monthly
  - Conductivity, Chlorine residual, HPC, pH, etc.

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Laboratory Grade Water

- Two most common methods:
  1. Distillation – heating water to produce steam, which is condensed to a receiving bottle

2. Deionization – passing water through a demineralization cartridge which removes contaminating ions

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Laboratory Grade Water

- Granular Activated Carbon
  - Primarily removes chlorine and organics
- Microfiltration
  - Cartridge filters 1-5 micron
  - Plugging monitored by differential pressure
    - Increase in pressure = filter plugging
- UV Sterilization
- Ultrafiltration
- Reverse Osmosis

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Key Points to Remember

- Volumetric pipets and flasks are most accurate
  - Do not blow out liquid
- Know how to read a meniscus
- Know how to properly clean glassware
  - Everyday washing
  - Acid washing

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Laboratory Safety – Review Questions

1. List at least 5 laboratory hazards.

2. Why should you never work alone in the laboratory?

3. You may add acid to water, but never add water to acid. True or False?

4. How would you dispose of a corrosive acid?

5. What does SDS stand for?

6. How long should SDS's be kept on file?

7. What is a signal word? List the 2 signal words that could be found on a chemical label and what each one represents.

8. What should you do if you get a chemical in your eyes?

9. What would you do if you spilled a concentrated acid on your hand?

10. List the most important types of Personal Protective Equipment required when working in the lab.

11. Any work that has the potential to generate hazardous or toxic vapors or fumes should be conducted where?

12. What common household products should you keep on hand to neutralize acids and bases?

13. How often should (plumbed) eye wash stations and emergency safety showers be flushed?

14. Chemicals should be stored in alphabetical order for quick access. True or false?

15. Why must acids and bases be stored in separate cabinets

16. How would you extinguish a small beaker fire?

17. List the 4 Classes of fire extinguishers mentioned in our presentation and the materials being consumed in each class.

18. What does P.A.S.S. stand for?

19. What could happen to the potable water source if proper backflow prevention measures are not installed or if those measures fail? And what is the best method of backflow prevention?

20. What is a teratogen?

Lab Policies

1. No horse play.
2. No shorts or open-toed shoes.
3. No smoking, eating, dipping or drinking in the lab.
4. Put broken glass in broken glass container, NOT IN THE TRASH.
5. Do not pipet by mouth.
6. Each day after class:
    - All used glassware will be washed in hot soapy water, rinsed in tap water, then DI water.
    - All counter top will be wiped clean with disinfectant.
    - Balance room must be clean.
7. Used pipets are placed in containers containing detergent immediately after use, tip up.
8. Acid spills must be cleaned up immediately.
9. Pipet bulbs must be cleaned immediately after over pipetting (getting liquid into the bulb).
10. Wear safety glasses when performing any experiment.
11. Wear aprons in the lab at all times.
12. Wear gloves when performing any experiment or washing glassware.
13. Wash your hands before leaving the laboratory.
14. Know where the eye wash stations are located and how to use them.
15. Know where the emergency shower is and how to use it.
16. Know where each fire extinguisher is located and how to use them.
17. Carefully read the Safety Data Sheets for all chemicals used in the laboratory.

U.S. Department of Labor

OSHA®
Occupational Safety and Health Administration

Job Safety and Health
IT'S THE LAW!

All workers have the right to:

- A safe workplace.
- Raise a safety or health concern with your employer or OSHA, or report a work-related injury or illness, without being retaliated against.
- Receive information and training on job hazards, including all hazardous substances in your workplace.
- Request an OSHA inspection of your workplace if you believe there are unsafe or unhealthy conditions. OSHA will keep your name confidential. You have the right to have a representative contact OSHA on your behalf.
- Participate (or have your representative participate) in an OSHA inspection and speak in private to the inspector.
- File a complaint with OSHA within 30 days (by phone, online or by mail) if you have been retaliated against for using your rights.
- See any OSHA citations issued to your employer.
- Request copies of your medical records, tests that measure hazards in the workplace, and the workplace injury and illness log.

This poster is available free from OSHA.

Employers must:

- Provide employees a workplace free from recognized hazards. It is illegal to retaliate against an employee for using any of their rights under the law, including raising a health and safety concern with you or with OSHA, or reporting a work-related injury or illness.
- Comply with all applicable OSHA standards.
- Report to OSHA all work-related fatalities within 8 hours, and all inpatient hospitalizations, amputations and losses of an eye within 24 hours.
- Provide required training to all workers in a language and vocabulary they can understand.
- Prominently display this poster in the workplace.
- Post OSHA citations at or near the place of the alleged violations.

FREE ASSISTANCE to identify and correct hazards is available to small and medium-sized employers, without citation or penalty, through OSHA-supported consultation programs in every state.

Contact OSHA. We can help.

1-800-321-OSHA (6742) • TTY 1-877-889-5627 • www.osha.gov

OSHA 3165-04R 2015

OSHA INFOSHEET

Health Effects from Contaminated Water in Eyewash Stations

Eyewash stations used in workplaces must be maintained to prevent injury and illness to workers. This InfoSheet provides updated information on eyewash station hazards.

Eyewash stations are critical emergency safety equipment intended to mitigate eye injuries when control methods do not prevent exposure to a physical or chemical irritant or a biological agent. The ANSI standard for eyewashes specifies that eyewashes must be capable of delivering tepid flushing fluid to the eyes not less than 1.5 liters per minute (0.4 gpm) for 15 minutes after a single movement and subsequent hands-free operation. Whether the eyewash station is permanently connected to a source of potable water (i.e., plumbed) or has self-contained flushing fluid, improper maintenance may present health hazards that can worsen or cause additional damage to a worker's eye.

Where are eyewash stations used?

Eyewash facilities are required in workplaces where corrosive chemicals are used (29 CFR 1910.151(c)), as well as in HIV and HBV research laboratories and production facilities (1910.1030(e)(3)(i)), and where there is any possibility that an employee's eyes may be splashed with solutions containing 0.1 percent or greater formaldehyde (1910.1048(i)(3)). They may also be found in research and production laboratories, in medical facilities and other workplaces with materials that may cause injury to or infection of the eyes.

How can improperly maintained eyewash stations cause infections?

Water found in improperly maintained eyewash stations is more likely to contain organisms (*e.g., Acanthamoeba*, *Pseudomonas*, *Legionella*) that thrive in stagnant or untreated water and are known to cause infections. When a worker uses an eyewash station that is not maintained, organisms in the water may come into contact with the eye, skin, or may be inhaled. Workers using eyewash stations after exposure to a hazardous chemical or material may have eye injuries that make the eye more susceptible to infection. Also, workers with skin damage or compromised immune systems (*e.g.,* transplant recovery, cancer, lupus) are at increased risk for developing illnesses from contaminated water. Early diagnosis is important to prevent infections from causing serious health effects, including permanent vision loss and severe lung diseases (*e.g.*, pneumonia).

The following are a few organisms that thrive in eyewash stations when not maintained properly and the health hazards they present. This list is not all inclusive. There are many other micro-organisms that live in stagnant water that are not listed below.

Acanthamoeba is a microscopic single cell organism (amoeba) that may cause eye infections (see Figure 1). This organism can live in treated water and is commonly found in mucous membranes (*e.g.*, nose, throat, eyes) and in neurological tissues (*e.g.*, brain) without causing harm to the person. On rare occasions, exposure to *Acanthamoeba results in harmful eye infections known as Acanthamoeba keratitis.* Along with keratitis, workers with compromised immune systems face a significantly higher risk for developing neurological infections (Granulomatous Amoebic Encephalitis) or whole body infections. Workers may also experience eye redness, pain, tearing, blurred vision, light sensitivity, and eye inflammation several days after the use of a contaminated eyewash station. Diagnosing *Acanthamoeba* keratitis is difficult because more common eye infections have similar symptoms.

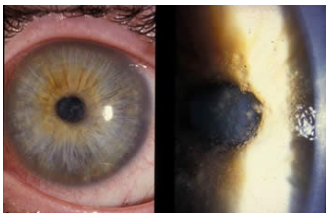

Photo courtesy of Dan B. Jones, M.D.

Figure 1. *Left, broad illumination; right, slit beam illumination. Early epithelial stage. Multifocal intraepithelial Acanthamoeba organisms.*

Pseudomonas infections are typically caused by a common bacteria species. *Pseudomonas aeruginosa* may cause infections to eyes, skin, muscle, lung, and other tissues. One symptom specific to *Pseudomonas aeruginosa* infection is green-blue pus in or around the infected area. If a pseudomonas infection spreads through the bloodstream (*i.e.*, septicemia), workers may become very sick with fevers, chills, confusion, shock, and even death. This bacterium has developed resistance to many antibiotics, which may make it harder to treat.

Figure 2. *Eyewash with protective covers.*

Legionella is a group of bacteria that are found in nature living with amoeba and may cause a serious lung infection. For example, since *Acanthamoeba* are effective hosts for *Legionella*, they may both be present in contaminated water. Although *Legionella* does not cause eye infections, inhaling water droplets containing the bacteria can cause Legionnaires' disease, a severe and fatal form of pneumonia. Workers with compromised immune systems, workers over the age of 55 or those with pre-existing lung diseases, such as Chronic Obstructive Pulmonary Diseases (COPD) are more at risk for infection. Legionnaires' disease symptoms occur 2 to 14 days after exposure, including coughing, breathlessness, high fever, muscle aches, and headaches, often requiring hospitalization.

For more information on Legionnaires' disease visit the OSHA Safety and Health Topics Page (www.osha.gov/SLTC/legionnairesdisease).

How can eyewash stations be maintained to prevent infections?

Eyewash station manufacturer instructions provide direction on how often and how long to activate specific plumbed systems to reduce microbial contamination and generally reference the American National Standards Institute (ANSI) standard Z358.1-2014. Self-contained eyewash units must be maintained and employers should consult the manufacturer's instructions for maintenance procedures. This includes flushing the system and using only solutions appropriate for flushing eyes.

Workers' Rights

Workers have the right to:

- Working conditions that do not pose a risk of serious harm.
- Receive information and training (in a language and vocabulary the worker understands) about workplace hazards, methods to prevent them, and the OSHA standards that apply to their workplace.
- Review records of work-related injuries and illnesses.
- File a complaint asking OSHA to inspect their workplace if they believe there is a serious hazard or that their employer is not following OSHA's rules. OSHA will keep all identities confidential.
- Exercise their rights under the law without retaliation, including reporting an injury or raising health and safety concerns with their employer or OSHA. If a worker has been retaliated against for using their rights, they must file a complaint with OSHA as soon as possible, but no later than 30 days.

For additional information on Workers' Rights, Employer Responsibilities, and other services OSHA offers, visit www.osha.gov.

Contact OSHA

For questions or to get information or advice, to report an emergency, fatality, inpatient hospitalization, amputation, or loss of an eye, or to file a confidential complaint, contact your nearest OSHA office, visit www.osha.gov, or call OSHA at 1-800-321-OSHA (6742), TTY 1-877-889-5627.

OSHA's On-site Consultation Program offers free and confidential advice to small and medium-sized businesses in all states across the country, with priority given to high-hazard worksites. On-site consultation services are separate from enforcement and do not result in penalties or citations.

For more information, to find the local On-site Consultation office in your state, or to request a brochure on Consultation Services, visit www.osha.gov/consultation, or call 1-800-321- OSHA (6742).

Many states operate their own OSHA-approved safety and health program. For further information, please visit www.osha.gov/dcsp/osp.

References

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OSHA® FactSheet

Laboratory Safety Chemical Hygiene Plan (CHP)

OSHA's Occupational Exposure to Hazardous Chemicals in Laboratories standard (29 CFR 1910.1450), referred to as the Laboratory standard, specifies the mandatory requirements of a Chemical Hygiene Plan (CHP) to protect laboratory workers from harm due to hazardous chemicals. The CHP is a written program stating the policies, procedures and responsibilities that protect workers from the health hazards associated with the hazardous chemicals used in that particular workplace.

Required CHP Elements

1. Standard operating procedures relevant to safety and health considerations for each activity involving the use of hazardous chemicals.
2. Criteria that the employer will use to determine and implement control measures to reduce exposure to hazardous materials [i.e., engineering controls, the use of personal protective equipment (PPE), and hygiene practices] with particular attention given to selecting control measures for extremely hazardous materials.
3. A requirement to ensure that fume hoods and other protective equipment are functioning properly and identify the specific measures the employer will take to ensure proper and adequate performance of such equipment.
4. Information to be provided to lab personnel working with hazardous substances include:
   - The contents of the Laboratory standard and its appendices.
   - The location and availability of the employer's CHP.
   - The permissible exposure limits (PELs) for OSHA regulated substances or recommended exposure limits for other hazardous chemicals where there is no applicable OSHA standard.
   - The signs and symptoms associated with exposures to hazardous chemicals used in the laboratory.
   - The location and availability of known reference materials on the hazards, safe handling, storage and disposal of hazardous chemicals found in the laboratory including, but not limited to, the Material Safety Data Sheets received from the chemical supplier.
5. The circumstances under which a particular laboratory operation, procedure or activity requires prior approval from the employer or the employer's designee before being implemented.
6. Designation of personnel responsible for implementing the CHP, including the assignment of a Chemical Hygiene Officer and, if appropriate, establishment of a Chemical Hygiene Committee.
7. Provisions for additional worker protection for work with particularly hazardous substances. These include "select carcinogens," reproductive toxins and substances that have a high degree of acute toxicity. Specific consideration must be given to the following provisions and shall be included where appropriate:
   - Establishment of a designated area.
   - Use of containment devices such as fume hoods or glove boxes.
   - Procedures for safe removal of contaminated waste.
   - Decontamination procedures.
8. The employer must review and evaluate the effectiveness of the CHP at least annually and update it as necessary.

Worker Training Must Include:

- Methods and observations that may be used to detect the presence or release of a hazardous chemical (such as monitoring conducted by the employer, continuous monitoring devices, visual appearance or odor of hazardous chemicals when being released, etc.).
- The physical and health hazards of chemicals in the work area.

- The measures workers can take to protect themselves from these hazards, including specific procedures the employer has implemented to protect workers from exposure to hazardous chemicals, such as appropriate work practices, emergency procedures, and personal protective equipment to be used.
- The applicable details of the employer's written CHP.

Medical Exams and Consultation

The employer must provide all personnel who work with hazardous chemicals an opportunity to receive medical attention, including any follow-up examinations which the examining physician determines to be necessary, under the following circumstances:

- Whenever a worker develops signs or symptoms associated with a hazardous chemical to which the worker may have been exposed in the laboratory, the worker must be provided an opportunity to receive an appropriate medical examination.
- Where exposure monitoring reveals an exposure level routinely above the action level (or in the absence of an action level, the PEL) for an OSHA regulated substance for which there are exposure monitoring and medical surveillance requirements, medical surveillance must be established for the affected worker(s) as prescribed by the particular standard.
- Whenever an event takes place in the work area such as a spill, leak, explosion or other occurrence resulting in the likelihood of a hazardous exposure, the affected worker(s) must be provided an opportunity for a medical consultation to determine the need for a medical examination.
- All medical examinations and consultations must be performed by or under the direct supervision of a licensed physician and be provided without cost to the worker, without loss of pay and at a reasonable time and place.

For additional information on developing a CHP, consult the following sources:

- View the complete standard at the OSHA Web site, www.osha.gov.
- Appendix A of 29 CFR 1910.1450 provides non-mandatory recommendations to assist in developing a CHP.

This is one in a series of informational fact sheets highlighting OSHA programs, policies or standards. It does not impose any new compliance requirements. For a comprehensive list of compliance requirements of OSHA standards or regulations, refer to Title 29 of the Code of Federal Regulations. This information will be made available to sensory-impaired individuals upon request. The voice phone is (202) 693-1999; the teletypewriter (TTY) number is (877) 889-5627.

For assistance, contact us. We can help. It's confidential.

OSHA FS-3461 8/2011
DSG

OSHA's Change Over to the Globally Harmonized System (GHS) of Classification, Labeling of Chemicals, and SDS

Fleming Training Center | TN Department of Environment & Conservation

1

Compliance Dates

Effective Dates

The table below summarizes the phase-in dates required under the revised Hazard Communication Standard (HCS):

Effective Completion Date	Requirement(s)	Who
December 1, 2013	Train employees on the new label elements and safety data sheet (SDS) format.	Employers
June 1, 2015* December 1, 2015	Compliance with all modified provisions of this final rule, except: The Distributor shall not ship containers labeled by the chemical manufacturer or importer unless it is a GHS label	Chemical manufacturers, importers, distributors and employers
June 1, 2016	Update alternative workplace labeling and hazard communication program as necessary, and provide additional employee training for newly identified physical or health hazards.	Employers
Transition Period to the effective completion dates noted above	May comply with either 29 CFR 1910.1200 (the final standard), or the current standard, or both	Chemical manufacturers, importers, distributors, and employers

2

Training

- Why?
  - Some suppliers/distributors are already using the new Safety Data Sheet format and labels
  - To ensure all employees are able to interpret the new labels and Safety Data Sheets
  - Unique to each facility and is provided by the **employer**
- Who?
  - Essentially any employee *potentially exposed* to chemicals as part of their routine job. That means everyone.
    - e.g. an employee occasionally picking up a bottle of Windex to wipe down a door would not need training; however, an employee who uses Windex regularly would

3

WHAT IS A HAZARDOUS CHEMICAL UNDER GHS?

Hazard Classification

4

Hazardous Chemical

- A chemical is defined as hazardous when it is classified as one of the following:
  - Health hazard
  - Physical hazard
  - Simple asphyxiant
  - Combustible dust
  - Pyrophoric gas
  - Hazard not otherwise classified

5

Health Hazard Classification

- A chemical is classified as a health hazard if it poses one of the following effects:
  - Acute oral toxicity (any route)
  - Skin corrosion or irritation
  - Serious eye damage or eye irritation
  - Respiratory or skin sensitization
  - Germ cell mutagenicity
  - Carcinogenicity
  - Reproductive toxicity
  - Specific target organ toxicity
  - Aspiration hazard

6

Physical Hazard Classification

- A chemical that poses one of the following hazardous effects:
  - Explosive
  - Flammable
  - Oxidizer
  - Self-reactive
  - Pyrophoric
  - Self-heating
  - Organic peroxide
  - Corrosive to metal
  - Gas under pressure
  - In contact with water emits flammable gas

7

Simple Asphyxiant Classification

- A chemical is classified as such if it displaces oxygen in the ambient atmosphere and can cause oxygen deprivation leading to unconsciousness and death
  - For example:
    - Nitrogen
    - Carbon dioxide
    - Hydrogen
    - Methane

8

Combustible Dust

- NFPA 654 (2006) and NEP Definitions
  - *Combustible Dust* A combustible particulate solid that presents a fire or deflagration hazard when suspended in air or some other oxidizing medium over a range of concentrations, regardless of particle size or shape
  - *Combustible Particulate Solid* Any combustible solid material, composed of distinct particles or pieces, regardless of size, shape or chemical composition

NFPA 69 (2002), and 499 (2004) Definitions

- *Combustible Dust.* Any finely divided solid material 420 microns* or less in diameter (i.e., material passing through a U.S. No 40 Standard Sieve) that presents a fire or explosion hazard when dispersed

9

Combustible Dusts

Agricultural Products	Cottonseed	Soybean dust	**Chemical Dusts**	Epoxy resin
Egg white	Garlic powder	Spice dust	Adipic acid	Melamine resin
Milk, powdered	Gluten	Spice powder	Anthraquinone	Melamine, molded (phenol-cellulose)
Milk, nonfat, dry	Grass dust	Sugar (10x)	Ascorbic acid	Melamine, molded (wood flour and mineral filled phenol-formaldehyde)
Soy flour	Green coffee	Sunflower	Calcium acetate	(poly) Methyl acrylate
Starch, corn	Hops (malted)	Sunflower seed dust	Calcium stearate	(poly) Methyl acrylate, emulsion polymer
Starch, rice	Lemon peel dust	Tea	Carboxy-methylcellulose	Phenolic resin
Starch, wheat	Lemon pulp	Tobacco blend	Dextrin	(poly) Propylene
Sugar	Linseed	Tomato	Lactose	Terpene-phenol resin
Sugar, milk	Locust bean gum	Walnut dust	Lead stearate	Urea-formaldehyde/ cellulose, molded
Sugar, beet	Malt	Wheat flour	Methyl-cellulose	(poly) Vinyl acetate/ ethylene copolymer
Tapioca	Oat flour	Wheat grain dust	Paraformaldehyde	(poly) Vinyl alcohol
Whey	Oat grain dust	Wheat starch	Sodium ascorbate	(poly) Vinyl butyral
Wood flour	Olive pellets	Xanthan gum	Sodium stearate	(poly) Vinyl chloride/ ethylene/vinyl acetylene suspension copolymer
	Onion powder		Sulfur	(poly) Vinyl chloride/ vinyl acetylene emulsion copolymer
Agricultural Dusts	Parsley (dehydrated)	**Carbonaceous Dusts**		
Alfalfa	Peach	Charcoal, activated	**Metal Dusts**	
Apple	Peanut meal and skins	Charcoal, wood	Aluminum	
Beet root	Peat	Coal, bituminous	Bronze	
Carrageen	Potato	Coke, petroleum	Iron carbonyl	
Carrot	Potato flour	Lampblack	Magnesium	
Cocoa bean dust	Potato starch	Lignite	Zinc	
Cocoa powder	Raw yucca seed dust	Peat, 22%H$_2$0		
Coconut shell dust	Rice dust	Soot, pine	**Plastic Dusts**	
Coffee dust	Rice flour	Cellulose	(poly) Acrylamide	
Corn meal	Rice starch	Cellulose pulp	(poly) Acrylonitrile	
Cornstarch	Rye flour	Cork	(poly) Ethylene (low-pressure process)	
Cotton	Semolina	Corn		

10

Pyrophoric Gas Classification

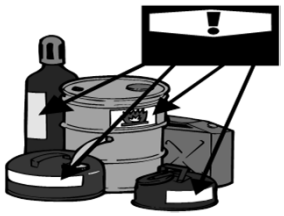
- A chemical in a gaseous state that will ignite spontaneously in air at a temperature of 130°F
  - For example:
    - Arsine
    - Silane
    - Metal carbonyls (dicobalt octacarbonyl, nickel carbonyl)
    - Diborane

11

Hazard Not Otherwise Classified Classification

- A chemical is classified as such when there is an adverse physical or health effect identified through evaluation of scientific evidence that does not meet the specified criteria for the physical and health hazard classes
- Not required on the label, but should be on the SDS
- Does not apply to adverse physical and health hazards under a GHS category that was not adopted by OSHA, such as acute toxicity Category 5

12



LABELING REQUIREMENTS

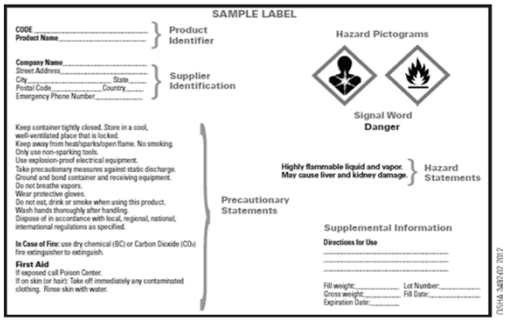
Labeling is the Law

13

Incoming Containers

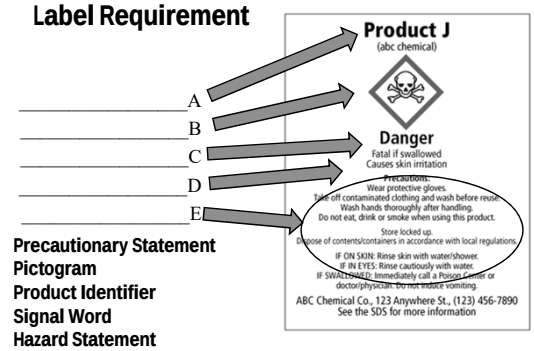
- The requirement to have labels is unchanged
- But the label content has changed
- The chemical manufacturer, distributor, or importer must label a container with
  - Product identifier
  - Signal word
  - Hazard statement(s)
  - Pictogram
  - Precautionary statement(s)
  - Name, address, telephone number of manufacturer, distributor or importer
  - Manufacturers, importers, will not ship containers without GHS labels after June 1, 2015
  - Distributors after December 1, 2015

14



15

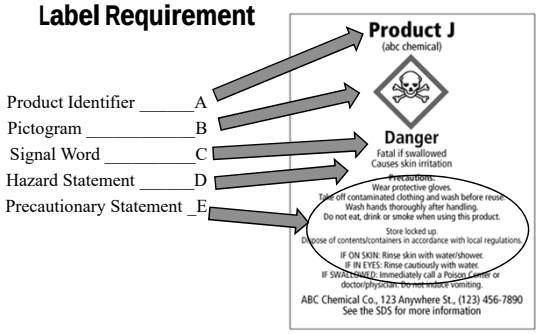
Manufacturer Container Label Requirement



Precautionary Statement
Pictogram
Product Identifier
Signal Word
Hazard Statement

16

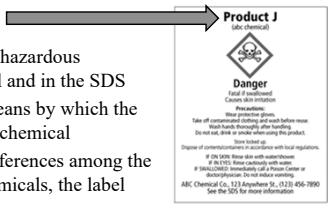
Manufacturer Container Label Requirement



17

Product Identifier

- The name used for a hazardous chemical on the label and in the SDS
- Provides a unique means by which the user can identify the chemical
- Shall permit cross-references among the list of hazardous chemicals, the label and the SDS



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Signal Word

- Used to indicate the relative level of severity of hazard and alert the reader to a potential hazard
- One, but not both, of the following
  - Danger—more severe hazard
  - Warning—less severe hazard

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Hazard Statement

- Assigned to a hazard class and hazard category and describes the nature of the hazard
- Examples
  - Fatal if swallowed
  - May cause damage to *kidneys* through prolonged or repeated exposure
  - May cause or intensify fire
  - Extremely flammable liquid or vapor
  - Heating may cause an explosion

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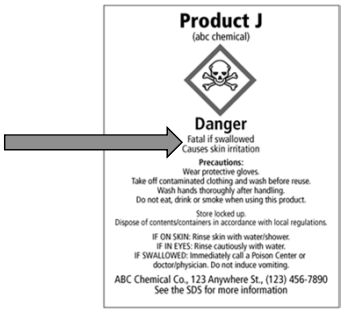
Hazard Statement



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Precautionary Statements

- A phrase that describes recommended measures that should be taken to minimize or prevent adverse effects resulting from exposure or improper storage or handling
- Prevention
- Response
- Storage
- Disposal
- They can be combined or consolidated to save space on the label

22

Precautionary Statement

23

Pictograms

- Nine are designated by GHS
- Eight are adopted by OSHA
- No duplicates or blank diamonds allowed on the label
- Correct name for the diamond is "squares-on-point"

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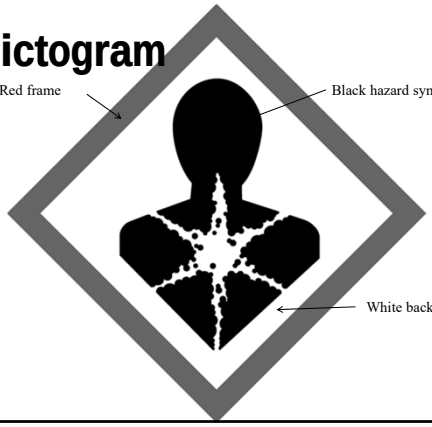
Pictogram



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Health Hazard

- Carcinogen
- Mutagenicity
- Reproductive Toxicity
- Respiratory Sensitizer
- Target Organ Toxicity
- Aspiration Toxicity

26

Skull and Crossbones

- Acute Toxicity

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Flame

- Flammables
- Pyrophorics
- Self-Heating
- Emits Flammable Gas
- Self Reactives
- Organic Peroxides

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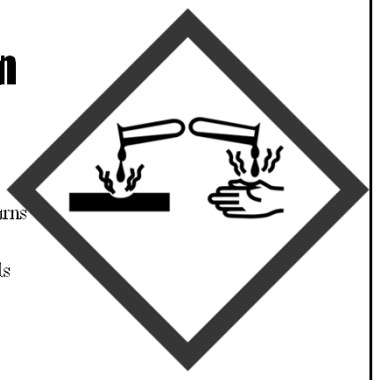
Flame Over Circle

- Oxidizers

29

Corrosion



- Skin Corrosion/Burns
- Eye Damage
- Corrosive to Metals

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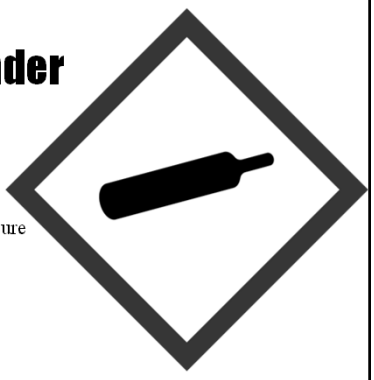
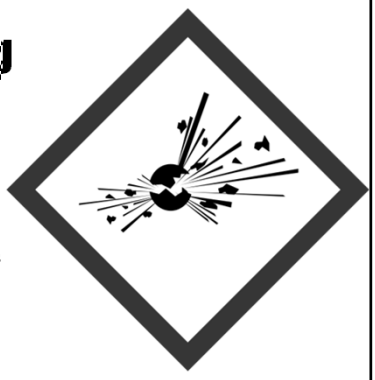
Gas Cylinder



- Gases Under Pressure

31

Exploding Bomb



- Explosives
- Self-Reactives
- Organic Peroxides

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Exclamation Mark

- Irritant (skin and eye)
- Skin Sensitizer
- Acute Toxicity-low
- Narcotic Effects
- Respiratory Tract Irritant
- Hazardous to Ozone Layer (-non-mandatory)
- (Low degree health hazard)

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Environmental (non-mandatory)

OSHA Does Not Enforce This One

- Aquatic Toxicity

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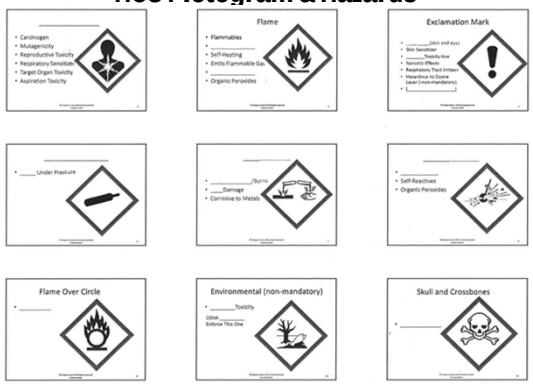
HCS Pictogram & Hazards



Pictogram

HCS Pictograms and Hazards

Health Hazard	Flame	Exclamation Mark
▪ Carcinogen ▪ Mutagenicity ▪ Reproductive Toxicity ▪ Respiratory Sensitizer ▪ Target Organ Toxicity ▪ Aspiration Toxicity	▪ Flammables ▪ Pyrophorics ▪ Self-Heating ▪ Emits Flammable Gas ▪ Self-Reactives ▪ Organic Peroxides	▪ Irritant (skin and eye) ▪ Skin Sensitizer ▪ Acute Toxicity ▪ Narcotic Effects ▪ Respiratory Tract Irritant ▪ Hazardous to Ozone Layer (Non-Mandatory) ▪ Low degree health hazard
Gas Cylinder	**Corrosion**	**Exploding Bomb**
▪ Gases Under Pressure	▪ Skin Corrosion/Burns ▪ Eye Damage ▪ Corrosive to Metals	▪ Explosives ▪ Self-Reactives ▪ Organic Peroxides
Flame Over Circle	**Environment (Non-Mandatory)**	**Skull and Crossbones**
▪ Oxidizers	▪ Aquatic Toxicity ▪ OSHA Does Not Enforce This One	▪ Acute Toxicity (fatal or toxic)

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Workplace Labels
(Transfer containers)

- The employer shall ensure that each container is labeled with either
  - Product identifier
  - Signal word
  - Hazard statement(s)
  - Pictogram

  Or
  - Product identifier and
  - Adequate information about the hazards
  - Employers must comply by June 1, 2016

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Transfer Container Labeling Exemption Continues

- Portable containers
  - Identity and hazard information (or product identifier, signal word, hazard statement, signal word, pictogram) must be transferred unless the portable container is:
    - Under the control at all times of the employee making the transfer from the labeled container and
    - Contents used up in one shift

Employers must comply by June 1, 2016

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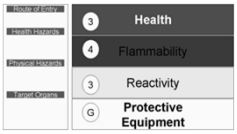
Alternative Labeling



- Permitted when employer's overall program proven effective
- Must ensure employees fully aware of hazards/use and understanding of labeling system
- Employer bears burden of establishing that employee awareness equals or exceeds conventional labeling system

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Workplace Labeling

- Can HMIS or NFPA system be used?
- While, the hazard category does not appear on the label, consider

GHS		HMIS/NFPA	
Category	Hazard	Category	Hazard
1	highest	1	slight
2	high	2	moderate
3	medium	3	serious
4	low →	4	severe

NFPA categories were intended for emergency response, not workplace hazards; only considers acute effects, does not consider chronic effects

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Labeling Effective Dates

- Chemical manufacturers, importers, and employers
  - Will not ship containers without GHS labeling/SDS by June 1, 2015
- Employers
  - By June 1, 2016
    - Update alternative workplace labeling and hazard communication program as necessary, and provide additional employee training for newly identified physical or health hazards.

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SAFETY DATA SHEETS (SDS)

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Safety Data Sheet Info

- In English
- New 16-section format
- Compliance date for chemical manufactures, imports and distributors —June 1, 2015
- **Example pH 7 below**

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Safety Data Sheet Sections

- Section 1 Identification
- Section 2 Hazard(s) identification
- Section 3 Composition/information on ingredients
- Section 4 First-aid measures
- Section 5 Fire-fighting measures
- Section 6 Accidental release measures
- Section 7 Handling and storage
- Section 8 Exposure controls/personal protection
- Section 9 Physical and chemical properties
- Section 10 Stability and reactivity
- Section 11 Toxicological information
- Section 12 Ecological information
- Section 13 Disposal considerations
- Section 14 Transport information
- Section 15 Regulatory information

Information in these sections will not be enforced by OSHA

- Section 16 Other information, including date of preparation or last revision

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Section 1

Section 1 – Chemical Product and Company Identification

Catalog Numbers: 40475
Product Identity: Buffer Soln. pH 7.00

Manufacturer's Name: AquaPhoenix Scientific, Inc., 9 Barnhart Dr., Hanover, PA 17331
Emergency Contact Number (24hr): InfoTrac (800) 535-5053

Identification Of The Substrate Or Mixture And Of The Supplier

- GHS product identifier
- Other means of identification
- Recommended use of the chemical and restrictions on use
- Supplier's details
  - Name, address, phone #
- Emergency phone number

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Section 2

Section 2 – Composition, Information on Ingredients

Sodium Phosphate, Dibasic, CAS# 7558-79-4, <3% w/v, ACGIH TLV: NA, OSHA PEL: NA
Potassium Phosphate, Monobasic, CAS# 7778-77-0, <2% w/v, ACGIH TLV: NA, OSHA PEL: NA
Water, purified, CAS# 7732-18-5, >95% w/v, ACGIH TLV: NA, OSHA PEL: NA

Hazards Identification

- GHS classification of the substance/mixture and any national or regional information
- GHS Label elements, including precautionary statements
  - Hazard symbols may be provided as a graphical reproduction of the symbols in black and white or the name of the symbol,
    - e.g. flame, skull and crossbones
- Other hazards which do not result in classification or are not covered by the GHS

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Section 3

Section 3 – Hazard Identification

Emergency Overview: Non-flammable, non-corrosive, non-toxic. Does not present significant health hazards. Wash areas of contact with water.
Target Organs: Eyes, skin.

Potential Health Effects
Eyes: May cause slight irritation.
Skin: May cause slight irritation.
Ingestion: Large doses may cause upset stomach
Inhalation: Not likely to be a hazard
Chronic Effect / Carcinogenicity: None (IARC, NTP, OSHA)

Composition/Information Ingredients

- Substance
  - Chemical identity
  - Common name, synonyms, etc.
  - CAS number, EC number, etc.
  - Impurities and stabilizing additives which are themselves classified and which contribute to the classification of the substance
- Mixture
  - The chemical identity and concentration or concentration ranges of all ingredients which are hazardous within the meaning of the GHS and are present above their cutoff levels

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Section 4

Section 4 – First Aid

Eyes: Immediately flush eyes with water for at least 15 minutes. Immediately get medical assistance.
Skin: Flush with water for 15 minutes. Get medical assistance if irritation develops.
Ingestion: Dilute with water or milk. Get medical assistance.
Inhalation: Remove to fresh air. Give artificial respiration if necessary. If breathing is difficult, give oxygen.

First Aid Measures

- Description of necessary measures, subdivided according to the different routes of exposure
  - i.e. inhalation, skin and eye contact, and ingestion
- Most important symptoms/effects, acute and delayed
- Indication of immediate medical attention and special treatment needed, if necessary

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Section 5

Section 5 – Fire Fighting Measures

Flash Point: NA
Extinguishing Media: Use means suitable to extinguishing surrounding fire.
Fire & Explosion Hazards: Not considered to be a fire or explosion hazard.
Fire Fighting Instructions / Equipment: Use normal procedures. Poisonous gases may be produced in fire. Use protective clothing. Use NIOSH-approved breathing equipment.
NFPA Rating: (estimated) Health: 1; Flammable: 0; Reactivity: 0

Firefighting Measures

- Suitable (and unsuitable) extinguishing media
- Specific hazards arising from the chemical
  - e.g. nature of any hazardous combustion products
- Special protective equipment and precautions for firefighters

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Section 6

Section 6 – Accidental Release Measures

Absorb with suitable material. Always obey local regulations.

Accidental Release Measures

- Personal precautions, protective equipment and emergency procedures
- Environmental precautions
- Methods and materials for containment and cleaning up

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Section 7

Section 7 – Handling and Storage

Handling: Wash hands after handling. Avoid contact with skin and eyes.
Storage: Protect from freezing and physical damage.

Handling and Storage

- Precautions for safe handling
- Conditions for safe storage, including any incompatibilities

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Section 8

Section 8 – Exposure Controls, Personal Protection

Engineering Controls: Normal ventilation is adequate.

Page 1 of 2 40475

Respiratory Controls: Normal ventilation is adequate.
Skin Protection: Chemical resistant gloves.
Eye Protection: Safety Glasses or goggles.

Exposure Controls/Personal Protection

- Control parameters
  - e.g. occupational exposure limit values or biological limit values
- Appropriate engineering controls
- Individual protection measures, such as PPE

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Section 9

Section 9 – Physical and Chemical Properties

Appearance: Clear, yellow liquid
pH: 5.8-8
Boiling Point: Approx 100C
Melting Point: Approx 0 C
Odor: Odorless
Solubility in Water: Infinite
Specific Gravity: Approx 1
Vapor Pressure: NA

Physical and Chemical Properties

- Appearance
- Odor
- Odor threshold
- pH
- Melting point/freezing point
- Initial boiling point and boiling range
- Flash point
- Flammability
- Upper/lower flammability or explosive limits
- Vapor pressure
- Vapor density
- Relative density
- Solubility(ies)
- Partition coefficient
- Autoignition temperature
- Decomposition temperature

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Section 10

Section 10 – Stability and Reactivity

Chemical Stability: Stable under normal conditions of use and storage.
Incompatibility: None Identified.
Hazardous Decomposition Products: Oxides of Phosphorus
Hazardous Polymerization: Does not occur

Stability and Reactivity

- Chemical stability
- Possibility of hazardous reactions
- Conditions to avoid
  - e.g. static discharge, shock or vibration
- Incompatible materials
- Hazardous decomposition products

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Section 11

Section 11 – Toxicological Information

LD50 orl-rat: 17 g/kg (Sodium Phosphate, Dibasic)
LC50 inhalation-rat: >4640 mg/kg (Potassium Phosphate, Monobasic)

Toxicological Information

- Concise but complete comprehensible description of the various toxicological (health) effects and the available data used to identify those effects
- Includes:
  - Information on the like routes of exposure
  - Symptoms related to the physical, chemical and toxicological characteristics
  - Delayed and immediate effects and also chronic effects from short and long term exposures
  - Numerical measures of toxicity
    - LD – Lethal does; amount ingested that kills 50% of test sample.

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Section 12

Information in this section will not be enforced by OSHA

Section 12 – Ecological Information

Ecotoxicity: NA

Ecological Information

- Eco-toxicity
- Persistence and degradability
- Bio-accumulative potential
- Mobility in soil
- Other adverse effects

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Section 13

Information in this section will not be enforced by OSHA

Section 13 – Disposal Considerations

Dilute with water.

All chemical waster generators must determine whether a discarded chemical is classified as hazardous waste.

Comply with all local, state, and federal regulations.

Disposal Considerations

- Description of waste residues and information on their safe handling and methods of disposal
  - Including the disposal of any contaminated packaging

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Section 14

Information in this section will not be enforced by OSHA

Transport Information

- UN number
- UN proper shipping name
- Transport hazard class(es)
- Packing group, if applicable
- Marine pollutant (yes/no)
- Special precautions which a user needs to be aware of or needs to comply with in connection with transport or conveyance either within or outside their premises

Section 14 – Transport Information

DOT - Not Regulated

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Section 15

Information in this section will not be enforced by OSHA

Regulatory Information

- Safety, health and environmental regulations specific for the product in question

Section 15 – Regulatory Information (not meant to be all inclusive)

OSHA Status: These chemicals are not considered hazardous by OSHA.
TSCA: The components of this solution are listed on the TSCA Inventory
SARA Title III Section 313: Not Applicable
RCRA Status: NA
CERCLA Reportable Quantity: Sodium Phosphate, Dibasic – 5,000 lbs.
WHMIS: NA

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Section 16

Other Information Including Information on Preparation and Revision of the SDS

Section 16 – Additional Information

Issue Date: 12/28/06
Revision Date: 6/5/08, 11/19/09

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EFFECTS ON OTHER STANDARDS

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Flammable Liquids

GHS FL Category	Flashpoint Deg F	Boiling Point Deg F	Old OSHA Class	Flashpoint Deg F	Boiling Point Deg F
1	**<73.4**	**≤95**	1A	<73	<100
2	**<73.4**	**<95**	1B	<73	≥100
3	**≥73.4 and ≤140**		1C II	≥73 and <100 ≥100 and <140	
4	**>140 and ≤199.4**		IIIA	≥140 and <200	
None			IIIB	>200	

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WHAT-TO-DO BOOKLET

A Template for Compliance With

(29 CFR 1910.1200 and 29 CFR 1926.59)

Hazard Communication Standard

And

(0800-1-1-.09)

The Tennessee Hazardous Chemical Right-To-Know Law

Revised November, 2012

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Resources

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

- CDC works 24/7 to protect America from health, safety and security threats, both foreign and in the U.S. Whether diseases start at home or abroad, are chronic or acute, curable or preventable, human error or deliberate attack, CDC fights disease and supports communities and citizens to do the same. **www.cdc.gov**

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Resources

- **OSHA (www.osha.gov)**

  - "No one should have to sacrifice their life for their livelihood, because a nation built on the dignity of work must provide safe working conditions for its people."
    -Secretary of Labor Thomas E. Perez

  - Under federal law, you are entitled to a safe workplace. Your employer must provide a workplace free of known health and safety hazards. If you have concerns, you have the right to speak up about them **without fear of retaliation**. You also have the right to:

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TN Department of Environment and Conservation

- **OSHA (www.osha.gov)**
  - Be trained in a language you understand
  - Work on machines that are safe
  - Be provided required safety gear, such as gloves or a harness and lifeline for falls
  - Be protected from toxic chemicals
  - Request an OSHA inspection, and speak to the inspector
  - Report an injury or illness, and get copies of your medical records
  - See copies of the workplace injury and illness log
  - Review records of work-related injuries and illnesses
  - Get copies of test results done to find hazards in the workplace

66

Resources

- **TOSHA**
  - Tennessee OSHA improves occupational safety and health through enforcement of the general industry, construction and agricultural occupational safety and health standards in workplaces. - See more at: https://www.tn.gov/workforce/section/tosha

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Resources

- **TOSHA**
  - TOSHA's mission is to assure the safety and health of Tennessee's working men and women
  - by promulgating and enforcing standards and regulations; providing training, outreach, and education;
  - establishing cooperative programs; and encouraging continual improvement in workplace safety and health
  - as well as the development of comprehensive safety and health management systems. Effective and
  - efficient use of resources requires careful, flexible planning. In this way, the overall goal of hazard
  - abatement and employee protection is best served.

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Resources

- **OSHA www.osha.gov**
- **CDC www.cdc.gov**
- **TOSHA https://www.tn.gov/workforce/article/standards-and-rules**
  - **Memphis Office 901-543-7259**
  - **Jackson Office 731-423-5641**
  - **Nashville Office 615-741-2793 1-800-249-8510**
  - **Knoxville Office 865-594-6180**
  - **Kingsport Office 423-224-2042**
  - **Chattanooga 423-634-6424**

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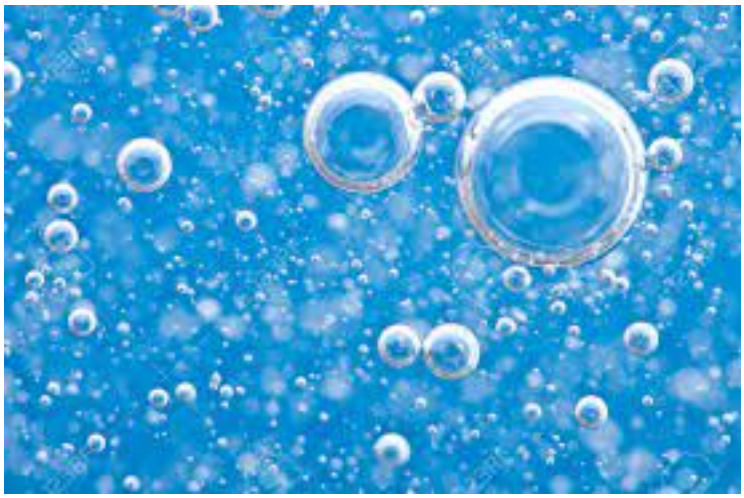
Section 2
Dissolved
Oxygen



Wastewater Laboratory Class

Dissolved Oxygen Measurement

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Definition

- Dissolved Oxygen
  - "DO" for short
  - Measurement of the amount of oxygen dissolved in a unit volume of water
  - Key parameter to monitor in both wastewater and drinking water treatment

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Dissolved Oxygen

- Useful for maintaining a body of water that is fit for swimming, fishing, and/or as a source of potable water
  - Receiving stream could also be used as a source for drinking water plant

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Why is DO important?

- Wastewater applications:
  1. Affects the health of treatment microorganisms and thus the speed and efficiency of the treatment process
  - optimum level of DO necessary for process
    - too low DO = bacteria die
    - too high DO = process becomes very costly

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Why is DO important?

- Wastewater applications:
  2. Sufficient DO must be present to protect fish and aquatic life in receiving streams
     - DO levels must be kept above permit limit minimum
     - Low DO can lead to fish kill
  3. DO measurements are used to determine the Biochemical Oxygen Demand (BOD), a measure of organic waste concentration

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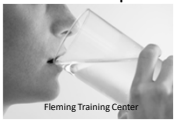
Why is DO important?

- Drinking water:
  1. Low or zero DO levels at the bottom of lakes or reservoirs often cause flavor and odor problems in drinking water
  2. Presence of DO in drinking water can contribute to corrosion of piping systems
  3. The flavor of water is improved by DO



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Dissolved Oxygen

- Dissolved oxygen levels in natural and wastewaters depend on the physical, chemical, and biochemical activities in the water body.

7

Saturation Concentration

- DO methods measure O_2, the same form of oxygen in air.
- There is always a two-way exchange of oxygen molecules between the gas phase and the liquid phase.
- When the rate of the molecules dissolving into the water equals the rate at which they volatize out, the system is in equilibrium and the concentration is called the ***Saturation Concentration.***

8

Saturation Concentration

- The DO saturation concentration decreases as salinity (TDS) increases

Table 9.13 Saturation concentrations for wastewater DO under different conditions

	Wastewater mg/L[a]	
Temperature	0 (sea level)	2,000 ft elevation
5°C (41°F)	12.8	11.9
10°C (50°F)	11.3	10.5
15°C (59°F)	10.1	9.4
20°C (68°F)	9.1	8.5
25°C (77°F)	8.3	7.7

[a]0.05% dissolved salts, 760 mmHg pressure. Calculated from: https://water.usgs.gov/software/DOTABLES/

Source: Operation of Wastewater Treatment, Vol 1, ed. 8

9

Saturation Concentration

- Amount of Oxygen that a given volume of water can hold depends on:

1. Air pressure (and thus altitude)
2. Temperature of the water*
3. The amount of other substances dissolved in the water (ex: salinity)

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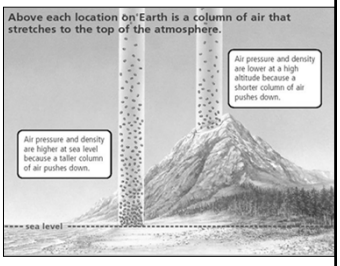
Barometric Pressure (BP)

- Barometric pressure = The pressure of the column of air above us
- The higher we go up, the less that pressure becomes
- Lower barometric pressures are found at higher elevations



11

Barometric Pressure

- Where to find barometric pressure:
  - Barometer (in lab), onboard barometer (built into meter), online (ex: weather app, airport, etc.)
- When you get a BP reading from the airport, it has been "corrected" to sea level
  - You need to uncorrect the BP before calibrating your DO probe!
  - In other words, you must determine the true barometric pressure

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Conversions - BP

- Your barometer may read in mm or in Hg (mercury)
  - Likewise, DO Tables can display either mm or in Hg
- To convert inches of mercury (Hg) to mm of mercury (Hg):
  inches of Hg X 25.4 = mm of Hg
- To convert mm of mercury (Hg) to inches of mercury (Hg):
  mm of Hg ÷ 25.4 = inches of Hg

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Why is BP important?

- You must compensate for barometric pressure when calibrating the instrument
- Weather condition variations are typically +/- 1% from week to week
  - Extreme weather can cause variations up to 4x larger
- You can see 1-2% variations in readings between weeks

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Cause of barometric pressure change	Period	Pressure change		
		mbars	In. Hg	In. H_2O
Diurnal variation	1 day	3–4	0.09–0.12	1.2–1.6
Weather systems	2–5 days	17–34	0.5 – 1.0	7–13
Severe storms	2–10 hours	34–68	1.0–2.0	13–27
Severe hurricane	2–5 days	166	5	68

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How to Uncorrect a Barometric Pressure

1. Determine the altitude (in feet) of your lab/facility
2. Determine the correction factor for your lab/facility
   CF = [760 – (Altitude x 0.026)] ÷ 760
3. Look up the barometric pressure (BP) for the day
   - Ex: Airport website, Weather Channel or app
4. Multiply the corrected BP times your Correction Factor (CF)

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Example

1. Fleming Training Center altitude = 543 feet
2. Plug that altitude into the Correction Factor equation:
   CF = [760 - (Altitude x 0.026)] ÷ 760
   = [760 - (543 x 0.026)] ÷ 760
   = [760-14.1] ÷ 760
   = 745.9 ÷ 760 = 0.9795
3. Use your cell phone to find the barometric pressure (Ex: Airport BP = 29.65 in Hg…remember, this is a *corrected* BP)
4. Multiply 29.65 x 0.9795 = 29.04

Therefore, your uncorrected BP is 29.04 in Hg

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Example (continued)

- You may have to convert inches of mercury (Hg) to mm of mercury (Hg):
  inches of Hg X 25.4 = mm of Hg
  Therefore, 29.04 X 25.4 = 737.6 mm Hg
- Your uncorrected BP is either 29.04 inches of Hg or 737.6 mm of Hg.

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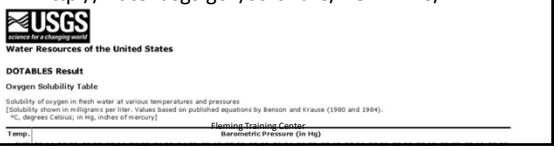
Example (continued)

- Once you have the uncorrected true BP, determine the oxygen solubility at that pressure and temperature
- Use the DO Saturation Table to obtain the maximum O_2 solubility at that temperature

http://water.usgs.gov/software/DOTABLES/


USGS science for a changing world

Water Resources of the United States

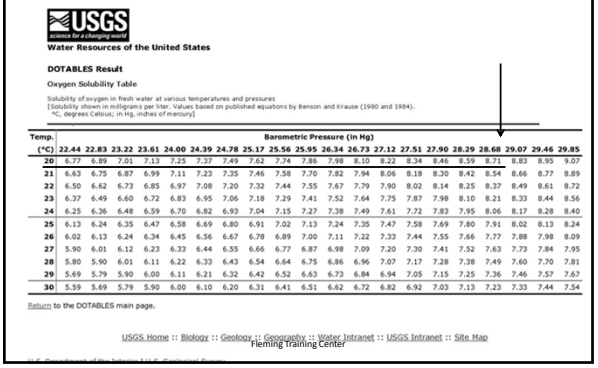
DOTABLES Result

Oxygen Solubility Table

Solubility of oxygen in fresh water at various temperatures and pressures
[Solubility shown in milligrams per liter. Values based on published equations by Benson and Krause (1980 and 1984).
°C, degrees Celsius; in Hg, inches of mercury]

Temp. | Barometric Pressure (in Hg)

Example (continued)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DOTABLES Result

Oxygen Solubility Table

Solubility of oxygen in fresh water at various temperatures and pressures
[Solubility shown in milligrams per liter. Values based on published equations by Benson and Krause (1980 and 1984).
°C, degrees Celsius; in Hg, inches of mercury]

Temp. (°C)	Barometric Pressure (in Hg)																		
	22.44	22.83	23.22	23.61	24.00	24.39	24.78	25.17	25.56	25.95	26.34	26.73	27.12	27.51	27.90	28.29	28.68	29.07	29.46	29.85
20	6.77	6.89	7.01	7.13	7.25	7.37	7.49	7.62	7.74	7.86	7.98	8.10	8.22	8.34	8.46	8.59	8.71	8.83	8.95	9.07
21	6.63	6.75	6.87	6.99	7.11	7.23	7.35	7.46	7.58	7.70	7.82	7.94	8.06	8.18	8.30	8.42	8.54	8.66	8.77	8.89
22	6.50	6.62	6.73	6.85	6.97	7.08	7.20	7.32	7.44	7.55	7.67	7.79	7.90	8.02	8.14	8.25	8.37	8.49	8.61	8.72
23	6.37	6.49	6.60	6.72	6.83	6.95	7.06	7.18	7.29	7.41	7.52	7.64	7.75	7.87	7.98	8.10	8.21	8.33	8.44	8.56
24	6.25	6.36	6.48	6.59	6.70	6.82	6.93	7.04	7.15	7.27	7.38	7.49	7.61	7.72	7.83	7.95	8.06	8.17	8.28	8.40
25	6.13	6.24	6.35	6.47	6.58	6.69	6.80	6.91	7.02	7.13	7.24	7.35	7.47	7.58	7.69	7.80	7.91	8.02	8.13	8.24
26	6.02	6.13	6.24	6.34	6.45	6.56	6.67	6.78	6.89	7.00	7.11	7.22	7.33	7.44	7.55	7.66	7.77	7.88	7.98	8.09
27	5.90	6.01	6.12	6.23	6.33	6.44	6.55	6.66	6.77	6.87	6.98	7.09	7.20	7.30	7.41	7.52	7.63	7.73	7.84	7.95
28	5.80	5.90	6.01	6.11	6.22	6.33	6.43	6.54	6.64	6.75	6.86	6.96	7.07	7.17	7.28	7.38	7.49	7.60	7.70	7.81
29	5.69	5.79	5.90	6.00	6.11	6.21	6.32	6.42	6.52	6.63	6.73	6.84	6.94	7.05	7.15	7.25	7.36	7.46	7.57	7.67
30	5.59	5.69	5.79	5.90	6.00	6.10	6.20	6.31	6.41	6.51	6.62	6.72	6.82	6.92	7.03	7.13	7.23	7.33	7.44	7.54

Return to the DOTABLES main page.

USGS Home :: Biology :: Geology :: Geography :: Water Intranet :: USGS Intranet :: Site Map

- **NOTE:** Pressure drops by 26 millimeters (mm, about 1 inch) for every 1000 feet above sea level. 26 ÷ 1000= 0.026
- That's why during the process, we multiply the altitude in feet by 0.026

Conversion - Temperature

- To convert from Celsius to Fahrenheit:

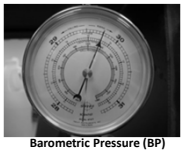
$$°F = [(°C)(1.8) + 32]$$

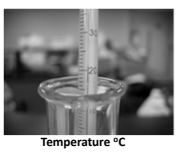
- To convert from Fahrenheit to Celsius:

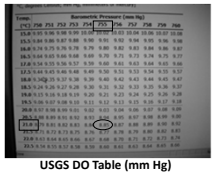
$$°C = \frac{(°F - 32)}{1.8}$$

Barometer on site

Easiest way to do this - Direct Read from Barometer & Thermometer in the lab to determine DO from USGS DO table

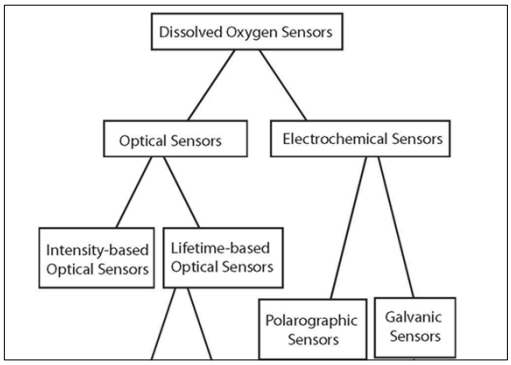

Barometric Pressure (BP) UNCORRECTED


Temperature °C


USGS DO Table (mm Hg)

Types of Measurement

- Electrode
  - (SM 4500 – O G - 2011)
- Optical probe (LDO)
  - (Hach Method 10360 Rev. 2011)
- Winkler titration
  - (Azide modification, SM 4500 – O (B – F) - 2011)



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Electrodes

- An electrode system where DO reacts at the cathode producing a measurable electrochemical effect.
- Effect can be galvanic (ability to conduct an electrical current), polarographic (electrochemical), or potentiometric (measurement of voltages).
- Most units are temperature compensated (thermistor or resistance thermometer)

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Types of Measurement

- DO probes and meters are the most commonly used method

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Electrode Method

- Polarographic probe
  - A voltage across 2 electrodes (in contact with electrolyte and separated from sample by a membrane) is provided by the meter, which causes oxygen molecules to be chemically changed to hydroxide ions through a reduction reaction…which means the DO is consumed by the probe
  - This reaction also consumes electrons and the meter reads the electron consumption as a current

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Electrode Method

- Oxygen gets inside the probe by diffusing through a plastic membrane at a rate that is proportional to the concentration in the water.
- Therefore, the concentration in the water determines the rate of oxygen flow into the probe, which determines the rate of oxygen consumption inside the probe and is read as a current by the meter and displayed as a concentration to the user

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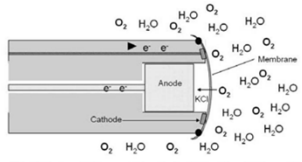

Figure 1. In electrochemical sensors, O_2 molecules diffuse through the membrane to the sensor at a rate proportional to the pressure difference across it.

Figure 3. On the right is a properly prepared cathode and anode. Note the light silver appearance of the anode and matte finish of the gold cathode.

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Electrode Method

- Meter calibrated to saturated conditions (water saturated air)
- Correction for altitude or barometric pressure
- Keep membrane clean
  - Clean probe after testing oily samples
  - Change membrane regularly
  - Erratic readings or inability to calibrate could indicate membrane failure

- Temperature calibration data verified

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Electrode Method

- Keep the membrane from drying out
  - As per manufacturer's instructions, store the probe in a saturated atmosphere or in a container with a wet sponge or paper towel
  - Do not store the probe submerged

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Dissolved Oxygen Analysis

- Analyze immediately
  - Maximum holding time = 15 minutes
- Preservation = none
- Sample should be measured in situ
- May be collected as a grab sample with minimal aeration into a B0D bottle with a glass stopper

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Remember - Hold Time

- 15 minutes – Note time of collection and time of analysis!

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Optical Method

Luminescent Dissolved Oxygen Probe

- No membrane
- No electrolyte to foul or poison
- Won't affect readings
- Accurate & stable readings

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Optical Method

- Blue light is shined on a special dye infused in a permeable membrane
- The light excites the atoms in the dye and when they fall back down to their normal energy state, they emit a red light
  - This is luminescence
- Oxygen molecules in the water interfere with the red light emission by limiting the intensity of the emitted red light and the length of time the luminescence occurs
- Both effects are directly related to the oxygen concentration

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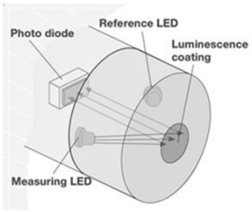
Optical Method

- In these sensors, the oxygen is not consumed, so stirring is usually not required
- Follow manufacturer's instructions, but sensors need to be periodically replaced
  - Replacement cycle often more than a year



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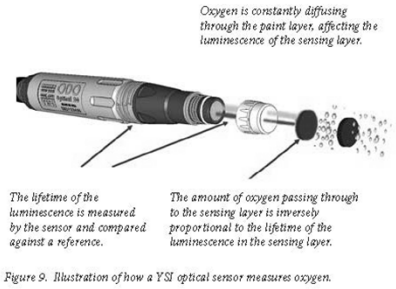

Figure 9. Illustration of how a YSI optical sensor measures oxygen.

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Winkler Method

- Classic wet chemistry procedure
- General principle: a series of reactions produces iodine in proportion to the amount of DO in the sample
- Using sodium thiosulfate with starch as the indicator, one can titrate the sample and determine the amount of DO
- Not commonly used in the industry any more
  - The Winkler method can be used to check the calibration or operation of a meter

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Winkler Method

- There are several modifications to the Winkler method since there are so many interferences
- The **Azide modification** effectively removes interference caused by nitrite, which is the most common interference in biologically treated effluents and incubated BOD samples

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Winkler Method

After addition of reagents, a manganese hydroxide floc appears if oxygen is present.

Titrate with 0.025M sodium thiosulfate.

Titrate until a pale straw color.

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Collecting DO samples

- Oxygen can dissolve from the atmosphere into the sample and change the reading
- If using a wide-mouth dipper or a bucket, you're likely to catch mainly water from the upper layer
  - Could have a higher DO than lower layers because it is in contact with the air

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Collecting DO Samples

- After collecting a sample, shield it from contact with the atmosphere
- Oxygen will dissolve into a container of water sitting open on a lab bench
- When taking DO readings in an aerated tank using a probe, do not place the probe directly over a diffuser
  - You want to measure the DO in the water, not the oxygen in the air supply to the aerator

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Taking D.O. Readings

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Common Deficiencies

- Samples for dissolved oxygen were collected in a bucket and then poured into the BOD bottle
- The D.O. probe was immersed in the water during calibration
- The D.O. probe had a water droplet on the end during calibration
- There was an air bubble under the membrane on the probe
- The meter was air calibrated by placing the probe on the counter

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Common Deficiencies

- New membranes and probes need a warm up time (electrochemical/polarographic)
- The calibration environment was not clean

Figure 6. The calibration bottle on the right is clean. The calibration bottle on the left actually has algae growing in it which could affect the calibration values.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- DOC
- Dup
- ICAL/CCV
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Demonstration of Capability
  - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
  - Hach Method 10360 9.2.1 – Prepare and measure four samples of air-saturated water according to section 7.2.
    - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
    - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
    - 7.2.3 – With a steady gentle stream of filtered air (≈ 10-40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Hach Method 10360 continued
  - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature (20 ± 3°C) for 30 minutes and note the barometric pressure [uncorrected] of the laboratory during preparation. The barometric temperature reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
  - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
  - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman…
- Real people language – prepare dilution water that is air-saturated and analyze four bottles and compare to the theoretical dissolved oxygen concentration.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Calibration
  - 7.1.1 – Add approximately 1 inch (2.54 cm) of reagent water to a clean BOD bottle and stopper).
  - 7.1.2 – Shake vigorously for ~ 10 seconds.
  - 7.1.3 – Allow for the BOD bottle and its contents to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
  - 7.1.4 – The stopper may now be removed from the BOD bottle and the LBOD probe inserted for calibration purposes.
- Real people language – **calibrate daily by following manufacturer's instructions**.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Calibration Verification (ICV) daily
  - 9.3.1 Upon air calibration, prepare a calibration verification standard (Section 7.2 – on the following slide) with each analytical batch of 20 samples or less in an 8 hour period

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Calibration Verification, Initial Precision and Recovery, and On-going Precision and Recovery (7.2)
  - 7.2.1 Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
  - 7.2.2 Allow water to equilibrate to room temperature (approximately 20± 3 °C
  - 7.2.3 With a steady stream of filtered air (≈10-40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Calibration Verification, Initial Precision and Recovery, and On-going Precision and Recovery (7.2)
  - 7.2.4 at the completion of aeration, let water re-equilibrate to room temperature (20± 3°C) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric reading is used in calculation and determination of the theoretical DO concentration for the preparation of air-saturated water
  - 7.2.5 Transfer the aerated water to a BOD bottle until overflowing and stopper
  - 7.2.6 Calculate the theoretical dissolved oxygen concentration using dissolved oxygen table.

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Continuing Calibration Verification (CCV) daily
  - 9.4.1 to demonstrate that the analysis system is in control, and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations
  - 9.4.2 prepare a precision and recovery standard (7.2) with each analytical batch
  - 9.4.3 Initially, and at the end of each analytical batch of samples, analyze a precision and recovery standard and compare the concentration recovery with the limits for ongoing precision and recovery

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DO Hach Method 10360, LDO Measurement Oct. 2011

- Duplicate
  - Real people language – analyze 2 samples for DO, grab sample in a bucket and dip probe twice to get two readings
  - Target value is to get close to the first value and have a small RPD
  - **2014 Update – For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum, then the minimum value should be reported even if falls outside your permit limit.**

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DO SM4500-O G – 2001 Membrane Electrode Method

- DOC
- Dup
- ICAL/CCV
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency

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DO SM4500-O G – 2016 Membrane Electrode Method

- Demonstration of Capability
  - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
  - Follow Hach Method 10360 9.2.1 – Prepare and measure four samples of air-saturated water according to section 7.2.
    - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
    - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
    - 7.2.3 – With a steady gentle stream of filtered air (≈ 10-40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.

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DO SM4500-O G – 2016 Membrane Electrode Method

- Hach Method 10360 continued
  - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature (20 ± 3°C) for 30 minutes and note the barometric pressure [uncorrected] of the laboratory during preparation. The barometric temperature reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.
  - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
  - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman…
- Real people language – prepare dilution water that is air-saturated and analyze four bottles and compare to the theoretical dissolved oxygen concentration.

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DO SM4500-O G – 2016 Membrane Electrode Method

- Calibration
  - Calibrate daily (day of) by following manufacturer's instructions
    - Using barometric pressure is best
- Duplicates of the sample
  - Run on a 5% basis, one for every 20 samples
  - Calculate %RPD, ≤ 20%
  - For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum, then the minimum value should be reported even if falls outside your permit limit.

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DO SM4500-O G – 2016 Membrane Electrode Method

- Continuing Calibration Verification (CCV) – **daily**
  - 7.2.1 – Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle
  - 7.2.2 – Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 ± 3°C.
  - 7.2.3 – With a steady gentle stream of filtered air (≈ 10-40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.
  - 7.2.4 – At the completion of aeration, let water re-equilibrate to room temperature (20 ± 3°C) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric temperature reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.

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DO SM4500-O G – 2016 Membrane Electrode Method

- Continuing Calibration Verification (CCV) continued
  - 7.2.5 – Transfer the aerated water to a BOD bottle until overflowing and stopper.
  - 7.2.6 – Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman…
  - 9.3.1 – Upon air calibration, prepare a calibration verification standard with each analytical batch of 20 samples or less in an 8 hour period.
  - 9.4.3 – Initially and at the end of each analytical batch of samples, analyze a dilution water sample that is air-saturated
  - Real people language – prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration.

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Temperature

- NIST (National Institute of Standards & Technology) traceable thermometer
- Scale marked in 0.1˚C
- Calibrate annually by checking against a NIST certified thermometer
- Corrections can be made up to ± 3˚C
  - SM 2550 B. Footnote

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Temperature

- Clean the probe end with deionized water
- Swirl the thermometer in the sample and allow at least one minute to equilibrate
- Suspend the thermometer away from the sides and bottom of container
- Record readings to nearest 0.5˚C
- May be measured in situ with probe if verified against NIST traceable thermometer

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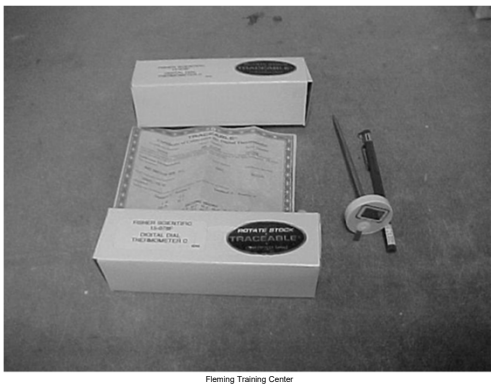

64

Temperature SM2550 B – 2000 Thermometric Measurement

- ICAL
  - Have thermometers verified **annually** by an NIST thermometer
- Corrective Action
- QC Frequency

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Any Questions?

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Dissolved Oxygen Calibration and Barometric Pressure

Most barometric pressures you find on TV or online (Ex: airport, radio, TV, weather websites, etc.) will provide you with a "corrected" barometric pressure, which means that it has been corrected to sea level. This is common because the aviation industry uses pressure altimeters to determine where the ground is (pressure is critical for aviation). If you have an altimeter (a device that matches the atmospheric pressure to the corresponding altitude) or a portable weather station, most likely you are already getting the uncorrected value.

You will need a USGS DOTABLES Oxygen Solubility Table (one is included in your book, or you can create one at: https://water.usgs.gov/software/DOTABLES/)

How to "UNCORRECT" the "corrected" barometric pressure:

1) Find the altitude (in feet) of your location
2) Calculate the Correction Factor (CF) using the following equation:
   CF = [760 – (Altitude x 0.026)] ÷ 760
3) Multiply the "corrected" barometric pressure by the CF. The result is the Uncorrected barometric pressure.
4) Depending on the USGS DOTABLES you are using, you may have to convert inches of mercury (Hg) to mm of mercury (Hg) with the following equation:
   Inches of Hg x 25.4 = mm of Hg

Now that you have your uncorrected barometric pressure, you will use that to determine the theoretical DO Saturation Point using the USGS DOTABLES Oxygen Solubility Table.

1) Determine the temperature and convert from Fahrenheit to Celsius (if necessary) using the following equation:
   Degrees Celsius = $\frac{(°\mathrm{F}-32)}{1.8}$
2) Find your uncorrected barometric pressure on the x- axis (along the top) of the table.
3) Find your temperature on the y-axis (down the left hand side) of the table.
4) Use your finger to slide down and over to the point where the x-axis and y-axis intersect. The number at that intersection is the theoretical DO.
5) You will compare the theoretical DO to what your DO probe is actually reading. The readings should be within +/- 0.2 mg/L or within 10% of each other. If not, you will need to recalibrate and/or initiate corrective action.

Dissolved Oxygen (DO) – Review Questions

1. What is the hold time for a DO sample?

2. List at least 5 reasons why DO levels are important with regards to water?

3. The amount of oxygen that a given volume of water can hold is a function of what 3 things?
    a)
    b)
    c)

4. What is barometric pressure?

5. Convert the following numbers into in (inches) Hg:
    a) 760 mm Hg
    b) 732 mm Hg
    c) 745 mm Hg

6. Convert the following numbers into mm Hg:
    a) 23.61 in Hg
    b) 25.56 in Hg
    c) 29.85 in Hg

7. Convert the following temperatures into °F:
    a) 17°C
    b) 20°C
    c) 29°C

8. Convert the following temperatures into °C:
    a) 55°F
    b) 65°F
    c) 70.5°F

9. You checked the local airport website and they are reporting the current barometric pressure as 29.46 in Hg. You know that this is a "corrected" BP, so now you must "uncorrect" it to determine the oxygen solubility. Use the equation listed below to find the correction factor that will be used to get the uncorrected BP. (The elevation/altitude of Fleming Training Center is 543 feet above sea level.)

   $$CF = [760 - (Altitude \times 0.026)] \div 760$$

   Use that correction factor to determine the uncorrected BP.

10. Now that you have calculated the uncorrected BP, you must use the USGS DOTABLE to find the oxygen solubility. The room temperature is 68°F and must be converted to Celsius. Use that information, in conjunction with the uncorrected BP, to determine the oxygen solubility.

11. Now try one on your own. Using the following information, determine the theoretical DO solubility to be used for probe calibration:
    a) Barometric Pressure from portable weather station on site = 29.00 in Hg
    b) Elevation of lab = 600 feet above sea level
    c) Temperature = 70°F

12. A BOD lab has been set up inside the weather station at Pikes Peak in Colorado. Using the following information, determine the theoretical DO solubility at that location. Do you suspect the analyst will have any problems with their BODs? Explain your answer.
    a) Corrected Barometric Pressure = 30.312 in Hg
    b) Elevation of lab = 14110 feet above sea level
    c) Average temperature = 36°C

Water Resources of the United States

DOTABLES Result

Oxygen Solubility Table

Solubility of oxygen in fresh water at various temperatures and pressures
[Solubility shown in milligrams per liter. Values based on published equations by Benson and Krause (1980 and 1984).
°C, degrees Celsius; in Hg, inches of mercury]

Temp.	Barometric Pressure (in Hg)																			
(°C)	22.44	22.83	23.22	23.61	24.00	24.39	24.78	25.17	25.56	25.95	26.34	26.73	27.12	27.51	27.90	28.29	28.68	29.07	29.46	29.85
0	10.95	11.14	11.33	11.52	11.71	11.90	12.10	12.29	12.48	12.67	12.86	13.05	13.24	13.44	13.63	13.82	14.01	14.20	14.39	14.59
1	10.64	10.83	11.01	11.20	11.39	11.57	11.76	11.95	12.13	12.32	12.51	12.69	12.88	13.06	13.25	13.44	13.62	13.81	14.00	14.18
2	10.35	10.53	10.71	10.89	11.08	11.26	11.44	11.62	11.80	11.98	12.16	12.35	12.53	12.71	12.89	13.07	13.25	13.43	13.62	13.80
3	10.07	10.25	10.43	10.60	10.78	10.96	11.13	11.31	11.49	11.66	11.84	12.02	12.19	12.37	12.55	12.72	12.90	13.08	13.25	13.43
4	9.81	9.98	10.15	10.32	10.50	10.67	10.84	11.01	11.18	11.36	11.53	11.70	11.87	12.04	12.22	12.39	12.56	12.73	12.90	13.08
5	9.55	9.72	9.89	10.06	10.22	10.39	10.56	10.73	10.89	11.06	11.23	11.40	11.57	11.73	11.90	12.07	12.24	12.40	12.57	12.74
6	9.31	9.47	9.64	9.80	9.96	10.13	10.29	10.45	10.62	10.78	10.95	11.11	11.27	11.44	11.60	11.76	11.93	12.09	12.25	12.42
7	9.08	9.24	9.40	9.55	9.71	9.87	10.03	10.19	10.35	10.51	10.67	10.83	10.99	11.15	11.31	11.47	11.63	11.79	11.95	12.11
8	8.85	9.01	9.16	9.32	9.48	9.63	9.79	9.94	10.10	10.26	10.41	10.57	10.72	10.88	11.04	11.19	11.35	11.50	11.66	11.81
9	8.64	8.79	8.94	9.10	9.25	9.40	9.55	9.70	9.86	10.01	10.16	10.31	10.47	10.62	10.77	10.92	11.08	11.23	11.38	11.53
10	8.43	8.58	8.73	8.88	9.03	9.18	9.33	9.47	9.62	9.77	9.92	10.07	10.22	10.37	10.52	10.67	10.81	10.96	11.11	11.26
11	8.24	8.38	8.53	8.67	8.82	8.96	9.11	9.25	9.40	9.55	9.69	9.84	9.98	10.13	10.27	10.42	10.56	10.71	10.86	11.00
12	8.05	8.19	8.33	8.47	8.62	8.76	8.90	9.04	9.19	9.33	9.47	9.61	9.75	9.90	10.04	10.18	10.32	10.47	10.61	10.75
13	7.86	8.00	8.14	8.28	8.42	8.56	8.70	8.84	8.98	9.12	9.26	9.40	9.54	9.68	9.81	9.95	10.09	10.23	10.37	10.51
14	7.69	7.83	7.96	8.10	8.23	8.37	8.51	8.64	8.78	8.92	9.05	9.19	9.33	9.46	9.60	9.74	9.87	10.01	10.14	10.28
15	7.52	7.65	7.79	7.92	8.06	8.19	8.32	8.46	8.59	8.72	8.86	8.99	9.12	9.26	9.39	9.53	9.66	9.79	9.93	10.06
16	7.36	7.49	7.62	7.75	7.88	8.01	8.14	8.28	8.41	8.54	8.67	8.80	8.93	9.06	9.19	9.32	9.45	9.58	9.72	9.85
17	7.20	7.33	7.46	7.59	7.72	7.84	7.97	8.10	8.23	8.36	8.49	8.61	8.74	8.87	9.00	9.13	9.26	9.38	9.51	9.64
18	7.05	7.18	7.30	7.43	7.56	7.68	7.81	7.93	8.06	8.19	8.31	8.44	8.56	8.69	8.81	8.94	9.07	9.19	9.32	9.44
19	6.91	7.03	7.15	7.28	7.40	7.52	7.65	7.77	7.90	8.02	8.14	8.27	8.39	8.51	8.64	8.76	8.88	9.01	9.13	9.25

Temp.	Barometric Pressure (in Hg)																			
(°C)	22.44	22.83	23.22	23.61	24.00	24.39	24.78	25.17	25.56	25.95	26.34	26.73	27.12	27.51	27.90	28.29	28.68	29.07	29.46	29.85
20	6.77	6.89	7.01	7.13	7.25	7.37	7.49	7.62	7.74	7.86	7.98	8.10	8.22	8.34	8.46	8.59	8.71	8.83	8.95	9.07
21	6.63	6.75	6.87	6.99	7.11	7.23	7.35	7.46	7.58	7.70	7.82	7.94	8.06	8.18	8.30	8.42	8.54	8.66	8.77	8.89
22	6.50	6.62	6.73	6.85	6.97	7.08	7.20	7.32	7.44	7.55	7.67	7.79	7.90	8.02	8.14	8.25	8.37	8.49	8.61	8.72
23	6.37	6.49	6.60	6.72	6.83	6.95	7.06	7.18	7.29	7.41	7.52	7.64	7.75	7.87	7.98	8.10	8.21	8.33	8.44	8.56
24	6.25	6.36	6.48	6.59	6.70	6.82	6.93	7.04	7.15	7.27	7.38	7.49	7.61	7.72	7.83	7.95	8.06	8.17	8.28	8.40
25	6.13	6.24	6.35	6.47	6.58	6.69	6.80	6.91	7.02	7.13	7.24	7.35	7.47	7.58	7.69	7.80	7.91	8.02	8.13	8.24
26	6.02	6.13	6.24	6.34	6.45	6.56	6.67	6.78	6.89	7.00	7.11	7.22	7.33	7.44	7.55	7.66	7.77	7.88	7.98	8.09
27	5.90	6.01	6.12	6.23	6.33	6.44	6.55	6.66	6.77	6.87	6.98	7.09	7.20	7.30	7.41	7.52	7.63	7.73	7.84	7.95
28	5.80	5.90	6.01	6.11	6.22	6.33	6.43	6.54	6.64	6.75	6.86	6.96	7.07	7.17	7.28	7.38	7.49	7.60	7.70	7.81
29	5.69	5.79	5.90	6.00	6.11	6.21	6.32	6.42	6.52	6.63	6.73	6.84	6.94	7.05	7.15	7.25	7.36	7.46	7.57	7.67
30	5.59	5.69	5.79	5.90	6.00	6.10	6.20	6.31	6.41	6.51	6.62	6.72	6.82	6.92	7.03	7.13	7.23	7.33	7.44	7.54

Return to the DOTABLES main page.

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U.S. Department of the Interior | U.S. Geological Survey
URL: http://water.usgs.gov/cgi-bin/dotables
Page Contact Information: Water Webserver Team
Page Last Modified: Wednesday, 16-Jan-2013 at 10:03:49 EST

DOTABLES Result

Oxygen Solubility Table

Solubility of oxygen in fresh water at various temperatures and pressures
[Solubility shown in milligrams per liter. Values based on published equations by Benson and Krause (1980 and 1984).
°C, degrees Celsius; mm Hg, millimeters of mercury]

Temp.	Barometric Pressure (mm Hg)																				
(°C)	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600
20	4.69	4.81	4.93	5.05	5.18	5.30	5.42	5.54	5.66	5.79	5.91	6.03	6.15	6.28	6.40	6.52	6.64	6.77	6.89	7.01	7.13
21	4.59	4.71	4.83	4.95	5.07	5.19	5.31	5.43	5.55	5.67	5.79	5.91	6.03	6.15	6.27	6.39	6.51	6.63	6.75	6.87	6.99
22	4.49	4.61	4.73	4.85	4.96	5.08	5.20	5.32	5.44	5.56	5.67	5.79	5.91	6.03	6.15	6.26	6.38	6.50	6.62	6.74	6.85
23	4.40	4.52	4.63	4.75	4.86	4.98	5.10	5.21	5.33	5.45	5.56	5.68	5.79	5.91	6.03	6.14	6.26	6.37	6.49	6.61	6.72
24	4.31	4.43	4.54	4.65	4.77	4.88	5.00	5.11	5.22	5.34	5.45	5.57	5.68	5.79	5.91	6.02	6.14	6.25	6.36	6.48	6.59
25	4.22	4.34	4.45	4.56	4.67	4.79	4.90	5.01	5.12	5.23	5.35	5.46	5.57	5.68	5.80	5.91	6.02	6.13	6.24	6.36	6.47
26	4.14	4.25	4.36	4.47	4.58	4.69	4.80	4.91	5.02	5.13	5.24	5.35	5.46	5.58	5.69	5.80	5.91	6.02	6.13	6.24	6.35
27	4.06	4.17	4.27	4.38	4.49	4.60	4.71	4.82	4.93	5.04	5.14	5.25	5.36	5.47	5.58	5.69	5.80	5.90	6.01	6.12	6.23
28	3.98	4.08	4.19	4.30	4.41	4.51	4.62	4.73	4.83	4.94	5.05	5.15	5.26	5.37	5.48	5.58	5.69	5.80	5.90	6.01	6.12
29	3.90	4.00	4.11	4.22	4.32	4.43	4.53	4.64	4.74	4.85	4.95	5.06	5.16	5.27	5.37	5.48	5.58	5.69	5.80	5.90	6.01
30	3.82	3.93	4.03	4.13	4.24	4.34	4.45	4.55	4.65	4.76	4.86	4.96	5.07	5.17	5.28	5.38	5.48	5.59	5.69	5.79	5.90
31	3.75	3.85	3.95	4.06	4.16	4.26	4.36	4.46	4.57	4.67	4.77	4.87	4.98	5.08	5.18	5.28	5.38	5.49	5.59	5.69	5.79
32	3.68	3.78	3.88	3.98	4.08	4.18	4.28	4.38	4.48	4.58	4.68	4.78	4.89	4.99	5.09	5.19	5.29	5.39	5.49	5.59	5.69
33	3.60	3.70	3.80	3.90	4.00	4.10	4.20	4.30	4.40	4.50	4.60	4.70	4.80	4.90	5.00	5.10	5.19	5.29	5.39	5.49	5.59
34	3.53	3.63	3.73	3.83	3.93	4.02	4.12	4.22	4.32	4.42	4.51	4.61	4.71	4.81	4.91	5.01	5.10	5.20	5.30	5.40	5.50
35	3.47	3.56	3.66	3.76	3.85	3.95	4.05	4.14	4.24	4.34	4.43	4.53	4.63	4.72	4.82	4.92	5.01	5.11	5.21	5.30	5.40
36	3.40	3.49	3.59	3.68	3.78	3.88	3.97	4.07	4.16	4.26	4.35	4.45	4.54	4.64	4.74	4.83	4.93	5.02	5.12	5.21	5.31
37	3.33	3.43	3.52	3.61	3.71	3.80	3.90	3.99	4.09	4.18	4.27	4.37	4.46	4.56	4.65	4.75	4.84	4.93	5.03	5.12	5.22
38	3.27	3.36	3.45	3.55	3.64	3.73	3.82	3.92	4.01	4.10	4.20	4.29	4.38	4.48	4.57	4.66	4.76	4.85	4.94	5.04	5.13

LDO Probe Calibration

			LDO Probe				Theoretical DO				Compare Meter DO with Theoretical DO		
Date	Initials	Instrument ID	Temp (on meter)	% DO (after calibration)	Meter BP	Meter DO	Temp °F/°C(room)	"Corrected BP" in Hg	"Uncorrected BP" in Hg	USGS Theoretical DO	LDO Meter DO	Theoretical DO	% Difference (+/- 0.2 mg/L or 10%)

Below is an example of an LDO calibration record that is included on the top portion of the CBOD benchsheet.

City of _____ WWTP CBOD Worksheet - Standard Methods BODs SM5210 B-2011 Permit # _____

Time of Sampling 6:50 Date 4-1-18 Initials J.O

Time Analysis Ran 9:15 Date 4-1-18 Initials J.O

Final Time Analysis Ran 8:10 Date 04-06-18 Initials SH

LDO Calibration

Date	Time	Temp (°C)	Cal. Reading (mg/L)	Meter mmHg	Lab mmHg	USGS DO Table
4-1-18	6:05	18.1	9.3	746	746	9.2
04.06-18	7:05	18.7	9.1	744	744	9.1

Hach Method 10360
Luminescence Measurement of Dissolved Oxygen in Water and Wastewater and for Use in the Determination of BOD_5 and $cBOD_5$
Revision 1.2
October 2011

1. Scope and Application

1.1 This method is for the measurement of dissolved oxygen (DO) in surface and ground water, municipal and industrial wastewater, and for use in Biochemical Oxygen $Demand_5$ (BOD_5) and carbonaceous Biochemical Oxygen $Demand_5$ ($cBOD_5$) determination.

1.2 The method may be used as a replacement for the modified Winkler and membrane electrode procedures for the measurement of DO in wastewater treatment processes such as aeration and biological nutrient basins, effluent outfalls, receiving water, and water for BOD_5 and $cBOD_5$ determination.

1.3 The method is for use in the United States Environmental Protection Agency's (EPA's) survey and monitoring programs for the measurement of DO and for the determination of BOD_5 and $cBOD_5$ under the Clean Water Act.

1.4 This method is capable of measuring DO in the range of 0.20 to 20 mg/L.

1.5 This method is restricted to luminescence probe technologies where calibration is performed by single-point water-saturated air (100% saturation).

2. Summary of Method

2.1 This luminescence-based sensor procedure measures the light emission characteristics from a luminescence-based reaction that takes place at the sensor-water interface. A light emitting diode (LED) provides incident light required to excite the luminophore substrate. In the presence of dissolved oxygen the reaction is suppressed. The resulting dynamic lifetime of the excited luminophore is evaluated and equated to DO concentration.

3. Interferences

3.1 There are no known agents that interfere with luminescence DO detection and quantification with this method.

4. Safety

4.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of any chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 17.5-17.6.

5. Equipment for the Measurement of Dissolved Oxygen

5.1 BOD bottle 300-mL with stoppers and plastic caps (Hach # 62016 and 241906, or equivalent).

5.2 Magnetic Stirring plate (optional)

5.3 Magnetic stirring device (optional)

5.4 Pipette, serological, 1-mL (Hach Catalog Number 919002, or equivalent)

5.5 Pipette, serological, 5-mL (Hach Catalog Number 53237, or equivalent)

5.6 Pipette, serological, 10-mL (Hach Catalog Number (53238, or equivalent)

5.7 Pipette Filler (Hach Catalog Number 1218900, or equivalent)

5.8 Meter and LBOD Probe (Hach Catalog Number 8508500) for DO measurement in BOD bottles, or equivalent as defined in Section 1.5 of this method)

5.9 Meter and LDO Probe (Hach Catalog Number 8505200 or 8506300) for DO measurement in open containers and water bodies, or equivalent as defined in Section 1.5 of this method)

5.10 Dispenser Cap, for Nitrification Inhibitor (Hach Catalog Number 4590)

5.11 Temperature controlled environment for BOD bottle incubation, 20 ± 1° C.

6. REAGENTS

6.1 Phosphate Buffer Solution - 0.17 g AR grade Ammonium Chloride, 8.5 g Potassium Phosphate Monobasic, 17.7 g Sodium Phosphate Dibasic, 21.7 g Potassium Phosphate Dibasic diluted to 1000 mL with deionized water, APHA, pH 7.2, (Hach Catalog Number 43149, or equivalent)

6.2 Calcium Chloride Solution - 27.5 g AR grade Calcium Chloride diluted to 1000 mL with deionized water, APHA, for BOD (Hach Catalog Number 42849, or equivalent)

6.3 Ferric Chloride Solution - 0.25 g Ferric Chloride diluted to 1000 mL with deionized water, APHA, for BOD (Hach Catalog Number 42953, or equivalent)

6.4 Glucose-glutamic Acid - Standard Solution, Voluette™ Ampoule, 300-mg/L, 150 mg glucose and 150 mg glutamic Acid to 1000 mL in deionized water, 10 mL (Hach Catalog Number 1486510, or equivalent) or, ezGGA Ampoules, 450 mg/L, 225 mg/L Glucose and 225 mg/L Glutamic Acid, (Hach Catalog Number 25144-20, or equivalent)

6.5 Magnesium Sulfate Solution - 22.5 g Magnesium Sulfate diluted to 1000 mL with deionized water, APHA, (Hach Catalog Number 43094, or equivalent)

6.6 Nitrification Inhibitor - (Hach Catalog Number 253334)

6.7 Potassium Iodide Solution (100 g AR grade Potassium Iodide diluted to 1000 mL with deionized water) (Hach Catalog Number 1228949, or equivalent)

6.8 Sodium Thiosulfate Solution – 0.025 N (Hach Catalog Number 35253, or equivalent)

6.9 Sodium Hydroxide Solution - 1 N (Hach Catalog Number 104532, or equivalent)

6.10 Sodium Hydroxide Pellets - ACS (Hach Catalog Number 18734, or equivalent)

6.11 Starch Indicator - 5.5 g AR grade Starch, and 1.25 g AR grade Salicylic Acid diluted to 1000 mL with deionized water (Hach Catalog Number 34932, or equivalent)

6.12 Sulfuric Acid Solution - 0.020 N (Hach Catalog Number 104532, or equivalent)

6.13 Sulfuric Acid Solution - 1.000 N (Hach Catalog Number 127053, or equivalent)

Note: The Phosphate Buffer Solution should be refrigerated to decrease the rate of biological growth.

7. Standards for Calibration

7.1 Initial LDO/LBOD Probe Calibration

7.1.1 Add approximately 1 inch (2.54 cm of reagent water to a clean BOD bottle and stopper.

7.1.2 Shake vigorously for ~ 10 seconds.

7.1.3 Allow for the BOD bottle and its contents to equilibrate to room temperature. Room temperature should be approximately 20 ± 3° C.

7.1.4 The stopper may now be removed from the BOD bottle and the LBOD probe inserted for calibration purposes.

7.1.5 The luminescence technology for measuring dissolved oxygen is a superior technique from that of Winkler titration and membrane potentiometric measurement and has no interferences associated with the oxygen detection process. Therefore, for calibration and measurement purposes, do not adjust the calibration luminescence measurement to that of Winker or membrane measurement readings.

Note: Section 7.1 is a suggested procedure for the preparation of water-saturated air. Other procedures for the preparation of water-saturated air may used that are equally effective.

7.2 Calibration Verification, Initial Precision and Recovery, and On-going Precision and Recovery

7.2.1 Add approximately 1500 mL of organic-free water or BOD dilution water to a 2-L beaker or PET bottle.

7.2.2 Allow the water to equilibrate to room temperature. Room temperature should be approximately 20 ± 3° C.

7.2.3 With a steady gentle stream of filtered air (≈ 10 – 40 mL per minute), aerate the water for a minimum of 30 minutes. Alternatively, vigorously shake the reagent water or BOD dilution water for several minutes.

7.2.4 At the completion of aeration, let water re-equilibrate to room temperature (20 ± 3° C) for 30 minutes and note the barometric pressure of the laboratory during preparation. The barometric temperature reading is used in the calculation and determination of the theoretical DO concentration for the preparation of air-saturated water.

7.2.5 Transfer the aerated water to a BOD bottle until overflowing and stopper.

7.2.6 Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman referenced in Section 17.2 of this method.

Note: Section 7.2 is a suggested procedure for the preparation of air-saturated water. Other procedures for the preparation of air-saturated water may used that are equally effective.

8. Sample Collection Preservation and Storage

8.1 See Title 40 of the Code of Federal Regulations Part 136.3, Table II (Section 17.3) for information regarding required sample collection containers, preservation techniques and holding times for collection of water for measurement of DO and for the determination of BOD_5 and $cBOD_5$.

9. Quality Control

9.1 It is recommended that each laboratory that uses this method be required to operate a formal quality assurance program (Reference 17.1). The minimum requirements of this program consist of an initial demonstration of laboratory capability and ongoing analyses of laboratory prepared water standards as a test of continued performance to assess accuracy and precision. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control. These procedures are described in Sections 9.3 and 9.4, respectively.

9.1.3 Accompanying QC for the determination of DO is required per analytical batch. An analytical batch is a set of samples processed during a contiguous 8-hour period, not to exceed 20 samples. Each analytical batch should be accompanied by a calibration verification and ongoing precision and recovery sample, resulting in a minimum of three analyses (1 CV, 1 sample, and 1 OPR). Perform additional CV and OPR for each batch that exceeds 20 samples.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Initial precision and recovery (IPR) - To establish the ability to generate acceptable precision and accuracy for the measurement of DO in water, the analyst shall perform the following operations:

9.2.1.1 Prepare and measure four samples of the IPR standard (Section 7.2) according to the procedure beginning in Section 11.

9.2.1.2 Using the results of the set of four analyses, compute the average percent recovery (X) and the standard deviation of the percent recovery (s) for DO. Use the following equation for calculation of the standard deviation of the percent recovery:

$$s = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1}}$$

where:

n = Number of samples
x = Concentration in each sample

9.2.1.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 4. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the precision limit or X falls outside the range for recovery, system performance is unacceptable. In this event correct the problem, and repeat the test.

9.3 Calibration Verification

9.3.1 Upon air calibration, prepare a calibration verification standard (Section 7.2) with each analytical batch of 20 samples or less in an 8 hour period. Analyze according to the procedure beginning in Section 11 and compare the recovery results to those in Table 4.

9.4 Ongoing Calibration and Precision and Recovery

9.4.1 To demonstrate that the analysis system is in control, and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations

9.4.2 Prepare a precision and recovery standard (Section 7.2) with each analytical batch according to the procedure beginning in Section 11.

9.4.3 Initially, and at the end of each analytical batch of samples, analyze a precision and recovery standard and compare the concentration recovery with the limits for ongoing precision and recovery in Tables 4 and 5. If the recovery is in the range specified, measurement process is in control and analysis of samples may proceed. If, however, the recovery is not in the specified range, the analytical process is not in control. In this event, correct the problem, recalibrate and verify the calibration and reanalyze analytical batch, repeating the ongoing precision and recovery test.

9.4.4 The laboratory should add results that pass the specification in Tables 4 and 5 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of the percent recovery (sr). Express the accuracy as a recovery interval from R 2sr to R + 2sr. For example, if R = 95% and sr = 5%, the accuracy is 85% to 105%.

9.5 Depending upon specific program requirements, field replicates may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

9.6 Glucose-Glutamic Acid Seed Strength Check

9.6.1 Many factors can influence the BOD analysis (toxicity from sample matrix, contaminated dilution water, poor quality seed, etc. In order to insure sufficient seeding in the BOD test, a glucose-glutamic acid check is performed in parallel with each day's BOD_5 and $cBOD_5$ test samples. Well prepared dilution water and an active seed will produce a BOD_5 of 198 ± 30 mg/L BOD (Reference 17.7).

9.6.1.1 Prepare in triplicate a 300-mL BOD bottle with 3.0 mL of the 300 mg/L Standard Solution of GGA (Section 6.4) or 1 ampoule (2.0 mL) of ezGGA (Section 6.4) with each day of samples prepared for BOD determination.

9.6.1.2 When using ezGGA ampoules, place the ezGGA ampoule in the ampoule breaker (provided with ezGGA ampoules) and rinse the assembly with reagent water. Hold the ampoule and breaker over the rim of the BOD bottle, break and allow the ampoule to fall into the BOD bottle. Leave the ampoule in the BOD bottle during the incubation period and reading of DO.

GGA Standard Solution

$$\frac{3.0\ \text{mL} \times 0.300\ \text{mg/mL GGA}}{300\ \text{mL final volume}} \times 1000\ \text{mL/L} = 3.0\ \text{mg/L GGA per bottle}$$

ezGGA Ampoule

1 ampoule (2.0 mL) x 0.450 mg/mL GGA x 1000 mL/L = 3.0 mg/L GGA per bottle
300 mL final volume

9.6.1.2 Add seed at three different volumes (typically 4 mL, 6 mL, and 8 mL,) to the GGA bottles. Other volumes may be required, depending on the strength of the seed being used.

9.6.1.3 Bring to volume with dilution water and analyze as described in Sections 12.9 and 12.10.

Note: GGA BOD_5 recovery results outside of 198 ± 30 mg/L should be investigated as to causation. If toxicity of dilution water has been ruled out as a probable cause for low recovery, it is likely that the seed is of low activity or poor quality. Either increase the seed amount or use a seed of higher quality. High GGA recoveries are generally due to incorrect amount of GGA Standard Solution.

10. Calibration and Standardization

10.1 Because of the possible diversity of future LDO instrument hardware and, no detailed operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements of this method and to maintain quality control data verifying instrument performance and analytical results.

10.2 Water-saturated air (Section 7.1) is used for instrument calibration.

10.3 Calibration verification (Section 7.2) is performed with air-saturated water prior to any DO sample measurements to the method specifications in Section 14.

11. Procedure for Measuring DO in Grab Samples, Outfalls, and Open Water Bodies

11.1 Instrument Setup

11.1.1 Follow the instrument manufacturer's instructions for instrument setup (Hach Document DOC022.53.80021 for Hach LDO IntelliCal™ Rugged and Standard Probes, Hach Document Number DOC022.53.80116 for LBOD probes, and Hach Catalog Number 5790018 for Hach LDO process probes.

Note: Manufacturer's instructions are only for instrument set and use. These instructions do not preclude the calibration and performance requirements of this method.

11.2 Measurement of DO

11.2.1 For samples in an open vessel, container, or water body, place the LDO probe into the water sample to be measured and stir gentle with probe or add a stir bar. Do not put the probe on the bottom or sides of the container. Stir the sample at a moderate rate or put the probe in flowing conditions. Read sample. The display will show "Stabilizing" and a progress bar as the probe stabilizes in the sample. The display will show the lock icon when the reading stabilizes.

11.3.1 For BOD_5 and $CBOD_5$ prepared samples, insert the LBOD probe into the BOD bottle for DO determination. Insure that there are no air bubbles that may have collected around the probe or sensor. Turn on the stir paddle and read sample. The display will show

"Stabilizing" and a progress bar as the probe stabilizes in the sample. The display will show the lock icon when the reading stabilizes.

12. Procedure for the Preparation and Determination of BOD_5 and $cBOD_5$ Samples

12.1 The BOD test is a 5-day test. Follow all steps carefully to make sure that the test does not have to be repeated.

12.2 The dilution water for this test must be fully air-saturated immediately before use and determined to not have an oxygen demand or any toxins. When incubated for 5 days at 20 ± 1° C, the dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L. Air-saturation is a function of water temperature and laboratory barometric pressure. Use an oxygen saturation table such as in Section 17.2 of this method to insure full air-saturation of dilution water.

12.3 Distilled Water Preparation

12.3.1 The distilled water must be prepared very carefully to make sure that no source of oxygen demand or toxins are added. The water that is used to prepare the dilution water must be of very high quality. The water must not have any organic compounds or any toxic compounds such as chlorine, copper, and mercury at a concentration level that would interfere would the BOD seed and inhibit microbiological growth of organisms.

12.3.2 For best results, use an alkaline permanganate distillation for preparing dilution water. Resin in ionization cartridges will occasionally release organic materials that have an oxygen demand.

12.4.3 Store the distilled water in clean jugs at a temperature of 20 ± 3° C. Fill the containers to about ¾ full and shake the jugs to saturate the water with air. Alternatively, saturate the water with air as described in Section 7.2.1. A small aquarium pump or air compressor can be used to saturate the water with air. Insure that the air is filtered and that the air filter does not grow bacteria.

12.4 Dilution Water Preparation

12.4.1 Using the distilled water prepared above in Section 12.4, select a BOD nutrient buffer pillow from the BOD nutrient buffer pillows in Table 1.

12.4.2 Add the contents of the BOD Nutrient Buffer Pillow to the distilled water in a jug with ample headspace. Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.

12.4.3 Alternatively, prepare the dilution water by adding 1 mL each of the following solutions per liter of distilled water prepared in Section 12.4:

Phosphate Buffer Solution - 0.1.7 g AR grade Ammonium Chloride, 8.5 g Potassium Phosphate Mono Basic, 17.7 g Sodium Phosphate Dibasic, 21.7 g Potassium Phosphate Dibasic to 1000 mL with deionized water, APHA, pH 7.2, (Hach Catalog Number 43149, or equivalent)

Calcium Chloride Solution - 27.5 g AR grade Calcium Chloride to 1000 mL with deionized water, APHA, for BOD (Hach Catalog Number 42849, or equivalent)

Ferric Chloride Solution - 0.25 g Ferric Chloride to 1000 mL deionized water, APHA, for BOD (Hach Catalog Number (42953, or equivalent)

12.5.4 Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.

***Note*:** Dilution water should be prepared immediately before use unless it can be demonstrated that the dilution water blank has no DO depletion greater than 0.2 mg/L.

12.5 Seed Preparation

12.6.1 Use raw sewage or other reliable sources for the bacterial seed that will yield 198 ± 30 mg/L BOD with the GGA check sample in Section 9.6. Potential seed sources include wastewater influent, primary effluent, soil, and domestic sewage.

12.6.2 Allow raw sewage to stand undisturbed at 20 ± 3° C for 24 to 36 hours before use.

12.6.3 When seeding samples with raw sewage, always pipette from the upper portion of the sewage.

12.7 Sample Size Selection Guide

12.7.1 Make an estimate of the sample volumes that are necessary for the test. At least 2.0 mg/L of DO should be consumed during the test and at least 1.0 mg/L of un-depleted DO should remain in the bottle.

12.7.2 Samples such as raw sewage will have a high BOD. Small sample volumes must be used because large samples will deplete all of the oxygen in the sample. Samples with a low BOD must use larger sample volumes to insure that adequate oxygen is depleted to give accurate results.

12.7.3 Refer to the Minimum Sample Volume in Table 2 to select the minimum sample volume. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the minimum sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the minimum sample volume is 15 mL.

12.7.4 Refer to the Maximum Sample Volume in Table 3 to select the maximum sample volume. At 1000 in elevation, with an estimated BOD_5 of 300 mg/L, the largest sample volume is 8 mL. For a BOD of 40 mg/L, the maximum volume of sample is 60 mL.

12.8 Sample Matrix Pretreatment

12.8.1 Determine the pH of each sample at a sample temperature of 20 ± 3° C. prior to BOD sample preparation. For samples that of have pH of less than 6 or greater than 8, adjust the pH accordingly with a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH). Strength of pH adjustment solution should be at a concentration that does not dilute the sample by greater than 0.5 percent.

12.8.2 For sample matrices that contain residual chlorine, de-chlorinate with a solution of Sodium Thiosulfate ($Na_2S_2O_3$).

12.8.2.1 Measure 100 mL of sample into a 250 mL Erlenmeyer flask. Using a 10-mL serological pipette and pipette filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100-g/L, to the flask.

12.8.2.2 Add three full droppers of Starch Indicator Solution and swirl to mix.

12.8.2.3 Fill a 25-mL burette with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.

12.8.2.4 Calculate the amount of 0.025 N Sodium Thiosulfate Solution to add to the sample:

mL 0.025 N Sodium Thiosulfate required = mL titrant used x volume of remaining sample divided by 100

12.8.2.5 Add the required amount of 0.025 N Sodium Sulfate Standard Solution to the sample. Mix thoroughly and wait 10 to 20 minutes before performing the BOD test.

***Note*:** Samples should be brought to a temperature of 20 ± 3° C. prior to making dilutions.

12.9 Sample Preparation

12.9.1 Select the sample volume as described in Section 12.7. Select a minimum of three different volumes for each sample.

12.9.1.1 If the minimum sample volume is 3 mL or more, determine the DO concentration in the undiluted sample; this determination can be omitted when analyzing sewage and settled effluents known to have dissolved oxygen content near 0 mg/L.

12.9.2 Stir the sample gently with a pipette. Use the pipette to add the determined sample volumes to the BOD bottles.

12.9.3 Add the appropriate seed to the individual BOD bottles as described in Section 12.6.

12.9.3.1 Separately, with each batch of BOD samples, prepare a seed sample with dilution water. Measure the BOD of the seed for subtraction from the sample BOD.

12.9.3.2 A seed that has a BOD_5 of 200 mg/L (a typical range for domestic sewage) will typically deplete at least 0.6 mg/L DO when added at a rate of 3 mL/L of dilution water.

12.9.4 If the test is for $cBOD_5$, add two potions of Nitrification Inhibitor (approximately 0.16 g) to each bottle. The oxidation of nitrogen-based compounds will be prevented.

12.9.5 Fill each bottle to just below the lip with dilution water.

12.9.5.1 Allow the dilution water to flow down the sides of the bottle to prevent air bubbles from becoming entrapped in the bottle.

12.9.6 Fill an additional BOD bottle with only dilution water. This will be the dilution water blank.

12.9.7 Stopper the bottles carefully to prevent air bubbles from becoming entrapped.

12.9.7.1 Tightly twist the stopper and invert the bottles several times to mix.

Note: The sample preparation procedures in Section 12.9 are designed for a BOD sample analysis volume of 300 mL. A 60-mL sample preparation volume may also be used.

12.10 Sample Analysis

12.10.1 Measure the initial dissolved oxygen concentration in each bottle with the LBOD probe within 30 minutes of sample preparation.

12.10.2 After the initial DO measurement, stopper the bottles carefully to prevent air bubbles from becoming entrapped.

12.10.2.1 Add dilution water to the lip of each BOD bottle to make a water seal.

12.10.3 Place a plastic cap over the lip of each bottle and incubate at 20 ± 1° C for five days.

12.10.4 After 5 days, measure the remaining dissolved oxygen concentration in each bottle with the LBOD probe.

12.10.4.1 At least 1.0 mg/L DO should have remained in each bottle.

12.10.4.2 Discard results of samples where the DO is depleted below 1.0 mg/L.

13. BOD and cBOD Calculations

13.1 When Dilution Water Not Seed (generally influent and primary treated influent to treatment)

$$BOD_5 \text{ or } cBOD_5, \text{ mg/L} = \frac{D_1 - D_2}{P}$$

where:

BOD_5 or $cBOD_5$ = BOD value from the 5-day test
D_1 = DO of diluted sample immediately after preparation, in mg/L
D_2 = DO of diluted sample after 5 day incubation at 20 ±1° C, in mg/L
P = Decimal volumetric fraction of sample used

13.2 When Dilution Water Requires Seed

$$BOD_5 \text{ or } cBOD_5, \text{ mg/L} = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

as defined above plus:

B_1 = DO of seed control before incubation, in mg/L
B_2 = DO of seed control after incubation, in mg/L
f = ratio of seed in diluted sample to seed in seed control (% seed in diluted sample/%seed in seed control) or, if seed material is added directly to sample or to seed-control bottles:
f = (volume of seed in diluted sample/volume of seed in seed control)

13.3 *Averaged Results*

13.3.1 Averaged results from different dilutions are acceptable if more than one sample dilution meets all of the following criteria:

13.3.1.1 The remaining un-depleted DO is at least 1 mg/L.

13.3.1.2 The final DO value is at least 2 mg/L lower than the initial prepared sample DO

13.3.1.3 There is no evidence of toxicity at higher sample concentrations

14. Method Performance for Dissolved Oxygen in Reference Water and GGA BOD_5 Recovery

Acceptance Criterion	Section	Limit
Initial DO Accuracy in Reagent Water	9.2.1	95% to 105%
Initial Precision in Reagent Water	9.2.1	2.1%
On-going DO Accuracy	9.4.1	95% to 105%

15. Pollution Prevention

15.1 There are no standards or reagents used in this method when properly disposed of, pose any threat to the environment.

16. Waste Management

16.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect air, water, and land by minimizing and control all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

16.2 For further information on waste management, consult "The Waste Management manual for Laboratory Personnel", and Less is Better: Laboratory Chemical Management for Waste Reduction", both available from the American Society's Department of Government relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

17. References

17.1 Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMSL-CI, Cincinnati, OH 45268, EPA-600-4-79-019, March 1979.

17.2 Hitchmen, M.L. (1978) *Chemical analysis*. Vol. 49. *Measurement of Dissolved Oxygen*. Wiley and sons, New York.

17.3 Title 40, Code of Federal Regulations (40 CFR), Part 136.

17.4 Protocol for EPA Approval of New Methods for Organic and Inorganic Analytes in Wastewater and Drinking Water (EPA-821-B-98-003, March 1999).

17.5 "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976)

17.6 "Safety in Academic Chemistry Laboratories," American Chemical Society, Committee on Chemical Safety, 3rd Edition, 1979.

17.7 "Standard Methods for the Examination of Water and Wastewater", 20th Edition; American Public health Association: 1015 Fifteenth Street, NW, Washington, D.C. 2005, 1998; Method 5210B.

18. Tables

18.1 Nutrient Buffer Preparation Options

Table 1 - BOD Nutrient Buffer Pillows

Volume of Dilution Water to Prepare	Hach BOD Nutrient Pillow Catalog Number
300 mL) add pillow to each BOD Bottle	1416066
3 liters	1486166
4 liters	2436466
6 liters	1486266
19 liters	1486398

Note: Hach BOD Nutrient Pillows are formulated with the same reagents adjusted for volume preparation as in Sections 6.1 through 6.3.

18.2 Sample Volume Selection Guides

Table 2 - Minimum Sample Volume Selection Guide

Sample Type	Estimated BOD mg/L	Minimum Sample Volume (mL)
Strong Waste	600	1
Raw and Settled Sewage	300	2
	200	3
	150	4
	100	6
	75	8
	60	10
Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Water	6	100
	4	200
	2	300

Table 3 – Maximum Sample Volume Selection Guide

BOD at Sea Level (mg/L)	BOD at 1000 ft Elevation (mg/L)	BOD at 5000 feet Elevation (mg/L)	Maximum Sample Volume (mL)
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

Note: Samples with higher concentrations of BOD should be pre-diluted.

18.3 Performance Criteria

Table 4 - Initial Precision and Recovery Method Performance

IPR Range	IPR DO Conc. (mg/L)	97.5% Lower Limit of Recovery (%)	97.5% Upper Limit of Recovery (%)	95% Upper Limit of Precision (%)
High	7.22 – 9.23	95.8	104.8	1.26

Table 5 - Calibration Verification Performance

CV DO Concentration	Average % Recovery	% Standard Deviation	% Relative Standard Deviation
7.22 mg/L – 9.23 mg/L	100.1	2.5	2.5

19. Glossary of Definitions and Purposes

The definitions and purposes are specified to this method but have been conformed to common usage as much as possible.

19.1 Units of Weight and Measure and their Abbreviations

19.1.1 Symbols
^{o}C degrees Celsius

19.1.2 Alphabetical characters
mg/L milligram per liter

19.2 Definitions, acronyms, and abbreviations

19.2.1 LDO® - Luminescence dissolved oxygen

19.2.2 LBOD® - Luminescence biochemical oxygen demand

19.2.3 BOD - Biochemical oxygen demand

19.2.4 BOD_5 - Biochemical oxygen demand, 5-day test

19.2.5 $cBOD_5$ - Carboneous biochemical oxygen demand, 5-day test

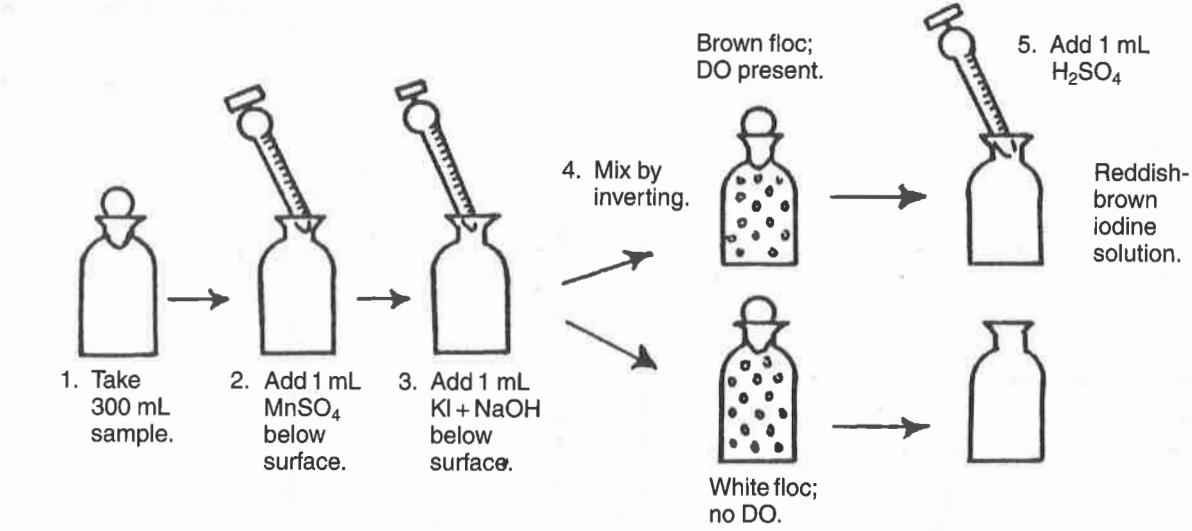
19.2.6 DO: Dissolved oxygen

19.2.75 CV: Calibration verification

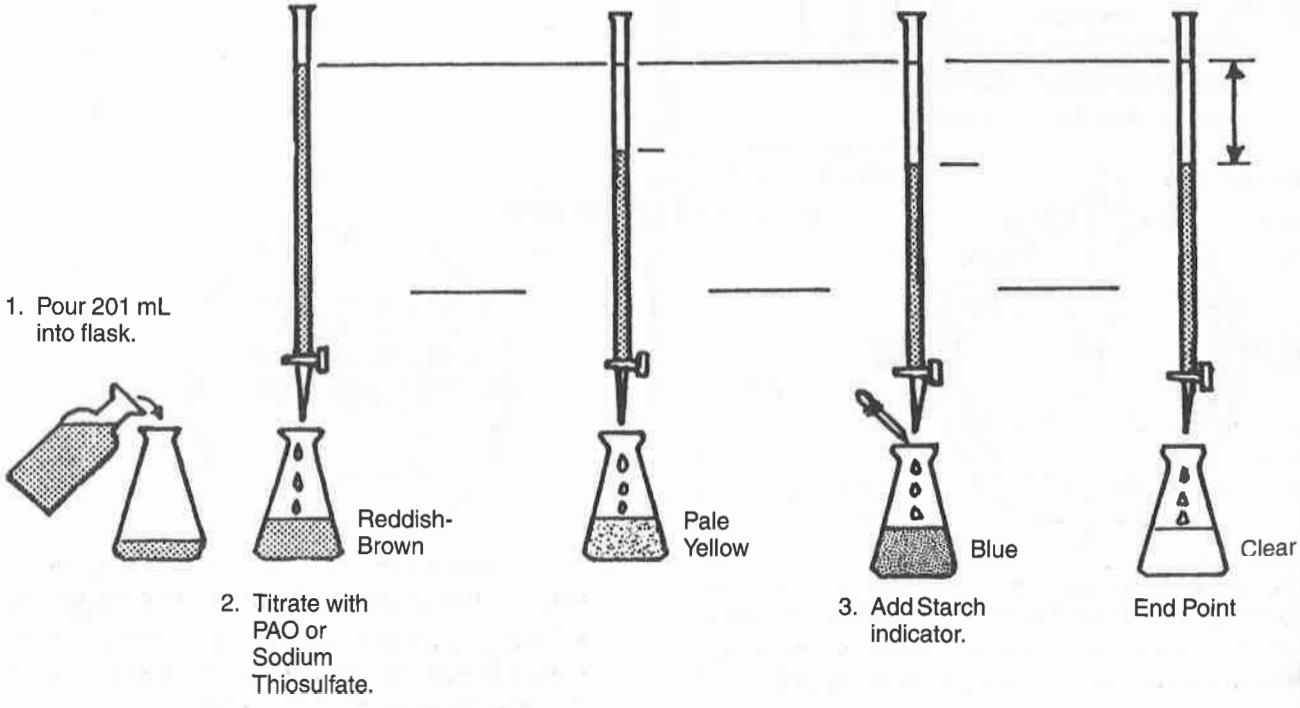
19.2.8 IPR: Initial precision and recovery

19.2.9 OPR: On-going precision and recovery

OUTLINE OF PROCEDURE FOR DO



Titration of Iodine Solution:



Section 3
Biochemical Oxygen Demand

BIOCHEMICAL OXYGEN DEMAND
BOD

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OUTLINE

- Introduction to BOD
  - What is BOD?
  - Why is BOD Important?
  - BOD Calculations
- Laboratory
  - Set up BOD Bottles
  - Measure Initial DO
- How is BOD Run?

2

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WHAT IS BOD?

- Biochemical oxygen demand testing is used to determine the relative oxygen requirements
  - Changes in dissolved oxygen concentration are used as an indirect measure of organic content.
- The BOD test is an estimate of the "food" available in the sample.
  - The more "food" present, the more DO will be required

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WHAT IS BOD?

- BOD measures the molecular oxygen:
  - Biochemically degrade organic material (carbonaceous demand)
    - In the presence of free oxygen, aerobic bacteria use the organic matter in wastewater as "food."
  - Oxidize inorganic material (e.g. sulfides and ferrous iron)
  - Measure the amount of oxygen used to oxidized reduced forms of nitrogen (nitrogenous demand) unless an inhibitor is added to prevent such reduction

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HISTORY OF BOD

- The Royal Commission on River Pollution, which was established in 1865, and the formation of the Royal Commission on Sewage Disposal in 1898 led to the selection in 1908 of BOD_5 as the definitive test for organic pollution of rivers.
- Five days was chosen as an appropriate test period because it was supposedly the longest time that river water took to travel from source to ocean in the U.K.
  - A 5-day duration for BOD determination has no theoretical grounding but is based on historical convention

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HISTORY OF BOD

- Thames River - London, England
  - Waste dumped in Thames River took 5 days to reach ocean

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History of BOD

- Tchobanoglous and Schroeder (1985) provide the following background: "In a report prepared by the Royal Commission on Sewage Disposal in the United Kingdom at the beginning of the century, it was recommended that a 5-day, 18.3°C, BOD value be used as a reference in Great Britain. These values were selected because British rivers do not have a flow time to the open sea greater than 5 days and average long-term summer temperatures do not exceed 18.3°C. The temperature has been rounded upward to 20°C, but the 5-day time period has become the universal scientific and legal reference."

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History of BOD

- In 1912, the commission also set a standard of 20 mg/L BOD_5 as the maximum concentration permitted in sewage works discharging to rivers, provided that there was at least an 8:1 dilution available at dry weather flow.
- The United States includes BOD effluent limitations in its secondary treatment regulations.
- Secondary sewage treatment is generally expected to remove 85 percent of the BOD measured in sewage and produce effluent BOD concentrations with a 30-day average of less than 30 mg/L and a 7-day average of less than 45 mg/L.

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BOD Timeline

- 1849: Forchamer determines the amount of potassium permanganate that a sample of polluted water consumes. (The first chemical oxygen demand experiment.)
- 1857: Brodie made the connection that pollutants could be removed from wastewater by oxidation.
- 1868: Frankland observes the absorption of gas by sealed containers of polluted water in an experimental setup that looks like the modern multiple dilution BOD test performed in BOD bottles.

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BOD Timeline

- 1884: Dupre proposes that microorganisms are the cause of the difference (amount of gas absorbed) and that oxygen is the link. The process cannot be proven until Winkler develops his titration procedure for determination of dissolved oxygen in 1888.
- 1889: The first proposed standard method for the determination of the oxygen-consuming capacity of a polluted water
- 1897: Separated oxygen demand into carbonaceous and nitrogenous components
- 1917: Third edition of Standard Methods contains BOD test

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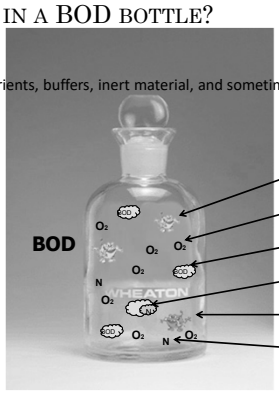
What is in a BOD bottle?

Plus water, nutrients, buffers, inert material, and sometimes interfering compounds



Bacteria

Oxygen

Organic Material

Nitrogen-containing Organic material

Nitrifying Bacteria

Nitrogen (ammonia, ammonium, nitrites)

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What is BOD?

- All three must be present in order to have BOD.

, Food, and O_2

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WHAT IS BOD?

(Bacteria) + Food + Oxygen (O_2) → Carbon Dioxide (CO_2)

Food - Organic material (carbon), exerts carbonaceous oxygen demand (cBOD)

Examples of food: $C_6H_{12}O_6$ – Glucose $C_4H_7O_4N$ – Glutamic Acid

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WHAT IS BOD?

(Bacteria) + Ammonia or Nitrite (NO_2^-) + Oxygen (O_2) → Nitrate(NO_3^-)

Food – Reduced forms of nitrogen, exert nitrogenous oxygen demand

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WHAT IS BOD?

Carbonaceous Oxygen Demand (cBOD) + Nitrogenous Oxygen Demand = **BOD**

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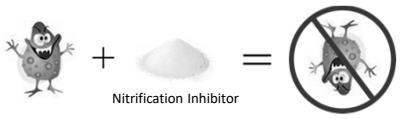
BOD

- The total BOD includes both carbonaceous and nitrogenous components
- If your permit requires CBOD (Carbonaceous BOD) only, you must add nitrification inhibitor
  - This prevents the oxidation of nitrogen compounds



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WHAT IS BOD?

- Dissolved oxygen levels are monitored before and after an incubation period, to determine the amount of oxygen depletion.

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WHAT IS BOD?

- Incubation conditions:
  - Temperature – 20 ± 1°C
  - Time – 5 days
  - In the dark

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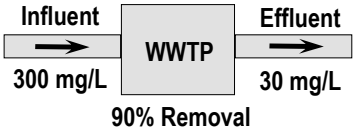
Why is BOD Important?

- Wastewater treatment plants (WWTP) aim to remove BOD from the influent (raw) water.
  - BOD can be used for process control and to measure the efficiency of a WWTP.
- BOD Removal Efficiency
  - BOD of effluent versus influent



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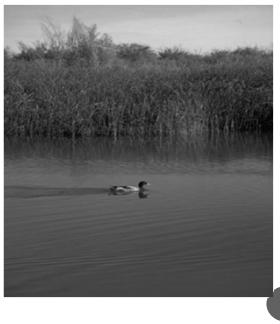
Why is BOD Important?

- BOD measurements help in monitoring the effect of effluent on the dissolved oxygen concentration of the receiving water body.
- BOD may be regulated by permit requirements.



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BOD Test Requirements

- Multiple sample dilutions
- Dilution water blank
- Seed control
- Seed blank
- Glucose/Glutamic Acid Standard
- Duplicates

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Sample Volume Dilution Estimation

- Industrial wastes = 0.1 - 1.0 %
- Raw/settled sewage = 1.0 - 5.0 %
- Oxidized effluent = 5.0 - 25 %
- Polluted river water = 25 - 100 %

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How is BOD Measured?

- Dilution Method

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BOD Calculations

- Using the dilution method, three values must be known in order to calculate BOD:
  - Initial DO
  - Final DO
  - Volume of sample

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BOD Dilution Method

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BOD – Dilution Method

- Glassware
- Preparing dilution water
- Seeding dilution water
- Sampling and sample handling
- Determining range and sample volumes
- Obtaining data points
- Running Standards

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Glassware

- Glassware must be extremely clean!
  - Have a set of glassware dedicated to BOD testing.
  - Clean glassware thoroughly before each use
    - Clean with a dilute bleach solution
    - Rinse at least 3x with DI water
    - Clean with 1:1 sulfuric acid
    - Rinse at least 3x with DI water
    - Allow to air dry

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Dilution Water

- Source water
- Reagents
- Method criteria
- Failure factors
- Other considerations

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Dilution Water Source Water

- 23nd Ed. Standard Methods 5210B 4.C
  - Serves to check the quality of unseeded dilution water and the cleanliness of bottles
  - May be distilled, tap or receiving stream
    - As long as they meet depletion of < 0.2 mg/L
    - Deionized water often contains enough organics and microorganisms to cause the dilution-water QC check to fail
  - Free of heavy metals
    - Ex: copper
  - Do not store for >24 hr after adding nutrients

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Dilution Water Reagents

- Use clean glassware, tubing (medical grade) and bottles
- Distilled water should be used in preparing reagents in advance (sterilized is preferred)
- Discard if any sign of precipitation or biological growth
- Commercial reagents are acceptable

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Dilution Water Method Criteria

- Preferably run two blanks, beginning and ending of sample set
- Dilution water blanks must meet quality control limits, < 0.2 mg/L DO (preferably <0.1 mg/L)
  - Otherwise discard and prepare fresh solution
- No seed or nitrification inhibitor is added for dilution water blank
- Total of two blanks
  - One dilution water blank at beginning
  - One dilution water blank at end

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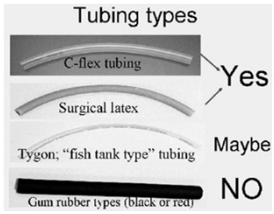
Dilution Water Failure Factors

- Check out this site for more information: http://dnr.wi.gov/regulations/labcert/BODDH2O.html
- Tubing constructed of oxygen-demand leaching material (consider medical grades)



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Dilution Water Failure Factors

- Slime growth in delivery tubing
- Poor water quality/improperly maintained system
- Poorly cleaned bottles or dilution water storage container
- Improperly calibrated probe

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Dilution Water Failure Factors

- Poor quality air for aeration.
- Deionizer systems can leach organics and grow bacteria.
  - Must be cleaned on a regular basis.
  - Try reverse osmosis (RO)/polisher combination systems that will produce ASTM Type 1 water.

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Dilution Water Other Considerations

- Store bought distilled water
  - What are the containers made of?
  - QA/QC of water? What's in it?
- If it ain't broke, don't fix it.
  - If the water that you purchased works satisfactorily and shows good QA/QC, continue using it.
- Use an all glass still, avoid metal stills.
  - Copper toxicity

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Preparing Dilution Water

- Store distilled water in a BOD incubator until the temperature reaches 20°C.
- Water is then saturated with oxygen and is ready for use.
  - Needs to be ≥ 7.5
  - Saturate with DO by shaking in a partially filled bottle or
  - By aerating with filtered air

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Preparing Dilution Water

- After adding buffer and nutrients, shake the bottle vigorously to ensure saturation.
- Use immediately!
  - Storage of prepared dilution water for more than 24 hours after adding nutrients, minerals and buffer is not recommended unless dilution water blanks consistently meet quality control limits

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Seeding Dilution Water

- Add seed to the dilution water (if needed)
- Seed can be added to:
  - Bulk dilution water preparation (good)
  - Individual bottles (better but expensive)

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Seeding Dilution Water

- When do you need to seed a sample??
- Seed may be necessary when testing:
  - Oxidized effluents
  - Toxic effluents
  - Samples with insufficient microorganisms
  - Samples collected after disinfection
  - ***cBOD samples***
- Preferable to sample BEFORE any disinfection
  - If sampling after any disinfection, samples MUST be seeded

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Seeding Dilution Water

- Sources of seed:
  - Settled domestic wastewater
  - Effluent from primary clarifiers
  - Diluted mixed liquor from an aeration basin
  - Undisinfected effluent
  - Receiving water from below the point of discharge
  - Purchased BOD seed

- When effluent of mixed liquor from a biological treatment process is used as a seed source, inhibition of nitrification is recommended

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Seeding Dilution Water

- How much seed should be added?
  - Seed should contribute an oxygen demand ranging from 0.6-1.0 mg/L.
  - The amount of seed added should be adjusted from this range to that required to provide glucose-glutamic acid (GGA) check results of 198 ± 30.5 mg/L
  - For example, if 1 mL of seed is needed to achieve 198 ± 30.5 mg/L, then use 1 mL in each BOD bottle receiving the test wastewater

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Seeding Dilution Water

- How is BOD of the seed determined?
  - Run seed control to determine the BOD of the seed.
  - Seed controls are run as if they were samples
    - Bottles containing dilution water and specific volumes of seed

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Seed Control Factor

- Divide the DO depletion by the volume of seed in mL for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results.

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Sampling and Sample Handling

- Sample Pre-Treatment
  - Composite samples kept at 1-6 °C
  - Recommended Hold Time = Analysis must be run within 2 hours of collection (grab or end of 24-hour compositing period)
    - Refrigerate if unable to do so, ≤6° C
    - 48-hour holding time (40 CFR 136, Table II) @ ≤ 6°C
  - Sample Temperature (20 ± 3 °C)
  - Sample (Dilution) pH (between 6.0-8.0)
    - If not, adjust pH to between 6.5 and 7.5
  - Check residual chlorine
    - If present, (1) quench chlorine, (2) seed samples
  - Samples Supersaturated? (DO > 9 mg/L at 20 °C)
    - Warm; shake or aerate to remove O_2

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Determining Range and Sample Volume

- Run a series of sample dilutions, at least 3 dilutions
  - Want 2 mg/L oxygen depletion in the first dilution
    - Minimum sample volume
  - Want 1 mg/L oxygen remaining in the last dilution
    - Maximum sample volume
  - Bring sample to 20 ± 3°C before checking initial DO

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Determining Range and Sample Volume

- Fill bottles to top without adding bubbles
- If > 67% (200 mL) sample after dilution, add nutrients, mineral and buffer solutions directly to the sample at a rate of 1 mL/L (0.3 mL/300-mL bottle) or commercial prepared product
- Add NI to partially filled sample bottle for cBOD.
  - Seeding is required
- After preparing dilution, measure initial DO within 30 minutes

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Determining Range and Sample Volume

<u>First Dilution</u>
2mg/L oxygen demand

8mg/L
6mg/L

<u>Last Dilution</u>
1mg/L oxygen remaining

1mg/L

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Determining Range and Sample Volume

- Selection of sample volumes used in the test depends on two factors:
  - Type of sample
  - Elevation

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Determining Range and Sample Volume

Table 2 Determining Minimum Sample Volume

Sample Type	Estimated BOD mg/L	mL of Sample*
Strong Trade Waste	600	1
Raw and Settled Sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Waters	6	100
	4	200
	2	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

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Determining Range and Sample Volume

Table 3 Determining Maximum Sample Volume

Estimated BOD at			mL of Sample*
sea level	1000 ft.	5000 ft.	
2460	2380	2032	1
1230	1189	1016	2
820	793	677	3
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

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Determining Range and Sample Volume

- If our sample is approximately 300mg/L BOD, what should the minimum and maximum sample volumes be?

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Determining Range and Sample Volume

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Oxidized Effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted River Waters	6	100
	4	200
	2	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

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41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

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Determining Range and Sample Volume

- If our sample is approximately 300mg/L BOD, what should the minimum and maximum sample volumes be?
  - Minimum volume = 2mL
  - Maximum volume = 8mL

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Determining Range and Sample Volume

Calculation of Range of Acceptable BOD results

$$\text{BOD Sample} = \frac{\text{Depletion}}{\text{Sample Volume (SV)}} \times 300\text{mL}$$

Lowest (minimum) acceptable results – lowest acceptable depletion 2.0 minimum depletion criterion

$$\text{Minimum BOD} = \frac{\text{Minimum Depletion (2.0)}}{\text{SV}} \times 300\text{mL}$$

$$\text{Minimum BOD To be reported} = \frac{600}{\text{SV}}$$

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Determining Range and Sample Volume

Calculation of Range of Acceptable BOD results

Highest (maximum) acceptable results – highest acceptable depletion
Lowest Acceptable Residual 1.0 minimum residual criterion
(Assume average initial DO to be 8.0 mg/L)

Maximum BOD = $\frac{\text{Initial Dissolved Oxygen} - 1.0}{\text{Sample Volume (SV)}} \times 300\text{mL}$

Maximum BOD = $\frac{7.0}{SV} \times 300\text{mL}$

Maximum BOD to be reported = $\frac{2100}{SV}$

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Determining Range and Sample Volume

mL used	Minimum BOD (2.0 mg/L Depletion)	Maximum BOD (assuming 8 mg/L initial DO)
1	600	2100
2	300	1050
3	200	700
4	150	525
5	120	420
6	100	350

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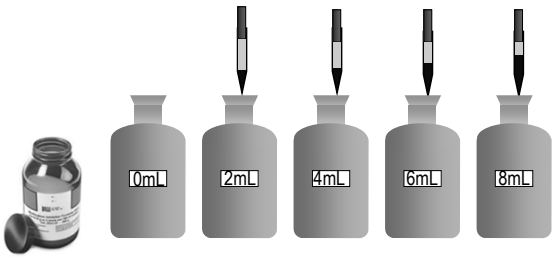
Determining Range and Sample Volume



Can add nitrification inhibitor (cBOD) and seed after bottles are $^2/_3$ full

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Determining Range and Sample Volume

- Nitrification:
  - Nitrosomonas + $NH_3 + O_2 \rightarrow NO_2^-$

    organism oxidizes ammonia into nitrite as a metabolic process
  - Nitrobacter + $NO_2^- + O_2 \rightarrow NO_3^-$

    bacteria of this family derive their energy from oxidizing ammonia to nitrite, or by oxidizing nitrite to nitrate.

    - Nitrogenous demand observed if these microbiologically mediated reactions occur.

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Determining Range and Sample Volume

- Nitrification inhibitor
  - Prevents Nitrosomonas from oxidizing ammonia to nitrite, preventing nitrogenous oxygen demand in the sample (CBOD measurement).
  - TCMP – 2-chloro-6-(trichloromethyl)pyridine

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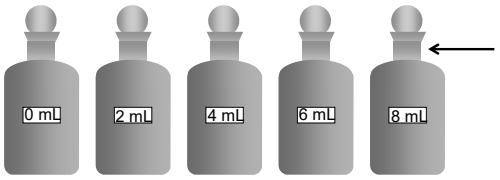
Determining Range and Sample Volume

- Fill bottles past the neck with dilution water and invert to mix (no air bubbles).



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Obtaining Data Points

- Dissolved oxygen concentration must be measured prior to incubation
  - Winkler titration
    - Duplicate bottles must be prepared
  - Dissolved oxygen meter

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Obtaining Data Points

- Measure DO
- Prior to measurement, prepare probe by:
  - Polarizing
  - Polishing
  - Calibrating
    - Water saturated air versus Winkler titration

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Calibration

- Winkler titration - best; most accurate
  - Relies on chemistry
- Probe: Air-saturated water
  - Reagent water at 20°C shaken/aerated to saturate
  - Maximum DO at 20°C ~ 9.00 mg/L
  - Meter result shouldn't vary greatly from the saturation point
  - Correct for pressure and/or altitude differences

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Calibration

- Probe: Water-saturated air (most common)
  - Air-calibration chamber → calibrate at sample temperature.
  - Minimizes errors caused by temperature differences.
  - Keep interior of the chamber just moist -- not filled with water.
  - Typical for probes
  - Probe is stored in a constant humidity environment
  - Container should be sealed somehow (to maintain constant humidity)

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Obtaining Data Points

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Obtaining Data Points

- After measuring DO, replace any lost volume in the bottle with dilution water.
- Replace stopper – watch for air bubbles!

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Obtaining Data Points

- Fill area in around stopper with dilution water.
- Cover with plastic BOD bottle cap.

You need a water seal!

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Obtaining Data Points

- Transfer samples to 20^0C incubator and incubate for 5 days in the dark.

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Obtaining Data Points

- After 5 days, remove samples from incubator and measure final DO.

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Obtaining Data

- Plug data into equation:

$$BOD_5, mg/L = \frac{(Initial\ DO - Final\ DO)}{P}$$

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Obtaining Data

- Plug data into equation:
- P = Sample volume/300

$$P = 2/300 = 0.00667$$

$$BOD_5, mg/L = \frac{(7.3 - 5.2)}{0.00667}$$

$$BOD_5, mg/L = 315\ mg/L$$

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Obtaining Data

- If bottles were seeded:

$$BOD_5, mg/L = \frac{(D_1 - D_2) - (B_1 - B_2)\ f}{P}$$

D = DO of sample

B = DO of seed

P = Sample volume/300

F = Ratio of seed in sample to seed in control

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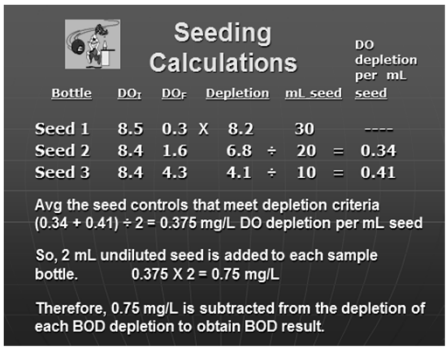
Obtaining Data



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BOD Solids Handling Bias

- Homogeneous
  - Mix sample while removing aliquot
- Use wide bore pipette
- Pipette as fast as possible to prevent loss of solids
- Pipette each sample dilution separately

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BOD QC Checks

- Sample depletion criteria
  - Depletion should be greater than 2.0 mg/L
  - Final DO should be > 1.0 mg/L
- Seed control should also meet sample depletion criteria
  - Seed contribution should be between 0.6 and 1.O mg/L
  - Blank's DO depletion <0.2 mg/l
  - GGA range approx. 198 ± 30.5 mg/L

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BOD Quality Control

- Solubility of oxygen in water at 20^0C is 9.2 mg/L
- Super saturation = initial DO > 9.0 mg/L
- Average all dilutions that meet QC criteria
- Suspect toxicity if smallest sample concentration yields greatest uptake
- What if no samples meet criteria?
  - Report qualified results

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Common Sources Of Error

- Not adjusting pH to within 6.0 – 8.0
  - Adjustment not required if effluent is between 6.0-8.0
  - Otherwise, adjust sample temperature to 20 $\pm$ 3°C, then adjust pH to 7.0 to 7.2 (H_2SO_4 or NaOH)
  - If pH is adjusted, samples must be seeded
- Improper calibration of DO meter
- Incubation temperatures not constant
- Initial DOs above saturation

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Common Sources Of Error

- Depletion criteria not met
  - Not depleting 2.0 mg/L
  - Final DO <1.0 mg/L
- Subtracting blanks
- Not seeding when required
- Seed strength not constant
- Not analyzing GGA samples
- Not evaluating for toxicity
- Improper calculations
- Water quality issues

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Running Standards

- Standard Methods – 6 mL/300 mL bottle
- Commercial solutions may contain other GGA concentrations; adjust doses accordingly
  - Add nitrification inhibitor if seed is obtained for a source that is nitrifying
  - 5-day BOD of 198 mg/L with a standard deviation of 30.5 mg/L
- Each lab may establish own control limits by performing at least 25 GGA checks
- When nitrification inhibitors are used, GGA test results outside the control-limit range often indicate that incorrect amounts of seed were used

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BOD – Take Home Messages

- BOD is an indirect measure of organic content.
- BOD is measured by oxidizing organics using microorganisms (under specific conditions) and directly measuring the amount of oxygen consumed in the process.

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- DOC
- LRB (Blank)
- LFB (Standard = GGA)
- Dup
- ICAL/CCV
- Control Charts
- Corrective Action
- QC Acceptance
- Batch Size (20)
- QC Frequency (depends on permit)

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Minimum DO depletion (including seed bottles) of 2.0 mg/l
- Minimum residual DO of at least 1.0 mg/l
- Dilution water quality check (nutrient, mineral, buffer) must not be more than 0.2 mg/l (0.1 is preferred)
- Seed control of three dilutions. Smallest to give at least 2.0 mg/l depletion and the largest to at least 1.0 mg/l residual….

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Demonstration of Capability (DOC)
  - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
  - Real people language: Each operator running this test needs to analyze 4 samples of GGA at a concentration of 198 ± 30.5 mg/L
  - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
  - Recommend backup analyst do this once a year.

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Method Blanks
  - Real people language: analyze dilution water
    - One at the beginning and one at end
  - Run daily (day of)
  - Target value is less than 0.20 mg/L (preferably less than 0.10 mg/L)

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Laboratory Fortified Blank
  - Real people language: analyze a Glucose/Glutamic Acid (GGA) standard at a concentration of 198 ± 30.5 mg/L
  - Run on a 5% basis, one for every 20 samples

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Duplicate
  - Analyze 2 samples for BOD or CBOD
    - Example, if you run 6, 9 and 12 mL on your raw/influent sample, run a second 9 mL sample.
    - You would end up with a total of 4 bottles for your raw/influent sample
  - Run on a 5% basis, one for every 20 samples
  - Calculate %RPD, ≤ 20%
  - 2014 Update - For reporting purposes, average results that meet method criteria.

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Initial Calibration (ICV)
  - Calibrate daily (day of) by following manufacturer's instructions
    - Using barometric pressure is best

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Continuing Calibration (CCV)
  - Prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L).
  - Same as DO CCV if using a different probe

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- Corrective Action - 1020 B.5., B.8,. & B.15.
- 5210 B.7.b. – Identify results in the test reports when any of the following quality control parameters is not met:
  - Dilution water exceeds 0.20 mg/L (5210B.6c)
  - Glucose-glutamic acid check falls outside of acceptable limits (5210B.6b)
  - Test replicates show more than 30% difference between high and low values
  - Seed control samples do not meet the above criteria in all dilutions (5210B.6d) or
  - Minimum DO is less than 1.0 mg/L (5210B.7a3)

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BOD5/cBOD5 SM5210 B – 2016 & Hach Method 10360

- QC Acceptance Criteria
  - Blanks < 0.20 mg/L
  - GGA = 198 ± 30.5 mg/L (if running cBOD
  - RPD < 20%
  - Minimum of three dilutions for each sample, at least one sample must have valid data with at least 2.0 mg/L depletion and a residual of 1.0 mg/L

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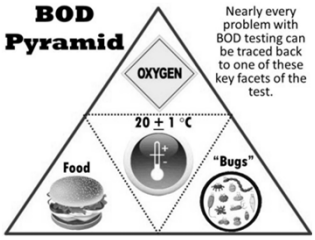
ANY QUESTIONS?



Source: https://dnr.wi.gov/regulations/labcert/BODAbout.html

TDEC - Fleming Training Center

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Biochemical Oxygen Demand (BOD) – Review Questions

Laboratory portion:

1. Why are dilution water Blanks included with each set of BOD tests?

2. What is the standard we used in the BOD test and what is its expected result?

3. What are the required incubation conditions?

4. The Initial DO must be no greater than what?

5. When do you add the buffer/nutrient solution to your dilution water?

6. When 4 examples of when it is necessary to add seed to a sample.

7. How much dilution water did we add to our Polyseed capsule?

8. Why is it necessary to check the pH of your sample and make adjustments if it falls outside of the 6.0 -8.0 range? If you make pH adjustments, are you required to seed the sample?

9. Why is it necessary to dechlorinate a BOD sample?

10. After the stopper is added to the full BOD bottle, the area around it is filled in with dilution water and then covered with a plastic cap. What is the purpose of the dilution water?

Classroom portion:

11. What two forms of oxygen demand make up BOD?

12. What is being measured when you add Nitrification Inhibitor to a sample?

13. List potential sources of error that could lead to dilution water failure.

14. What is the Seed Correction Factor (SCF)?

15. What is the acceptable range for SCF? In other words, the seed should contribute how much DO uptake per BOD bottle?

16. Do the following BOD sample results meet the sample dilution criteria requirements for valid BOD_5 results? If no, explain why not.

    Blank depletion = 0.25 mg/L

    Initial DO = 8.5 mg/L

    Final DO = 6.9 mg/L

17. Fill in the missing information:
    Sample Dilution Criteria for valid BOD_5 results

    - Blank depletion must be:
    - Initial DO must be:
    - Samples must deplete at least:
    - Samples must have at least:

BOD_5 of Soda Lab
23rd Edition Standard Methods 5210B-2016

Step 1: Set up Dilution Water

1. Aerated DI water
2. Hach BOD Nutrient Buffer Pillows (1 buffer for 4 liters of water)

Add buffer pillow to the aerated DI water and mix well.

Step 2: Set up Polyseed

1. 1000 mL beaker
2. Stir plate with stir bar
3. Seed material (1 polyseed capsule)
4. Dilution Water

To prepare the seed, add one polyseed capsule to 500 mL dilution water (DO NOT USE DI Water). Add stir bar and stir for one hour. After the hour has elapsed let seed solution settle for 15 min and pour supernatant into another 500 mL beaker making sure not to pour any solid material into the new beaker. Add clean stir bar and keep stirring for the remainder of the BOD setup.

Step 3: Prepared Diluted Soda Sample

1. 1 mL Volumetric Pipette
2. 100 mL Volumetric Flask
3. DI water
4. 6N NaOH

Need to create a 1:100 dilution. Put 1 mL of soda into the 100 mL volumetric flask and fill to the line with DI water to make dilution.
*** At the end of the “5 day” test we will multiply our results by 100***

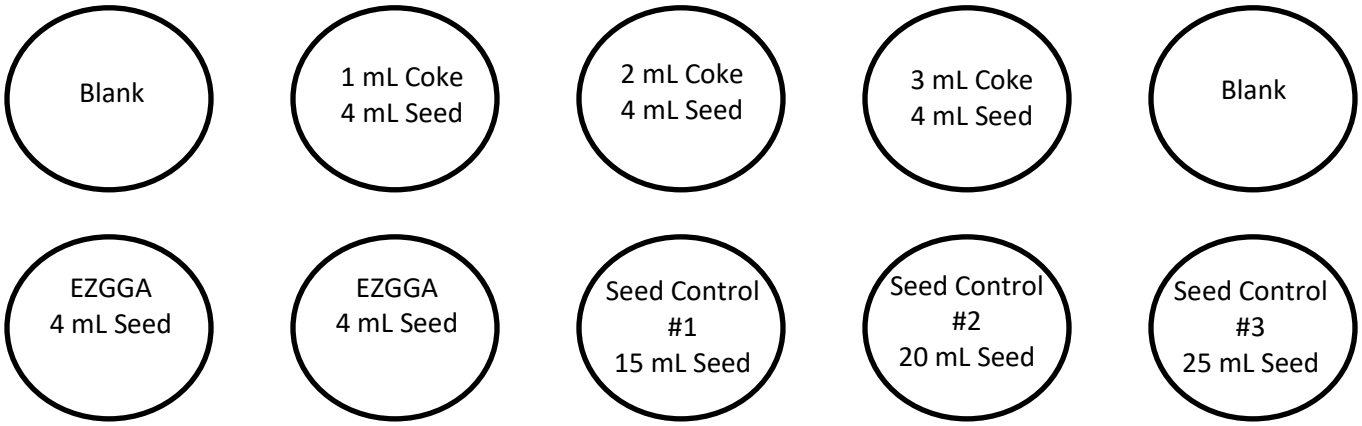
Adjust pH to 6.5 to 7.5 using 6N NaOH with a glass stirring rod.

At this point we will also set up 2 COD samples with our soda sample (look at Hach Method 8000 for instructions)

Step 4: Set up BOD Bottles

1. Calibrated LDO probe/meter
2. 10 BOD bottles, stoppers, and caps with plastic tote
3. 25 mL graduated cylinder
4. 100 (or 1,000) mL graduated Cylinder
5. 2 GGA (Glucose Glutamic Acid) Standard Ampules (EZGGA)

You will set up 10 BOD bottles: 3 sample dilutions, 2 blanks, 2 GGA standards, and 3 seed controls:



After filling bottles with proper sample fill with BOD dilution water to middle of the neck of the bottle. Read the DO of each bottle and record the number on the bench sheet. Replace any dilution water that is removed from the samples while taking the DO. Want to insure there is a water seal at the top of the stopper.

Incubate the samples in the dark at 20^{o}C +/- 1 for 5 days.

Step 5: Take off Samples

After incubation period remove the bottles from the incubator and record the final DO on the bench sheet.

Notes:

1. Rinse all glassware with DI water before adding anything to the bottles
2. Seed is not added to blank bottles
3. Since we diluted the original soda sample, we will need to factor that into our calculations (multiply result by 100)

5210 BIOCHEMICAL OXYGEN DEMAND (BOD)*

5210 A. Introduction

1. General Discussion

Biochemical oxygen demand (BOD) testing is used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters; its widest application is in measuring waste loadings to treatment plants and in evaluating the plants' BOD-removal efficiency. BOD testing measures the molecular oxygen used during a specified incubation period to

- biochemically degrade organic material (carbonaceous demand),
- oxidize inorganic material (e.g., sulfides and ferrous iron), and/or
- measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless an inhibitor is added to prevent such reduction.

The seeding and dilution procedures provide an estimate of BOD at pH 6 to 8.

The methods below measure oxygen consumed in a 5-d period (5210B), oxygen consumed after 60 to 90 d of incubation (5210C), and continuous oxygen uptake (5210D). Other BOD methods published elsewhere may use shorter or longer incubation periods; tests to determine oxygen-uptake rates; and/or alternative seeding, dilution, and incubation conditions to mimic receiving-water conditions, thereby estimating the environmental effects of wastewaters and effluents.

The ultimate BOD (UBOD) test measures the oxygen required to totally degrade organic material (ultimate carbonaceous demand) and/or to oxidize reduced nitrogen compounds (ultimate nitrogenous demand). UBOD values and appropriate kinetic descriptions are needed in water-quality modeling studies [e.g., UBOD:BOD_5 ratios for relating stream assimilative capacity to regulatory requirements; definition of river, estuary, or lake deoxygenation kinetics; and instream ultimate carbonaceous BOD (UCBOD) values for model calibration].

A number of factors (e.g., soluble versus particulate organics, settleable and floatable solids, oxidation of reduced iron and sulfur compounds, or lack of mixing) may affect the accuracy and precision of BOD measurements. Presently, there are no effective adjustments or corrections to compensate for these factors.

2. Carbonaceous Versus Nitrogenous BOD

Microorganisms can oxidize reduced forms of nitrogen, such as ammonia and organic nitrogen, thus exerting nitrogenous demand. Nitrogenous demand historically has been considered an interference in BOD testing; adding ammonia to dilution water contributes an external source of nitrogenous demand. The interference from nitrogenous demand can now be prevented by an inhibitory chemical,[1] but if it isn't used, the measured oxygen demand is the sum of carbonaceous and nitrogenous demands.

Measurements that include nitrogenous demand generally are not useful for assessing the oxygen demand associated with organic material. Nitrogenous demand can be estimated directly from ammonia nitrogen, and carbonaceous demand can be estimated by subtracting the theoretical equivalent of the nitrite and nitrate produced in uninhibited test results. However, this method is cumbersome and subject to considerable error. Chemical inhibition of nitrogenous demand provides a more direct, reliable measure of carbonaceous demand.

How much nitrogenous compounds oxidize during the 5-d incubation period depends on the concentration and type of microorganisms that can carry out this oxidation. Such organisms quite often are present in raw or settled primary sewage in adequate numbers to oxidize enough reduced nitrogen forms to contribute oxygen demand in the 5-d BOD test. Most biological treatment plant effluents contain enough nitrifying organisms to cause nitrification in BOD tests. Because nitrogenous compounds can oxidize in such samples, nitrification inhibition (as directed in 5210B.5*e*) is recommended for secondary-effluent samples, samples seeded with secondary effluent, and polluted-water samples.

3. Reference

1. YOUNG, J.C. 1973. Chemical methods for nitrification control. *J. Water Pollut. Control Fed.* 45:637.

* Approved by Standard Methods Committee, 2016.
Joint Task Group: James C. Young (chair), Victor D. Hahn, Robert V. Menegotto, Devon A. Morgan, Robin S. Parnell, Lisa M. Ramirez, Debra A. Waller.

5210 B. 5-Day BOD Test

1. General Discussion

The BOD test is an indirect measurement of organic matter; it measures the change in DO concentration caused by microorganisms as they degrade organic matter in a sample held in a stoppered bottle incubated for 5 d in the dark at 20°C. Analysts measure DO before and after incubation, and compute BOD using the difference between DO measurements. Because initial DO is determined shortly after dilution, all oxygen uptake occurring after this measurement is included in the BOD measurement.

For sampling and storage procedures, see 5210B.4*a*.

2. Apparatus

a. Incubation bottles: Use 60-mL glass bottles or larger (300-mL bottles with a flared mouth and ground-glass stopper are preferred). Clean bottles with a detergent, rinse thoroughly,

https://doi.org/10.2105/SMWW.2882.102

and drain before use. Alternatively, use disposable plastic BOD bottles that are capable of meeting all method quality-control (QC) checks.

b. Air incubator or water bath, thermostatically controlled at 20 ± 1°C. Exclude all light to prevent the possibility of photosynthetic production of DO.

c. Oxygen-sensitive membrane electrode, polarographic or galvanic, or *oxygen-sensitive optical probe* with appropriate meter.

3. Reagents

Discard reagents if there is any sign of precipitation or biological growth in the stock bottles. Commercial equivalents of these reagents are acceptable, and different stock concentrations may be used if doses are adjusted proportionally. Use reagent grade or better for all chemicals and use distilled or equivalent reagent-grade water (see Section 1080) to make all solutions.

a. Phosphate buffer solution: Dissolve 8.5 g monopotassium phosphate (KH_2PO_4), 21.75 g dipotassium phosphate (K_2HPO_4), 33.4 g disodium phosphate ($Na_2HPO_4 \cdot 7H_2O$, and 1.7 g ammonium chloride (NH_4Cl) in about 500 mL reagent-grade water and dilute to 1 L. The pH should be 7.2 without further adjustment. Alternatively, dissolve 42.5 g KH_2PO_4 and 1.7 g NH_4Cl in about 700 mL reagent-grade water. Adjust pH to 7.2 with 30% sodium hydroxide (NaOH) and dilute to 1 L.

b. Magnesium sulfate ($MgSO_4$) solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in reagent-grade water and dilute to 1 L.

c. Calcium chloride ($CaCl_2$) solution: Dissolve 27.5 g $CaCl_2$ in reagent-grade water and dilute to 1 L.

d. Ferric chloride ($FeCl_3$) solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in reagent-grade water and dilute to 1 L.

e. Acid and alkali solutions, 1N, to neutralize caustic or acidic waste samples.

1) Acid—Slowly and while stirring, add 28 mL conc sulfuric acid (H_2SO_4) to reagent-grade water. Dilute to 1 L.

2) Alkali—Dissolve 40 g NaOH in distilled water. Dilute to 1 L.

f. Sodium sulfite (Na_2SO_3) solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL reagent-grade water. This solution is unstable; prepare daily.

g. Nitrification inhibitor:

1) 2-chloro-6-(trichloromethyl) pyridine (TCMP)—Use pure TCMP or commercial preparations.*

2) Allylthiourea (ATU) solution—Dissolve 2.0 g allylthiourea ($C_4H_8N_2S$) in about 500 mL reagent-grade water and dilute to 1 L. Store at 4°C. The solution is stable for 2 weeks when stored at ≤6°C without freezing.

h. Glucose–glutamic acid (GGA) solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to reagent-grade water and dilute to 1 L. Prepare fresh immediately before use unless solution is maintained in a sterile container. Store all GGA mixtures at ≤6°C without freezing unless manufacturer recommendations state otherwise. Commercial preparations may be used but concentrations may vary. Discard solutions if evidence of contamination is indicated (e.g., growth occurs in the stock bottle or GGA test results are consistently low).

i. Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL reagent-grade water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

j. Source water for preparing BOD dilution water: Use demineralized, distilled, or equivalent reagent-grade water, tap, or natural water to make sample dilutions (see 5210B.4*c*).

4. Preparatory Procedures

a. Sampling and storage: Samples for BOD analysis may degrade significantly during storage between collection and analysis, resulting in low BOD values.

1) Grab samples—If analysis begins within 2 h of collection, cold storage is unnecessary. Otherwise, keep sample at ≤6°C between collection and analysis. Ideally, begin analysis within 6 h of sample collection; if impossible due to distance between sampling site and laboratory, then begin analysis within 24 h of collection. The recommended holding time is 24 h; however, the U.S. Environmental Protection Agency (EPA) allows for a 48-h holding time.

2) Composite samples—Limit compositing period to 24 h, and keep samples at ≤6°C during process. Store for the same time and temperature as grab samples, although in this case, holding time begins when the compositing period ends.

b. Sample preparation and pretreatment:

1) All samples—Check pH; if it is not between 6.0 and 8.0, adjust sample temperature to 20 ± 3°C, then adjust pH to between 6.5 and 7.5 using an H_2SO_4 or NaOH solution strong enough not to dilute sample by >0.5%. Exceptions may be justified with natural waters when BOD will be measured at *in situ* pH values. Dilution-water pH should not be affected by the lowest sample dilution. Always seed samples that have been pH-adjusted.

2) Samples containing residual chlorine compounds—If possible, avoid samples containing residual chlorine by sampling ahead of chlorination processes. If residual chlorine is present, dechlorinate sample. Sometimes chlorine will dissipate from sample within 1 to 2 h of standing in the light; this often occurs during transport and handling. If the chlorine residual does not dissipate in a reasonably short time, destroy it by adding Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution on a 100- to 1000-mL portion of neutralized sample by adding 10 mL 1 + 1 acetic acid or 1 + 50 H_2SO_4 and 10 mL potassium iodide (KI) solution (10 g/100 mL) per 1000 mL sample, and then titrating with Na_2SO_3 solution to the starch-iodine endpoint for residual. Add to neutralized sample the proportional volume of Na_2SO_3 solution determined by the above test, mix, and check sample for residual chlorine after 10 to 20 min. (NOTE: Excess Na_2SO_3 exerts an oxygen demand and reacts slowly with certain organic chloramine compounds that may be present in chlorinated samples.) Do not test chlorinated/dechlorinated samples without seeding.

3) Samples containing other toxic substances—Certain industrial wastes (e.g., plating wastes) contain toxic metals. Such samples often require special study and treatment.

4) Samples supersaturated with DO (Table 4500-O:I)—Samples with DO concentrations above saturation at 20°C may be collected in cold waters or in water where photosynthesis occurs. To prevent oxygen loss when incubating such samples, reduce

* Nitrification Inhibitor Formula 2533 (2% TCMP on sodium sulfate), Hach Co., Loveland, CO, or equivalent.

DO to saturation by bringing sample to about 20 ± 3°C in partially filled bottle while agitating by vigorous shaking or aerating with clean, filtered compressed air.

5) Samples containing hydrogen peroxide—Hydrogen peroxide remaining in samples from some industrial bleaching processes (e.g., those used at paper mills and textile plants) can cause supersaturated oxygen levels in samples collected for BOD testing. Mix such samples vigorously in open containers long enough to allow hydrogen peroxide to dissipate before setting up BOD tests. Check adequacy of peroxide removal by observing DO concentrations over time during mixing or by using peroxide-specific test strips. Mixing times can vary from 1 to 2 h, depending on the amount of hydrogen peroxide present. The peroxide reaction can be considered complete when DO no longer increases during a 30-min period without mixing.

c. Selection and storage of source water for BOD sample dilution: Obtain water from suitable source (i.e., distilled, tap, or reagent-grade water). Make sure water is free of heavy metals, specifically copper (<0.05 mg/L) and toxic substances [e.g., chlorine (<0.10 mg/L)] that can interfere with BOD measurements. Protect source-water quality by using clean glassware, tubing, and bottles. Deionized (DI) water often contains enough organics and microorganisms to cause the dilution-water QC check to fail (5210B.6*c*). DI water is not recommended unless dilution-water blanks consistently meet QC limits. Source water may be stored before use as long as the prepared dilution water (5210B.5*a*) meets QC criteria in the dilution-water blank (5210B.6*c*). Such storage may improve the quality of some source waters but may allow biological growth to deteriorate others. Storing prepared dilution water (5210B.5*h*) for >24 h after adding nutrients, minerals, and buffer is not recommended unless dilution-water blanks consistently meet QC limits. Discard stored source water if dilution-water blank shows >0.2 mg/L DO depletion in 5 d (5210B.6*c*).

d. Preparation of seed suspension: Each BOD bottle must contain a microorganism population that can oxidize biodegradable organic matter in the sample. Domestic wastewater, unchlorinated or other undisinfected effluents from biological wastewater treatment plants, and surface waters receiving wastewater discharges usually contain satisfactory microbial populations. Some samples (e.g., some untreated industrial wastes, disinfected wastes, high-temperature wastes, wastes with pH values <6 or >8, or wastes stored >6 h after collection) do not contain a sufficient microbial population. Seed such samples by adding a population of suitable microorganisms; the preferred seed comes from a sample-related biological wastewater treatment system or receiving water. In this case, use supernatant from settled domestic wastewater, effluent from primary clarifiers, diluted mixed liquor from an aeration basin, undisinfected effluent, or receiving water from below the discharge point. If using effluent or mixed liquor from a biological treatment process as a seed source, nitrification inhibition is recommended. Do not use seed from effluents that have been disinfected by chlorine or other means. Commercial seed sources may be used according to manufacturer's preparation instructions but are more likely to be unadapted to the wastewater constituents. Do not filter seed sources; filtering removes the seed microorganisms.

If adapted seed sources are unavailable, develop an acclimated seed in the laboratory by continuously aerating a sample of settled domestic wastewater and adding small daily increments of sample from the waste in question. Use a soil suspension, activated sludge, or a commercial seed preparation to obtain the initial microbial population. Determine the existence of a satisfactory population by testing the seed's performance in BOD tests on the sample. BOD values that increase during adaptation to a steady high value indicate successful seed acclimation.

5. Testing Procedure

a. Preparation of dilution water: Transfer desired working volume of source water (5210B.4*c*) to a suitably sized bottle (glass is preferred). Check to ensure that the DO concentration is at least 7.5 mg/L before using water for BOD tests. If not, add DO by shaking bottle or aerating it with organic-free filtered air. Alternatively, store the water in cotton-plugged bottles long enough for the DO concentration to approach saturation. Add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solution/L to prepared source water (5210B.4*c*). Mix thoroughly and bring temperature to 20 ± 3°C. Prepare dilution water immediately before use, unless dilution-water blanks (5210B.6*c*) show that the water is acceptable after longer storage times. If dilution-water blanks show a DO depletion >0.2 mg/L, then improve purification or use water from another source. Do not add oxidizing agents or expose dilution water to ultraviolet light to try to bring the dilution blank into range.

b. Sample temperature adjustment: Bring sample temperature to 20 ± 3°C before making dilutions.

c. Preparation of dilutions: Using dilution water prepared as in ¶ *a* above, make at least three dilutions of prepared sample estimated to produce, at the end of the test, at least one dilution that would result in a residual DO of ≥1.0 mg/L and a DO uptake of ≥2.0 mg/L after a 5-d incubation. Two dilutions are allowed if experience with a particular sample source produces at least one bottle with acceptable minimum DO depletions and residual limits (5210B.6*a*). Individual laboratories should evaluate the need for more than three dilutions when historical sample data are unavailable. A more rapid analysis, such as COD (Section 5220), may be correlated approximately with BOD and serve as a guide in selecting dilutions. In the absence of prior knowledge, use the following percentages of wastewater when preparing dilutions: 0.01 to 1.0% for strong industrial wastes, 1 to 5% for raw and settled wastewater, 5 to 25% for biologically treated effluent, and 25 to 100% for polluted river waters. The number of bottles to be prepared for each dilution depends on DO technique and number of replicates desired. Prepare dilutions in volumetric containers (Class A glass or equivalent) and then transfer to BOD bottles, or else prepare directly in BOD bottles. Either dilution method can be used to transfer sample to respective BOD bottles.

1) Dilutions prepared in volumetric containers—Using a wide-tipped pipet or graduated cylinder, add desired amount of prepared sample to individual volumetric cylinders or flasks. Mix sample well immediately before pipetting to avoid solids loss via settling. For dilutions greater than 1:300, make a primary dilution before making final dilution in volumetric cylinders or flasks. Fill cylinders or flasks at least two-thirds full with dilution water and sample without entraining air. Add appropriate amounts of seed suspension (¶ *d* below) and nitrification inhibitor (¶ *e* below). Dilute to final level with dilution water (¶ *a* above). Mix well but avoid entraining air. Siphon mixed dilution

into a suitable number of BOD bottles, taking care not to let solids settle in cylinder or flask during transfer. When a cylinder or flask contains >67% of sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions (5210B.3*a–d*) directly to diluted sample at a rate of 1 mL/L (0.30 mL/300-mL bottle), or use commercially prepared solutions designed to dose the appropriate container size.

2) Dilutions prepared directly in BOD bottles—Using a wide-tip volumetric pipet or graduated cylinder, add desired sample volume to individual BOD bottles. Mix sample well immediately before pipetting to avoid solids loss via settling. For dilutions greater than 1:300, make a primary dilution before making final dilution in the bottle. Fill each BOD bottle approximately two-thirds full with dilution water and/or sample without entraining air. Add appropriate amounts of seed suspension (¶ *d* below) and nitrification inhibitor (¶ *e* below) to individual BOD bottles. Fill remainder of BOD bottle with dilution water. When a bottle contains >67% of sample after dilution, nutrients may be limited in the diluted sample and subsequently reduce biological activity. In such samples, add the nutrient, mineral, and buffer solutions (5210B.3*a–d*) directly to diluted sample at a rate of 1 mL/L (0.30 mL/300-mL bottle), or use commercially prepared solutions designed to dose the appropriate bottle size.

d. Addition of seed suspension: If seeding is used, add seed suspensions to dilution vessels or individual BOD bottles before final dilution, as described in ¶ *c* above. Do not add seed directly to wastewater samples before dilution if they contain toxic materials. Generally, 1 to 3 mL of settled raw wastewater or primary effluent or 1 to 2 mL of a 1:10 dilution of mixed liquor/300-mL bottle will provide enough microorganisms. Do not filter seed suspension before use. Agitate seed suspension during transfer to ensure that the same quantity of microorganisms is added to each BOD bottle. Always record the exact volume of seed suspension added to each bottle. The DO uptake attributable to added seed generally should be between 0.6 and 1.0 mg/L, but adjust seed amount as needed to provide GGA check results of 198 ± 30.5 mg/L. For example, if 1 mL seed suspension is required to achieve 198 ± 30.5 mg/L BOD in the GGA check, then use 1 mL in each BOD bottle receiving the test wastewater.

e. Addition of nitrification inhibitor: Samples that may require nitrification inhibition[1] include, but are not limited to, biologically treated effluents, samples seeded with biologically treated effluents, and river waters. Note the use of nitrification inhibition and the related chemical used when reporting results. (NOTE: TCMP is the preferred nitrification inhibitor but requires handling and transfer in a solid form. ATU is not always effective in inhibiting nitrification within the 5-d incubation period, and concentrations >2 mg/L may increase carbonaceous BOD (CBOD) measurements and/or adversely affect the azide modification of the iodometric method.) Seed all samples to which nitrification inhibitor has been added.

1) Nitrification inhibition using TCMP—Add 10 mg TCMP/L to diluted sample, 3 mg TCMP to each 300-mL bottle, or proportional amounts to other sized bottles after initial sample dilution but before final filling of bottles with dilution water. Do not add TCMP to BOD bottles before they are at least two-thirds filled with diluted sample. (NOTE: TCMP dissolves slowly and can float on top of sample if not mixed well.) Some commercial TCMP formulations are not 100% TCMP; adjust dosage appropriately.

2) Nitrification inhibition using ATU—Add 1 mL ATU solution [5210B.3*g*2)]/L diluted sample or 0.3 mL/300-mL test bottle. Do not add ATU to BOD bottles until they are at least two-thirds filled with diluted sample.

f. Sealing bottles: Completely fill each bottle by adding enough dilution water so insertion of stopper leaves no bubbles in the bottle. Mix sample by turning bottle manually several times unless immediately using a DO probe with a stirrer to measure initial DO concentration. As a precaution against drawing air into the dilution bottle during incubation, use a water seal. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of special BOD bottles. Place a paper or plastic cup or foil cap over flared mouth of bottle to reduce evaporation of water seal during incubation.

g. Determination of initial DO: Use the azide modification of the iodometric method (Section 4500-O.C), membrane-electrode method (Section 4500-O.G), or optical-probe method (Section 4500-O.H) to determine initial DO on all sample dilutions, dilution-water blanks, and, where appropriate, seed controls. Replace any displaced contents with enough diluted sample or dilution water to fill the bottle, stopper all bottles tightly, and water seal before beginning incubation. After preparing dilution, measure initial DO within 30 min. If using the membrane-electrode method or optical probe method, calibrate DO probe daily by following the manufacturer's calibration procedure. Make frequent calibration checks daily to ensure accurate DO readings and, ideally, perform a Winkler titration as needed to verify calibration. If using the azide modification of the titrimetric iodometric method, prepare an extra bottle for initial DO determination for each sample dilution.

h. Sample incubation: Incubate at 20 ± 1°C the stoppered and sealed BOD bottles containing desired dilutions (¶ *c* above), seed controls (5210B.6*d*), dilution-water blanks (5210B.6*c*), and GGA checks (5210B.6*b*). Exclude light to avoid algae growth in bottles during incubation.

i. Determination of final DO: After 5 d ± 6 h of incubation, determine DO in all sample dilutions, blanks, and checks as in 5210B.6*g*, using the azide modification of the titrimetric method (Section 4500-O.C), membrane-electrode method (Section 4500-O.G), or optical-probe method (Section 4500-O.H).

6. Quality Control Checks

The QC practices considered to be an integral part of each method are summarized in Table 5020:I.

a. Minimum residual DO and minimum DO depletion: Only bottles (including seed controls) whose DO depletion is ≥2.0 mg/L and residual DO is ≥1.0 mg/L after 5 d of incubation are considered to produce valid data, because ≥2.0 mg oxygen uptake/L is required to give a meaningful measure of oxygen uptake and ≥1.0 mg/L must remain to ensure that waste constituents' oxidation rates were not limited by insufficient DO. However, there are exceptions—for reporting purposes only—when testing undiluted samples and all bottles' DO depletion is <2.0 mg/L and residual DO is <1.0 mg/L (see 5210B.7).

b. Glucose–glutamic acid check: The GGA check is the primary basis for establishing the BOD test's accuracy and precision, as well as the principal measure of seed quality and set-up procedure. Together with each batch of BOD or CBOD samples, check seed effectiveness and analytical technique by using pro-

cedures in 5210B.5 to make BOD measurements on an equal weight mixture of glucose and glutamic acid as follows: Add sufficient amounts of standard glucose and glutamic acid solutions (5210B.3*h*) to give 3.0 mg glucose/L and 3.0 mg glutamic acid/L in each of three test bottles (20 mL GGA solution/L seeded dilution water, or 6.0 mL/300-mL bottle). Commercial solutions may contain other GGA concentrations; adjust doses accordingly. Add nitrification inhibitor if seed is obtained from a source that is nitrifying, and also to all CBOD GGA checks. Evaluate data as described in 5210B.8. The resulting average BOD/CBOD for the three bottles, after correcting for dilution and seeding, must fall into the control-limit range established in 5210B.8*a*. If the average value falls outside this range, evaluate the cause and make appropriate corrections. Consistently high values can indicate too much seed suspension, contaminated dilution water, or nitrification; consistently low values can indicate poor seed quality or quantity or else the presence of a toxic material. If low values persist, prepare a new GGA mixture and check the dilution-water and seed sources.

c. Dilution-water-quality check: With each batch of dilution water, incubate two or more bottles of dilution water containing nutrient, mineral, and buffer solutions but no seed or nitrification inhibitor. Dilution water checks must be analyzed with each batch of samples; the dilution-water blank serves as a check on the quality of unseeded dilution water and cleanliness of incubation bottles. Determine initial and final DO for each bottle (5210B.5*e* and *i*), and average results. The average DO uptake in 5 d must not be >0.2 mg/L and preferably ≤0.1 mg/L before making seed corrections. If average dilution-water blank is >0.2 mg/L, record the data and clearly identify such samples in data records.

d. Seed control: Determine the seed suspension's BOD as for any other sample. This is the *seed control.* Ideally, make three dilutions of seed so the smallest quantity depletes ≥2.0 mg/L DO and the largest quantity leaves ≥1.0 mg/L DO residual after 5 d of incubation. Determine DO uptake per milliliter of seed by dividing the DO depletion by the volume of seed in milliliters for each seed control bottle with a 2.0 mg/L depletion and >1.0 mg/L minimum residual DO, and averaging the results. Seed dilutions showing widely varying depletions per milliliter of seed (±30%) suggest the presence of toxic substances or large particulates in the seed suspension; check or change the seed source.

7. Data Analysis and Reporting

a. Calculations:

1) For each test bottle with at least 2.0 mg/L DO depletion and at least 1.0 mg/L residual DO—before seed correction, calculate BOD as follows:

$$\text{BOD}_5, \text{mg/L} = \frac{(D_1 - D_2) - (S)V_S}{P}$$

where:

D_1 = DO of diluted sample immediately after preparation, mg/L,
D_2 = DO of diluted sample after 5 d incubation at 20°C, mg/L,
S = oxygen uptake of seed [Δ DO/mL seed suspension added per bottle (5210B.6*d*) ($S = 0$ if samples are unseeded)],
V_s = volume of seed in respective test bottle, mL, and
P = decimal volumetric fraction of sample used; $1/P$ = dilution factor.

2) If DO depletion is <2.0 mg/L and sample concentration is 100% (no dilution except for seed, nutrient, mineral, and buffer solutions), actual seed-corrected DO depletion may be reported as the BOD even if it is <2.0 mg/L.

3) When all dilutions result in a residual DO <1.0, select the bottle with the highest DO concentration (usually the greatest dilution) and report:

$$\text{BOD}_5, \text{mg/L} > \frac{(D_1 - D_2) - (S)V_S}{P}$$

4) If all dilutions result in DO depletion <2.0 mg/L and the sample was diluted, select the bottle with the largest volume of sample (the least dilution) and calculate the report as if the dilution had depleted 2.0 mg/L:

$$\text{BOD}_5, \text{mg/L} < \frac{(D_1 - D_2) - (S)V_S}{P}$$

In the above calculations, do not make corrections for DO uptake by the dilution-water blank during incubation.

b. Reporting: Average test results for all qualified bottles in each dilution series. Report the result as BOD_5 if nitrification is not inhibited; report it as CBOD_5 if nitrification is inhibited. Samples with large differences between the computed BOD for different dilutions (e.g., the highest value is >30% larger than the lowest value) may indicate a toxic substance or analytical problems. When the effect becomes repetitive, investigate to identify the cause. Toxicity should be claimed only after thorough investigation using respirometric (5210D) or equivalent methods. Identify results in the test reports when any of the following QC conditions occur:

- dilution-water blank average is >0.2 mg/L (5210B.6*c*),
- GGA check falls outside acceptable limits (5210B.6*b*),
- test replicates show >30% difference between highest and lowest values,
- none of the seed control samples meet the above criteria (5210B.6*d*), or
- all dilutions result in a residual DO <1.0 mg/L [5210B.7*a*3)].

8. Precision and Bias

There is no measurement for establishing the BOD test's bias. The GGA check prescribed in 5210B.6*b* is intended to be a reference point for evaluating dilution-water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed GGA solution provided the following results:

Number of months:	14	
Number of triplicates:	421	
Average monthly recovery:	204	mg/L
Average monthly standard deviation:	10.4	mg/L

In a series of interlaboratory studies,[2] each involving 2 to 112 laboratories (and as many analysts and seed sources), 5-d BOD measurements were made on synthetic-water samples containing a 1:1 mixture of GGA ranging from 3.3 to 231 mg/L total concentration. The regression equations for mean value, X, and standard deviation, S, from these studies were:

X = 0.658 (added concentration, mg/L) + 0.280 mg/L

S = 0.100 (added concentration, mg/L) + 0.547 mg/L

a. Control limits: Applying the above equations to the 300-mg/L GGA primary standard yields an average 5-d BOD of 198 mg/L with a standard deviation of 30.5 mg/L. Because many factors affect BOD tests in multi-laboratory studies, resulting in extremely variable test results, one standard deviation (as determined by interlaboratory tests) is recommended as a control limit for individual laboratories. Alternatively, each laboratory may establish its own control limits by performing at least 25 GGA checks (5210B.6*b*) over several weeks or months and calculating the mean and standard deviation. Use the mean ±3 standard deviations as the control limit for future GGA checks. Compare calculated control limits to the single-laboratory tests presented above and to interlaboratory results. If any GGA test results are outside the acceptable control-limit range, identify them clearly in all data records, investigate source of the problem, and make appropriate corrections.

When nitrification inhibitors are used, GGA test results outside the control-limit range often indicate that incorrect amounts of seed were used. Adjust the amount of seed added to the GGA test so results fall within range (5210B.6*b*).

b. Working range and reporting limit: The *working range* is equal to the difference between the maximum initial DO (7 to 9 mg/L) and minimum DO residual of 1 mg/L corrected for seed and multiplied by the dilution factor, including any intermediate dilutions performed (5210B.5*c*).

Reporting limits are established by the minimum DO depletion and minimum DO residuals as follows:

- The lower reporting limit for unseeded samples that require no dilution—except for nutrient, mineral, and buffer solutions ($S = 0$; $P = 1.0$)—is equal to the DO measurement method's detection limit (~0.1 mg/L).
- The lower reporting limit for seeded samples that require no dilution—except for seed, nutrient, mineral, and buffer solutions ($S > 0$; $P = 1.0$)—is the difference between sample DO depletion and seed correction.

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5210 C. Ultimate BOD Test

1. General Discussion

The ultimate BOD test is an extension of the 5-d dilution BOD test (5210B) but with a number of specific test requirements and differences in application. Be familiar with the 5210B procedure before conducting tests for UBOD.

a. Principle: The method consists of placing a single sample dilution in full, airtight bottles and incubating under specified conditions for an extended period, depending on wastewater, effluent, river, or estuary quality.[1] DO is measured (with probes) initially and intermittently during the test. From the DO versus time series, UBOD is calculated by an appropriate statistical technique. For more accuracy, run tests in triplicate.

Bottle size and incubation time are flexible to accommodate individual sample characteristics and laboratory limitations. Incubation temperature, however, is 20°C. Most effluents and some naturally occurring surface waters contain materials whose oxygen demands exceed the DO available in air-saturated water; in such cases, either dilute sample or monitor DO frequently to ensure that low DO or anaerobic conditions do not occur. Reaerate sample when DO concentrations approach 2 mg/L.

Because bacterial growth requires nutrients (e.g., nitrogen, phosphorus, and trace metals), the necessary amounts may be added to dilution water, along with a buffer to keep pH in the bacterial-growth range and enough seed for an adequate bacterial population. (No specific nutrient or buffer formulations are included here because of the wide range of water and wastewater characteristics and varied applications of UBOD data.) That said, if the result will be used to estimate the oxidation rate of naturally occurring surface waters, adding nutrients and seed probably will accelerate the decay rate and produce misleading results. If only UBOD is desired, adding supplemental nutrients that accelerate decay and shorten test duration may be advantageous. When using nutrients, also add them to the dilution-water blank.

How much nitrogenous compounds will oxidize during the prescribed incubation period depends on how many relevant oxidizing microorganisms are present. These organisms may be too scarce in wastewaters to oxidize significant quantities of reduced nitrogen, but abundant in naturally occurring surface waters. Results may be erratic when a nitrification inhibitor is used,[2] so do NOT use one unless prior experimental evidence on

TABLE 5210:I. UBOD RESULTS FOR WASTEWATER SAMPLE

Day	(1) Average DO* *mg/L*	(2) Average Blank DO† *mg/L*	(3) Accumulated DO Consumed by Sample‡ *mg/L*	(4) Average NO_3-N *mg/L*	(5) NBOD *mg/L*§	(6) CBOD *mg/L*‖
0	8.1	–	0	0.0	0	0
3	5.6	–	2.5	–	0	2.5
5	3.5/8.0	–	4.6	0.0	0	4.6
7	6.2	–	6.4	–	0.23	6.2
10	3.2/8.2	–	9.4	0.10	0.46	8.9
15	4.3	–	13.3	–	0.58	12.7
18	2.7/8.1	–	14.9	0.15	0.69	14.2
20	6.6	–	16.4	–	0.80	15.6
25	5.4	–	17.6	0.20	0.92	16.7
30	2.6/8.2	–	20.4	–	0.92	19.5
40	5.3	–	23.3	0.20	0.92	22.4
50	3.1/8.0	–	25.5	–	0.92	24.6
60	4.5	–	29.0	–	0.92	28.1
70	3.3/8.1	–	30.2	–	0.92	29.3
80	5.4	–	32.9	0.20	0.92	32.0

* Two readings indicate concentrations before and after re-aeration.
† None was used.
‡ Column (1) – blank correction (none needed in the example).
§ Column (4) × 4.57 (linear interpolation between values).
‖ [Column (3) – Column (5)] × dilution factor.
UCBOD = 34.5 mg/L; CBOD decay rate = 0.03/d (calculated with first-order equation from 5210C.4).

the particular sample suggests that it is acceptable.* Monitor nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) to compute the oxygen equivalency of the nitrification reaction. When these values are subtracted from the DO versus time series, the CBOD time series can be constructed.[3]

b. Sampling and storage: See 5210B.4*a*.

c. Quality control: The QC practices considered to be an integral part of each method are summarized in Table 5020:I.

2. Apparatus

a. Incubation bottles: 2-L or larger glass bottles with ground-glass stoppers;† 4- to 10-L glass serum bottles are available. Alternatively, use nonground-glass bottles with nonbiodegradable plastic caps as a plug insert. Do not reuse plugs because they become discolored with continued use. Replace plugs every 7 to 14 d. Do not use rubber stoppers that may exert an oxygen demand.

Clean bottles with a detergent and wash with dilute hydrochloric acid (HCl) (3*N*) to remove surface films and precipitated inorganic salts; rinse thoroughly with DI water before use. Cover top of bottles with paper after rinsing to prevent dust from collecting.

Use a water seal to avoid drawing air into sample bottle during incubation. If bottle does not have a flared mouth, construct a water seal by making a watertight dam around the stopper (or plug) and fill with water from the reservoir as necessary. Cover dam with clean aluminum foil to retard evaporation. If using a 2-L BOD bottle, fill reservoir with sample and cover with a polyethylene cap before incubation.

Place a clean magnetic stirring bar in each bottle to mix contents before making DO measurement or taking a subsample. Do not remove magnets until test is complete.

Alternatively, use a series of 300-mL BOD bottles (5210B.2*a*) if larger bottles are unavailable or incubation space is limited.

b. Reservoir bottle: 4-L or larger glass bottle. Close with plastic screw cap or non-rubber plug.

c. Incubator or water bath, thermostatically controlled at 20 ± 1°C. Exclude all light to prevent the possibility of photosynthetic production of DO.

d. Oxygen-sensitive membrane electrode: See Section 4500-O.G.2.

3. Procedure

a. River water samples: Preferably fill large BOD bottle (>2 L, or else 6 or more 300-mL BOD bottles) with sample at 20°C. Add no nutrients, seed, or nitrification inhibitor if in-bottle decay rates will be used to estimate in-stream rates. Do not dilute sample unless pretesting or experience shows that ultimate BOD will be high (>20 mg/L).

Measure DO in each bottle, stopper it, and make an airtight seal. Incubate at 20°C in the dark.

Measure DO in each bottle at intervals of at least 2 to 5 d over 30 to 60 d (minimum of 6 to 8 readings), or longer under special circumstances. To avoid oxygen depletion in samples containing ammonia nitrogen (NH_3-N), measure DO more frequently until nitrification has taken place. If DO falls to about 2 mg/L, re-aerate as directed below. Replace sample lost by the cap and

* Some analysts have reported satisfactory results with 2-chloro-6-(trichloromethyl) pyridine (Nitrification Inhhibitor, Formula 2533, Hach Co., Loveland, CO, or equivalent).
† Wheaton 2-L BOD bottle No. 227580, 1000 North Tenth St., Millville, NJ, or equivalent.

DO-probe displacement by adding 1 to 2 mL sample from the reservoir bottle.

When DO approaches 2 mg/L, re-aerate. Pour a small amount of sample into a clean vessel and re-aerate the remainder directly in the bottle by vigorous shaking or bubbling with purified air (medical grade). Refill bottle from the storage reservoir and measure DO. This concentration becomes the initial DO for the next measurement. If using 300-mL BOD bottles, empty all of the bottles into a clean vessel, re-aerate, and refill the small bottles.

Analyze for NO_2^--N + NO_3^--N (see Sections 4500-NO_2^- and 4500-NO_3^-) on Days 0, 5, 10, 15, 20, and 30. Alternatively, determine NO_2^--N and NO_3^--N each time DO is determined, thereby producing corresponding BOD and nitrogen determinations. If the ultimate demand occurs after 30 d, make additional analyses at 30-d intervals. Remove 10 to 20 mL from the bottle for these analyses, and refill bottle as necessary from the reservoir bottle. Preserve NO_2^--N + NO_3^--N subsample with H_2SO_4 to pH <2 and refrigerate. If the UBOD test's goal is to assess UBOD and not to provide data for rate calculations, measure NO_3^--N concentration only at Day 0 and on the last day of the test (kinetic rate estimates are not useful when the nitrification reaction is not followed).

Calculate oxygen consumption during each time interval and make appropriate corrections for nitrogenous oxygen demand. Correct by using 3.43 × the NH_3^--N to NO_2^--N conversion plus 1.14 × the NO_2^--N to NO_3^--N conversion to reflect the stoichiometry of NH_4^+ oxidation to NO_2^- or NO_3^-.

When using a dilution-water blank, subtract the blank's DO uptake from the total DO consumed. High-quality reagent water without nutrients typically will consume a maximum of 1 mg DO/L in a 30- to 90-d period. If the dilution water's DO uptake is >0.5 mg/L for a 20-d period or 1 mg/L for a 90-d period, report the magnitude of the correction and try to obtain higher-quality dilution water for subsequent UBOD tests.

When weekly DO consumption drops below 1 to 2% of the total accumulative consumption, calculate UBOD using a nonlinear regression method.

b. Wastewater treatment plant samples: Use high-quality reagent water (see Section 1080) for dilution water. Add no nitrification inhibitors if decay rates are desired. If seed and nutrients are necessary, add the same amounts of each to the dilution-water blank. Use minimal sample dilution. As a general rule, the diluted sample's UBOD should be in the range of 20 to 30 mg/L. Dilution to this level probably will require two or three sample re-aerations during incubation to prevent DO concentrations from falling below 2 mg/L.

Use 2-L or larger BOD bottles (alternatively, multiple 300-mL BOD bottles) for each dilution. Add desired volume of sample to each bottle and fill with dilution water.

Fill a BOD bottle with dilution water to serve as a dilution-water blank. Treat blank the same as all samples. Follow procedure given in ¶ *a* above and incubate for at least as long as UBOD test.

4. Calculations

An example of results obtained for an undiluted wastewater sample, without seed and nutrients, is given in Table 5210:I .

UBOD can be estimated by using a first-order model described as follows:

$$BOD_t = UBOD(1 - e^{-kt})$$

where:

BOD_t = oxygen uptake measured at time t, mg/L, and
k = first-order oxygen uptake rate.

The data in Table 5210:I were analyzed via a nonlinear regression technique applied to the above first-order model.[4] However, a first-order kinetic model may not always be the best choice. Significantly better statistical fits usually are obtained with alternative kinetic models, including sum of two first-order and logistic function models.[1,3–8]

5. Precision and Bias

UBOD-test precision was assessed via a series of replicate tests in a single laboratory. Interlaboratory studies have not been conducted.

Reference	Replicate No.	UBOD *mg/L*	Precision Summary*
2	1	154	μ = 151 mg/L
	2	154	
	3	145	CV = 3.5%
5	1	10.3	
	2	11.1	
	3	9.6	μ = 10.0 mg/L
	4	9.9	
	5	9.8	CV = 5.8%
	6	9.6	
6	1	12.8	μ = 12.4 mg/L
	2	12.6	
	3	12.6	CV = 4.4%
	4	11.6	

* μ = mean.
CV = coefficient of variation.

Bias was assessed by determining the BOD of a known concentration of glucose (150 mg/L) and glutamic acid (150 mg/L). This solution has a UBOD of 321 to 308 mg/L, depending on the extent of nitrification. The results of the study, conducted in triplicate, were:

Estimated* UBOD *mg/L*	Theoretical BOD *mg/L*	Percent Difference
276	308/321	–10/–14
310	308/321	+1/–3
303	308/321	–2/–6

* By statistical model.

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5210 D. Respirometric Method

1. General Discussion

a. Principle: Respirometric methods directly measure the oxygen consumed by microorganisms in an air- or oxygen-enriched environment in a closed vessel under constant temperature and agitation.

b. Uses: Respirometry measures oxygen uptake more or less continuously over time. Respirometric methods are useful for assessing the biodegradation of specific chemicals; the treatability of organic industrial wastes; the effect of known amounts of toxic compounds on a test wastewater's or organic chemical's oxygen-uptake reaction; the concentration at which a pollutant or a wastewater measurably inhibits biological degradation; the effect of various treatments (e.g., disinfection, nutrient addition, and pH adjustment) on oxidation rates; the oxygen requirement for essentially complete oxidation of biologically oxidizable matter; the need for using adapted seed in other biochemical oxygen-uptake measurements (e.g., the dilution BOD test); or a sludge's stability.

Respirometric data typically will be used comparatively (i.e., direct comparisons of oxygen-uptake rates in two test samples or in a test sample and a control). Because of inherent differences among uses, seed cultures, instruments, and applications of results, no single procedure for respirometric tests is applicable to all cases. Therefore, only basic recommendations and guidelines for overall test setup and procedure are given. Follow manufacturer's instructions for operating specific commercial instruments.

c. Types of respirometers: Four principal types of commercial respirometers are available: manometric, volumetric, electrolytic, and direct-input. Manometric respirometers relate oxygen uptake to the pressure change due to oxygen consumption when volume is constant. Volumetric respirometers measure oxygen uptake in incremental gas-volume changes when pressure is constant (at the time of reading). Electrolytic respirometers monitor how much oxygen is produced when water electrolyzes to maintain constant oxygen pressure in the reaction vessel. Direct-input respirometers deliver oxygen from a pure-oxygen supply to a sample via on-demand metering in response to minute pressure differences.

Most respirometers have been instrumented to permit data collection and processing via computer. Reaction-vessel contents are mixed by a magnetic or mechanical stirring device or by bubbling the reaction vessel's gaseous phase through its liquid phase. All respirometers remove carbon dioxide (CO_2) produced during biological growth by suspending a concentrated adsorbent (granular or solution) in the closed reaction chamber or by recirculating the gas phase through an external scrubber.

d. Interferences: Evolution of gases other than CO_2 may introduce errors in pressure or volume measurements; this is uncommon in the presence of DO. Incomplete CO_2 absorption will introduce errors if appropriate amounts and concentrations of alkaline absorbent are not used. Temperature fluctuations or inadequate mixing will introduce error. Fluctuations in barometric pressure can cause errors with some respirometers. Become familiar with the limits of the instrument used.

e. Minimum detectable concentration: Most commercial respirometers can detect oxygen demand in increments as small as 0.1 mg, but test precision depends on the total amount of oxygen consumed at the time of reading, the precision of pressure or volume measurement, and the effect of temperature and barometric-pressure changes. The upper limits of oxygen-uptake rate are determined by the ability to transfer oxygen into solution from the gas phase, which typically is related to mixing intensity. Transfer limits typically range from <10 mg O_2/L/h for low-intensity mixing to >100 mg O_2/L/h for high-intensity mixing.

f. Relationship to dilution BOD: Variations in waste composition, substrate concentration, mixing, and oxygen concentrations from one wastewater source to another generally preclude use of a general relationship between oxygen uptake by respirometers and the 5-d BOD at 20°C (see 5210B). Reasonably accurate correlations may be possible for a specific wastewater. The incubation period for respirometric measurements need not be 5 d because equally valid correlations can be made between the 5-d BOD and respirometric oxygen uptake at any time after 2 d.[1,2] Correlations between respirometric measurements and 5-d BOD for municipal wastewaters seem to occur at about 2 to 3 d incubation; however, correlations between respirometric measurements and 5-d BOD for industrial wastes and specific chemicals are less certain. Respirometric measurements also can provide an indication of UBOD (see 5210C). In many cases, it is

reasonable to consider that the 28- to 30-d oxygen uptake is essentially equal to UBOD.[3]

More commonly, respirometers are used as a diagnostic tool. The continuous oxygen-consumption readout in respirometric measurements indicates lag, toxicity, or any abnormalities in the biodegradation reaction. A change in the normal shape of an oxygen-uptake curve in the first few hours may help identify a toxic or unusual waste entering a treatment plant in time to adjust operations appropriately.

g. Relationship to other test methods and protocols: This method supports most of the protocols and guidelines established by the European Organization for Economic Co-operation and Development[3] (OECD) that require oxygen-uptake measurements.

h. Sampling and storage:

1) Grab samples—If analysis is begun within 2 h of sample collection, cold storage is unnecessary. Otherwise, keep sample ≤6°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible, store ≤6°C and report storage temperature and duration. Never start analysis >24 h after grab-sample collection.

2) Composite samples—Keep samples ≤6°C during compositing. Limit compositing period to 24 h. Store using the same criteria as for grab samples; holding time begins when the compositing period ends.

2. Apparatus

a. Respirometer system: Use commercial apparatus and check manufacturer's instructions for specific system requirements, reaction vessel type and volume, and instrument operating characteristics.

b. Incubator or water bath: Use a constant-temperature room, incubator chamber, or water bath to control temperature to ±1°C. Exclude all light to prevent any algae in sample from forming oxygen. Use red, actinic-coated bottles for analysis outside of a darkened incubator.

3. Reagents

The following reagent formulations produce 1-L solutions, but smaller or larger volumes may be prepared according to need. Discard any reagent showing signs of biological growth or chemical precipitation. Stock solutions can be sterilized by autoclaving to provide longer shelf life.

a. Distilled water: Use only high-quality water distilled from a block tin or all-glass still (see Section 1080) or equivalent reagent-grade water. DI water may be used but often contains high bacterial counts. The water must contain <0.01 mg heavy metals/L and be free of chlorine, chloramines, caustic alkalinity, organic material, or acids. Make all reagents with this water. When other waters are required for special-purpose testing, state clearly their source and quality characteristics.

b. Phosphate buffer solution, 1.5*N:* Dissolve 207 g sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$) in water. Neutralize to pH 7.2 with 6*N* potassium hydroxide (KOH) (¶ *g* below) and dilute to 1 L.

c. Ammonium chloride solution, 0.71*N:* Dissolve 38.2 g NH_4Cl in water. Neutralize to pH 7.0 with KOH. Dilute to 1.0 L; 1 mL = 10 mg N.

d. Calcium chloride solution, 0.25*M:* Dissolve 27.7 g $CaCl_2$ in water and dilute to 1 L; 1 mL = 10 mg Ca.

e. Magnesium sulfate ($MgSO_4$) solution, 0.41M: Dissolve 101 g $MgSO_4 \cdot 7H_2O$ in water and dilute to 1 L; 1 mL = 10 mg Mg.

f. Ferric chloride solution, 0.018*M:* Dissolve 4.84 g $FeCl_3 \cdot 6H_2O$ in water and dilute to 1 L; 1 mL = 1.0 mg Fe.

g. Potassium hydroxide solution, 6*N:* Dissolve 336 g KOH in about 700 mL water and dilute to 1 L. **CAUTION: Add KOH to water slowly and use constant mixing to prevent excessive heat buildup. Alternatively, use commercial solutions containing 30 to 50% KOH by weight.**

h. Acid solutions, 1*N:* Add 28 mL conc H_2SO_4 or 83 mL conc HCl to about 700 mL water. Dilute to 1 L.

i. Alkali solution, 1*N:* Add 40 g NaOH to 700 mL water. Dilute to 1 L.

j. Nitrification inhibitor: Reagent-grade TCMP or equivalent.[3]*

k. Glucose–glutamic acid solution: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 15.0 g glucose and 15.0 g glutamic acid to distilled water and dilute to 1 L. Neutralize to pH 7.0 using 6*N* KOH (¶ *g* above). This solution may be stored for up to 1 week at 4°C.

l. Electrolyte solution (for electrolytic respirometers): Use manufacturer's recommended solution.

m. Sodium sulfite solution, 0.025*N:* Dissolve 1.575 g Na_2SO_3 in about 800 mL water. Dilute to 1 L. This solution is not stable; prepare daily or as needed.

n. Trace element solution: Dissolve 40 mg $MnSO_4 \cdot 4H_2O$, 57 mg H_3BO_3, 43 mg $ZnSO_4 \cdot 7H_2O$, 35 mg $(NH_4)_6Mo_7O_{24}$, and 100 mg Fe-chelate ($FeCl_3$-EDTA) in about 800 mL water. Dilute to 1 L. Sterilize at 120°C and 200 kPa (2 atm) pressure for 20 min.

o. Yeast extract solution:[3] Add 15 mg laboratory- or pharmaceutical-grade brewer's yeast extract to 100 mL water. Make this solution fresh immediately before each test in which it is used.

p. Nutrient solution:[3] Add 2.5 mL phosphate buffer solution (¶ *b* above), 0.65 mL ammonium chloride solution (¶ *c* above), 1.0 mL calcium chloride solution (¶ *d* above), 0.22 mL magnesium sulfate solution (¶ *e* above), 0.1 mL ferric chloride solution (¶ *f* above), 1 mL trace element solution (¶ *n* above), and 1 mL yeast extract solution (¶ *o* above) to about 900 mL water. Dilute to 1 L. This solution and those of ¶s *n* and *o* above are specifically formulated for use with the OECD method.[3] (NOTE: A 10:1 concentrated nutrient solution can be made and diluted accordingly.)

4. Procedure

a. Instrument operation: Follow respirometer manufacturer's instructions for assembling, testing, calibrating, and operating the instrument. NOTE: The manufacturer's stated maximum and minimum measurement limits are not always the same as the instrument's output limits. Make sure test conditions are within measurement limits.

b. Sample volume: The sample volume or concentration of organic chemicals to be added to test vessels is a function of

* Formula 2533, Hach Chemical Co., Loveland, Colo., or equivalent. NOTE: Some commercial formulations are not pure TCMP. Check with supplier to verify compound purity and adjust dosages accordingly.

expected oxygen-uptake characteristics and the instrument's oxygen-transfer capability. Small volumes or low concentrations may be required for high-strength wastes. Large volumes may be required for low-strength wastes to improve accuracy.

c. Data recording interval: Set instrument to give data readings at suitable intervals. Intervals typically range from 15 min to 6 h.

d. Sample preparation:

1) Homogenization—If sample contains large settleable or floatable solids, homogenize it with a blender and transfer representative test portions while all solids are in suspension. If concerned about changing sample characteristics, skip this step.

2) pH adjustment—Neutralize samples to pH 7.0 using H_2SO_4 (5210D.3*h*) or NaOH (5210D.3*i*) without diluting the sample >0.5%.

3) Dechlorination—Avoid analyzing samples containing residual chlorine by collecting them ahead of chlorination processes. If residual chlorine is present, aerate as described in ¶ *d*5) below or let stand in light for 1 to 2 h. If a chlorine residual persists, add Na_2SO_3 solution. Determine required volume of Na_2SO_3 solution by adding 10 mL 1 + 1 acetic acid or 1 + 50 H_2SO_4 and 10 mL potassium iodide solution (10 g/100 mL) to a portion of sample. Titrate with 0.025*N* Na_2SO_3 solution to the starch-iodine endpoint (see Section 4500-Cl.B). Add to the neutralized sample a proportional volume of Na_2SO_3 solution determined above, mix, and after 10 to 20 min check for residual chlorine. Reseed the sample (see ¶ *h* below).

4) Samples containing toxic substances—Certain industrial wastes contain toxic metals or organic compounds. These often require special study and treatment.[3]

5) Initial oxygen concentration—If samples contain DO concentrations smaller or larger than the desired concentration, agitate or aerate with clean and filtered compressed air for about 1 h immediately before testing. Minimum and maximum actual DO concentrations will vary with test objectives. In some cases, pure oxygen may be added to respirometer vessels to increase oxygen levels above ambient.

6) Temperature adjustment—Bring samples and dilution water to desired test temperature (±1°C) before making dilutions or transferring to test vessels.

e. Sample dilution: Use distilled water or water from other appropriate sources free of organic matter. In some cases, receiving-stream water may be used for dilution. Add desired sample volume to test vessels using a wide-tip volumetric pipet or other suitable volumetric glassware. Add dilution water to bring sample to about 80% of desired final volume. Add appropriate amounts of nutrients, minerals, buffer, nitrification inhibitor (if desired), and seed culture as described in ¶s *f–h* below. Dilute sample to desired final volume. The number of test vessels needed to prepare for each dilution depends on test objectives and number of replicates desired.

f. Nutrients, minerals, and buffer: Add enough ammonia nitrogen to provide a COD:N:P ratio of 100:5:1 or a TOC:N:P ratio of 30:5:1. Add 2 mL each of calcium, magnesium, ferric chloride, and trace mineral solutions to each liter of diluted sample, unless sufficient amounts of these minerals are present in the original sample. Phosphorus requirements will be met by the phosphate buffer, if used (1 mL/50 mg/L COD or UBOD of diluted sample usually is sufficient to maintain pH between 6.8 and 7.2). Be cautious in adding phosphate buffer to samples containing metal salts because metal phosphates may precipitate and show less toxic or beneficial effect than when phosphate is not present. For OECD-compatible tests, substitute the nutrient, mineral, and buffer amounts listed in 5210D.3*p* for the above nutrient/mineral/buffer quantities.

g. Nitrification inhibition: If nitrification inhibition is desired, add 10 mg TCMP/L sample in the test vessel. Samples that may nitrify readily include biologically treated effluents, samples seeded with biologically treated effluents, and river waters.[4]

h. Seeding: See 5210B.4*d* for seed preparation. Use enough seed culture to prevent major lags in the oxygen-uptake reaction but not so much that the seed's oxygen uptake exceeds about 10% of the seeded sample's oxygen uptake.

Determine the seeding material's oxygen uptake in the same way as for any other sample. This is the seed control. Typically, seed volume in the seed control should be 10 times the volume used in seeded samples.

i. Incubation: Incubate samples at 20°C or other suitable temperature ±1.0°C. Take care that the stirring device does not raise sample temperature.

5. Calculations

To convert instrument readings to oxygen uptake, refer to manufacturer's procedures.

Correct oxygen uptake for seed and dilution as follows:

$$C = [A - B(S_A/S_B)](1000/N_A)$$

where:

C = corrected oxygen uptake of sample, mg/L,
A = measured oxygen uptake in seeded sample, mg,
B = measured oxygen uptake in seed control, mg,
S_A = volume of seed in Sample A, mL,
S_B = volume of seed in Sample B, mL, and
N_A = volume of undiluted sample in Sample A, mL.

6. Quality Control

The QC practices considered to be an integral part of each method are summarized in Table 5020:I.

Periodically use the following procedure to check distilled water quality, instrument quality, instrument function, and analytical technique by making oxygen-uptake measurements using a GGA mixture as a standard check solution.

Adjust water for sample formulation to test temperature and saturate with DO by aerating with clean, organic-free filtered air. Protect water quality by using clean glassware, tubing, and bottles.

Prepare a *test solution* by adding 10 mL GGA solution (5210D.3*k*); 6 mL phosphate buffer (5210D.3*b*); 2 mL each of ammonium chloride (5210D.3*c*), magnesium sulfate (5210D.3*e*), calcium chloride (5210D.3*d*), ferric chloride (5210D.3*f*), and trace element solution (5210D.3*n*) to approximately 800 mL water. Add 10 mg TCMP/L to inhibit nitrification. Add sufficient seed from a suitable source (5210D.4*h*) to give a lag time <6 h (usually 25 mL supernatant from settled primary effluent/L test

solution is sufficient). Dilute to 1 L. Adjust temperature to 20 ± 1°C.

Prepare a *seed blank* by diluting 500 mL or more of seed solution to 800 mL with distilled water. Add the same amount of buffer, nutrients, and TCMP as in the test solution, and dilute to 1 L. Adjust temperature to 20 ± 1°C.

Place test solution and seed-blank solution in separate reaction vessels of respirometer and incubate for 5 d at 20°C. Run at least three replicates of each. The seed-corrected oxygen uptake after 5 d incubation should be 260 ± 30 mg/L; if the value is outside this range, repeat the test using a fresh seed culture and seek the cause of the problem.

7. Precision and Bias

a. Precision: No standard is available to check the accuracy of respirometric oxygen uptake measurements. To obtain laboratory precision data, use a GGA mixture (5210D.6) with a known theoretical maximum oxygen-uptake value. Tests with this and similar organic compound mixtures have shown that the standard deviation (expressed as the coefficient of variation, C_v) is approximately 5% for samples with total oxygen uptakes of 50 to 100 mg/L and 3% for more concentrated samples.[1,2] Individual instruments have different readability limits that can affect precision. The minimum response or sensitivity of most commercial respirometers ranges from 0.05 to 1 mg oxygen. Check manufacturer's specifications for a given instrument's sensitivity.

b. Control limits: To establish laboratory control limits, perform a minimum of 25 GGA checks over several weeks or months and calculate the mean and standard deviation. If measured oxygen uptake in 5 d at 20°C is outside the 260 ± 30 mg/L range, re-evaluate procedure to identify the source of error. Forother samples, use the mean ±3 standard deviations as the control limit.

c. Working range and detection limits: The working range and detection limits are established by each commercial instrument's limits. Refer to manufacturer's specifications.

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Operator:_____ Checked by: _____

pH (6-8) √ _____
T°C (20±3°C) √ _____
Initial DO ≤ 9.0 before dilution √ _____
Initial Blank DO at least 7.5 ____
Source of seed material Polyseed

CBOD / BOD WORKSHEET for SOFT DRINK

Incubator Temperature °C (20±1°C)	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5

Seed used per bottle 4 mL	1:100 SOFT DRINK			DUP	GGA			BLANKS	
mL. used OR % Concentration (P)	1	2	3		6	6		300ml 100%	300ml 100%
Bottle Number									
Initial DO (D_1)									
(-) Final DO (D_2)									
Oxygen Demand ($D_1 - D_2$)									
(-) Depletion due to seed ($B_1 - B_2$)f									
Net depletion ($D_1 - D_2$) - ($B_1 - B_2$)f									
Dilution Factor (1/P) Vol/300mL= P	300	150	100		50	50			
5-Day BOD (mg/L)									
	Avg. mg/L		Avg. mg/L		Avg. mg/L		Avg. mg/L	Avg. mg/L	

	Seed Control			
mL. used OR % Concentration (P)	15	20	25	
Initial DO (B_1)				
Final DO (B_2)				
Oxygen Demand				
Demand / volume Depletion due to seed ($B_1 - B_2$)f				

For seeded samples: BOD_5, mg/L = $\frac{(D1 - D2) - (B1 - B2)\,f}{P}$

Sample Dilutions Criteria

REQUIREMENTS FOR VALID BOD_5 RESULTS

Blank depletion must be ≤ **0.2 mg/L** DO

Initial DO must be ≤ **9.0 mg/L**

Samples **must deplete** at least **2.0 mg/L** DO

Samples must have at least 1.0 mg/L DO **remaining** at the end of the incubation period

InterLab®

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PolySeed® Application Procedure
BOD_5 Seed Inoculum

PolySeed® is a blend of broad spectrum bacteria designed specifically as a seed inoculum for the Biochemical Oxygen Demand (BOD_5) test as conducted in accordance to ***Standard Methods of the Examination of Water and Wastewater***. **PolySeed®** is an EPA approved BOD_5 seed inoculum that has been used to seed both municipal and industrial wastes for almost 35 years.

Overview: The following are the most important parts of the BOD_5 test. **First,** the BOD water must be made properly and stored at 20° C. **Next,** the PolySeed® solution must be properly rehydrated and tested to determine its' effect on the test (i.e., the Seed Control Factor – SCF). **Finally,** the seed inoculum must be tested against a known Glucose-Glutamic Acid (GGA) standard. With these tests in order, a very reliable and accepted BOD_5 test can be performed.

1st Step: Control – Dilution Water ("BOD Water"): Prepare the dilution water (some call it "BOD Water" or "Blank Water") in accordance with *Standard Methods*. Be sure to use fresh deionized water and remineralize with the appropriate nutrients and chemicals. Store the Control-Dilution water at 20° C until ready to use. Run a control ***"Blank"*** on the neat Control-Dilution water at 20° C along with the actual BOD test. To insure an acceptable final test the ***"Blank"*** must have an oxygen depletion of less than 0.2 mg/liter over the 5-day period. If you have any questions, refer to *Standard Methods,* InterLab's e-Guide Videos or our Frequently Asked Questions ("FAQ") page available at www.polyseed.com.

2nd Step: Seed Solution (i.e. "PolySeed® Solution"): To make the seed solution, place the entire contents of one PolySeed® capsule (discard the gelatin capsule) into 500ml of "DILUTION WATER" prepared in accordance to *Standard Methods* **(do not use DI water by itself)**. Normal dilutions are one (1) PolySeed® capsule to 500ml of BOD water; however, the concentration of seed can be adjusted to compensate for variations in BOD water and established internal laboratory testing protocol. This seeded dilution water will be referred to as the "PolySeed® solution". ***Note: Bran, which acts as the carrier for the microorganisms, will neither dissolve nor inhibit microbial activity, but must be settled out of the PolySeed® solution prior to use.***

Next, aerate and stir the PolySeed® solution for one (1) hour. Finally, decant the supernatant carefully so as not to allow any bran in the biological solution. Pour the decanted PolySeed® solution in a clean 500ml beaker with a sterile stir bar, place on magnetic stirrer and gently stir for the remainder of the test. (Note: Our lab uses a Nalgene separatory funnel for this purpose) For best results, the PolySeed® solution should be used within six (6) hours of rehydration of the capsule.

05/2009

3rd Step: Seed Control Factor ("SCF"): After following Step 2, carefully draw an aliquot from the PolySeed® solution. It is best to prepare the seed control using 15, 20, 25 & 30ml of PolySeed® solution; however, these aliquots may vary depending upon laboratory procedures. The resulting DO uptake should fall between 0.60 and 1.0 (see calculations below).

At the end of the 5-day test period calculate the **SEED CONTROL FACTOR ("SCF")** of the PolySeed® solution per *Standard Methods* by using [(D1 - D2) x f] where:

D1 = DO of seed control before incubation, mg/L
D2 = DO of seed control after incubation, mg/L and,
f = (Volume of seed in diluted sample)/(volume of seed in seed control)

Note:This can be automatically calculated using InterLab's BOD calculator.

4th Step: Glucose-Glutamic Acid Standard: After the glucose-glutamic acid (GGA) standard solution is prepared (refer to *Standard Methods* or our FAQ page at www.polyseed.com), use 4ml of PolySeed® solution for each BOD_5 bottle. Again, make sure there is no undissolved bran in the pipette. No other seed is required. (Note: PolySeed® solution volume can be adjusted to compensate for variations in DI water, laboratory procedures and established internal laboratory testing protocol.)

5th Step: BOD Sample Analysis: Prepare the live BOD samples in accordance with *Standard Methods*. Insure that the PolySeed® Solution is prepared and stirred in accordance with Step 2 above. Add 4mls of PolySeed® solution (this volume can be adjusted for varying BOD water) to each BOD_5 bottle when preparing the wastewater samples. No other seed is required. Follow *Standard Methods* procedures for incubation, seed correction, GGA, and dilution water preparation. When reporting results using PolySeed® it is best to use the BOD calculator located at www.polyseed.com or hand calculations in accordance with *Standard Methods*.

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05/2009

Instruction Sheet

ezGGA Solution

Use the following instructions to add the BOD Standard Solution, 150 mg/L glucose and 150 mg/L glutamic acid (after dilution), to a BOD bottle.

DO measurement with the LBOD probe

1. Add the necessary seed to a 300-mL BOD bottle.
2. Fill the BOD bottle with dilution water until the water level is approximately ¼ inch up the ground glass portion of the neck. (See dimension "x" in illustration).
3. Put the 2-mL BOD standard ampule into the ampule breaker and rinse the assembly with deionized water.
4. Hold the ampule and breaker over the rim of the BOD bottle.
5. Use the ampule breaker to open the ampule and allow it to fall into the BOD bottle. Leave ampule in the BOD bottle during incubation period.
6. Follow the general procedure for the BOD test.
7. Calculate the BOD concentration of the standard solution. The 2 mL in the vial is equivalent to 6 mL as prepared by Standard Methods. Calculate the BOD concentration as though there were 6 mL added to the bottle instead of 2 mL. The dilution factor for this standard is 50x.

BOD dilution water preparation

Source: http://dnr.wi.gov/regulations/labcert/BODDH2O.html

The BOD dilution water nutrient buffer solutions can also be a source of contamination. If you prepare your own solution make sure you store the phosphate buffer in a refrigerator. Discard any solution if it becomes cloudy or you observe any "chunks" floating in the solution. Using single-use nutrient buffer pillows will avoid many of these pitfalls.

Nutrient Solutions:

1. Magnesium sulfate solution: 22.5 g $MgSO_4 \cdot 7H_20$. Dilute to 1 L.
2. Calcium chloride solution: 27.5 g $CaCl_2$. Dilute to 1 L.
3. Ferric Chloride solution: 0.25 g $FeCl_3 \cdot 6H_20$. Dilute to 1 L.
4. Phosphate buffer: 8.5 g KH_2P0_4, 21.75 g K_2HP0_4, 33.4 g $Na_2HP0_4 \cdot 7H_20$, and 1.7 g NH_4Cl. Dilute to 1 L.

The pH should be 7.2.
Store in 4°C refrigerator.
Check before each use for contamination (discard any reagent with growth).

- Add 1 mL each of PO4 buffer; $MgSO_4$, $CaCl_2$, and $FeCl_3$ per Liter or the contents of one buffer pillow (buy the right size!).
- Before use bring dilution water temperature to 20 ± 1°C.
- Saturate with DO:
    - shake or aerate with organic-free filtered air
    - store in cotton-plugged bottles "long enough to become saturated"

Dilution water misconceptions

There are many misconceptions regarding dilution water preparation. Many have taught that dilution water cannot be mixed, aerated or shaken immediately before use because the water will be supersaturated. This is **not** true. Dilution water at room temperature (17-23°C) can be rigorously mixed, shaken or even aerated with compressed air minutes before use without fear of super-saturation. Excess oxygen will physically dissipate almost immediately. In fact, to the contrary... shaking dilution water after it has equilibrated to room temperature is required to bring the water to saturation.

Here are some simple tips for dilution water preparation:

Dilution water should be prepared immediately before use. Without the phosphate buffer, you can prepare dilution water days/weeks ahead of time. Phosphate buffer is key reagent because phosphorus is the limiting nutrient in stimulating growth so it must be added the day the water is to be used.

- Use only high quality distilled or deionized water
- Allow water to equilibrate for >24 hours at 20°C in an incubator or temperature controlled room (17-23°C) before use.
- Add the nutrient buffer solutions the day the water is to be used
- Avoid contaminating the water while allowing the water to become oxygenated. *Always cover the bottle with a cotton plug, sponge or clean paper towel*.
- If you use compressed air, filter the air.
- **Do not** allow anything but glass or Teflon come in contact with the water.

Never allow "fish tank" (tygon) tubing or air stones to come in contact with the water. Tygon **will** leach oxygen demanding material over time and air stones provide an excellent surface for bacterial growth. If they are used, you may inoculate your BOD dilution water with bacteria.

If you wish to age your dilution water, **do not** add the single-use nutrient buffers until the day the water is to be used. Please note that you should not need to age lab reagent water if it is prepared properly.

Excessive depletion in dilution water

Excessive DO depletion in BOD blanks is one of the most common problems that plague all wastewater testing laboratories. Even the best labs occasionally have problems meeting the DO depletion in the BOD blanks (< 0.2 mg/L). The following are the most common causes for excessive depletion in blanks:

- Tubing is constructed of oxygen-demand leaching material
- Correct tubing not being used throughout the lab
- Slime growth in delivery tubing
- Poor water quality/improperly maintained system
- Poorly cleaned BOD bottles or dilution water storage container. **NOTE: Glass is best!**
- Contamination during aeration
- Poorly calibrated DO Probe

Solving dilution water quality problems

Bottled water

Generally avoid using "grocery store" distilled water. This water is stored in plastic bottles which can leach oxygen demanding materials. Some analysts have had great luck using "grocery store" distilled water. If you have good luck with a particular brand, don't change. However, be aware that you can't control how the water is stored. If the water sits on a shelf for several months in a hot warehouse, it's going to be a problem. Nothing leaches organic matter from plastic like hot water.
Try doing a blank using dilution water prepared from a jug of distilled water that's been in the back seat of your vehicle for a week during a particularly hot July!

Those that have successfully used "grocery store" water generally have a system. Some will go directly to the factory and buy fresh water. They then date the water, store it in a cool, dry place and discard the water after it is a certain age (e.g., expiration date). **Bottom Line: Use it if it's working for you; but if it isn't, consider other alternatives.**

Aging dilution water

You should not have to age dilution water —as Standard Methods suggests— if the water is prepared properly. If you have to age to improve quality, you should be concentrating your efforts on improving the preparation process.

Deodorizers and Dilution Water

Avoid using auto-dispensing deodorizers in the laboratory. You may be tempted to use deodorizers because wastewater labs can have a bit of an odor. However, don't use them! They typically use alcohol as a carrier which has a very high BOD.

Water purification systems

All lab reagent water systems, much like expensive instruments, are prone to problems if they are not maintained properly. Follow the manufacturer's recommendations for cleaning and disinfecting stills, water polishers and deionizer systems.

Simple deionizer systems can work well but can quickly be overgrown with bacteria and mold and can leach organics. Again, regular scheduled maintenance is the key to keeping a system working optimally.

A simple deionizer system can and has worked very well for many laboratories. However, you must use quality virgin or nuclear-grade resins. Poorer grade or frequently 're-generated' household softener resins WILL leach oxygen demanding material and will NOT work for BOD testing. Don't let a manufacturer talk you into lower quality resins to save money. This is a "penny wise and dollar foolish" approach. Don't sacrifice quality for a few bucks.

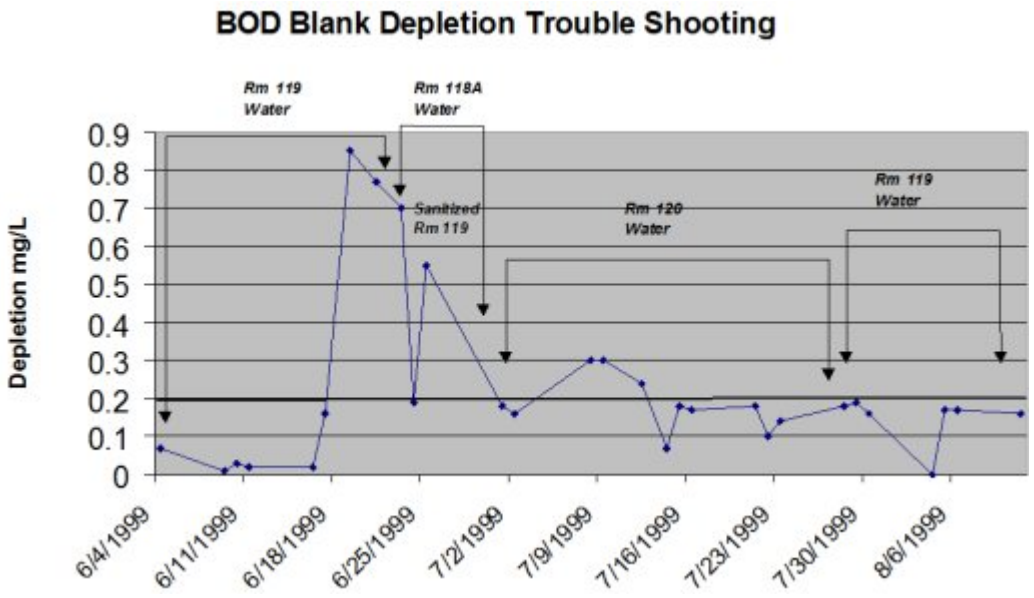
Activated charcoal in deionizer systems are also prone to contamination problems. They can quickly become contaminated with bacteria and mold, and can slough-off oxygen demanding material.

Chlorinated water directly feeding ion exchange systems can breakdown and leach oxygen demanding material. The solution is to pass the water through activated charcoal cartridge prior to resin.

SLH's dilution water experiences

The Wisconsin State Laboratory of Hygiene (WSLH) built a state-of-the-art laboratory in 1999. They also installed a state-of-the-art laboratory reagent water system. However, the manufacturer sold them poorer grade anion and cation exchange resins which allowed them to be "low bidder". The WSLH was unable to get BOD blanks to pass the depletion criteria (i.e., <0.2 mg/L) using this water. They spent months troubleshooting the water system. After many months of systematically troubleshooting the system, the WSLH discovered the problem and switched to virgin or nuclear grade resins. The above graph illustrates the problems they experienced.

So be aware that even the best systems will not produce water good enough for BOD testing unless the ion exchange resins are used. End of line water polishing units will NOT remove all of the organics leached from the poorer quality resin even though manufactures may claim they will. Quality lab reagent water is the foundation of all laboratories. Remember, you get what you pay for. Don't scrimp and shortchange your lab by buying a "cheap" system.



Source: Wisconsin State Laboratory of Hygiene.

Dilution water- simplest solutions

There are a number of simple solutions to obtaining quality lab reagent water to prepare BOD dilution water.

Obtain water from another laboratory or vendor.

Probably the easiest approach is to obtain water from another laboratory that has a track record of producing consistently high quality BOD dilution water. It may be easier to haul water once a week than maintaining a lab water system, particularly if you have a small laboratory.

Purchase water from a source that has proven success.

You may also find a commercial source of water that is of consistent quality. If you find a particular brand and supplier that works for you, stick with it! **Don't fix it if it isn't broken**.

Buy an all glass laboratory still and distill your own water.

If you only need a limited amount of water (e.g., < 5 gallons per week), consider buying an all glass still. They are less costly than other water purification systems and tend to produce quality reagent water providing they are maintained properly.

Buy a bench-top water RO and polisher combo that will produce ASTM Type I water.

Bench-top reverse osmosis (RO)/polisher combination systems that will produce ASTM Type I water are also available. These systems work well. However, they tend to be expensive (> $1,000) and require regular maintenance to be effective.

Solving: glassware cleanliness concerns

- Use a good lab-grade non-phosphate detergent and bleach
- Rinse thoroughly with tap water followed by distilled water
- Allow to dry before storing.
- Always cover glassware and store in a clean, dry place.
- Commercial lab dishwashers with non-phosphate, lab grade detergents and acid rinse solutions (non-phosphate) will also work well.
- Use BOD QC samples (blanks, duplicates and GGA) to evaluate washing effectiveness.

Alternate glassware cleaning protocol without bleach

Bleach-free alternate cleaning method

DO NOT MIX hydrochloric acid (HCl) and bleach: It will produce poisonous chlorine gas!!!!.

- Use a good laboratory grade non-phosphate detergent
- Rinse thoroughly with tap water followed dilute HCl (10% solution; 100 mL HCl per liter of water).
- Rinse again with tap water followed by distilled water.
- Allow to dry before storing.
- Always cover glassware and store in a clean, dry place.

Table 7 – Convincing Evidence of "Toxicity"

Source	Bottle	Sample Vol. (mL)	Seed Vol (mL)	Initial DO (mg/L)	Final DO (mg/L)	Depletion (mg/L)	Dilution	BOD (mg/L)
Influent	1	20	1	8.9	3.9	5	15	66
	2	10	1	8.9	4.3	4.6	30	120
	3	5	1	8.9	4.6	4.3	60	222
	4	2	1	8.9	3.9	5.0	150	750
	5	10*	1	8.9	5.7	3.2	30	780
	6	5*	1	8.9	6.0	2.9	60	1380
	7	2*	1	8.9	5.7	3.0	150	3900***
	8	1*	1	8.9	7.0	1.9**		

sample conc. ↑

* After ten-fold dilution of entire sample

** Does not meet criterion of at least 2.0 mg/L DO depletion

*** Value to be reported

Example # 1A

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Incubator Temperature ^{o}C	20	20.1	20	19.9	20

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)				EFFLUENT				UP STREAM			DOWN STREAM			BLANKS	
Seeded or (unseeded)				DUP				DUP			DUP			DUP		
(ml. used) OR % Concentration (P for 100% = 1.0, 75% - 0.75)	15	24	30		75	150	300								300	302
Bottle Number	8	12	27		38	39	40								54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9								8.5	8.5
Final DO (D_2)	0.5	0.5	0.6		6.9	7.0	7.4								8.4	8.4
Oxygen Demand	8.0	7.9	7.7		1.9	1.8	1.5								0.1	0.1
Seed contribution																
Dilution Factor (1/P)	20	12.5	10		4	2	1									
5-Day BOD (mg/L)	(160)	98.75	77		7.6	3.6	1.5									

>160 mg/L <1.5 mg/L

For unseeded samples: $BOD_5, mg/L = (D_1 - D_2)\frac{1}{P}$

None of the dilutions met the 2.0 mg/L minimum depletion. Using 100% sample concentration, multiply 1.5 by the total volume of 300, divided by the highest amount of sample. <1.5 x 300/300 = <1.5 mg/L

The detection limit rules at the end of the method deal with how low can you go after you subtract initial – final DO. 3rd bullet at the very end of SM 5210B-20018.b. says detection limit is ~0.1. Because there is a 100% sample dilution in the series, use 1.5 (SM 5210-2001B 7.a.2.) and report as <1.5 mg/L.

Refer to SM 5210B-2001 7.a.3. For diluted influent unseeded, report >160 mg/L (with a greater than as the qualifier due to excessive depletion).

• The lower limit for unseeded samples that require no dilution ($S = 0$; $P = 1.0$) is equal to the detection limit of the DO measurement method (~0.1 mg/L).

3) When all dilutions result in a residual DO < 1.0, select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

Example # 1B

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)				EFFLUENT				UP STREAM			DOWN STREAM			BLANKS	
Seeded or (unseeded)				DUP				DUP			DUP			DUP		
(mL) used OR % Concentration (P for 100% = 1.0, 75% - 0.75)	15	24	30		75	150	250								300	302
Bottle Number	8	12	27		38	39	40								54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9								8.5	8.5
Final DO (D_2)	0.5	0.5	0.6		6.9	7.0	7.4								8.4	8.4
Oxygen Demand	8.0	7.9	7.7		1.9	1.8	1.5								0.1	0.1
Seed contribution																
Dilution Factor (1/P)	20	12.5	10		4	2	1.2									
5-Day BOD (mg/L)	160	98.75	77		7.6	3.6	1.5									

>160 mg/L <2.4 mg/L

For unseeded samples: $BOD_5, mg/L = (D_1 - D_2) \frac{1}{P}$

None of the dilutions met the 2.0 mg/L minimum depletion and there is no 100% sample concentration. Therefore, multiply 2.0 by the total volume of 300, divided by the highest amount of sample (250). <2.0 x 300/250 = <2.4 mg/L

The detection limit rules at the end of the method deal with how low can you go after you subtract initial – final DO. 3rd bullet at the very end of SM 5210B-20018.b. says detection limit is ~0.1.

• The lower limit for unseeded samples that require no dilution ($S = 0$; $P = 1.0$) is equal to the detection limit of the DO measurement method (~0.1 mg/L).

Refer to SM 5210B-2001 7.a.3. For diluted influent unseeded, report >160 mg/L (with a greater than as the qualifier due to excessive depletion).

3) When all dilutions result in a residual DO < 1.0, select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

Example # 2

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)				EFFLUENT				UP STREAM			DOWN STREAM			BLANKS	
Seeded or (unseeded)				DUP				DUP			DUP			DUP		
(mL) used OR % Concentration (P - 100% = 1.0)	15	24	30		75	150	200								300	302
Bottle Number	8	12	27		38	39	40								54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9								8.5	8.5
Final DO (D_2)	1.2	1.1	1.0		6.9	7.0	7.4								8.4	8.4
Oxygen Demand	7.3	7.3	7.3		1.9	1.8	1.5								0.1	0.1
Seed contribution																
Dilution Factor (1/P)	20	12.5	10		4	2	1.5									
5-Day BOD (mg/L)	146	91.2	73		7.6	3.6	2.25									

>146 mg/L >7.6 mg/L

For unseeded samples: BOD_5, mg/L = $(D_1 - D_2)\frac{1}{P}$

None of the dilutions met the 2.0 mg/L minimum depletion. There's evidence of toxicity in sample. Report as (greater than) > 7.6 mg/L

All the dilutions met the 1.0 mg/L final DO. However, there appears to be some **toxicity** in the influent. SM 5210B 7.b. (see below) So, use largest dilution and report as (greater than) >146.

Samples showing large differences between the computed BOD for different dilutions, for example, greater than 30%, may indicate the presence of a toxic substance or analytical problems. When the effect becomes repetitive, investigate to identify the cause. Identify results in the test reports when any of

Example # 3

Location Collected: effluent flume **Before Cl_2** √ **After Cl_2** _____ **Sample Seeded:** no
Initial Date/time: 12/14/05 2:00pm **Final Date/time:** 12/19/05 2:30pm **Sample Type:** grab **Date/Time Sample Collected:** 12/13/05 10:00

	INFLUENT (RAW)			DUP	EFFLUENT			DUP	UP STREAM	DUP	DOWN STREAM	DUP	BLANKS	
mL. used OR % Concentration (P)	15	24	30		75	150	300						300	302
Bottle Number	8	12	27		38	39	40						54	55
Initial DO (D_1)	8.5	8.4	8.3		8.8	8.8	8.9						8.5	8.5
Final DO (D_2)	4.5	2.4	0.8		0.5	0.5	0.5						8.4	8.4
Oxygen Demand	4.0	6.0	7.5		8.3	8.3	8.4						0.1	0.1
Seed contribution														
Dilution Factor (1/P)	20	12.5	10		4	2	1							
5-Day BOD (mg/L)	80	75			31									

Avg. 78 mg/L > 31 mg/L

For unseeded samples: BOD_5, mg/L = $(D_1 - D_2)\frac{1}{P}$

Refer to 5210B-2001 7.a.3.

3) When all dilutions result in a residual DO <1.0, select the bottle having the lowest DO concentration (greatest dilution) and report:

$$\text{BOD, mg/L} > \frac{(D_1 - D_2) - (S)V_s}{P}$$

where:
D_1 = DO of diluted sample immediately after preparation, mg/L,
D_2 = DO of diluted sample after 5 d incubation at 20°C, mg/L,
S = oxygen uptake of seed, Δ DO/mL seed suspension added per bottle (¶ 6*d*) (S = 0 if samples are not seeded),
V_s = volume of seed in the respective test bottle, mL, and
P = decimal volumetric fraction of sample used; $1/P$ = dilution factor.

Where S = 0 for unseeded samples

The effluent is a bit tricky, but since final DO <1, set final DO at 1 and use greatest dilution (lowest DO concentration).

8.8 – 1 = 7.8 x 4 = greater than (>) 31 (rounded to whole value). The greater than (>) sign is used as the qualifier due to excessive depletion.

Example # 4

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Incubator Temperature ^{o}C	20	20	20	20	20

Location Collected: inf/eff flows **Initial Date:** 3-10-05 13:16 **After Cl_2:** √ **Sample Seeded:** √
Sample Temp: 4^{oC} **Final Date:** 3-15-05 14:28 **Before Cl_2:** **Time Sample Collected:** 07:30

	INFLUENT (RAW) 2 mL SEED			DUP	EFFLUENT 2 mL SEED				UP STREAM			DOWN STREAM			BLANKS	
				DUP				DUP			DUP			DUP		
mL. used OR % Concentration (P)	6	9	12		150	200	300	300							300ml 100%	300ml 100%
Bottle Number	326	337	292		307	311	312	313							8.14	8.16
Initial DO (D_1)	8.09	8.02	7.93		8.89	8.91	8.98	8.97							7.97	8.00
(-) Final DO (D_2)	4.39	2.73	0.72		7.16	7.09	7.04	7.02							0.17	0.16
Oxygen Demand ($D_1 - D_2$)	3.70	5.29	7.21		1.73	1.82	1.94	1.95								
(-) Depletion due to seed $(B_1 - B_2)f$	1.66	1.66	1.66		1.66	1.66	1.66	1.66								
Oxygen Demand after seed correction	2.04	3.63	5.55		0.07	0.06	0.28	0.29								
Dilution Factor (1/P)	50	33.3	25		2.0	1.5	1.0	1.0								
5-Day BOD (mg/L)	102	120.9														

See next page for seed calculations

Avg. 111 mg/L Avg. 0.29 mg/L

For unseeded samples: $BOD_5, mg/L = \frac{(D1 - D2)}{P}$ **For seeded samples**: $BOD_5, mg/L = \frac{(D1 - D2) - (B1 - B2)\,f}{P}$

For the 4th case, BOD, only 1mL of seed should have been used; 2mL is too high. But, otherwise it appears the green influent is calculated correctly. Average the 2 values meeting depletion criteria before rounding to whole number.

The effluent is tricky, but because you have a 100% sample (5201B 7.a.2.), you can use actual depletion even though <2, so sample would be 0.29.

> 2) If DO depletion is less than 2.0 mg/L and sample concentration is 100% (no dilution except for seed, nutrient, mineral, and buffer solutions), actual seed-corrected, DO depletion may be reported as the BOD even if it is less than 2.0 mg/L.

	Seed Control			
mL. used OR % Concentration (P)	5	7	9	Avg
Initial DO (B_1)	8.15	8.17	8.16	
Final DO (B_2)	3.57	2.42	1.32	
Oxygen Demand	4.58	5.75	6.84	
$(B_1-B_2)f$	1.83	1.64	1.52	1.66
Oxygen Demand per mL of seed material	4.58/5=0.92	5.75/7=0.82	6.84/9=0.76	Avg 0.83

√ The DO uptake attributable to seed added to each bottle generally should be between 0.6 and 1.0 mg/L. However, the seed added should be adjusted from this range to that required to provide acceptable GGA results.

f Value: $f = \frac{\text{(volume of seed in sample dilutions)}}{\text{(volume of seed in seed control)}}$

Glucose-Glutamic Acid (GGA) Test

5210B-2001

Intended use for evaluation of dilution water quality, seed effectiveness, and analytical technique

Use 6 mL of BOD Standard and 2 mL of seed (typically 2 mL)

	GGA std			
mL GGA used	6	6		
mL seed	2	2		
Bottle Number	296	297		
Initial DO (D_1)	8.21	8.22		
(-) Final DO (D_2)	2.15	2.32		
Oxygen Demand (depletion) ($D_1 - D_2$)	6.06	5.90		
(-) Depletion due to seed $(B_1 - B_2)f$	1.66	1.66		
Net depletion due to GGA $(D_1 - D_2)$ - $(B_1 - B_2)f$	4.40	4.24		
Dilution Factor (300/6)	50	50		
5-Day BOD (mg/L)	220	212		

Avg. 216 mg/L

GGA std BOD values should lie within the range of 198 ± 30.5 mg/L. (167.5-228.5 mg/L)

1. Weak seed usually causes LOW GGA results.
2. Some sewage seeds are relatively inactive and yield LOW GGA results.
3. Soap contamination typically yields HIGH GGA results.
4. Distilled water contaminated with copper yields LOW GGA results.
5. Too much seed usually causes HIGH GGA results
6. BOD bottles that are not properly rinsed is indicated by HIGH GGA results and would also result in high blank depletion

Seeding Calculations

Bottle	DO_I	DO_F	Depletion		mL seed		DO depletion per mL seed
Seed 1	8.5	0.3	X 8.2		30		----
Seed 2	8.4	1.6	6.8	÷	20	=	0.34
Seed 3	8.4	4.3	4.1	÷	10	=	0.41

Avg the seed controls that meet depletion criteria
(0.34 + 0.41) ÷ 2 = 0.375 mg/L DO depletion per mL seed

So, 2 mL undiluted seed is added to each sample bottle. 0.375 X 2 = 0.75 mg/L

Therefore, 0.75 mg/L is subtracted from the depletion of each BOD depletion to obtain BOD result.

➢ Test Ranges / Requirements:

Water:

Deionized / Distilled water:

pH	5-6
Conductivity	<1micromho

BOD Water:

pH	6.8 - 7.2
Conductivity	125-165 micromhos

BOD Test:

Blanks	<.2 mg/L
Seed Corrections (SCF)	0.6 -1.0 mg/L
Standard (GGA)	168-228 mg/L

BOD ☑ Checklist

➢ Equipment:

DO Meter:

- ☐ Meter turned on at least 30 min prior to use
- ☐ Membrane good
- ☐ Calibrated according to manufacturers instructions
- ☐ Clean probe with Deionized water before use

BOD Bottles:

- ☐ Cleaned with a lab grade soap
- ☐ Triple rinsed with Deionized water
- ☐ Acid wash using 10% HCl solution

Tubing / Air Stones:

- ☐ Check for signs of mold in tubing
- ☐ Replace tubing at least once a month
- ☐ Replace air stones at least once a week
- ☐ Rinse tubing with Deionized water after every use
- ☐ Allow tubing to dry thoroughly before storing it

➢ Dilution Water:

- ☐ Only use Deionized or Distilled water for your test
- ☐ Store source water no more than 24 hrs in advance
- ☐ Keep source water at 20° ± 1°C
- ☐ Saturate source water before use
- ☐ Add nutrients immediately before use

➢ Nutrients:

If you make your own nutrients:

- ☐ Discard nutrients if there is any sign of precipitation or biological growth in the stock bottles
- ☐ Store nutrients at room temperature

Commercial nutrients:

- ☐ Use within the expiration date
- ☐ Store according to manufacturers instructions

➢ PolySeed:

- ☐ 500 mls of BOD water per Capsule (may be adjusted to achieve required results)
- ☐ Aerate and stir for 1 hour
- ☐ Let the solution settle 15 minutes
- ☐ Decant solution into a clean beaker (pour all but 20mls into a clean beaker) to eliminate the bran
- ☐ Place solution back on stir plate and gently stir throughout the test
- ☐ Use at least 4 dilutions for your seed control (15ml, 20ml, 25ml, 30ml)
- ☐ Use 4 ml of PolySeed in your GGA's and Samples
- ☐ Use solution with in 6 hours

➢ Glucose-Glutamic Acid:

If you make your own GGA:

- ☐ Use only reagent-grade glucose and reagent-grade glutamic acid
- ☐ Dry at 103°C for 1 hour
- ☐ Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1L
- ☐ Prepare fresh immediately before use

If you use commercial GGA:

- ☐ Check expiration date
- ☐ Check manufacturers instructions for proper use

Biological Oxygen Demand (BOD) – Overview

Author Unknown

Biochemical Oxygen Demand (BOD) refers to the amount of oxygen that would be consumed if all the organics in one liter of water were oxidized by bacteria and protozoa (ReVelle and ReVelle, 1988).

The first step in measuring BOD is to obtain equal volumes of water from the area to be tested and dilute each specimen with a known volume of distilled water which has been thoroughly shaken to insure oxygen saturation.

After this, an oxygen meter is used to determine the concentration of oxygen within one of the vials. The remaining vial is than sealed and placed in darkness and tested five days later. BOD is then determined by subtracting the second meter reading from the first.

The range of possible readings can vary considerably: water from an exceptionally clear lake might show a BOD of less than 2 ml/L of water. Raw sewage may give readings in the hundreds and food processing wastes may be in the thousands.

Background Information

Microorganisms such as bacteria are responsible for decomposing organic waste. When organic matter such as dead plants, leaves, grass clippings, manure, sewage, or even food waste is present in a water supply, the bacteria will begin the process of breaking down this waste. When this happens, much of the available dissolved oxygen is consumed by aerobic bacteria, robbing other aquatic organisms of the oxygen they need to live. Biological Oxygen Demand (BOD) is a measure of the oxygen used by microorganisms to decompose this waste. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste. In this case, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. As the waste is consumed or dispersed through the water, BOD levels will begin to decline.

Nitrates and phosphates in a body of water can contribute to high BOD levels. Nitrates and phosphates are plant nutrients and can cause plant life and algae to grow quickly. When plants grow quickly, they also die quickly. This contributes to the organic waste in the water, which is then decomposed by bacteria. This results in a high BOD level.

When BOD levels are high, dissolved oxygen (DO) levels decrease because the oxygen that is available in the water is being consumed by the bacteria. Since less dissolved oxygen is available in the water, fish and other aquatic organisms may not survive.

Test Procedure

The BOD test takes **5 days to complete** and is performed using a dissolved oxygen test kit. The BOD level is determined by comparing the DO level of a water sample taken immediately with the DO level of a water sample that has been incubated in a dark location for 5 days. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level.

1. Take 2 samples of water
2. Record the DO level (ppm) of one immediately using the method described in the dissolved oxygen test.
3. Place the second water sample in an incubator in complete darkness at 20^{o}C for **5 days**. If you don't have an incubator, wrap the water sample bottle in aluminum foil or black electrical tape and store in a dark place at room temperature (20^{o}C or 68 °F).
4. After 5 days, take another dissolved oxygen reading (ppm) using the dissolved oxygen test kit.
5. Subtract the Day 5 reading from the Day 1 reading to determine the BOD level. Record your final BOD result in *ppm*.

What to Expect

BOD Level *(in ppm)*	**Water Quality**
1 - 2	**Very Good** There will not be much organic waste present in the water supply.
3 - 5	**Fair: Moderately Clean**
6 - 9	**Poor: Somewhat Polluted** Usually indicates organic matter is present and bacteria are decomposing this waste.
100 or greater	**Very Poor: Very Polluted** Contains organic waste.

NOTE: Generally, when BOD levels are high, there is a decline in DO levels. This is because the demand for oxygen by the bacteria is high and they are taking that oxygen from the oxygen dissolved in the water. If there is no organic waste present in the water, there won't be as many bacteria present to decompose it and thus the BOD will tend to be lower and the DO level will tend to be higher.

At high BOD levels, organisms such as macro invertebrates that are more tolerant of lower dissolved oxygen (i.e. leeches and sludge worms) may appear and become numerous. Organisms that need higher oxygen levels (i.e. caddisfly larvae and mayfly nymphs) will NOT survive.

Source: http://www.polyseed.com/users/other_resources.php

BOD Test Requirements Tim Loftus

Legend has it that the 5-day BOD (Biological Oxygen Demand) test was developed in England. Sewage was dumped in a river and it took five days for it to reach the ocean, hence the five-day incubation requirement in the BOD method.

Obviously, the BOD test is not the most scientifically based test we perform. As such, it has many problems associated with it. The most significant problem is that the results come five days after the fact. By that time you've already discharged any problem wastewater. Then the variability of the seed must be constantly monitored. Sometimes the bacteria wants to work, sometimes they don't. Again, by the time you find out, it is five days too late. Some analysts support the use of respirometry to give more timely results, but regulatory agencies have not fully accepted the methods yet so NPDES permits continue to specify that we perform the standard 5-day BOD test.

There is no absolute BOD value of a sample as there would be for say copper or lead. BOD results are test defined. In other words, BOD values are based on the parameters of the test method, not on any "true" BOD value. Below are the requirements that must be met for a BOD analysis to be valid (i.e. be in a form that can be used to compare BOD values with other sources). By meeting these requirements, it means that you have the method correct. It does not cover the additional problems of sample toxicity or errant dilutions. For information on how to set up and calculate a BOD analysis, refer to Standard Methods for the Examination of Water and Wastewater, 18th edition.

The BOD blank (a BOD bottle full of dilution water containing only the required nutrients, but not any seed) must not show a DO, or dissolved oxygen, depletion of more than 0.2 mg/L after the five day incubation period. A drop of more than 0.2 mg/L indicates some type of contamination or calibration error.

The seed, or the microorganisms added to industrial wastes or disinfected wastewater effluent samples to break down the organic compounds, should contribute 0.6 to 1.0 mg/L DO uptake per BOD bottle.

A glucose-glutamic acid standard, made according to Standard Methods, should produce a BOD result of 198 mg/L +/- 30.5. However, it's best to determine your own average and standard deviation so you can develop a more accurate and useful range and control chart.

Ideally, sample dilutions should show about a 50% DO decrease after the 5-day incubation period. At a minimum, there should be at least a 2.0 mg/L DO change between the initial and the final reading. There should also be a residual DO of at least 1.0 mg/L.

The blank, seed determination and glucose-glutamic acid standard should be run every time a BOD analysis is performed. If any of these basic BOD requirements are not met, then the test is considered invalid and remedial action is needed.

It's important to keep accurate records of these measurements so that you can monitor trends. You may find, for example, that seed viability changes with the seasons. Knowing this, adjustments in the amount of seed added to each bottle can be made to meet the 0.6 to 1.0 mg/L seed uptake requirement.

For all its shortcomings, the 5-day BOD test is here for a while. It has a lot of variability and must meet a number of requirements to be valid. However, it is possible to consistently meet these requirements, but it takes work and careful monitoring of everything that goes into the analyses.

The information in this article is based on an EPA accepted test method for NPDES monitoring. As usual, check your federal, state, and local regulations. You may have additional regulations or reporting requirements that you must meet.

This article was written under the auspices of the New England Water Environment Association (a chapter of the Water Environment Federation) Laboratory Practices Committee. Please visit the NEWEA website at www.newea.org for membership information and other opportunities.

Additional Techniques and Hints for Accurate BOD Results (2nd of 3 BOD articles)
Tim Loftus

The previous BOD article reviewed what quality control measures indicate a "good" BOD run. These measures include test-defined limits for the blank, standard, and seed, as well as limits on dissolved oxygen (DO) residuals at the end of the analysis. This article will cover additional techniques and hints to get accurate and valid BOD results.

As with any biological system, pH affects the efficiency of the bacteria breaking down organic matter in the sample. Adjust the pH of all samples for BOD analysis to between 6.5 and 7.5 SU using 1 N sulfuric acid or 1 N sodium hydroxide.

Any sample that has been chlorinated, even if no chlorine residual is left, must be seeded with viable bacteria so that the organic strength, or BOD, of the sample can be measured. Samples that show chlorine residual must also be dechlorinated using sodium sulfite (see Standard Methods for the recipe). But be careful, excess sodium sulfite in the sample will exert an oxygen demand giving false high BOD readings. It's important to remember that the dechlorinating agent for coliform/E. coli analysis cannot be used for BODs. It is not the same chemical.

Most of us use electronic dissolved oxygen probes to measure the DO in the BOD bottles. These probes usually calibrate to an air setting rather than DO saturated water. If your probe is an air calibration type, calibrate to the barometric pressure in your lab rather than to 760 mm (sea level) or to a calculated air pressure based on your topographic elevation (which is commonly done). Air pressure often changes daily and sometimes hourly. Most likely the air pressure is not the same the day of a BOD setup and five days later when the BODs are read again. This will be important when measuring the BOD blank. Since the DO change of the blank should not exceed 0.2 mg/L, you can see where calibration accuracy would aid in validating the analysis.

Bubbles in a BOD bottle also invalidate that bottle's DO measurement. Algae in a BOD sample and left out on a lab bench exposed to sunlight can be a source of bubbles. Always put the BOD bottle in a dark incubator soon after the initial DO is measured and the bottle sealed. But a more common source of bubbles is from dirty glassware. Even though we should try to fill BOD bottles with sample and dilution water as bubble free as possible, there seems to always be tiny bubbles generated. If the glassware is not thoroughly cleaned, then the bubbles stick to the side of the glass and will eventually collect near the bottle's seal during the five-day incubation period.

Another source of bubbles can come from aerated dilution water or from samples that are at a lower temperature than 20 degrees C. Since cold water will hold more dissolved air, aerating cold dilution water will give a higher oxygen content than if the dilution water was aerated at 20 C. After placing the samples in an incubator at 20 C, the water will warm and not be able to hold as much DO. As a result, bubbles may form in the bottles. This can also happen with a low dilution sample, such as an effluent composite sample that was collected at 4 C and not warmed to temperature. It's important to always warm samples to 20 C, then shake the sample to remove excess dissolved oxygen before setting up for BOD. If your laboratory has heating problems, as they all seem to have, try storing the dilution water in your incubator overnight to stabilize the temperature to 20 C. This will help remove excess dissolved oxygen from the dilution water.

As with all analyses performed in your lab, always record the actions of what you do to samples on the data sheet or in a bound notebook specifically for that analysis. For these BOD examples, record sample temperature, pH of BOD sample (before and after adjustment), chlorine residual and amount of dechlorinating agent used (if needed), and the barometric pressure that the BOD probe was calibrated to.

BODs are a lot of work to do. And it's often harder to get them to come out right. With the right techniques and some foresight of potential problems, your results will not only be accurate, but will be valid as well.

The information in this article is based on an EPA accepted test method for NPDES monitoring. As usual, check your federal, state, and local regulations. You may have additional regulations or reporting requirements that you must meet.

This article was written under the auspices of the New England Water Environment Association (a chapter of the Water Environment Federation) Laboratory Practices Committee. Please visit the NEWEA website at www.newea.org for membership information and other opportunities.

BOD Techniques (3rd of 3 BOD articles)

Tim Loftus

The first article of this series reviewed what quality control measures are needed to validate a Biological Oxygen Demand, or BOD, analysis. The second article covered pH adjustment, dechlorinating samples, calibrating dissolved oxygen (DO) probes to the barometric pressure, and sources of bubbles in the BOD bottles. This third and final article in the series will focus on the results of BOD analyses.

You've set up the perfect BOD analysis. The sample was pH adjusted properly, there were no bubbles in the bottle after five days, your blank, standard, and seed all came out within acceptable limits. Then why are there three different results for the three dilutions of the same sample?

There are a several reasons that these types of errors happen. The first is incorrect dilution choices. The second is the fault of the sample (yes, we can blame the sample sometimes!). However, both types of errors can be reduced or even eliminated with a little bit of planning.

Dilution errors:

All samples must be mixed well prior to diluting in BOD bottles. If the sample has large particulate matter, as found in raw wastewater for example, you must ensure that these particles do not settle out while multiple dilutions are made. You may need to continuously mix the sample to keep the particles evenly distributed. Otherwise the BOD dilutions can give widely varied results. If a series of dilutions for a sample shows two results that are close and one result that is way off, report the average of the two that are close in value. For example: A series of raw wastewater BOD dilutions calculate to 235 mg/L, 245 mg/L, and 390 mg/L. Average the 235 mg/L and 245 mg/L results. Most likely the high value (390 mg/L) was the result of an uneven distribution of oxygen-demanding particles in the sample dilution.

Choose dilutions that produce a DO decrease in the BOD bottle of at least 2 mg/L, but retains an oxygen residual of more than 1 mg/L after a five-day incubation period. If the DO in a bottle did not decrease by more than 2 mg/L, drop that measurement and calculate the remaining dilutions. Likewise, if the oxygen residual in a bottle is less than 1 mg/L, drop that measurement and calculate the remaining dilutions. For example: the initial DO for four dilutions of a single sample is 8.5 mg/L. After a five-day incubation the oxygen residual is 7.9 mg/L, 5.7 mg/L, 4.3 mg/L, and 0.5 mg/L (representing a dissolved oxygen change of 0.6 mg/L, 2.8 mg/L, 4.2 mg/L, and 8.0 mg/L respectively). Since the first dilution did not decrease the required 2 mg/L, do not use this dilution in the calculation of the sample's average BOD value. Also, the fourth dilution left an oxygen residual less than 1 mg/L and should not be used in the calculation of the sample's average BOD value.

Occasionally a series of dilutions will all be bad. Either none of the dilutions will give a DO decrease of 2 mg/L or all dilutions will use up almost all the dissolved oxygen in the bottle. If the sample is "weak" in BOD and all the dilutions use very little oxygen (a change of less than 2 mg/L), take the lowest dilution and calculate it out as if it had dropped 2 mg/L. For example, the initial DO of a BOD sample is 8.5 mg/L for three dilutions of 3%, 4%, and 5%. After a five-day incubation, the residual oxygen level is 8.0 mg/L, 7.5 mg/L and 7.0 mg/L (for a DO change of 0.5 mg/L, 1.0 mg/L, and 1.5 mg/L respectively). Since none of the sample dilutions meet the 2 mg/L DO change, calculate the lowest diluted sample (the 5% diluted sample) as if the change

was 2 mg/L rather than the 1.5 mg/L measured value. Then report the result as “less than (<) this calculated value.”

Calculations where all dilutions have an oxygen residual of less than 1 mg/L are done similarly. But in these cases, calculate the result using the highest diluted sample as if the oxygen residual is 1 mg/L and report as “greater than (>) this calculated value.”

While the greater than (>) and less than (<) designations on results are the best you can do under the circumstances, it is best to avoid it all in the first place by developing a series of dilutions where at least one sample will fall within the proper range. Even if you know, for example, that the BOD result for the final effluent is less than the permit limit, you should still strive for accuracy. You cannot add, subtract, multiply, or divide “less than” or “greater than” signs. Especially when these sample results must be used in calculations to determine plant loadings or removal rates.

Blame the sample:

Sometimes the sample may be toxic to the bacteria, or seed, that break down the wastes. This is often seen as decreasing BOD results on a sample coinciding with decreasing dilution rates. For example, three dilutions (1%, 2%, 3%) of an industrial wastewater sample gives results of 450 mg/L, 375 mg/L, and 250 mg/L respectively. This indicates a level of toxicity in the sample. In these cases, calculate the BOD value using the most diluted sample (450 mg/L) since this shows the least effect of toxicity.

This article concludes the series on BOD analyses. It takes lots of knowledge, time, and experience to get the test to work properly. And then there will be times that the bugs in the BOD bottle will do as they want anyway. Hopefully, the information presented in these articles will help you in your laboratory to achieve greater BOD accuracy. As usual, check your federal, state, and local regulations. You may have additional regulations or reporting requirements that you must meet.

This article was written under the auspices of the New England Water Environment Association (a chapter of the Water Environment Federation) Laboratory Practices Committee. Please visit the NEWEA website at www.newea.org for membership information and other opportunities.

BOD – 10 minute COD

1. A ten-minute COD is run on each sample to get an approximate value for calculating the BOD sample sizes.

2. Effluents and clear samples are usually run in the low (0 – 150) range COD vials.

3. Influents and other turbid samples are usually run in the high (0 – 1500) range COD vials.

4. Two mL of deionized water is placed in one each of the high and low COD vials to be run as blanks (for zeroing the spectrophotometer).

5. Two mL of sample is placed into the appropriate COD vial(s).

6. The vials are cooked in the COD reactor (at 150°C) for 10 minutes and then allowed to cool.

7. Read the vials on the spectrophotometer.

8. If a low COD reads greater than 150, it should be re-run using the 0 – 1500 range vial.

9. If a high COD reads greater than 1500, a dilution should be made and reanalyzed.

Sample Size Calculation:

Use the following calculation for the high-range COD vials:

$$\frac{1500}{COD} = X \text{ } \textit{(Sample Size in mL)}$$

This answer is in milliliters of sample to use for the BOD. Use the following guide to determine the other two dilutions for a three-dilution BOD:

If COD is < 100, then use X, 2X, and 4X sample sizes.
If COD is > 100, then use ½ X, X, and 2X sample sizes.

Example:

If the 10-minute COD was 300, then 1500/300 = 5 mL. Because the COD was greater than 100, then the dilutions would have 2.5mL, 5 mL, and 10 mL of sample.

If the 10-minute COD was 50, then 1500/50 = 30 mL. Because the COD was less than 100, then the dilutions would have 30 mL, 60 mL, and 120 mL of sample.

Note: For the low-range COD, divide the reading into *150* (not 1500) and use greater-than or less-than *10* as the determinant (not > or < 100).

Section 4
Chemical Oxygen Demand

1

Chemical Oxygen Demand (COD)

Approved Methods Under
the Clean Water Act (NPDES)

TDEC - Fleming Training Center

Fleming Training Center | TN Department of Environment & Conservation

1

2

TDEC - Fleming Training Center

Why Test For COD?

- To measure the organic matter content of a sample that is susceptible to oxidation by strong chemical oxidants.
- Can measure the strength of wastes that are too toxic for the BOD test.

2

3

TDEC - Fleming Training Center

Sample Collection

- Plastic or glass bottles
- Bottles must be thoroughly cleaned
  - Even a trace amount of organic matter may cause gross error
- Representative sample (100 mL)
- Composite sample
- Analyze as soon as possible (same day) OR
- Cool <6°C, preserve with H_2SO_4 to pH<2
- Max holding time: 28 days (after preservation)

3

4

TDEC - Fleming Training Center

Sample Preparation

- Homogenize samples that contain solids
- Use wide tip pipet to ensure a representative sample is added
- Sample must be well mixed before it is heated
- Always wear goggles and gloves!
  - Corrosive and toxic reagents
  - Contain mercury – requires special disposal

4

5

TDEC - Fleming Training Center

COD vs. BOD

- COD
  1. Is a good estimate of the first-stage oxygen demand for most municipal wastewaters
  2. Measures the strength of the organic waste that is too toxic for BOD
  3. Results are quicker
  4. Results can be used to estimate BOD

5

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TDEC - Fleming Training Center

COD vs. BOD

- COD should be considered an independent measurement of organic matter
  - Rather than a substitute for BOD
- Indirect measure of amount of organics in water sample

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COD vs. BOD (Advantages)

- Faster than BOD
  - 2 hours instead of 5 days
- Fewer interferences
  - Chloride
- Stable methodology yielding repeatable results
- Easy to run

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COD vs. BOD (Disadvantages)

- Does not measure the rate of biodegradability of organic matter
- Cannot be used to predict the effects of an effluent on the DO in receiving waters
- Difficult to predict the treatability of a particular wastewater by biological processes
- Toxic and corrosive reagents
  - Special care when handling
  - Special disposal

8

9

Can COD be correlated to BOD?

- It depends…
  - May or may not exist depending on sample composition, seasonal variation, and other factors
1. Must collect empirical data
   - COD and BOD data for the same water sample collected over the same period of time
2. Must graph data
   - Graph COD and BOD data to determine whether or not a correlation exists

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Summary of Method

- The method oxidizes organic substances in the wastewater sample using:
  - Potassium dichromate in 50% sulfuric acid
  - Silver sulfate (catalyst)
  - Mercuric sulfate (removes interferences)
  - Sample heated/digested for 2 hours
- Chloride is the primary interference
  - Samples with high chloride must be diluted

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Method

- The COD test measures the amount of dichromate (oxidant) consumed in the breakdown of organic matter
  - More oxidant consumed = high levels of organics
  - Less oxidant consumed = low levels of organics

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Selection of Method

- Titrimetric (blue-green to reddish):
  - EPA Method 410.3
  - SM 5220B or C – 2011
- Spectrophotometric/Colorimetric (manual or automated):
  - EPA Method 410.4, Rev. 2.0
  - SM 5220D - 2011
  - Hach Method 8000

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Laboratory Apparatus

- Titrimetric Method:
  - Reflux ground-glass apparatus
  - Heat source
  - Buret
- Spectrophotometric/ Colorimetric Method:
  - Digestion tubes
  - Hot block (150°C)
  - Spectrometer 420nm and/or 600 nm

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Calculations

- Titrimetric
  - COD, mg/L = $\frac{(A-B) \times N \times 8000}{S}$
    - A = mL of titrant for blank
    - B = mL of titrant for sample
    - N = Normality of titrant
    - S = mL of sample used

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Calculations (continued)

- Colorimetric:
  - Direct reading in mg/L from instrument
  - Results in mg/L COD are defined as the milligrams of O_2 consumed per liter of sample
  - If different sample volumes used then,

$$\text{COD, mg/L} = \frac{(\text{mg } O_2 \text{ in final vol.})(1000)}{\text{mL sample}}$$

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Quality Assurance/Quality Control

- Titrimetric Method EPA 410.3:
  - DI Blank
  - Chloride check
  - Standardize Titrant
  - $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$
- Hach Method 8000:
  - Pre-programmed calibration curve
  - Accuracy Check Standard (potassium acid phthalate)
  - DI Blank

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Spectrophotometric Methods - QA/QC

- EPA Method 410.4/SM5220D - 2011
  - Initial Demonstration of Performance:
    - Linear Calibration Range (LCR)
    - Quality Control Sample (QCS)
    - Method Detection Limit (MDL)

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Colorimetric Methods - QA/QC

- EPA Method 410.4/SM5220D - 2011
  - Laboratory Performance:
    - Laboratory Reagent Blank (LRB)
    - Laboratory Fortified Blank (LFB)
    - Instrument Performance Check (IPC)
  - Analyte Recovery & Data Quality:
    - Matrix Spike, Duplicate & Reference Standard

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COD Permitted

- Title 40 CFR Part 136 still requires an MDL study be performed as with other applicable analyses. Regulatory requirements do sometimes supersede the specifications of consensus body analysis methods.

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COD Permitted

- The calibration curve preparation discussed in Standard Method 5220 D-2011 4.c. must be performed at least monthly.
- While this may result in more frequent checks than allowed in the method, monthly checks are consistent with current Division and EPA Region 4 guidance for another colorimetric method, the DPD Colorimetric Method for total residual chlorine analyses.

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COD Permitted

- With regard to the range of standards necessary, Standard Methods does not specify any required concentrations. Therefore, the laboratory may choose five standard concentrations best representing the analytical range of interest.

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COD Audit Checklist

- Sample collection & preservation
- Holding time
- Approved Method
- Heat regulated devices checked for accuracy
- Apparatus e.g. ground-glass used (titrimetric)
- Glassware cleaned properly
- QC samples analyzed
- Results reported correctly

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COD Take home messages

- COD is an indirect measure of organics
- COD is measured by oxidizing organics with a strong oxidant (dichromate) and measuring the amount of oxidant consumed
- Correlation between COD and BOD is sample specific – must conduct a study!
- COD will always be higher than BOD
  - Includes both biodegradable and non-biodegradable substances

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COD – Review Questions

1. What does COD measure? And it is a good estimate of what?

2. What type of container should be used to collect samples for COD? Why is it important for the sample containers to be thoroughly cleaned and free of contaminants?

3. What type of sample should be used in the COD test?

4. The COD vials we used in lab class contain a toxic reagent that requires special disposal, what is that reagent and what it its purpose in the test?

5. List at least 4 advantages of the COD test as compared to the BOD test.

6. Why is the COD test more stable than the BOD test?

7. List at least 3 disadvantages to the COD test as compared to the BOD test.

8. What must be done to determine a correlation between the COD and BOD at your facility?

9. What is the oxidant used in the COD test?

10. Using the colorimetric method, how are the results reported? Explain how those results are defined.

11. COD is always higher than BOD; why?

Oxygen Demand, Chemical

USEPA[1] Reactor Digestion Method[2] — Method 8000

0.7 to 40.0[3] mg/L COD (ULR); 3 to 150 mg/L COD (LR); 20 to 1500 mg/L COD (HR); 200 to 15,000 mg/L COD (HR Plus)

Scope and application: For water and wastewater. Digestion is required.

[1] Ranges 3 to 150 mg/L COD and 20 to 1500 mg/L COD are USEPA approved for wastewater analyses (Standard Method 5220 D), Federal Register, April 21, 1980, 45(78), 26811-26812.
[2] Jirka, A.M.; Carter, M.J., Analytical Chemistry, 1975, 47(8), 1397.
[3] The ULR is only available with spectrophotometers that can measure at a wavelength of 350 nm.

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the instruments that use them.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for test tubes

Instrument	Adapters	Light shield
DR6000, DR5000	—	—
DR3900	—	LZV849
DR3800, DR2800, DR2700	—	LZV646
DR1900	9609900 (D[1])	—
DR900	4846400	Cover supplied with the instrument

Before starting

Install the instrument cap on the DR900 cell holder before ZERO or READ is pushed.
DR3900, DR3800, DR2800 and DR2700: Install the light shield in Cell Compartment #2 before this test is started.
The reagent that is used in this test is corrosive and toxic. Use protection for eyes and skin and be prepared to flush any spills with running water.
The reagents that are used in this test contain mercury. Collect the reacted samples for safe disposal.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number is on the container label. Refer to Blanks for colorimetric determination on page 4.
Store unused (light sensitive) vials in a closed box.
If the samples contain high concentrations of chloride, refer to the Alternate reagents section.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

[1] The D adapter is not available with all instrument versions.

Items to collect

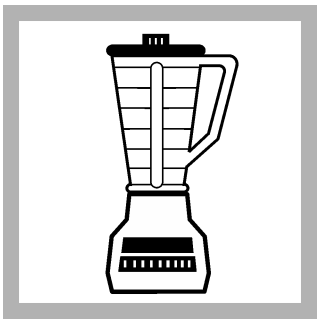
Description	Quantity
Beaker, 250-mL	1
Blender	1
COD Digestion Reagent vials	varies
DRB200 Reactor	1
Light shield or adapter (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Magnetic stirrer and stir bar	1
Opaque shipping container for storage of unused, light-sensitive reagent vials	varies
Pipet, TenSette, 0.1- to 1.0-mL, with pipet tips (for use with the 200–15,000 mg/L range)	1
Pipet, volumetric, 2.00-mL	2
Pipet filler safety bulb	1
Test tube rack	2

Refer to Consumables and replacement items on page 7 for order information.

Sample collection and storage

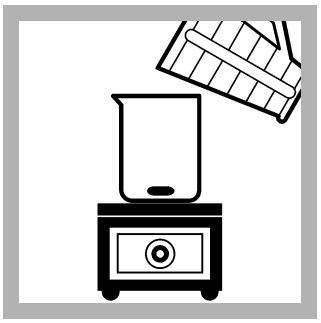
- Collect samples in clean glass bottles. Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples that contain solids to get a representative sample.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated sulfuric acid (approximately 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at 2–6 °C (36–43 °F) for a maximum of 28 days.
- Correct the test result for the dilution caused by the volume additions.

Reactor digestion procedure

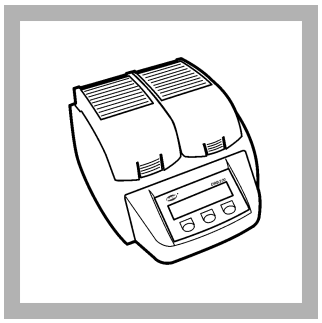


1. Put 100 mL of sample in a blender. Blend for 30 seconds or until homogenized.

For samples with large amounts of solids, increase the homogenization time. If the sample does not contain suspended solids, go to step 3.



2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.



3. Set the DRB200 Reactor power to on. Preheat to 150 °C.

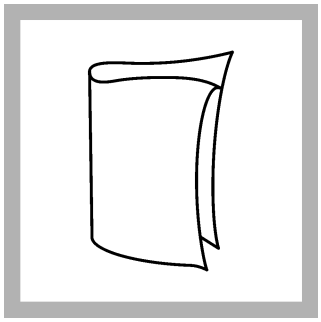
Refer to the DRB200 User Manual for selecting pre-programmed temperature applications.

4. Prepare the sample: Remove the cap from a vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of sample to the vial.

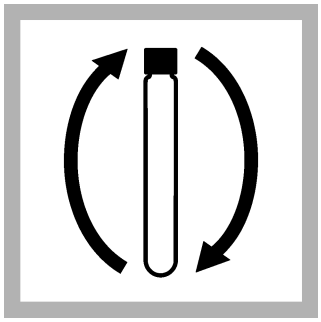
For 250–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of sample to the vial.

5. Prepare the blank: Remove the cap from a second vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of deionized water to the vial.

For 250–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of deionized water to the vial.

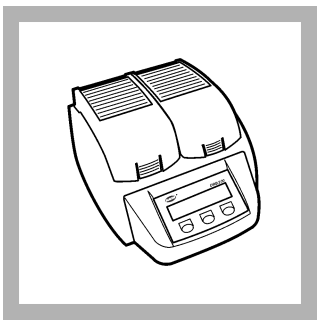


6. Close the vials tightly. Rinse the vials with water and wipe with a clean paper towel.

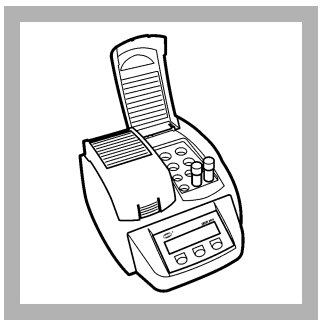


7. Hold the vials by the cap, over a sink. Invert gently several times to mix. **The vials get very hot during mixing.**

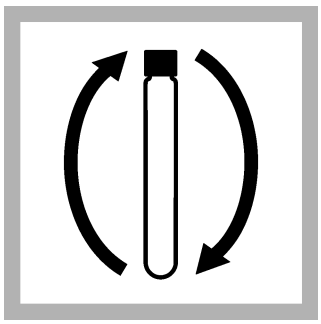
8. Put the vials in the preheated DRB200 reactor. Close the lid.



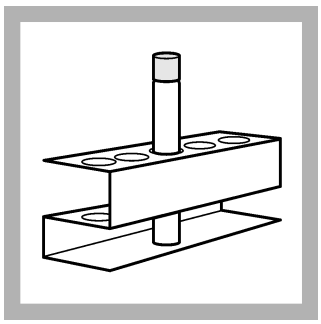
9. Heat the vials for 2 hours.



10. Set the reactor power to off. Let the vials cool in the reactor for approximately 20 minutes to 120 °C or less.

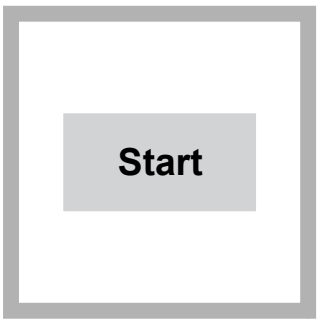


11. Invert each vial several times while it is still warm.

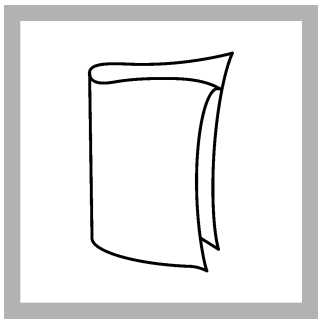


12. Put the vials in a tube rack to cool to room temperature.

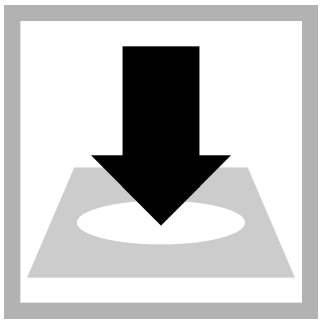
Colorimetric procedure



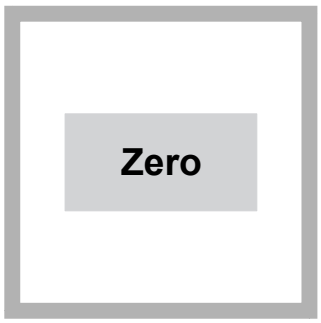
1. Start program **431 COD ULR**, **430 COD LR** or **435 COD HR**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.



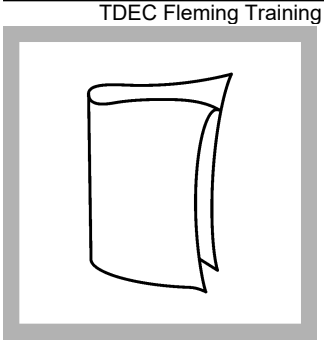
2. Clean the blank sample cell.



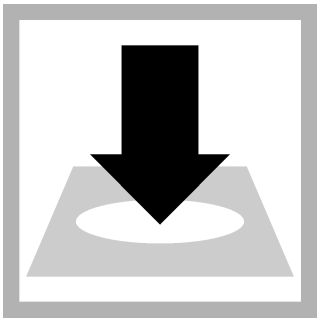
3. Insert the blank into the cell holder.



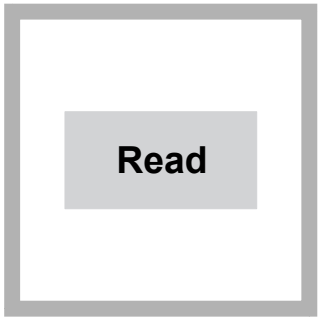
4. Push **ZERO**. The display shows 0 or 0.0 mg/L COD.



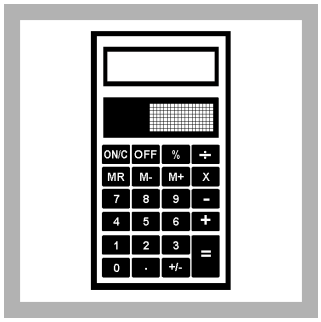
5. Clean the prepared sample cell.



6. Insert the prepared sample into the cell holder.



7. Push **READ**. Results show in mg/L COD.



8. If using High Range Plus COD digestion reagent vials, multiply the result by 10. For the most accurate results with samples near 1500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.

Blanks for colorimetric determination

The blank vial can be used again and again for measurements that use the same lot of reagent vials. Measure the absorbance of the blank vial over time and prepare a new blank vial when the absorbance changes.

1. Put the instrument in the absorbance mode at the applicable wavelength. Refer to Table 3 on page 6.
2. Add 5 mL of deionized water into an empty vial.
3. Put the vial in the instrument and zero the instrument.
4. Put the blank vial that is used in the test procedure into the instrument and record the absorbance value.
5. Keep the blank vial in the dark.
6. Prepare a new blank when the absorbance has changed by approximately 0.01 absorbance units.

Interferences

Chloride is the primary interference in this test procedure. Each COD vial contains mercuric sulfate that removes chloride interference to the level specified in Column 1 of Table 2. Dilute samples that have higher chloride concentrations to the level given in Column 2.

Note: *For best results, use the low range and ultra-low range vials for samples that have high chloride concentrations (near maximum concentration) and low COD concentrations.*

If sample dilution causes the COD concentration to be too low for accurate measurements, add 0.50 g of mercuric sulfate ($HgSO_4$) to each COD vial before the sample is added. The additional mercuric sulfate will increase the maximum chloride concentration to the level given in Column 3.

Note: *Bromide interference is not removed with mercuric sulfate.*

Table 2 Chloride concentration limits in the sample

Vial range	Column 1 (maximum mg/L Cl^-)	Column 2 (mg/L Cl^- for diluted samples)	Column 3 (maximum mg/L Cl^- with mercuric sulfate)
ULR[2] (0.7–40.0 mg/L)	2000	1000	N/A
LR (3–150 mg/L)	2000	1000	8000

[2] The ULR is only available for spectrophotometers that can measure at a wavelength of 350 nm.

Table 2 Chloride concentration limits in the sample (continued)

Vial range	Column 1 (maximum mg/L Cl^-)	Column 2 (mg/L Cl^- for diluted samples)	Column 3 (maximum mg/L Cl^- with mercuric sulfate)
HR (20–1500 mg/L)	2000	1000	4000
HR Plus (200–15,000 mg/L)	20,000	10,000	40,000

Accuracy check

Standard solution method

Items to collect:

- 1000 mg/L COD standard solution
- 100-mL volumetric flask, Class A
- Volumetric pipets, Class A and pipet filler
- Deionized water
- Potassium acid phthalate (KHP), dried overnight at 120 °C (HR Plus only)

0.7 to 40.0 mg/L ULR

1. Prepare a 30-mg/L COD standard solution as follows:
   a. Use a pipet to add 3.00 mL of the 1000 mg/L standard solution into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
2. Use the test procedure to measure the concentration of the standard solution.
3. Compare the expected result to the actual result.
   Note: *The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

3 to 150 mg/L LR

1. Prepare a 100-mg/L COD standard solution as follows:
   a. Use a pipet to add 10 mL of the 1000 mg/L standard solution into a 100-mL volumetric flask.
   b. Dilute to the mark with deionized water. Mix well.
2. Use the test procedure to measure the concentration of the standard solution.
3. Compare the expected result to the actual result.
   Note: *The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

20 to 1500 mg/L HR

1. Use the test procedure with a 300-mg/L, 800 mg/L or 1000 mg/L COD standard solution to measure the concentration of the standard solution.
2. Compare the expected result to the actual result.
   Note: *The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

200 to 15,000 mg/L HR Plus

1. Prepare a 10,000 mg/L COD standard solution as follows:
   a. Dissolve 8.500 g of dried KHP in 1000-mL of organic-free deionized water.
2. Use the test procedure to measure the concentration of the standard solution.
3. Compare the expected result to the actual result.
   Note: *The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.*

Alternate reagents

Mercury-free COD2 Reagents are available as a mercury-free alternative. These reagents are fully compatible with test procedures and stored programs in the instruments. Chloride and ammonia determinations are recommended for accurate results.

NOTICE

COD2 reagents are not approved for USEPA reporting purposes. Because COD2 reagents do not contain mercury as a masking agent, they exhibit a positive interference from chloride. More information is available for use with specific applications.

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% Confidence Interval)	Sensitivity Concentration change per 0.010 Abs change
431 (ULR)	30 mg/L COD	28.8–31.2 mg/L COD	0.5 mg/L COD
430 (LR)	80 mg/L COD	77–83 mg/L COD	3 mg/L COD
435 (HR)	800 mg/L COD	785–815 mg/L COD	23 mg/L COD
435 (HR Plus)	8000 mg/L COD	7850–8150 mg/L COD	230 mg/L COD

Summary of method

The results in mg/L COD are defined as the milligrams of O_2 consumed per liter of sample under the conditions of this procedure. The sample is heated for 2 hours with sulfuric acid and a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}).

When the 0.7–40.0 or the 3–150 mg/L colorimetric method is used, the amount of Cr^{6+} that remains is measured. When the 20–1500 mg/L or 200–15,000 mg/L colorimetric method is used, the amount of Cr^{3+} that is produced is measured. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

Test results are measured at the wavelengths that are specified in Table 3.

Table 3 Range-specific test wavelengths

Range in mg/L COD	Wavelength
0.7–40.0 mg/L	350 nm (for applicable instruments)
3–150 mg/L	420 nm
20–1500	620 nm (610 nm for colorimeters)
200–15,000 mg/L	620 nm (610 nm for colorimeters)

Pollution prevention and waste management

Reacted samples contain mercury, silver and chromium and must be disposed of as a hazardous waste. Dispose of reacted solutions according to local, state and federal regulations. Users in the United States can use the ez COD Recycling Service for disposal of COD vials. Refer to Consumables and replacement items on page 7.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
COD, Ultra Low Range, 0.7–40 mg/L	1–2 vials	25/pkg	2415825
COD, Low Range, 3–150 mg/L	1–2 vials	25/pkg	2125825
COD, High Range, 20–1500 mg/L	1–2 vials	25/pkg	2125925
COD, High Range Plus, 200–15,000 mg/L	1–2 vials	25/pkg	2415925
Water, deionized	varies	4 L	27256

Alternate reagents and package sizes

Description	Quantity/test	Unit	Item no.
COD2, Low Range, 0–150 mg/L COD	1–2 vials	25/pkg	2565025
COD2, High Range, 0–1500 mg/L COD	1–2 vials	25/pkg	2565125
COD2, High Range, 0–1500 mg/L COD	1–2 vials	150/pkg	2565115
COD2, High Range Plus, 0–15,000 mg/L COD	1–2 vials	25/pkg	2834325
COD Digestion Reagent Vials, 3–150 mg/L COD	1–2 vials	150/pkg	2125815
COD Digestion Reagent Vials, 20–1500 mg/L COD	1–2 vials	150/pkg	2125915
COD Digestion Reagent Vials, ULR 0.7–40.0 mg/L	1–2 vials	150/pkg	2415815
COD Digestion Reagent Vials, HR plus, 200–15,000 mg/L	1–2 vials	150/pkg	2415915

Required apparatus

Description	Quantity/test	Unit	Item no.
Blender, 2-speed, 120 VAC option	1	each	2616100
OR			
Blender, 2-speed, 240 VAC option	1	each	2616102
DRB200 Reactor, 110 VAC option, 15 x 16-mm wells	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 VAC option, 15 x 16-mm wells	1	each	LTV082.52.40001
Pipet filler, safety bulb	1	each	1465100
Pipet, volumetric, Class A, 2.00 mL	1	each	1451536

Recommended standards and apparatus

Description	Unit	Item no.
Beaker, 250 mL	each	50046H
COD Standard Solution, 300 mg/L	200 mL	1218629
COD Standard Solution, 300 mg/L	500mL	1218649

Recommended standards and apparatus (continued)

Description	Unit	Item no.
COD Standard Solution, 800 mg/L	200 mL	2672629
COD Standard Solution, 1000 mg/L	200 mL	2253929
Oxygen Demand Standard (BOD, COD, TOC), 10-mL ampules	16/pkg	2833510
Pipet, TenSette®, 0.1–1.0 mL	each	1970001
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette® Pipet, 0.1–1.0 mL	1000/pkg	2185628
Potassium Acid Phthalate (KHP), ACS	500 g	31534
Stir bar, octagonal	each	2095352
Stirrer, electromagnetic, 120 VAC, with electrode stand	each	4530001
Stirrer, electromagnetic, 230 VAC, with electrode stand	each	4530002
Test tube rack, stainless steel	each	1864100
Wipes, disposable	70/pkg	2096900

Optional reagents and apparatus

Description	Unit	Item no.
Balance, analytical, 80 g x 0.1 mg 100–240 VAC	each	2936701
Flask, volumetric, Class A, 1000 mL glass	each	1457453
Flask, volumetric, Class A, 100 mL, glass	each	1457442
Pipet, volumetric, Class A, 3 mL	each	1451503
Pipet, volumetric, Class A, 10 mL	each	1451538
Sulfuric Acid, ACS	500 mL	97949
Wastewater Influent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4^{3-}, COD, SO_4^{2-}, TOC	500 mL	2833149
EZ COD™ Recycling Service with 5-gal bucket-mail back option (For US customers only. 20 and 55 gallon sizes are also available.)	each	2895405
EZ COD™ Recycling Service with 5-gal bucket- pick up option. (For US customers only. 20 and 55 gallon sizes are also available.)	each	2895405P
Finger cots	2/pkg	1464702
Gloves, chemical resistant, size 9–9.5	pair	2410104[3]
Paper, for weighing, 100 x 100 mm	500/pkg	1473885
Safety goggles, vented	each	2550700
Wastewater Effluent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4^{3-}, COD, SO_4^{2-}, TOC	500 mL	2833249

[3] Other sizes available

Understanding Laboratory Wastewater Tests:
I. ORGANICS (BOD, COD, TOC, O&G)

Since the implementation of the Clean Water Act and subsequent creation of the United States Environmental Protection Agency (USEPA) in the early 1970s, industrial, institutional and commercial entities have been required to continually improve the ***quality*** of their process wastewater effluent discharges.

At the same time, population and production increases have increased water use, creating a corresponding rise in wastewater ***quantity***. This increased water use and process wastewater generation requires more efficient removal of by-products and pollutants that allows for effluent discharge within established environmental regulatory limits.

The determination of wastewater ***quality*** set forth in environmental permits has been established since the 1970s in a series of laboratory tests focused on four major categories:

1. **Organics** – A determination of the concentration of carbon-based (i.e., organic) compounds aimed at establishing the relative "strength" of wastewater (e.g., Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), and Oil and Grease (O&G)).

2. **Solids** – A measurement of the concentration of particulate solids that can dissolve or suspend in wastewater (e.g., Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Volatile Solids (TVS), and Total Fixed Solids (TFS)),

3. **Nutrients** – A measurement of the concentration of targeted nutrients (e.g., nitrogen and phosphorus) that can contribute to the acceleration of eutrophication (i.e., the natural aging of water bodies), and

4. **Physical Properties and Other Impact Parameters** – Analytical tests designed to measure a varied group of constituents directly impact wastewater treatability (e.g., temperature, color, pH, turbidity, odor).

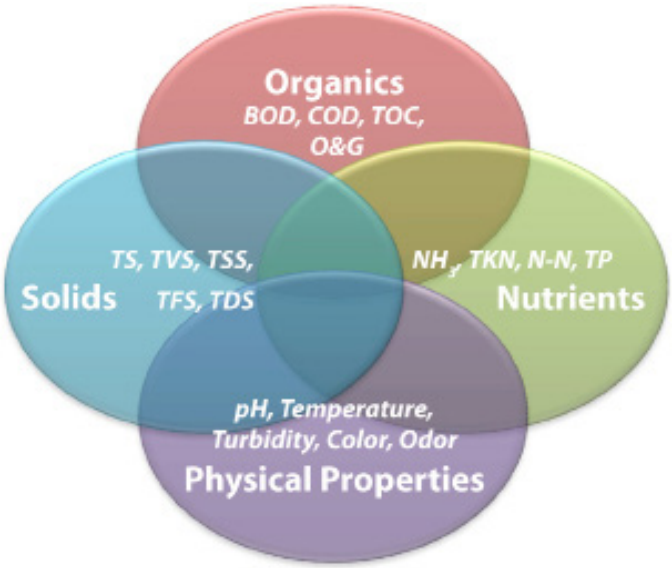


Figure 1. Interaction of wastewater analytical categories and laboratory tests

Although wastewater analytical tests are often separated into categories, it is important to understand that these tests are not independent of each other (Figure 1). In other words, a contaminant identified by one test in one category can also be identified in another test in a separate category. For example, the organics in a wastewater sample represented by BOD will also be represented in the spectrum of solids, either as suspended (TSS) or dissolved (TDS) particulates. For most people a complete understanding of the standard methods required to accurately complete critical wastewater analytical tests is not necessary. However, a fundamental understanding of the theory behind and working knowledge of the basic procedures used for each test, and the answers to commonly asked questions about each test can be a valuable tool for anyone involved in generating, monitoring, treating or discharging process wastewater.

This publication is designed to provide a solid working knowledge of one of the major wastewater analytical test categories: **ORGANICS.**

ORGANICS (BOD, COD, TOC, O&G)

Analytical tests aimed at establishing the concentration (typically in mg/L or ppm) of organic (i.e., carbon-containing) matter have traditionally been used to determine the relative "strength" of a wastewater sample. Today there are four common laboratory tests used to determine the gross amount of organic matter (i.e., concentrations > 1.0 mg/L) in wastewater:

1. **BOD (biochemical oxygen demand)** - *See Page 3*
2. **COD (chemical oxygen demand)** - *See Page 5*
3. **TOC (total organic carbon)** - *See Page 7*
4. **O&G (oil and grease)** - *See Page 8*

Is My Wastewater "High-Strength"?

Wastewater generated by commercial, industrial and institutional facilities is typically referred to as "high-strength" compared to typical household wastewater. Table 1 shows the typical concentrations (*mg/L*) of organics found in untreated domestic wastewater. This table can be used to understand how non-sanitary process wastewater compares to typical domestic wastewater.

Table 1. Typical concentrations of organics in untreated domestic wastewater.

Constituents	Unit	Typical Concentration		
		Low	Medium	High
BOD (biochemical oxygen demand)	mg/L	110	190	350
COD (chemical oxygen demand)	mg/L	250	430	800
TOC (total organic carbon)	mg/L	80	140	260
O&G (oil and grease)	mg/L	50	90	100

Adapted from Metcalf & Eddy, Inc., 2003

Wastewater Analytics Acronyms

Organics:
BOD – biochemical oxygen demand
COD – chemical oxygen demand
TOC – total organic carbon
O&G – oil and grease

Solids:
TS – total solids
TSS – total suspended solids
TDS – total dissolved solids
TVS – total volatile solids
TFS – total fixed solids

Nutrients:
NH_3 – ammonia
TKN – total Kjeldahl nitrogen
N-N – nitrite/nitrate
TP – total phosphorus

1. BOD (BIOCHEMICAL OXYGEN DEMAND)

- BOD is the traditional, most widely used test to establish concentration of organic matter in wastewater samples (i.e., relative strength).
- BOD is based on the principle that if sufficient oxygen is available, aerobic biological decomposition (i.e., stabilization of organic waste) by microorganisms will continue until all waste is consumed.
- The BOD test is also known as "BOD5" since it is based on the accurate measure of DO (dissolved oxygen) at the beginning and end of a five-day period in which the sample is held in dark, incubated conditions (i.e., 20°C or 68°F).
- The change in DO concentration over five days represents the "oxygen demand" for respiration by the aerobic biological microorganisms in the sample.
- The five-day completion window is an inherent disadvantage of the test because wastewater treatment system personnel cannot use it to make real-time operational adjustments.
- An extended UBOD (ultimate BOD) test that measures oxygen consumption after 60 days or more is sometimes required in wastewater permits.

BOD Test Procedures

1. To ensure proper biological activity during the BOD test, a wastewater sample:
   - **a.** Must be free of chlorine. If chlorine is present in the sample, a dechlorination chemical (e.g, sodium sulfite) must be added prior to testing.
   - **b.** Needs to be in the pH range of 6.5 - 7.5 S.U. If the sample is outside this range, then acid or base must be added as needed.
   - **c.** Needs to have an existing adequate microbiological population. If the microbial population is inadequate or unknown, a "seed" solution of bacteria is added along with an essential nutrient buffer solution that ensures bacteria population vitality.
2. Specialized 300 mL BOD bottles designed to allow full filling with no air space and provide an airtight seal are used. The bottles are filled with the sample to be tested or dilution (distilled or deionized) water and various amounts of the wastewater sample are added to reflect different dilutions. At least one bottle is filled only with dilution water as a control or "blank."
3. A DO meter is used to measure the initial dissolved oxygen concentration (mg/L) in each bottle, which should be a least 8.0 mg/L. Each bottle in then placed into a dark incubator at 20°C for five days.
4. After five days (± 3 hours) the DO meter is used again to measure a final dissolved oxygen concentration (mg/L), which ideally will be a reduction of at least 4.0 mg/L.
5. The final DO reading is then subtracted from the initial DO reading and the result is the BOD concentration (mg/L). If the wastewater sample required dilution, the BOD concentration reading is multiplied by the dilution factor.

What is DO (dissolved oxygen)?

- As the name implies, a DO test measures the concentration of oxygen dissolved in a water or wastewater sample.
- DO measurement most often takes place using an electronic meter fitted with a specialized DO probe.
- The concentration of DO in a water sample is significantly influenced by:

  - **Temperature**: As water temperature *increases*, DO *decreases* (i.e., as water gets warmer, it holds less oxygen) (Table 2).

  - **Salinity**: As water salinity *increases*, DO *decreases* (i.e., as water gets saltier, it holds less oxygen).

  - **Atmospheric Pressure**: As pressure *increases*, DO also *increases* (i.e., water holds less oxygen as you increase altitude).

Table 2. Effect of temperature on oxygen saturation at 1 atmospheric pressure (i.e., sea level)*

Temperature		Concentration (mg/L) of DO at Saturation
°C	°F	
0	32	14.6
5	41	13.1
10	50	11.3
15	59	10.1
20	68	9.1**
25	77	8.2

** Adapted from Metcalf & Eddy, 2003*
*** BOD test method temperature*

How can my wastewater have a BOD of 1,500 mg/L when clean water at 68°F can only contain 9.1 mg/L of DO (Table 2)?

The answer is ***serial dilution*** – a procedure that allows for the stepwise reduction in concentration (usually 10-fold) of full strength wastewater in DI (deionized) water, as illustrated in Figure 2 below.

After dilution, the resulting difference between the initial and final DO reading simply has to be multiplied by the dilution factor to determine the final BOD result. For example: 1.0 mL of a full strength wastewater sample added to 9.0 mL of DI water results in a 0.1 dilution of the wastewater. The DO concentration (mg/L) reduction must then be multiplied by 10 to determine the final BOD concentration.

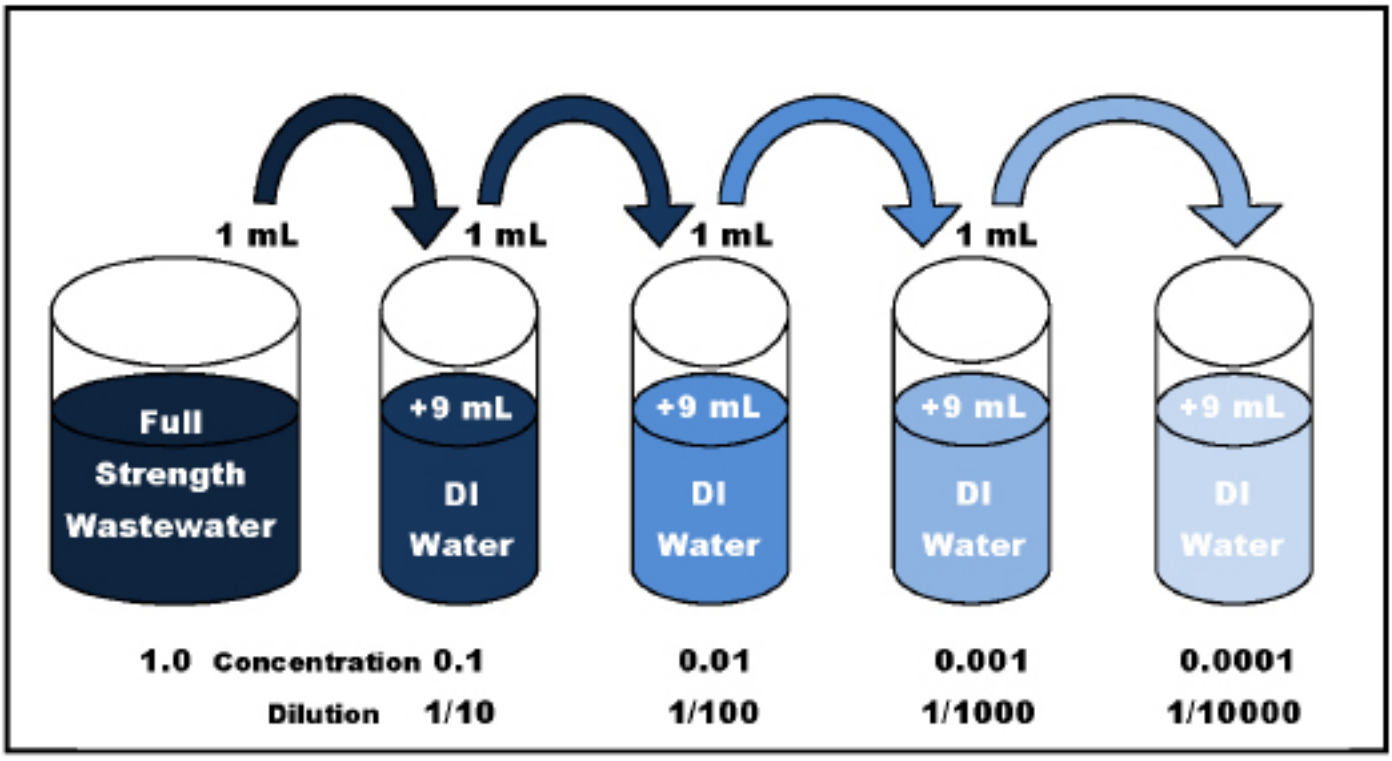


Figure 2. Logarithmic (10-Fold) Serial Dilution of Wastewater

My lab reports lists my results in "ppm." What does that mean?

Most wastewater test results will be reported in either milligrams per liter (mg/L) or parts per million (ppm). The good news is these two units are equal and thus are interchangeable! However, make sure you always note the units reported. Some wastewater parameters (e.g., heavy metals) are often reported in smaller units such as micrograms per liter (µm/L) or parts per billion (ppb).

APHA Standard Methods for BOD Measurement

5210 B.	5-Day BOD Test [1, 2]
5210 C.	Ultimate BOD Test
5210 D.	Respirometric Method

[1] 5210 B. is the only EPA approved BOD method
[2] Most popular method

2. COD (CHEMICAL OXYGEN DEMAND)

- COD is the most popular alternative test to BOD for establishing the concentration of organic matter in wastewater samples.
- The COD test only takes a few hours to complete, giving it a major advantage over the 5-day BOD test. Wastewater treatment system personnel can use COD as an almost real-time operational adjustment parameter.
- COD can test wastewater that is too toxic for the BOD test.
- The COD test should be considered an **independent** measure of the organic matter in a wastewater sample rather than a **substitute** for the BOD test.
- The COD test uses a chemical (potassium dichromate in a 50% sulfuric acid solution) that "oxidizes" both organic (predominate) and inorganic substances in a wastewater sample, which results in a higher COD concentration than BOD concentration for the same wastewater sample since only organic compounds are consumed during BOD testing.
- The most popular current testing method for COD involves using sealed and heated (i.e., closed reflux) low-range (3 - 150 ppm) or high-range (20 - 1500 ppm) pre-prepared vials that change color from orange to green based on the amount of oxidation and that are read using a laboratory colorimeter that measures the relative color change.

COD Test Procedures

1. Prior to completing the COD test, a series of known standards are prepared using KHP (potassium hydrogen phthalate). Most wastewater samples will fall in the high range, so standards of 100, 250, 500 and 1000 mg/L are typically prepared. COD standards can also be purchased.
2. A COD reactor/heating (150°C) block and a colorimeter are turned on so that both instruments are allowed to stabilize.
3. Pre-prepared low-range (3 - 150 ppm) or high-range (20 - 1500 ppm) vials are selected for the COD test based on expected results. Both ranges can be used if expected results are unknown.
4. One vial is marked as a "blank," and three or four vials are marked with known standard levels. Two vials are then marked for the wastewater sample to make a duplicate run. **Note**: If multiple wastewater samples are being run, at least 10% of samples are duplicated.
5. 2 mL of liquid are added to each vial. In the case of the "blank," 2 mL of DI water are added. 2 mL of each standard are added to the corresponding vials. If the wastewater sample is tested at full strength, then 2 mL is added to the corresponding vial. If dilution is required, then serial dilutions are performed and 2 mL of the diluted sample are added to the corresponding vial.
6. Each vial is mixed well and placed into the reactor block for two hours. After two hours, the vials are removed from the block to a cooling rack for about 15 minutes.
7. The colorimeter is set and calibrated per the specific instructions for that unit (i.e., proper wavelength, blank and standards) and each vial is placed in the unit and the COD concentration read.
8. If the sample was diluted, the corresponding multiplication is made.

Selected References

APHA. 2005. Standard methods for the Examination of Water and Wastewater. 21st Edition. American Public Health Association, Washington, D.C.

CSUS. 1993. Operation of Wastewater Treatment Plants. Volume 2. 4th Edition. California State University, Sacramento, CA.

Metcalf & Eddy, Inc. 2003. Wastewater Engineering: Treatment and Reuse. 4th Edition. McGraw-Hill, New York, NY.

Can I use my COD results to predict my BOD?

YES. Although COD should be considered an **independent** test from BOD, and will generate a higher concentration reading than BOD for a particular wastewater sample, it is generally accepted that COD and BOD share an empirical relationship. Extensive observation of the COD and BOD levels on the same wastewater has shown that the COD to BOD ratio of a particular wastewater will remain constant over time.

For example, food processing wastewater will generally have a COD:BOD ratio of ~2:1, while textile wastewater that can contain dyes will often have a much higher COD:BOD ratio of ~5:1.

To establish the COD:BOD ratio for your wastewater, simply have both COD and BOD run on several wastewater samples. Divide the COD concentration by the BOD concentration for each sample and average the results. For example, below is the COD:BOD ratio developed using three wastewater samples from a food processor:

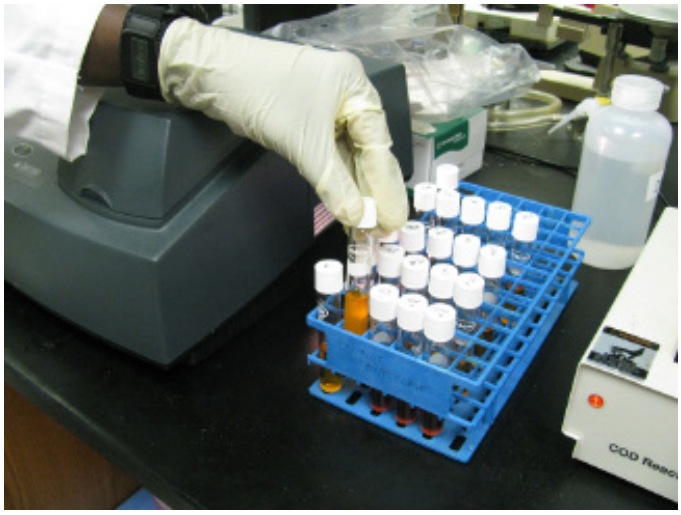
The important point is that once you have established an average COD:BOD ratio for your wastewater stream, then the relatively simple and quick COD test can be used to predict BOD with relative reliability.

Sample 1:	COD = 2,150 mg/L	BOD = 1,100 mg/L
	$\frac{COD}{BOD} = \frac{2,150}{1,100}$	= 1.95
Sample 2:	COD = 1,990 mg/L	BOD = 1,050 mg/L
	$\frac{COD}{BOD} = \frac{1,990}{1,050}$	= 1.89
Sample 3:	COD = 1,850 mg/L	BOD = 997 mg/L
	$\frac{COD}{BOD} = \frac{1,850}{997}$	= 1.86
	(1.95 + 1.89 + 1.86) / 3 = 1.9	
	COD:BOD Ratio = 1.9:1	

Note: *Three samples are used for this example, but 3 samples are too few to calculate an accurate ratio. It is recommended that a minimum of 10 samples be used to develop an initial ratio and that the ratio is consistently updated based on additional periodic sample results.*

APHA Standard Methods for COD Measurement	
5220 B.	Open Reflux Method
5220 C.	Closed Reflux, Titrimetric Method [1]
5220 D.	Closed Reflux, Colorimetric Method [1, 2]

[1] *EPA Approved Method*
[2] *Most popular method*



WARNING! COD…Hazardous Waste

Along with the potassium dichromate in 50% sulfuric acid solution, pre-prepared COD vials also contain silver sulfate as a catalyst and mercuric sulfate to eliminate chloride interference. Thus, COD vials are considered ***hazardous waste*** and must be handled and disposed of in an approved manner.

Do Not Dispose of COD Vial Contents Down the Drain!

Most pre-prepared COD vial vendors will have a return policy for used COD vials so that used vials can be returned to the vendor sealed in the original containers for proper disposal.

3. TOC (TOTAL ORGANIC CARBON)

- The TOC test is gaining popularity because it only takes 5 - 10 minutes to complete.
- Like COD, the TOC test can be used to rapidly estimate BOD concentration once a consistent TOC to BOD ratio is established on a particular wastewater stream (See **"CAN I USE MY COD RESULTS TO PREDICT MY BOD?"** in the COD section).
- At the heart of the TOC test is a **carbon analyzing** instrument that measures the total organic carbon in a wastewater sample.
- Various heat and oxygen, ultraviolet radiation and chemical oxidant-based methods are available to measure TOC that are specific to the carbon analyzing instrument utilized.
- In the TOC test, organic carbon is converted to carbon dioxide (CO_2) and typically measured with an infrared analyzer.

TOC TEST PROCEDURES

TOC test procedures are relatively simple and straightforward, but are specific to the type of carbon-analyzing instrument utilized in the laboratory. Thus, no "typical" TOC procedure exists. The instrument manufacturer's procedures should be followed accurately to achieve the best results.

APHA STANDARD METHODS FOR TOC MEASUREMENT	
5310 B.	High-Temperature Combustion Method
5310 C.	Persulfate-Ultraviolet or Heated-Persultfate Oxidation Method
5310 D.	Wet-Oxidation Method

CONCENTRATION VERSUS LOADING

Concentration (most often reported in wastewater samples as mg/L or ppm) tells how much of a substance (e.g., mg of BOD) is present in a known volume of wastewater (e.g., 1 Liter). However, concentration isn't the whole story since it does not tell how much (i.e., mass or weight) of a substance is going down the drain – commonly referred to as **loading**.

While wastewater pollutant **concentrations** are typically reported as mg/L or ppm, wastewater pollutant **loadings** are typically calculated and reported as pounds per day (lbs/d) and are calculated using the following formula:

Flow	X	Concentration	X	8.34	=	Lbs/day
Million gallons per day (MGD)		mg/L or ppm		Weight (Lbs) of 1 gallon of water (MGD)		

The importance of understanding both concentration and loading can be highlighted by comparing two fictitious industrial plants. Plant A discharges effluent with a BOD level of 250 mg/L, while Plant B's discharge is 1000 mg/L to a city's sewer system. Simply looking at the difference in concentration between the two plants would lead us to believe that Plant B contributes a much higher amount of organics (four times as much) into the sewer. However, we need to take into account that Plant A is a large industrial manufacturer that discharges 1,000,000 gallons of wastewater per day (1.0 MGD), while Plants B is a much smaller facility only discharging 50,000 gallons each day (0.05 MGD). Plugging these values into the **loadings formula** gives the following results:

As this example shows, Plant B's BOD concentration is four times higher than Plant A. **But,** the loadings formula shows that Plant A produces five times more BOD by weight than Plant B.

(Flow) (Concentration) (8.34) = Lbs/day	
Plant A:	(1.0 MGD) (250 mg/L) (8.34) = **2085 Lbs/day**
Plant B:	(0.05 MGD) (1000 mg/L) (8.34) = **417 Lbs/day**

4. O&G (OIL AND GREASE)

- O&G consists of a group of related constituents that are of special concern in wastewater treatment due to their unique physical properties and highly concentrated energy content.
- The term O&G (oil and grease) has become the popular term replacing the term FOG (fat, oil and grease), although both terms refer to the same wastewater constituents.
- O&G constituents in wastewater can come from plants and animals (e.g, lard, butter, vegetable oils and fats) as well as petroleum sources (e.g., kerosene, lubricating oils).
- O&G are generally hydrophobic (i.e., "water-hating") and thus have low solubility in wastewater, resulting in relatively low biodegradability by microorganisms.
- O&G becomes more soluble (i.e., more easily dissolved) in wastewater at high temperatures and will form emulsions (i.e., oil-water mixtures) that will often separate back out of wastewater as temperatures become cooler; thus, O&G are notorious for causing sewer collection system problems (e.g., blockages, pump failures).
- **WARNING! Since O&G adheres to plastic, only GLASS sample collection containers can be used to collect O&G samples!**

O&G Test Procedures

1. A clean flask is dried, cooled and weighed.
2. A 1L wastewater sample is acidified (typically using hydrochloric or sulfuric acid) to a pH $\leq$ 2.
3. The acidified wastewater sample is then transferred to a 2L separatory funnel.
4. 30 mL of the extraction chemical (e.g., *n-Hexane*) are then added to the funnel and the funnel is shaken vigorously for two minutes.
5. The wastewater/extraction chemical layers are allowed to separate in the funnel (the lighter water layer will be on the top and heavier extraction chemical layer will be on the bottom). The bottom layer of extraction chemical is drained into the flask prepared in Step 1.
6. Steps 4/5 are repeated twice more to extract O&G.
7. The contents of the flask (i.e., the extraction chemical containing O&G) are then heated so that the extraction chemical is distilled into another container.
8. The flask (containing the extracted O&G) is reweighed. The original weight of the flask is subtracted and the total O&G weight in mg is calculated. The results provide the O&G concentration in mg/L.

APHA Standard Methods for O&G Measurement	
5520 B.	Liquid-Liquid, Partition-Gravimetric Method [1]
5520 C.	Partition-Infrared Method
5520 D.	Soxhlet Extraction Method
[1] *EPA Approved Method*	

Other Publications in the
Understanding Laboratory Wastewater Tests Series:

- **II: Solids** (TS, TSS, TDS, TVS, TFS)
- **III: Nutrients** (NH_3, TKN, N-N, TP)
- **IV: Physical Properties** (pH, Color, Turbidity, Temperature, Odor)
- **V. Wastewater Sampling**

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Section 5
Solids

Analysis of Solids in Wastewater

1

Why do I care about Solids?

- Major function of STP is reduction organic loading of wastewater for safe discharge to receiving stream
  - Biological treatment: monitored through BOD and COD (Demands)
  - Sedimentation: monitored through total suspended solids (TSS)

2

Wastewater Solids Removal

- Most suspended solids are organic
- Oxygen Demand
- Serve as refuge for harmful bacteria
- Unsightly appearance

3

Solids Testing

- Why should I care about solids tests?
  - Assessment of compliance
  - Control of Biological and Physical treatment process
    - Poor data = Poor decisions

4

Definitions

- Total Solids
  - All residue left after drying
- Dissolved Solids
  - The portion of TS which pass through a 2.0um filter
- Suspended Solids
  - The portion retained on the 2.0um filter
- Fixed Solids
  - The portion of TS, DS, TSS which remains after ignition at 550°C
- Volatile Solids
  - The portion which burned away at 550°C

5

Standard Methods 2540B-G

- Sources of error
  - Sampling
  - Sub sampling
  - Measuring
  - Filter/Bowl preparation
  - Filter/Bowl handling
- Remedies
  - MIX WELL
  - Measure quickly

6

Standard Methods 2540B-G

- Temperatures
  - Each Method has a specified drying Temp.
  - Minimize opening desiccator
  - Weigh quickly, dry samples attract moisture
- Rinse Water
  - Type III, distilled or deionized water

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Standard Methods 2540B-G

- Dry samples to constant weight
  - Entails multiple cycles of drying, cooling, and weighing for each sample
- Make sure samples are cooled to ambient temperature
- Remove excess water from filter before placing them on weighing dishes or pans
  - Excess water will cause filters to adhere to a weighing dish or pan

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Standard Methods 2540B-G

- Sampling
  - Glass or plastic containers, watch for particles adhering to container walls, especially plastic.
- Begin test ASAP (preferred)
- Sampling Holding
  - Preserve at <6°C
  - Hold <24 hours preferred
    - 2 hour window when bringing samples to room temp
  - Max holding time: 7 days

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Method Choice

- Methods B-F
  - Potable, surface, saline, domestic and industrial wastewater up to 20,000mg/L
- Method G
  - Solid and Semisolid samples >2%
  - Biosolids, per 40 CFR 503

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Total Solids 2540-B

- Temperature 103-105°C
- Calculations based on sample volume

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Dissolved Solids 2540-C

- Temperature 180°C
- Non-regulatory at 103-105°C

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Total Suspended Solids 2540-D

- Temperature 103-105°C
- Interferences
  - Limit sample size to ≤ 200mg of residue
    - Excessive filter residue may form a water entrapping crust
    - Increase filter diameter or decrease sample volume with prolonged filtration
- Glass fiber filter 22-125mm diameter.
  - Whatman 934AH 1.5um
  - Gelman A/E 1.0um
  - Millipore AP40
  - E-D Scientific Grade 161

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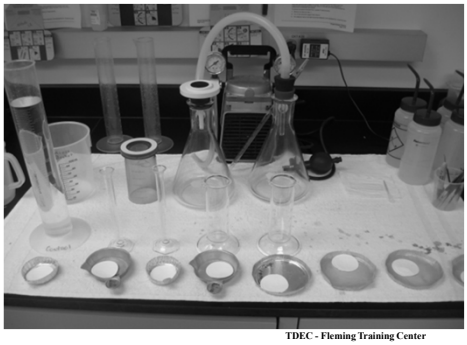
TSS SM2540 D – 2015 Dried at 103-105°C



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TSS SM2540 D – 2015 Dried at 103-105°C

- Filter preparation:
  - Handle all filters carefully, with the wrinkled side up
  - Transfer filters using forceps to grip the residue-free edge
  - Weigh only the filters, not the support pants or dishes, unless a Gooch crucible is used
  - Insert filter with wrinkled side up

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TSS SM2540 D – 2015 Dried at 103-105°C

- Filter preparation:
  - Pre-wash approved glass fiber filter by rinsing three times with 20mL of deionized (DI) water
  - Dry in weighing pans at 103 – 105° C for 1 hour and cool in desiccator
  - Adequate filter preparation is demonstrated by negligible weight loss or gain for method blanks
  - Choose a sample volume to yield between 2.5 - 200 mg dried residue
    - Filtration >10 min, increase filter size or decrease sample size

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TSS SM2540 D – 2015 Dried at 103-105°C

- Sample Analysis:
  - Stir or mix sample and use a pipet or graduated cylinder to transfer sample
  - Assemble filtering apparatus and filter
  - Wet filter with small amount of distilled water to seal before applying the sample
  - Sample must be well mixed before applying to filter

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TSS SM2540 D – 2015 Dried at 103-105°C

- Sample Analysis – continued:
  - Filter sample
    - Transfer sample with applied vacuum
  - Wash filter and apparatus three times with 10 mL of DI water
  - Transfer filter into support pans
  - Dry in oven at 103-105 ° C for at least1 hr and cool in desiccator
  - Repeat drying, cooling, weighing cycle until weight change is <0.5 mg

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TSS SM2540 D – 2015 Dried at 103-105°C

- Fats, Oil, and Grease
  - May interfere due to difficulty in drying to a constant weight in time range.
- Relative percent difference (RPD) of duplicates should not exceed 10%
- Weigh to a constant weight, within 4% or 0.0005g, whichever is less with at least a second drying/cooling/weighing step.

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Total Suspended Solids Analysis
Procedure to Omit Re-drying/Re-cooling/Re-weighing

How to acquire acceptable results for the total suspended solids comparability data:

- The maximum holding time for a total suspended solids sample prior to analysis is 7 days if stored at temperatures of 6°C and below (not 0°C). (*40CFR part 136, Table II*)
- EPA recommends that 4-7 different samples, in duplicate, be collected and analyzed for this procedure in order to prove that the step for "reheating, recooling, and reweighing" is unnecessary. "Different" could mean samples collected 4-7 consecutive days or 4-7 samples run in one day. These 4-7 samples are dried ***overnight*** at 103-105°C.
- The next morning, the filters are removed from the oven, allowed to cool in the desiccator and weighed.
- The samples are then returned to the drying oven for one hour, recooled and reweighed.
- The resulting data should be examined to determine if the difference between the overnight values and the redried values are less than 4% or 0.5 mg, whichever is less. If so, the redrying step may be omitted for a normal set of samples.
- This procedure excludes atypical samples. (i.e. high fat, oil and grease samples).
- The operator may choose not to perform this study and continue to follow the procedure for redrying/recooling/reweighing as stated the method (Std Methods, 2540 D).

The study should be re-evaluated at least once per year or whenever a change in sample characteristics occurs and kept on file at the treatment plant.

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TSS SM2540 D – 2015 Dried at 103-105°C

- Uses
  - Influent and effluent regulatory tests
  - Mixed Liquor Suspended Solids MLSS
  - Return Activated Sludge RAS
  - Clarifier Core Suspended Solids
  - Stream samples
  - Some digester solids tests

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TSS SM2540 D – 2015 Dried at 103-105°C

Sample	Common Range, mg/L
Influent	150 – 400
Primary Effluent	60 – 150
Secondary Effluent	10 – 60
Tertiary Effluent	0.0 – 3.0
MLSS	1,000 – 5,000
RAS	2,000 – 12,000

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TSS Calculations

- TSS in mg/L is equal to the amount of residue retained on the filter in mg per liter of sample
- Formula: TSS mg/L = $\frac{(A - B) \times 1{,}000{,}000}{\text{sample volume mL}}$
- Where: A = wt. of filter + dried residue (g)
  B = initial wt. of filter (g)
  1,000,000 = conversion factor
  (1 g = 1000 mg & 1L = 1000 mL)

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TSS Calculations

- Wt. of filter + residue = A = 1.0215 g
- Wt. of filter = B = 1.0160 g
- Sample volume = 200
- Mg/L = $\frac{(1.0215 - 1.0160)(1{,}000{,}000)}{200}$
  $= \frac{(0.0055)(1{,}000{,}000)}{200}$
  $= \frac{5500}{200}$
  = 27.5 mg/L

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TSS Calculations

- **ppm** is an abbreviation of **parts per million**. **ppm** is a value that represents the part of a whole number in units of 1/1000000.

  1 ppm = 1mg/l = 1ug /ml = 1000ug/L

- **Parts** can be any measure, pints, gallons, or a drop of water (grape juice, antifreeze, etc.). The size of the sample doesn't matter. It's the RATIO of the tested parts to the total number of parts that's important.

- Now think about a milligram. It is 1/1000th of a gram, making it 1/1,000,000th of a kilogram. **Put another way, a liter of water weighs 1,000,000 milligrams.** One million milligrams… see where this is going?

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TSS Calculations

- Let's say you're given 4000 mg/L of suspended solids and you want to determine the % of solids. This is measurement is the same as 4000 ppm (parts per million).
- Divide the ppm by 1,000,000 then multiply by 100 to get %.

$$\frac{4{,}000}{1{,}000{,}000} = \frac{4}{1000}$$

$$= 0.004 \times 100$$

$$= 0.4\%$$

- Alternatively, divide the % value by 100 and multiply by 1,000,000 to get **ppm**.

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TSS – Portable and Inline

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TSS SM2540 D – 2015 Dried at 103-105°C

- DOC
- LRB
- LFB
- Dup
- ICAL
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency

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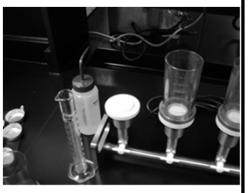
TSS SM2540 D – 2015 Dried at 103-105°C

- Demonstration of Capability (DOC)
  - Run a laboratory-fortified blank (LFB) at least four times and compare to the limits listed in the method
  - Real people language: each operator running this test need to analyze 4 samples of Total Suspended Solids Standards
  - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
  - Recommend backup analyst do this once a year.



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TSS SM2540 D – 2015 Dried at 103-105°C

- Blanks
  - Filter 100 mL of deionized/distilled water through a pre-washed/pre-dried/pre-weighed filter with each batch of 20 or fewer samples
    - Run on a 5% basis, one for every 20 samples
    - Should be less than 2.5 mg/L
- Laboratory Fortified Blank
  - Real people language: analyze a TSS standard that can be prepared from recipe (next slide) or bought premade
    - Run on a 5% basis, one for every 20 samples

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TSS SM2540 D – 2015 Dried at 103-105°C

- To prepare TSS check samples from dry reference material:
  - Dry the reference material* in the desiccator
  - On an analytical balance, weigh 0.1000 gram of the dry powder, put it in a 1000 mL volumetric flask, bring it to the mark with distilled or deionized water and shake well until well suspended.
  - Measure 100 mL and process as usual for environmental samples.
  - A difference of 10 mg should be obtained.
  - Calculation: $\frac{(A - B)\ (1000)}{\text{Vol. used}} = \frac{(10\ mg)\ (1000)}{100\ mL} = 100\ mg/L$

*Example of material available from Fisher

- Celite 545 Filter Aid (Powder), Fisher Chemical, 500 gram bottle – Cat#C212-500

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TSS SM2540 D – 2015 Dried at 103-105°C

- Standard Methods 2020.B.2.a
  - Check instrument balances daily
- Standard Methods 9020.B.4.b
  - Service balances annually or more often as conditions change or problems occur
    - Check balances routinely, preferably daily before use, with at least two working weights that bracket the normal usage range (e.g. ANSI/ASTM Class 1 or NIST Class S accompanied by appropriate certificate) for accuracy, precision and linearity.
    - Record results along with date and technician's initials
    - Recertify reference weights as specified in the certificate of calibration or at least every 5 years.

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TSS SM2540 D – 2015 Dried at 103-105°C

- Initial Calibration
  - Check balances daily (day of) with at least 2 working weights that bracket the normal usage range and record results on bench sheet or separate log book
- Duplicates
  - Run on a 5% basis, one for every 20 samples
    - Relative percent difference (RPD) should not exceed 10%
  - For reporting purposes, average sample and duplicate.

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TSS SM2540 D – 2015 Dried at 103-105°C

- Create and maintain control charts for Laboratory Fortified blanks.
  - 2021 update – all labs need to be maintaining control charts
  - follow QC Acceptance Criteria below.
    - Blanks < 2.5 mg/L
    - LFB ± 15%
    - RPD < 10%

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SS SM2540 F – 2015 Settleable Solids

- Important process control test for rates and volume of sedimentation
- Report as mL/L
  - Minimum Detection Limit 0.1mL/L
  - Adjust for water layers if present
- Basin control: Imhoff cone
  - Sample volume: 1 L
  - Solids settle 45 min
  - Gently stir sides
  - Solids settle another 15 minutes
  - Read after a total of 60 min

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SS SM2540 F – 2015 Settleable Solids

Sample	Common Range, mL/L
Influent	8 – 20
Primary Effluent	0.1 – 3
Secondary Effluent	0.1 – 0.5

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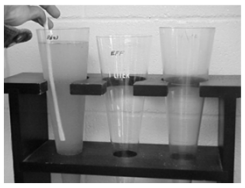
SS SM2540 F – 2015 Settleable Solids

- Dup
- Corrective Action
- Batch Size
- QC Frequency



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SS SM2540 F – 2015 Settleable Solids

- Duplicates
  - For example, pour up 1000 mL of effluent into Imhoff then pour up another 1000 mL of effluent in another Imhoff. Wait 45 min, stir, wait 15 min, read. Figure RPD for both samples.
  - Calculate RPD, (less than 20%)
  - Run on a 5% basis (see batch size for more information).
  - For reporting purposes, average samp and duplicate.

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SS SM2540 F – 2015 Settleable Solids

- QC Acceptance Criteria below.
  - RPD < 20%
  - Reporting Limit = lowest graduation mark on Imhoff cone

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Total Solids 2540-G

- For solid and semisolid materials
  - Required or Biosolids per 40 CFR 503
- Calculations based of wet and dry weight.

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Fixed and Volatile 2540-E

- An additional step of the Total, Suspended, or Dissolved Solids test.
- Sample is ignited at 550°C for 1 hr.
- Fixed Solids or Ash remains
- Volatile Solids were burned away.

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Balance Operation

- Key to quality solids tests
- Care of Balance
  - Vibration, heat, sunlight
  - Dust, dirt, moisture
- Calibration
  - Outside contractor
  - In house, "S" class weights

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Solids – Review Questions

Laboratory portion:

1. What is one of the biggest sources of error associated with this test?

2. Should samples be brought to room temperature before analysis?

3. Always analyze samples in duplicate. True or False?

4. What type of filter did we use in lab?

5. The filter must be placed with which side facing up?

6. During filter preparation, how much water is applied to the filter while the vacuum is on?

7. Briefly describe what happens in the drying-cooling-weighing cycles that the filters must go through.

8. The above drying-cooling-weighing cycle is repeated until what?

9. Where should the prepared filters be stored until use?

10. When transferring or moving the filter, why is it important to only use tweezers (and not touch the filter with your fingers)?

11. When you are beginning the sample analysis, after the filtering apparatus is assembled, and the vacuum is turned on, why do you add a small amount of DI water to the filter?

12. After you have added your homogenous sample to the filter, how much DI water is required for the rinse?

13. How long do continue to apply suction after you have finished rinsing your filter and the sides of the filtering apparatus with DI water?

14. The filters with sample residue also go through the weighing-cooling-drying cycle until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less. True or False?

Classroom portion:

15. Using Standard Methods, write out the definitions for the following words:

    a. Total suspended solids =

    b. Total dissolved solids =

    c. Fixed solids =

    d. Volatile solids =

    e. Settleable solids =

16. You want to choose a sample volume that will yield between _____ and _____ mg dried residue.

17. If complete filtration takes more than 10 minutes, what should you do?

18. List some examples of why it is so important to have good solids removal in the wastewater treatment process?

2540 SOLIDS*

2540 A. Introduction

Solids refer to matter suspended or dissolved in potable, surface, and saline waters, as well as domestic and industrial wastewaters. Solids may adversely affect water or effluent quality in a number of ways. Waters with high dissolved solids generally are of inferior palatability and may induce an unfavorable physiological reaction in the transient consumer, so a 500 mg dissolved solids/L limit is desirable for drinking waters. Highly mineralized waters also are unsuitable for many industrial applications. Waters high in suspended solids may be aesthetically unsatisfactory for bathing and other purposes.

Solids analyses are important for controlling water and wastewater treatment processes and assessing compliance with regulatory requirements.

1. Terminology

Fixed solids—the total, suspended, or dissolved solids remaining in a sample after ignition for a specified time at a specified temperature. [Determinations of fixed and volatile solids do not distinguish precisely between inorganic and organic matter because some inorganic compounds can be lost during ignition. Organic matter can be better characterized via total organic carbon (Section 5310), BOD (Section 5210), and COD (Section 5220) methods.]

Settleable solids—the material in a sample that settles out of suspension within a defined period. This may include floating material, depending on the technique used (e.g., 2540F.3*b*).

Total dissolved solids (TDS)—the portion of total solids in a water sample that passes through a filter with a nominal pore size of 2.0 μm (or smaller) under specified conditions.

Total solids—the material left in a sample vessel after evaporation and subsequent oven drying at a defined temperature. *Total solids* includes both total suspended and total dissolved solids, which are physically separated via filtration. Whether a solids particle is filtered into the "suspended" or "dissolved" portion principally depends on a filter's thickness, area, pore size, porosity, and type of holder, as well as the physical nature, particle size, and amount of solids being filtered.

Total suspended solids (TSS)—the portion of total solids in an aqueous sample retained on the filter. NOTE: Some clays and colloids will pass through a 2-μm filter.

Volatile solids—the total, suspended, or dissolved solids lost from a sample after ignition for a specified time at a specified temperature. [Determinations of fixed and volatile solids do not distinguish precisely between inorganic and organic matter because some inorganic compounds can be lost during ignition. Organic matter can be better characterized via total organic carbon (Section 5310), BOD (Section 5210), and COD (Section 5220) methods.]

2. Sources of Error and Variability

Sampling, subsampling, and measuring two- or three-phase samples may introduce significant errors. Maintain sample homogeneity during transfer, and handle carefully to ensure sample integrity. If part of a sample adheres to the container, consider this in evaluating and reporting results. During drying, some samples form a crust that prevents water evaporation; special handling is required to deal with this issue (see below).

Take special care with viscous samples, which might entrain air during mixing and can be difficult to transfer in accurate volumes. Also, take special care with samples with high dissolved solids levels, which are difficult to rinse completely during filtration.

Since total dissolved solids (TDS) and total suspended solids (TSS) are different fractions of the same sample being analyzed, the apparatus and rinsing techniques used may adversely affect TSS and/or TDS results. To avoid this, take care to keep the TDS fraction from migrating into the unrinsed edge of the filter beneath the funnel and being retained as "TSS" weight. If this is suspected, then investigate the filter apparatus seal's effectiveness and/or consider additional rinses.

If using a pipet to measure and transfer sample, place the pipet tip in the center (depth and width) of the well-mixed container. If using a magnetic stir plate and stir bar, set the speed to shear larger particles and blend sample to a more uniform particle size; then pipet sample from mid-depth midway between the container's wall and vortex. Centrifugal force may separate particles of different sizes and densities, resulting in poor precision when sample-withdrawal point varies. Avoid using a magnetic stirrer with samples containing magnetic particles. When using a graduated cylinder, transfer samples to the cylinder immediately after shaking or stirring to avoid any settling of the sample's solids.

Drying temperature, heating duration, and sample matrix can affect weight loss due to volatilization of organic matter, mechanically occluded water, water of crystallization, and gases from heat-induced chemical decomposition. They also can affect weight gains due to oxidation. When liquid samples are put directly into any oven heated above boiling temperature, they may splatter and lose some of the parameter of interest. Therefore, samples can be evaporated to dryness or near dryness at a temperature below boiling using a steam bath, hot plate, or oven before being dried to constant weight per method specifications.

Pay close attention to all samples during post-drying desiccation. Open desiccator as few times as possible to minimize the entry of moist air. If samples are stronger desiccants than the desiccants used in the desiccator, they may take on water. In general, weigh samples as soon as possible after removal from desiccator to minimize water absorption from the atmosphere.

Residues dried at 103–105°C may retain both water of crystallization and some mechanically occluded water. Occluded water, organic matter, and carbonate loss [as carbon dioxide (CO_2)] may slow drying time significantly. Residues dried at 180 ± 2°C should lose mechanically occluded water, but may also lose organic matter and some salts.

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* Approved by Standard Methods Committee, 2015.
Joint Task Group: Michael F. Delaney (chair), Osman M. Aly, David Berwanger, Marianne R. Guzman, Scott A. Jacobs, Keith A. Kibbey, Kim J. Laird, Patty R. Lee, Meaza G. Mariam-Woods, Devon A. Morgan, Lisa M. Ramirez, William R. Ray, Elizabeth J. Robinson, David A. Smith, Zachary B. Smith, J. Mitchell Spears, Mark M. Ultis, Stan K. Van Wagenen, Mark Wyzalek, Meifang Zhou.

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Dry samples to constant weight if possible; this entails multiple cycles of drying, cooling, and weighing for each sample. Results for residues with high oil or grease content may be questionable because such samples are difficult to dry to constant weight in a reasonable timeframe. Any samples that fail to reach a constant weight must be qualified by indicating the number of drying cycles and the final weight change.

When weighing dried samples, be alert to weight changes due to air exposure and/or sample degradation. Make sure samples are cooled to ambient temperature before weighing. Also, remove excess water from glass-fiber filters before placing them on weighing dishes or pans. Excess water will cause filters to adhere to a weighing dish or pan during drying, leading to tearing or loss of material when filter is lifted for weighing, thereby skewing results. This is especially critical for the low-level TSS method. See each method for further discussion of specific interferences.

Use medium-quality reagent water (or better) to rinse filters and filtered solids and to clean labware. Special samples may require a higher-quality water (see Section 1080).

When an analysis deviates from the stated procedures for any reason, record the variations and present them with the results so the reasons for not following the method are documented.

3. Sample Handling and Preservation

Use borosilicate glass, plastic, or fluoropolymer [e.g., polytetrafluoroethylene (PTFE), Teflon®] bottles, so long as suspended solids in sample do not adhere to container walls. Begin analysis as soon as possible, but in no case hold samples >7 d. Settleable solids must be analyzed within 48 h. Between collection and analysis, refrigerate (do NOT freeze) sample at <6°C to minimize microbiological decomposition of solids. Bring samples to room temperature before beginning analysis.

4. Selection of Method

Methods 2540B–F are suitable for determining solids in potable, surface, and saline waters, as well as domestic and industrial wastewaters. The analytical range for 2450B–D is 2.5 to 200 mg/L for a 1000-mL sample, but may be extended by using a small sample volume for analysis. Method 2540G is suitable for determining solids in soils and sediments, as well as solid and semisolid materials produced during water and wastewater treatment.

5. Quality Control (QC)

The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

Analyze ≥5% of all samples in duplicate or at least one duplicate sample with each batch of ≤20 samples. The laboratory may plot duplicate determinations on a control chart for evaluation. Typically, the relative percent difference (RPD) of duplicates should not exceed 10%, but RPDs may vary considerably due to sample matrix and concentration.

Analyze one method blank (MB) per batch of 20 samples for each method except settleable solids (2540F). Blank analysis includes all container- and filter-preparation steps and procedures except sample addition. If any MB measurements are at or above the reporting level, take immediate corrective action (see Section 1020B.5). This may include re-analyzing the sample batch.

Include one laboratory-fortified blank (LFB) per batch of 20 samples for all tests except settleable solids (2540F) and total, fixed, and volatile solids in solid and semisolid samples (2540G). Plot the percent recoveries on a control chart for laboratory evaluation. Laboratories may purchase known standards or prepare in-house working controls for use.

6. Bibliography

THERIAULT, E.J. & H.H. WAGENHALS. 1923. Studies of representative sewage plants. *Pub. Health Bull.* No. 132.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes; Pub. 600/4-79-020, rev. Mar. 1983. Environmental Monitoring and Support Lab., Cincinnati, Ohio.

2540 B. Total Solids Dried at 103–105°C

1. General Discussion

a. Principle: Evaporate a well-mixed sample in a pre-weighed dish and dry it to constant weight in a 103–105°C oven. The increase compared to the empty pre-weighed dish weight represents total solids. This result may not be the actual weight of dissolved and suspended solids in wastewater samples.

To meet the LFB requirement (2540A.5), a total solids standard can be created as follows: Dry, grind, and sieve a soil for use as a working control. This control may or may not be mixed with other reagents (e.g., Celite 545 or Sigmacell Cellulose Type 20) and may have water added according to the laboratory's procedures.

b. Interferences: Highly mineralized water with a significant concentration of calcium, magnesium, chloride, and/or sulfate may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing. Exclude large, floating particles or submerged agglomerates of nonhomogeneous materials from sample if they are not desired in the final result.

Optionally, disperse visible floating oil and grease with a blender or homogenizer before withdrawing a sample portion for analysis. If oil and grease sticks to blender sides and blades, thus potentially affecting sample composition, note this in the lab report.

Residues dried at 103–105°C may retain both water of crystallization and some mechanically occluded water. There will be CO_2 loss when bicarbonate converts to carbonate during drying. Usually, very little organic matter will volatilize. It may take a long time to attain constant weight because occluded-water removal is marginal at this temperature.

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Because excessive residue in the dish may form a water-trapping crust, limit sample to ≤200 mg residue.

2. Apparatus

a. Sample dishes: Dishes of approximately 90-mm dia and 100-mL capacity made of one of the following materials:

1) Porcelain,
2) Platinum,
3) High-silica glass (may react with highly alkaline samples),* or
4) Other material shown to be resistant to the sample matrix and weight stable at the required evaporation and drying temperatures. Aluminum is NOT appropriate for this purpose.†

*b. Wide-bore pipets,** Class B in glass, mechanical or electronic.

c. Graduated cylinders, Class A.

d. Steam bath (optional) for sample evaporation.

e. Hot plate or block (optional) for sample evaporation. Must be capable of maintaining a temperature <100°C without boiling samples.

f. Pre-drying oven (optional) for sample evaporation that operates at temperatures approximately 2°C below boiling to prevent splattering.

g. Drying oven that operates at 103–105°C.

h. Muffle furnace that operates at 550 ± 50°C.

i. Desiccator, which includes either a desiccant whose color changes in response to moisture concentration or an instrument for measuring moisture (e.g., a hygrometer).

j. Analytical balance, capable of weighing to 0.1 mg.

k. Magnetic stirrer with TFE stirring bar (optional).

l. Blender or homogenizer (optional).

m. Low-form beaker, Class B or better.

3. Procedure

a. Preparation of evaporating dish: If measuring volatile solids, then ignite clean evaporating dish at 550 ± 50°C for ≥15 min in a muffle furnace. If only measuring total solids, then heat clean dish at 103–105°C for ≥1 h. Cool dishes to ambient temperature and weigh. Store weighed dishes in desiccator or oven until needed.

b. Selection of sample size: Choose sample volume to yield between 2.5 and 200 mg dried residue. If necessary, successive sample portions may be added to the same dish after evaporation. Identify any sample that yields residue <2.5 mg or >200 mg, and report the value as described in Sections 1020 and 2020.

c. Sample analysis: Stir or mix sample and quantitatively transfer with a pipet or graduated cylinder to a pre-weighed dish. Evaporate samples to dryness on a steam bath, hot plate, or block, or in a drying oven. Make sure evaporation temperature is ≥2°C below boiling to prevent splattering. Dry evaporated sample for ≥1 h in a 103– 105°C oven. Cool dish in desiccator to ambient temperature, and weigh. Repeat cycle (drying for ≥1 h, cooling, desiccating, and weighing) until weight change is <0.5 mg.

4. Calculation

$$\text{mg total solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = final weight of dried residue + dish, mg, and
B = weight of dish, mg.

5. Precision

Single-laboratory duplicate analyses of 41 samples of water and wastewater were made with a standard deviation of differences of 6.0 mg/L.

6. Bibliography

Symons, G.E. & B. Morey. 1941. The effect of drying time on the determination of solids in sewage and sewage sludges. *Sewage Works J.* 13:936.

* Vycor, product of Corning Glass Works, Corning, NY, or equivalent.
† StableWeigh, Environmental Express, Charleston, SC, or equivalent.

2540 C. Total Dissolved Solids Dried at 180°C

1. General Discussion

a. Principle: Filter a well-mixed sample through a standard glass-fiber filter. Then, transfer the filtrate to a pre-weighed dish, evaporate it to dryness, and dry it to constant weight in an oven at 180 ± 2°C. The increase compared to the empty pre-weighed dish weight represents TDS.

These results may differ from the theoretical value for solids calculated from chemical analysis of sample. Approximation methods for correlating chemical and physical analyses are available.[1] The filtrate collected from the TSS determination (2540D) may be used to determine TDS.

To meet the LFB requirement (2540A.5), analysts can create a TDS standard as follows: Dry NaCl at 103–105°C for ≥1 h, weigh 50 mg, and dilute to 1 L with reagent water. This results in a 50-mg/L TDS standard.

b. Interferences: See 2540A.2. Highly mineralized waters with a considerable calcium, chloride, magnesium, and/or sulfate content may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing. Samples with high bicarbonate concentrations require careful, possibly prolonged drying at 180 ± 2°C to ensure that bicarbonate completely converts to carbonate.

Residues dried at 180 ± 2°C will lose almost all mechanically occluded water, but some water of crystallization may remain,

especially if sulfates are present. Organic matter may volatilize and be lost, but not completely removed. CO_2 loss occurs when bicarbonates convert to carbonates, and carbonates may be decomposed partially to oxides or basic salts. Some chloride and nitrate salts may be lost. In general, evaporating and drying water samples at 180 ± 2°C yields TDS values closer to those obtained by adding individually determined mineral species than the values obtained when drying at 103–105°C.

Because excessive residue in the dish may form a water-trapping crust, limit sample to ≤200 mg residue.

2. Apparatus

Apparatus listed in 2540B.2*a–l*, and in addition:

a. Glass-fiber filter disks, 22 to 125 mm dia, ≤2-μm nominal pore size without organic binder.*

b. Filtration apparatus: One of the following, suitable for the filter selected:

1) Membrane filter funnel—various capacities, to fit selected filter.
2) Gooch crucible—25- to 40-mL capacity, with Gooch crucible adapter.
3) Filtration apparatus with reservoir and coarse (40- to 60-μm) fritted disk as filter support.†

c. Suction flask with sufficient capacity for sample size selected.

d. Oven that operates at 180 ± 2°C.

3. Procedure

a. Preparation of glass-fiber filter disk: Insert disk with wrinkled side up into filtration apparatus. Apply vacuum and wash disk with three successive volumes of ≥20 mL reagent-grade water. Continue suction to remove all traces of water. If using commercially prepared glass-fiber filter disks, the washing step may be skipped if the manufacturer certifies that the filters meet this method's requirements.

b. Preparation of evaporating dish: If measuring volatile solids, ignite cleaned evaporating dish at 550 ± 50°C for ≥15 min in a muffle furnace. If only measuring TDS, then heat cleaned dish to 180 ± 2°C for ≥1 h in an oven. Cool dishes to ambient temperature and weigh. Store in desiccator or oven until needed.

* Whatman grade 934AH; Gelman type A/E; Millipore type AP40; Ahlstrom grade 161; Environmental Express Pro Weigh; or other products that give demonstrably equivalent results.
† Paul/Gelman No. 4201 magnetic, or equivalent.

c. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If filtration will take >10 min to complete, then increase filter size or decrease sample volume. Identify any sample that yields residue <2.5 mg or >200 mg, and report the value as described in Sections 1020 and 2020.

d. Sample analysis: Stir or mix sample and use a pipet or graduated cylinder to transfer a measured volume onto a glass-fiber filter with applied vacuum. Wash the entire exposed surface of filter with three successive volumes of ≥10 mL reagent-grade water. Allow complete drainage between washings, and continue suction until all traces of water are removed. Transfer total filtrate (with washings) to a pre-weighed evaporating dish and evaporate to dryness on a steam bath, hot plate, or block, or in a drying oven. If necessary, add successive portions to the same dish after evaporation. Dry evaporated sample for ≥1 h in an oven at 180 ± 2°C, cool in a desiccator to ambient temperature, and weigh. Repeat cycle (drying, cooling, desiccating, and weighing) until weight change is <0.5 mg.

If determining volatile solids, follow procedure in 2540E.

4. Calculation

$$\text{mg total dissolved solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = final weight of dried residue + dish, mg, and
B = weight of dish, mg.

5. Precision

Single-laboratory analyses of 77 samples of a known of 293 mg/L were made with a standard deviation of differences of 21.20 mg/L.

6. Reference

1. Sokoloff, V.P. 1933. Water of crystallization in total solids of water analysis. *Ind. Eng. Chem.,* Anal. Ed. 5:336.

7. Bibliography

Howard, C.S. 1933. Determination of total dissolved solids in water analysis. *Ind. Eng. Chem.,* Anal. Ed. 5:4.

U.S. Geological Survey. 1974. Methods for Collection and Analysis of Water Samples for Dissolved Minerals and Gases. Techniques of Water-Resources Investigations; Book 5, Chap. A1. Washington, D.C.

2540 D. Total Suspended Solids Dried at 103–105°C

1. General Discussion

a. Principle: Filter a well-mixed sample through a pre-weighed standard glass-fiber filter, and then dry the filter and the residue retained on it to a constant weight in a 103–105°C oven. The increase in filter weight represents TSS. To estimate an unknown sample matrix TSS concentration, calculate the difference between TDS and total solids.

To meet the LFB requirement (2540A.5), a TSS standard can be created as follows: weigh 100 mg of Sigmacell® Cellulose Type 20

or Celite 545, dilute to 1 L with reagent water, and stir for ≥15 min to mix well. This results in a 100 mg/L TSS standard.

b. Interferences: See 2540A.2 and B.1*b*. Unless representative of source, exclude large floating particles or submerged agglomerates of nonhomogeneous materials from sample. Limit sample size so it yields ≤200 mg residue, because excessive filter residue may form a water-entrapping crust.

If suspended solids clog the filter, prolonging filtration, consider increasing filter diameter or decreasing sample volume.

When samples contain high concentrations of dissolved solids, thoroughly wash all exposed filter surfaces to ensure that dissolved material is removed (see also 2540A.2). Prolonged filtration due to filter clogging may capture more colloidal materials, resulting in artificially high results.

Handle all filters carefully, with the wrinkled side up. Transfer filters using forceps to grip the residue-free edge. Weigh only the filters, not the support pans or dishes, unless a Gooch crucible is used.

2. Apparatus

Apparatus listed in 2540B.2 and C.2, except for evaporating dishes, steam bath, hot plate or block, and 180 ± 2°C drying oven. In addition:

a. Weighing dishes or pans, constructed of aluminum or other inert material, to hold filters.

b. Forceps.

3. Procedure

a. Preparation of glass-fiber filter disk: Insert filter with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive portions of ≥20 mL reagent-grade water. Continue suction to remove all traces of water. Remove filter from filtration apparatus and transfer to an inert weighing dish. If a Gooch crucible is used, remove crucible and filter combination. Dry in a 103–105°C oven for ≥1 h. Cool in desiccator to ambient temperature and weigh. Store filters (on inert dishes or pans) in desiccator or 103–105°C oven until needed. Adequate filter preparation is demonstrated by negligible weight loss or gain for method blanks.

If measuring volatile solids, ignite at 550 ± 50°C for ≥15 min in a muffle furnace. Cool to room temperature before proceeding. (Alternatively, the ignition step may be performed after washing and drying at 103–105°C for ≥1 h, but before weighing.)

If using commercially prepared glass-fiber filters, the ignition, washing, and weighing steps may be eliminated if the manufacturer certifies that the prepared filters meet this method's requirements. Verify filters using method blanks. Filters are verified if the measured weight differs from the manufacturer's weight by less than ±0.5 mg.

b. Selection of filter and sample sizes: Choose sample volumes to yield between 2.5 and 200 mg dried residue. If filtration takes >10 min to complete, increase filter size or decrease sample volume. Identify any sample that yields residue <2.5 mg or >200 mg, and report the value as described in Sections 1020 and 2020.

c. Sample analysis: Stir or mix sample and use a pipet or graduated cylinder to transfer a measured volume onto a glass-fiber filter with applied vacuum. Wash filter with at least three successive volumes of ≥10 mL reagent-grade water. Allow complete drainage between washings, and continue suction until all traces of water are removed. When filtering samples with high dissolved solids concentrations, additional washings may be required to ensure that dissolved material is removed from all exposed filter surfaces.

Using forceps, carefully remove filter from filtration apparatus and transfer to an inert weighing dish or pan as a support. If using a Gooch crucible, remove crucible and filter combination from the crucible adapter. Dry for ≥1 h in a 103–105°C oven, cool in a desiccator to ambient temperature, and weigh. Repeat the cycle (drying, cooling, desiccating, and weighing) until the weight change is <0.5 mg. If determining volatile solids, treat the residue according to 2540E.

4. Calculation

$$\text{mg total suspended solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = final weight of filter + dried residue, mg, and
B = weight of filter, mg.

5. Precision

The standard deviation was 5.2 mg/L (coefficient of variation 33%) at 15 mg/L, 24 mg/L (10%) at 242 mg/L, and 13 mg/L (0.76%) at 1707 mg/L in studies by two analysts of four sets of 10 determinations each.

Single-laboratory duplicate analyses of 50 samples of water and wastewater were made with a standard deviation of differences of 2.8 mg/L.

6. Bibliography

Degen, J. & F.E. Nussberger. 1956. Notes on the determination of suspended solids. *Sewage Ind. Wastes* 28:237.

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Trees, C.C. 1978. Analytical analysis of the effect of dissolved solids on suspended solids determination. *J. Water Pollut. Control Fed.* 50:2370.

2540 E. Fixed and Volatile Solids Ignited at 550°C

1. General Discussion

a. Principle: The residue from 2540B, C, or D is ignited to constant weight at 550 ± 50°C. The remaining solids are *fixed* total, dissolved, or suspended solids, while those lost to ignition are *volatile* total, dissolved, or suspended solids. This determination is useful in controlling wastewater treatment plant operations because it offers a rough approximation of the amount of organic matter present in wastewater solids, activated sludge, and industrial wastes.

b. Interferences: There may be negative errors in volatile solids calculations if volatile matter was lost during the drying step of 2540D.3*c*. If a sample contains far more fixed than volatile solids, then volatile solids determined by this procedure may be subject to considerable error; try estimating volatile solids via another test [e.g., total organic carbon (Section 5310)].

Highly alkaline residues may react with any silica in sample or crucibles.

Usually, about 15 min of ignition is required for up to 200 mg residue. However, longer ignition times may be needed if drying heavier residues or more than one sample.

To avoid breakage due to drastic temperature change, dishes and filters may be partially cooled in any oven, steam bath, or hot plate listed in 2540 or else in ambient air until most of the heat dissipates. Transfer to a desiccator for final cooling in a dry atmosphere. Do not overload desiccator. Weigh dish or filter as soon as it has cooled to ambient temperature.

2. Apparatus

See 2540B.2, C.2, and D.2.

3. Procedure

Bring a muffle furnace to 550 ± 50°C. Insert a dish or filter containing residue produced by 2540B, C, or D into furnace. Ignite for at least 15 min at 550 ± 50°C, cool in a desiccator to ambient temperature, and weigh. Repeat cycle (igniting, cooling, desiccating, and weighing) until weight is constant or weight change is <0.5 mg.

4. Calculation

$$\text{mg volatile solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

$$\text{mg fixed solids/L} = \frac{(B - C) \times 1000}{\text{sample volume, mL}}$$

where:

A = final weight of residue + dish or filter before ignition, mg,
B = final weight of residue + dish or filter after ignition, mg, and
C = weight of dish or filter, mg.

5. Precision

The standard deviation was 11 mg/L at 170 mg/L volatile total solids in studies by three laboratories on four samples and 10 replicates. Bias data on actual samples cannot be obtained.

2540 F. Settleable Solids

1. General Discussion

a. Principle: The settleable solids in surface and saline waters, and in domestic and industrial wastes, may be determined and reported based on either volume (mL/L) or weight (mg/L).

b. Interferences: The volumetric procedure (Imhoff cone) generally has a practical lower measurement limit between 0.1 and 1.0 mL/L, depending on sample composition. The settled-sample-level reading may be affected by sample foaming, sample separation, or pockets of liquid between large settled particles. When measuring heavy sludges whose measured settleable solids may be >100 mL/L, analysts may use a 1000-mL, Class A graduated cylinder instead of a cone. (Imhoff cones typically either lack graduation marks at that level or have marks scaled per 100 mL; a graduated cylinder may offer better resolution.)

If the settled matter contains pockets of liquid between large settled particles, then estimate the liquid volume, subtract it from settled-solids volume, and note in the lab report. If settleable and floating solids separate, do not estimate the floating material as settleable matter. If biological or chemical floc is present, the gravimetric method (2540F.3*b*) is preferred.

2. Apparatus

a. Volumetric:
1) Imhoff cone.
2) Graduated cylinder, Class A.
3) Stir-rod, made of glass or other inert material.

b. Gravimetric:
1) Apparatus listed in 2540D.2.
2) Glass vessel, minimum 9-cm-dia. A standpipe, graduated cylinder, or other vessel may be used as long as it satisfies the 9-cm-dia requirement and can hold the required sample volume.

3. Procedure

a. Volumetric: Fill an Imhoff cone or graduated cylinder to the 1-L mark with a well-mixed sample. Settle for 45 min, then gently agitate sample near the cone sides with a rod or by

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spinning. Allow sample to settle for another 15 min, and record volume of settleable solids in the cone as mL/L.

b. Gravimetric:

1) Determine TSS as in 2540D.

2) Stir or mix sample and quantitatively transfer at least 1 L of well-mixed sample into a 9-mm-dia glass vessel to fill vessel to ≥20 cm deep. Use a vessel with a larger diameter and larger sample volume if necessary. Let stand quiescent for 1 h. Then without disturbing the settled or floating material, carefully siphon 250 mL from center of container at a point halfway between the liquid surface and the surface of the settled material. Determine TSS (mg/L) of supernatant (2540D); these are the nonsettleable solids.

4. Calculation

$$\text{mg settleable solids/L} = \text{mg TSS/L} - \text{mg nonsettleable solids/L}$$

5. Precision and Bias

Precision and bias data are currently unavailable.

6. Bibliography

FISCHER, A.J. & G.E. SYMONS. 1944. The determination of settleable sewage solids by weight. *Water Sewage Works* 91:37.

2540 G. Total, Fixed, and Volatile Solids in Solid and Semisolid Samples

1. General Discussion

a. Applicability: This method can be used to determine total solids and its fixed and volatile fractions in such solid and semi-solid samples as river and lake sediments; sludges separated from water and wastewater treatment processes; and sludge cakes from vacuum filtration, centrifugation, or other sludge-dewatering processes.

b. Interferences: The determination of both total and volatile solids in these materials is subject to negative error due to loss of ammonium carbonate and volatile organic matter during drying. Although this is also true for wastewater, the effect tends to be more pronounced with sediments—especially sludges and sludge cakes. The mass of organic matter recovered from sludge and sediment requires a longer ignition time than that specified for wastewaters, effluents, or polluted waters. Carefully observe specified ignition time and temperature to control losses of volatile inorganic salts if these are a problem. Take all weight measurements quickly because wet samples tend to lose weight via evaporation. After drying or ignition, residues often are hygroscopic, rapidly absorbing moisture from the air. Highly alkaline residues may react with silica in samples or silica-containing crucibles.

2. Apparatus

All of the apparatus listed in 2540B.2 except the magnetic stirrer and pipets. A balance capable of weighing to 10 mg may be used.

3. Procedure

a. Total solids:

1) Preparation of evaporating dish—If measuring volatile solids, ignite a clean evaporating dish at 550 ± 50°C for ≥15 min in a muffle furnace. If only measuring total solids, heat dish for ≥1 h in a 103–105°C oven. Cool in desiccator to ambient temperature and weigh. Store in desiccator or 103–105°C oven until needed.

2) Sample analysis

a) Fluid samples—If sample contains enough moisture to flow readily, then stir or shake to homogenize, transfer approximately 25–50 g to a prepared evaporating dish, and weigh (dish plus sample). Evaporate to dryness on a water bath, on a hot plate or block, or in a drying oven, then dry the evaporated sample at 103–105°C for ≥1 h, cool to ambient temperature in a desiccator, and weigh. Repeat cycle (drying, cooling, desiccating, and weighing) until the weight change is <50 mg.

b) Solid samples—If sample consists of discrete pieces of solid material (e.g., dewatered sludge), then take care to obtain a representative sample whose particle size will not impede drying. One of the following manual-processing options may be used:

- take cores from each piece with a No. 7 cork borer and mix crumbled cores together well, or
- pulverize entire sample coarsely on a clean surface by hand (covered with clean gloves) or using a clean mortar and pestle.

Manually process samples as quickly as possible to prevent moisture loss. Processing via mechanical grinding is not recommended because moisture levels could drop during processing.

Transfer approximately 25–50 g to a prepared evaporating dish and weigh. Then, place in a 103–105°C oven for ≥1 h, cool to ambient temperature in a desiccator, and weigh. Repeat cycle (drying, cooling, desiccating, and weighing) until weight change is <50 mg.

b. Fixed and volatile solids: Transfer dried residue from 2540G.3*a*2)a) or b) to a cool muffle furnace, heat furnace to 550 ± 50°C, and then allow ignition to occur for ≥1 h. If residue contains large amounts of organic matter, consider first igniting it over a gas burner under an exhaust hood with enough air to lessen losses due to reducing conditions and to avoid odors in the laboratory. Alternatively, use a muffle furnace in a hood and open the door periodically to ensure air flow.

Cool in desiccator to ambient temperature, and weigh. Repeat cycle (igniting, cooling, desiccating, and weighing) until the weight change is <50 mg.

4. Calculation

$$\%\ \text{total solids} = \frac{(A - B) \times 100}{C - B}$$

$$\%\ \text{volatile solids} = \frac{(A - D) \times 100}{A - B}$$

https://doi.org/10.2105/SMWW.2882.030

$$\% \text{ fixed solids} = \frac{(D - B) \times 100}{A - B}$$

where:

A = final weight of dried residue + dish, mg,
B = weight of dish, mg,
C = weight of wet sample + dish, mg, and
D = final weight of residue + dish after ignition, mg.

5. Precision and Bias

Precision and bias data are currently unavailable.

6. Bibliography

Goodman, B.L. 1964. Processing thickened sludge with chemical conditioners. Pages 78 *et seq. in* Sludge Concentration, Filtration and Incineration. Univ. Michigan Continued Education Ser. No. 113, Ann Arbor.

Gratteau, J.C. & R.I. Dick. 1968. Activated sludge suspended solids determinations. *Water Sewage Works* 115:468.

https://doi.org/10.2105/SMWW.2882.030

Solids, Non-filterable Suspended, Total and Volatile

USEPA Gravimetric Method[1, 2] — Method 8158 and Method 8164

Scope and application: For water and wastewater.

[1] USEPA accepted.
[2] Adapted from *Standard Methods for the Examination of Water and Wastewater*, Section 2540B.

Test preparation

Before starting

Analyze samples as soon as possible for best results.
For the best accuracy, use as much filtered sample as possible (step 11). Samples that contain more than 15 mg of solids will clog the fiber filter disc. Adjust the correct volume of the water sample to get accurate results. Some completed tests will show if adjustments are necessary.
Always use tweezers with fiber filter discs. Moisture from fingers can add moisture to the fiber filter disc and cause a weighing error.
For Volatile Non-filterable Solids (or Residue) (VNR) preheat the muffle furnace below the recommended temperature. Do not put the watch glass directly in a 550 °C (1022 °F) muffle furnace because it can break. Put the watch glass in a 100 °C (212 °F) preheated muffle furnace and then increase the temperature to 550 °C (1022 °F) for 15 minutes.
The Total Non-filterable Solids (or Residue) (TNR) are the same as the Total Suspended Solids (TSS).

Items to collect

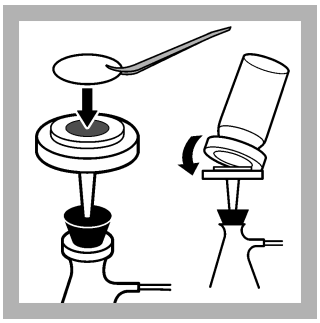
Description	Quantity
Analytical balance	1
Cylinder, graduated, 100 mL	1
Desiccator with desiccant	1
Drying oven	1
Filter flask	1
Filter holder	1
Filter, 47-mm	1
Furnace, muffle	1
Rubber policeman for 3/16 in. rod (user-supplied)	1
Tongs	1
Tweezers	1
Watch glass	1
Watch glass	1
Water, deionized	varies

Refer to Consumables and replacement items on page 5 for order information.

Sample collection and storage

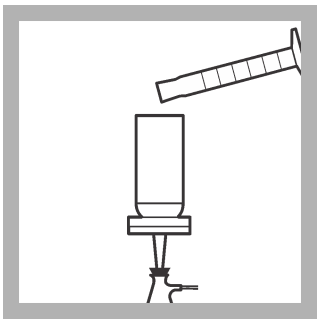
- Collect samples in clean glass or plastic bottles.
- To preserve samples for later analysis, keep the samples at or below 6 °C (43 °F) for up to 7 days.
- Let the sample temperature increase to room temperature before analysis.

Test procedure—Total Non-filterable Solids, Method 8158

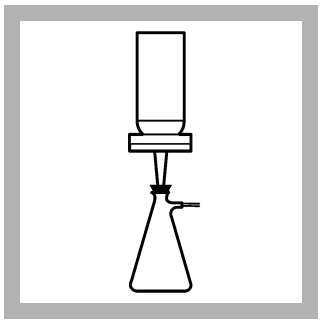


1. Use tweezers to put a fiber filter disc in the filter holder.

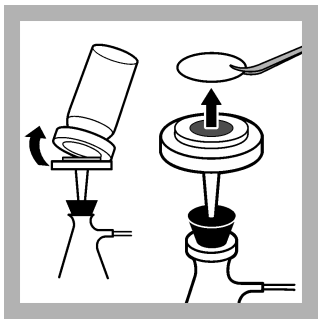
Put the filter holder assembly in the filtering flask.



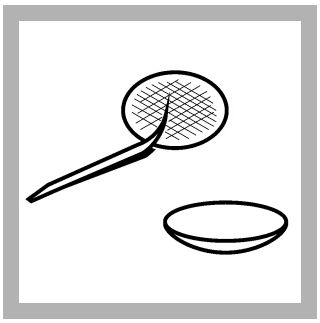
2. Use a graduated cylinder to add 100 mL of deionized water to the filtering flask.



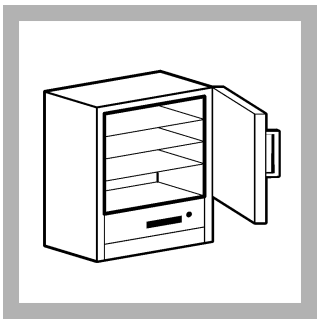
3. Apply vacuum to the flask until all of the water is pulled through the filter.



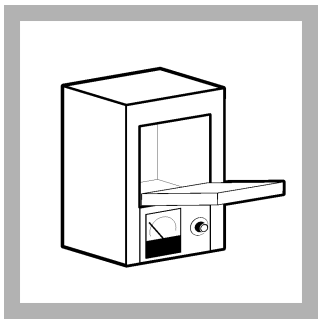
4. Slowly release the vacuum from the filtering system. Remove the fiber filter disc from the filter holder.



5. Put the fiber filter disc in a watch glass.



6. Put the watch glass with the fiber filter disc in a preheated drying oven at 103–105 °C (217–221 °F) for 1 hour.

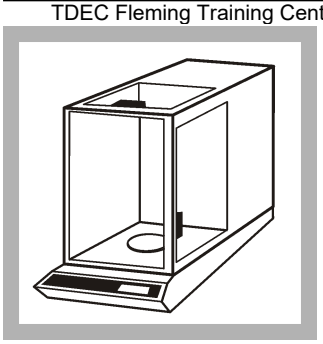


7. If Volatile Non-filterable Solids are also measured, use tongs to put the watch glass with the fiber filter disc into a preheated muffle furnace at 550 °C (1022 °F) for 15 minutes. Discard this step if Volatile Non-filterable Solids are not measured.

Note: *Do not put the watch glass directly in a 550 °C (1022 °F) muffle furnace because it can break. Put the watch glass in a 100 °C (212 °F) preheated muffle furnace and then increase the temperature to 550 °C (1022 °F) for 15 minutes.*

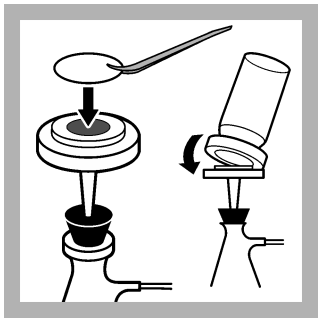
8. Use metal tongs to remove the watch glass with the fiber filter disc from the drying oven or muffle furnace and put in a desiccator. Immediately cover the desiccator. Do not seal the desiccator until the watch glass temperature has decreased a little, because pressure from the hot air inside can push the cover off.

Let the fiber filter disc and watch glass temperature decrease to room temperature.

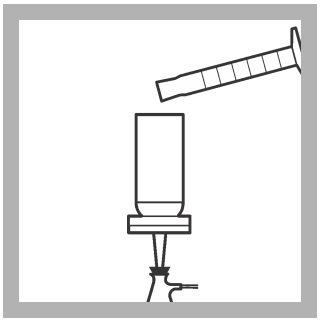


9. Remove the watch glass with the fiber filter disc from the desiccator and put it adjacent to the analytical balance.

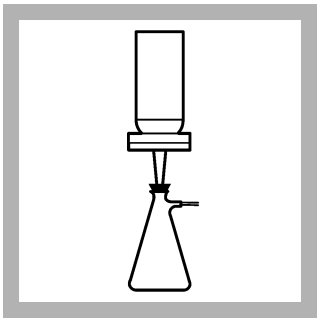
Use tweezers to remove the fiber filter disc from the watch glass. Weigh the fiber filter disc to the nearest 0.1 mg (0.0001 g). Record this mg value as B.



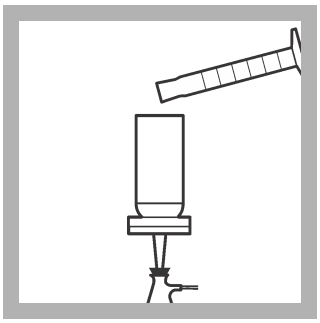
10. Put the fiber filter disc in the filter holder/filtering system again. Use deionized water to bond the fiber filter disc to the filter holder.



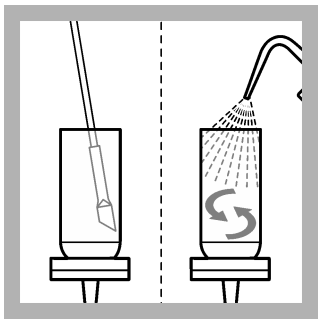
11. Use a graduated cylinder to add 100 mL (or more, if the solids content is low) of well-mixed, representative water sample.



12. Apply vacuum to the flask until all of the water is pulled through the filter.



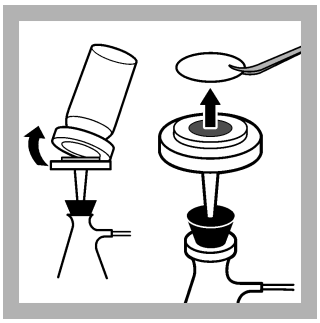
13. Add three different 10-mL aliquots of deionized water. Wait until each aliquot is pulled through the filter before the next one is added.



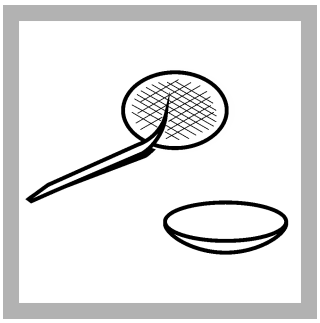
14. Move all of the remaining material that stays on the sides or bottom lip of the filter holder on the filter.

Use a rubber policeman on the end of a stirring rod as a scraper to remove the solids.

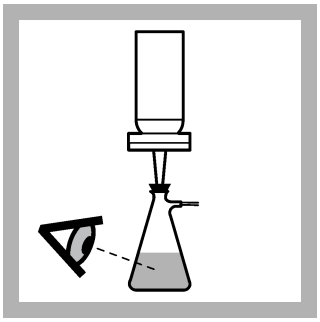
Use small amounts of deionized water to pull the solids down on the fiber filter disc.



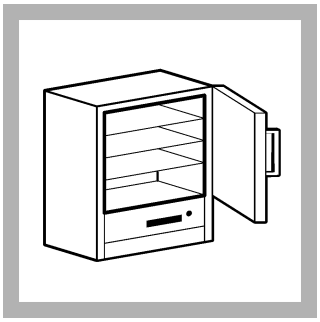
15. Slowly release the vacuum from the filtering system. Remove the fiber filter disc from the filter holder.



16. Put the fiber filter disc in a watch glass.



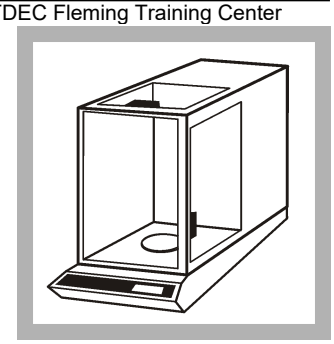
17. Examine the filtrate (filtered water in flask) to make sure that the solids are caught on the fiber filter disc.



18. Put the watch glass with the fiber filter disc in a preheated drying oven at 103–105 °C (217–221 °F) for 1 hour.

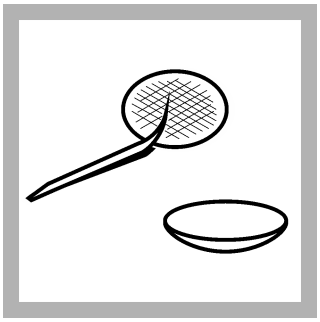
19. Use metal tongs to remove the watch glass with the fiber filter disc from the drying oven or muffle furnace and put in a desiccator. Immediately cover the desiccator. Do not seal the desiccator until the watch glass temperature has decreased a little, because pressure from the hot air inside can push the cover off.

Let the fiber filter disc and watch glass temperature decrease to room temperature.

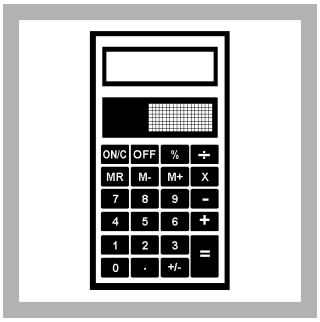


20. Remove the watch glass with the fiber filter disc from the desiccator and put it adjacent to the analytical balance.

Use tweezers to remove the fiber filter disc from the watch glass. Weigh the fiber filter disc to the nearest 0.1 mg (0.0001 g). Record this mg value as A.



21. Put the fiber filter disc back on the watch glass to measure Volatile Nonfilterable Residue. If not, discard the disc. If Volatile Nonfilterable Residue is measured, make sure to not lose any of the suspended matter on the disc.



22. Calculate the test results:

(A – B) ÷ L sample = mg/L Total Non-filterable Residue (TNR)

Where:
A = Weight (mg)[1] of fiber filter disc with solids

B = Weight (mg) of empty fiber filter disc

Example:

A = 95.5 mg

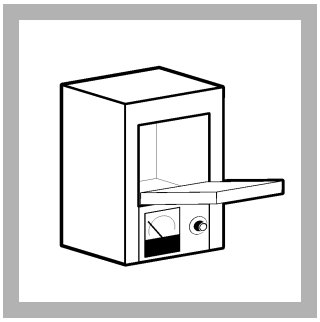
B = 81.5 mg

Volume of sample = 0.100 L

(95.5 mg – 81.5 mg) ÷ 0.100 = 140 mg/L TNR

[1] Weight in mg = grams × 1000

Test procedure—Volatile Non-filterable Solids, Method 8164

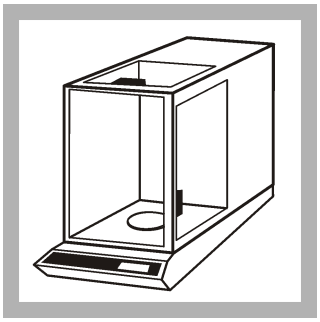


1. Put the watch glass and filter disc from the Total Non-filterable Solids procedure (step 21) in a preheated muffle furnace at 550 °C (1022 °F) for 15 minutes.

Note: *Do not put the watch glass directly in a 550 °C (1022 °F) muffle furnace because it can break. Put the watch glass in a 100 °C (212 °F) preheated muffle furnace and then increase the temperature to 550 °C (1022 °F) for 15 minutes.*

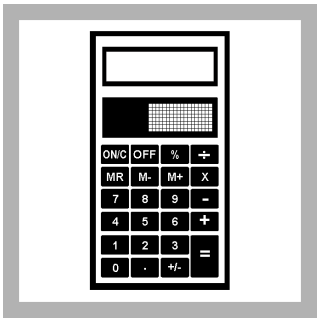
2. Use metal tongs to remove the watch glass with the fiber filter disc from the drying oven or muffle furnace and put in a desiccator. Immediately cover the desiccator. Do not seal the desiccator until the watch glass temperature has decreased a little, because pressure from the hot air inside can push the cover off.

Let the fiber filter disc and watch glass temperature decrease to room temperature.



3. Remove the watch glass with the fiber filter disc from the desiccator and put it adjacent to the analytical balance.

Use tweezers to remove the fiber filter disc from the watch glass. Weigh the fiber filter disc to the nearest 0.1 mg (0.0001 g). Record this mg value as C.



4. Calculate the test results:

(A – C) ÷ sample volume in L = mg/L Volatile Non-filterable Residue (VNR)

Where:
A = Weight (mg) of fiber filter disc with solids (step 20)

C = Weight (mg) of fiber filter disc with solids

Example:

A = 95.5 mg

C = 91.2 mg

Volume of sample = 0.100 L

(95.5 mg – 91.2 mg) ÷ 0.100 = 43 mg/L VNR

Summary of method

A glass fiber filter disc is used as a filter in a filtering flask. Deionized water is pulled with vacuum through the filter. The fiber filter disc is dried to a constant weight in an oven at 102-105 °C (217–221 °F) to determine the weight of the empty disc. A well-mixed filtered sample is dried in the same fiber filter disc to a constant weight in an oven at 102-105 °C (217–221 °F). The weight difference between the empty disc and the disc with the remaining materials shows the Total Non-filterable Solids. To measure the Volatile Non-filterable Solids, the fiber filter disc is put in a muffle furnace at 550 °C (1022 °F) to remove all of the volatile material. The weight difference between the disc and the disc with remaining materials shows the Volatile Non-filterable Solids.

Consumables and replacement items

Required reagents and apparatus

Description	Quantity/test	Unit	Item no.
Aspirator, vacuum pump	1	each	213100
Balance, analytical, 80 g x 0.1 mg, 100–240 VAC	1	each	2936701
Bottle, wash, 500 mL	1	each	62011
Cylinder, graduated, 100 mL	1	each	50842
Desiccant, indicating Drierite	1	each	2088701
Desiccator, without stopcock	1	each	1428500
Desiccator plate, ceramic	1	each	1428400
Filter discs, glass fiber, 47 mm	1	100/pkg	253000
Filter holder, 47-mm, magnetic base	1	each	1352900

Required reagents and apparatus (continued)

Description	Quantity/test	Unit	Item no.
Flask, filtering, glass, 1000 mL	1	each	54653
Furnace, muffle 240 VAC, 50/60 Hz	1	each	1429624
Furnace, muffle, 120 VAC, 50/60 Hz	1	each	1429600
Oven, laboratory, 240 VAC/50 Hz	1	each	1428902
Oven, laboratory, 120 VAC/60 Hz	1	each	1428900
Stopper, rubber, one-hole, number 8	1	6/pkg	211908
Tongs	1	each	56900
Tubing, rubber, 7.9 mm x 2.4 mm	varies	12 ft	56019
Tweezers, plastic	1	each	1428200
Watch glass, 100 mm	1	each	57870
Water, deionized	varies	4 L	27256

Optional reagents and apparatus

Description	Unit	Item No.
Ammonium Hydroxide, 58%	500 mL	10649
Sampling bottle with cap, low density polyethylene, 500-mL	12/pkg	2087079
Brush	each	68700
Pump, vacuum, hand-operated	each	1428300
Pump, vacuum, 1.2 CFM, 220 VAC	each	2824801
Pump, vacuum, 1.2 CFM 115 V	each	2824800
Stirring rod, glass	3/pkg	177001

Total Suspended Solids in Water Samples

Environmental waters may contain a variety of solid or dissolved impurities. In quantifying levels of these impurities, suspended solids is the term used to describe particles in the water column. Practically, they are defined as particles large enough to not pass through the filter used to separate them from the water. Smaller particles, along with ionic species, are referred to as dissolved solids. In considering waters for human consumption or other uses, it is important to know the concentrations of both suspended and dissolved solids. The most common pollutant in the world is "dirt" in the form of TSS.

First, let's consider some implications of total suspended solids (TSS).

-High concentrations of suspended solids may settle out onto a streambed or lake bottom and cover aquatic organisms, eggs, or macro-invertebrate larva. This coating can prevent sufficient oxygen transfer and result in the death of buried organisms.

-High concentrations of suspended solids decrease the effectiveness of drinking water disinfection agents by allowing microorganisms to "hide" from disinfectants within solid aggregates. This is one of the reasons the TSS, or turbidity, is removed in drinking water treatment facilities.

-Many organic and inorganic pollutants sorb to soils, so that the pollutant concentrations on the solids are high. Thus, sorbed pollutants (and solids) can be transported elsewhere in river and lake systems, resulting in the exposure of organisms to pollutants away from the point source.

Lab Procedures:

Preparing your filters

1) Rinse three filters with 20-30 mL DI to remove any solids that may remain from the manufacturing process. Place the filters in separate, labeled aluminum weight pans, dry them in a 104ºC oven for 30 minutes, place them (filter and pan) in a desiccator, and obtain a constant weight by repeating the oven and desiccation steps.

Obtaining the TSS measurement

2) Filter 100.mL of sample through each pre-weighed filter.

3) Place each paper in its aluminum weight pan in the 104ºC oven for 1 hour. Cool the filter and pan in a desiccator and obtain a constant weight by repeating the drying and desiccation steps. (This step will be completed after your normal lab meeting time.)

Calculation:

$$\text{TSS mg/L} = \frac{(\text{average weight from step 3 in g} - \text{average inital weight from step 1 in g})(1000\text{mg/L})}{\text{sample volume in L}}$$

Hints for success:

-Always, ALWAYS completely mix your sample before removing any solution/ suspension. The soil/sediment particles will settle and bias your results if you do not completely mix the sample every time you remove an aliquot.

-Perform all measurements in triplicate.

-Carefully clean all containers and pre-wash all filters with DI water prior to use. As the procedures notes, you must heat filters to the maximum temperature that you will use experimentally, before filtering. Also as noted in the procedures, you must obtain a constant weight (generally within 0.5 mg) before you end each experiment. (Fingerprints and dust weigh enough to significantly affect your results.)

-Your balances have been calibrated, but for best results you should still use the same balance for every measurement. Even if the calibration on a balance is slightly off, the change in weight will probably be accurate.

Section 6
Turbidity

TURBIDITY

Wastewater Laboratory

Fleming Training Center | TN Department of Environment & Conservation

1

1

Turbidity

- A measure of the clarity of water
- It is an expression of the optical property that causes light to be scatter and absorbed in water
- It is caused by particulate, such as silt, clay, organic matter, algae and other microorganisms
- Amount of light absorbed is proportional to the concentration of particulate in the sample

TDEC - Fleming Training Center 2

2

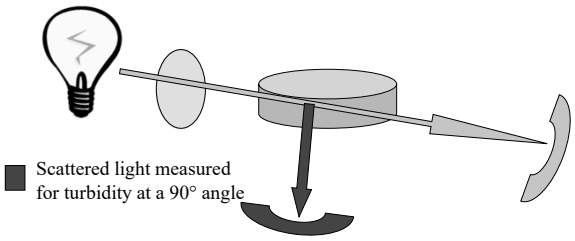
Turbidity

- Caused by suspended and colloidal matter in water
- It is expression of light that is scattered or absorbed through a sample
- Does not indicate the number or size of particles in a sample
- General indicator of overall effluent water quality and a good process control test for operator

TDEC - Fleming Training Center 3

3

Turbidimeters



Scattered light measured for turbidity at a 90° angle

Light source from tungsten lamp passing through three precisely aligned lenses, the light is focused in a narrow, collimated beam

4

4

Importance

- Supports growth of microorganisms
- Reduces effectiveness of chlorination
- Interferes with chemical and microbiological analysis
- Is unacceptable for aesthetic reasons
- Is related to coagulation and filtration
- Is unacceptable for most industrial water

TDEC - Fleming Training Center 5

5

Measuring

- Use an instrument for measuring and comparing turbidity of liquids
- Nephelometers are instruments which measure turbidity by comparing the amount of light in a sample to the amount of light scattered by a standard
- The amount of scattered light is measured and converted to units of turbidity or NTU's (Nephelometric Turbidity Units)

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6

Instruments

Hach 2100N Laboratory
Benchtop Turbidimeter

Hach 2100Q
Portable Turbidimeter

7

7

Measuring Notes

- Always cap the sample cell to prevent spillage into instrument
- Close the sample compartment lid during measurement
- Do not leave sample cell in the cell compartment for extended periods of time
- Leave the instrument on 24 hours a day if instrument is used regularly

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8

Measuring Notes

- Always use clean, scratch free sample cells and caps
- Always use silicone oil
- Measuring samples immediately to prevent changes in sample characteristics
- Remove air bubbles in sample cells
- Discard sample cells with scratches

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9

Calibrations

- Use Gelex Secondary Turbidity Standards for periodic checks
- Primary Stable Cal Standards
  - Formazin Solution Primary Standards and Procedure for making solutions
- Record keeping requirements and recommendations for operators
- Calibrate at least quarterly

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10

Turbidity – Review Questions

1. What is the definition of turbidity?

2. What causes turbidity?

3. What are colloidal solids?

4. List some examples of activities that contribute to turbidity in receiving waters.

5. Turbidity measurements can detect the exact number and size of particles in the sample. True or False?

6. List 5 examples of why turbidity is an important process control parameter for wastewater operators.

7. Turbidimeters use which unit of measurement?

8. What is the purpose of applying silicone oil to your sample cell?

9. Why should turbidity samples be measured immediately?

10. What material is used for making primary calibration standards for the turbidimeter?

2100N LABORATORY TURBIDIMETER QUICK REFERENCE GUIDE

NEPHELOMETRIC MEASUREMENT PROCEDURE

1. Collect a representative sample in a clean container. Fill the sample cell to the line (approximately 30 mL). Take care to handle the sample cell by the top. Cap the sample cell. (*Note: Instrument warm-up stabilization time with Ratio on is 30 minutes and with Ratio off is 60 minutes. Typical application is to leave the instrument on 24 hours a day.)*

2. Hold the sample cell by the cap, and wipe to remove water spots and finger prints.

3. Apply a thin bead of silicone oil from the top to the bottom of the cell—just enough to coat the cell with a thin layer of oil. Using the oiling cloth provided, spread the oil uniformly. Then, wipe off the excess. The cell should appear nearly dry with little or no visible oil. (*Note: See Section 2.3.2 Applying Silicone Oil in the instrument manual.)*

4. Place the sample cell in the instrument cell compartment, and close the cell cover. (*Note: For immediate update of the display, press* **ENTER.**)

5. If necessary, insert the EPA filter. Select manual or automatic ranging by pressing the **RANGE** key.

6. Select the appropriate **SIGNAL AVERAGING** setting (on or off) by pressing the **SIGNAL AVG** key.

7. Select the appropriate **RATIO** setting (on or off) by pressing the **RATIO** key. (*Note: Values >40 NTU require Ratio on*.)

8. Select the appropriate measurement unit (NTU, EBC or NEPH) by pressing the **UNITS/EXIT** key.

9. Read and record the results.

CALIBRATION

Preparing Recommended Formazin Dilutions

Hach Company recommends use of 20-, 200-, 1000- and 4000-NTU Formazin standards for calibration of the Model 2100N Turbidimeter. Prepare all Formazin dilutions immediately before calibration, and discard the dilutions after use. While 4000-NTU stock solutions are stable for up to one year, diluted solutions deteriorate more rapidly. Prepare dilutions of 20, 200 and 1000 NTUs according to the directions in *Table 2 (Formazin Standard Preparation)* in *Section 3* of the Instrument Manual. The dilution water also is used to make an initial blank measurement (refer to *Section 3.2 Calibration* in the Instrument Manual).

NOTE

The calibration is based on a first order linear equation consisting of up to three independent variables. Unpredictable results may occur if standards other than the recommended calibration points are used. The factory-suggested calibration points are those determined by Hach Company chemists and engineers to provide the best calibration accuracy. Use of standards other than those specified may result in less accurate calibrations.

Calibrating with Formazin Standards

The electronic and optical design of the 2100N Turbidimeter provides long-term stability and minimizes the need for frequent calibration. The three-detector ratioing optical system compensates for electronic and optical system variations between calibrations. When data is used for USEPA reporting, recalibrate at least every 90 days, or as stipulated by the regulating authority. Refer to *Section 3.2 Calibration* in the Instrument Manual.

1. Fill a clean sample cell to the line (≅ 30 mL) with dilution water. Wipe the cell clean and apply a thin film of silicone oil.

2. Place the sample cell into the cell holder, and *close the cell cover.*

3. Press the **CAL** key. The S0 annunciator lights. The NTU value of the dilution water used in the previous calibration is displayed.

4. Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. This result is stored and used to compensate for the turbidity of the dilution water.

5. The instrument automatically increments to the next standard, displays the expected NTU value (e.g., 20.00 NTU), and the S1 annunciator lights. Remove the sample cell from the cell holder.

6. Fill a clean sample cell to the line with well-mixed, 20-NTU Formazin standard. Wipe the sample cell clean, and apply a thin film of silicone oil on its surface. Place it into the cell holder, and *close the cell cover.*

7. Press the **ENTER** key. The display counts down from 60 to 0, and makes a measurement. The instrument automatically increments to the next standard, the display shows 200.0 NTU, and the S2 annunciator lights. Remove the sample cell from the instrument.

8. Fill a clean sample cell to the line with well-mixed, 200-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it into the cell holder, and *close the cell cover.* Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The instrument automatically increments to the next standard, the display shows 1000 NTU, and the S3 annunciator lights. Remove the sample cell from the instrument.

9. Fill a clean sample cell to the line with well-mixed, 1000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and *close the cell cover.* Press the **ENTER** key. The instrument display counts down from 60 to 0, and then makes a measurement. The display automatically increments to the next standard, the display shows 4000 NTU, and the S4 annunciator lights. Remove the sample cell from the instrument.

10. Fill a clean sample cell to the line with well-mixed, 4000-NTU Formazin standard. Wipe the cell clean and apply a thin film of silicone oil to the surface. Place it in the cell holder and *close the cell cover.* Press the **ENTER** key. The instrument counts down from 60 to 0, and then makes a measurement. The display automatically increments back to the dilution water standard. The S0 annunciator lights, and the previously measured value of the dilution water is displayed.

11. Press the **CAL** key. The instrument makes calculations based on the new calibration data, stores the new calibration and returns the instrument to the measurement mode.

Reviewing the Calibration Sequence

Press the **CAL** key and then use the **UP ARROW** key to scroll through the standards to review calibration data currently in effect. If the instrument is connected to a printer, pressing the **PRINT** key prints all of the calibration data in effect. Press the **UNITS/EXIT** key to return to the operating mode without altering the current calibration data.

Using Gelex® Secondary Turbidity Standards

Periodically, as experience or regulating authorities indicate, verify the instrument calibration using Gelex Secondary Standards. If the reading in the range of use is not within 5% of the standard's assigned value, recalibrate using Formazin primary standards (refer to *Section 3.2.5 Using Gelex Secondary Turbidity Standards* in the Instrument Manual).

1. Calibrate the instrument with Formazin (refer to *Section 3.2 Calibration* in the Instrument Manual).

2. Verify that the instrument is set for the NTU mode, Ratio on and Automatic Ranging.

3. Thoroughly clean the outside of the Gelex vials, and apply a thin coating of silicone oil.

4. Place the lowest NTU Gelex Standard in the sample compartment with the triangle on the vial aligned with the index mark on the instrument sample compartment. Close the sample cell cover.

5. Press the **ENTER** key. Record the value displayed. Remove the standard from the instrument, and mark this value on the vial with a water soluble marker.

6. Repeat steps 3 through 5 for the other Gelex standards.

NOTE

Reassign new values to the Gelex standards each time the instrument is calibrated with Formazin.

ERROR CODES

Error codes may result from instrument malfunction or operator error. **Errxx** error codes are cleared from the display by pressing the **ENTER** key. The meter continues operating in the error condition; a calibration in progress can be continued. Any calibration being calculated (at the time the message appears) is discarded; the old calibration is retained. *Table 1* lists the error codes displayed for specific conditions.

Table 1. Error Codes

Code	Probable Cause	Corrective Action
Err01	Dilution water calculated to be >0.5 NTU	Start calibration over with higher quality dilution water, or filter the water with a membrane filter before use.
Err02	Two calibration standards have the same value, or their difference is less than 60.0 NTU. Standard 1 is too low (<10 NTU)	Recheck preparation of standards and repeat calibration.
Err03	Low light error	Reinsert sample. Check that lamp is on. Dilution may be necessary.
Err04	Memory malfunction	Switch instrument off and back on with I/O. Call Hach Service.
Err05	A/D over-range	Contact Hach Service.
Err06	A/D under-range	Contact Hach Service.
Err07	Light leak	Contact Hach Service.
Err08	Bad lamp circuit	Contact Hach Service.
Err09	Printer timeout error	Check that external printer is properly connected. Check that external printer is selected (on-line).
Err10	System voltage out of range	Switch instrument off and back on with I/O. Call Hach Service.
Err11	System loop test error	Switch instrument off and back on with I/O. Call Hach Service.

Diagnostic Functions

The diagnostic mode accesses system function information that is useful primarily when the instrument function is in doubt. Hach service technicians use the information for precise troubleshooting, speeding repairs, and avoiding unnecessary service returns.

Access diagnostic information by pressing and holding the **RIGHT ARROW** key for 3 seconds. Use the **ARROW** keys to edit the display to read the diagnostic code number of interest. Press the **ENTER** key to display the diagnostic value. More information may be obtained by purchasing the instrument service manual, or contacting the service center nearest you.

Diagnostic Codes

Code	Display	Description
00	bP on/bP of	Keyboard Beeper On/Off
01	FS Pr/SL Pr	Fast/Slow Print Device
21	Pr In	Printer Test
22	*	Display Test
23	*	Keyboard Test
24	*	Memory Test

Refer to *Table 6 Diagnostic Codes* in *Section 8 Troubleshooting* of the instrument manual for a list of diagnostic codes.

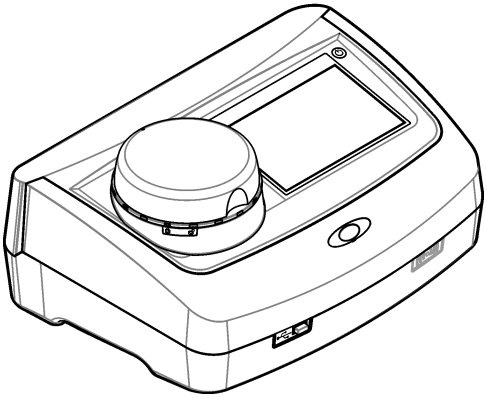
HACH COMPANY
WORLD HEADQUARTERS
P.O. BOX 389
Loveland, Colorado 80539
Telephone: (970) 669-3050
FAX: (970) 669-2932

DOC022.53.80489

TU5200

11/2021, Edition 6

Basic User Manual



Section 1 Additional information

An expanded user manual is available on the manufacturer's website.

Section 2 Specifications

Specifications are subject to change without notice.

Specification	Details
Measurement method	Nephelometry with the scattered light collected at a 90° angle to the incident light and 360° around the sample vial.
Primary compliance method	DIN EN ISO 7027
Dimensions (W x D x H)	41 x 28 x 12.5 cm (16 x 11 x 7.7 in.)
Weight	2.37 kg (5.23 lb)
Enclosure	IP20
Protection class	Instrument: III; Power supply: I
Pollution degree	2
Overvoltage category	II
Power requirements	Instrument: 15 VDC, 2 A; Power supply: 100–240 VAC ± 10%, 50/60 Hz
Operating temperature	10 to 40 °C (50 to 104 °F)
Storage temperature	–30 to 60 °C (–22 to 140 °F)
Humidity	5 to 95% relative humidity, non-condensing
Environmental conditions	Indoor use
Altitude	2000 m (6562 ft) maximum
Display	17.8 mm (7 in.) color touch screen
Laser	**Class 1 laser product:** Contains a non user-serviceable class 1 laser.
Optical light source	850 nm, maximum 0.55 mW
Measurement units	NTU, FNU, TE/F, FTU, EBC, mg/L, mNTU[1] or mFNU
Range	0 to 1000 FNU, FNU, TE/F, FTU; 0 to 100 mg/L; 0 to 250 EBC
Accuracy	± 2% of reading plus 0.01 NTU from 0 to 40 FNU ± 10% of reading from 40 to 1000 FNU based on Formazin primary standard at 25 °C (77 °F)
Linearity	Better than 1% for 0 to 40 NTU on Formazin at 25 °C (77 °F)
Precision	< 40 NTU: 0.002 NTU or 1% (the larger value); > 40 NTU: 3.5% based on Formazin primary standard at 25 °C (77 °F)
Stray light	< 0.01 FNU

[1] 1 mNTU = 0.001 NTU

Specification	Details
Calibration options	**StablCal®:** 1-point calibration (20 FNU) for 0 to 40 FNU measurement range; 2-point calibration (20 and 600 FNU) for 0 to 1000 FNU (full) measurement range **Formazin:** 2-point calibration (20 FNU and dilution water) for 0 to 40 FNU measurement range; 3-point calibration (20 FNU, 600 FNU and dilution water) for 0 to 1000 FNU (full) measurement range **Degrees:** 3-point calibration (20 and 100 mg/L and dilution water) for 0 to 100 mg/L (full) measurement range **SDVB:** 3-point calibration (20 FNU, 600 FNU and dilution water) for 0 to 1000 FNU (full) measurement range **Custom:** 2- to 6-point custom calibration for a measurement range of 0 FNU to the highest calibration point.
Verification options	Glass verification rod (secondary turbidity standard) < 0.1 NTU, StablCal or Formazin (0.1 to 40 NTU)
Verification (RFID or Link2SC®)	Process and laboratory measurements are compared with RFID or Link2SC for verification of the measurement value.
Certifications	CE compliant; US FDA accession number: 1420492-xxx. This product complies with IEC/EN 60825-1 and to 21 CFR 1040.10 in accordance with Laser Notice No. 50. Australian RCM.
Warranty	1 year (EU: 2 years)

Section 3 General information

In no event will the manufacturer be liable for direct, indirect, special, incidental or consequential damages resulting from any defect or omission in this manual. The manufacturer reserves the right to make changes in this manual and the products it describes at any time, without notice or obligation. Revised editions are found on the manufacturer's website.

3.1 Safety information

The manufacturer is not responsible for any damages due to misapplication or misuse of this product including, without limitation, direct, incidental and consequential damages, and disclaims such damages to the full extent permitted under applicable law. The user is soley responsible to identify critical application risks and install appropriate mechanisms to protect processes during a possible equipment malfunction.

Please read this entire manual before unpacking, setting up or operating this equipment. Pay attention to all danger and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment.

Make sure that the protection provided by this equipment is not impaired. Do not use or install this equipment in any manner other than that specified in this manual.

3.1.1 Use of hazard information

⚠ DANGER
Indicates a potentially or imminently hazardous situation which, if not avoided, will result in death or serious injury.

⚠ WARNING
Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

⚠ CAUTION
Indicates a potentially hazardous situation that may result in minor or moderate injury.

NOTICE
Indicates a situation which, if not avoided, may cause damage to the instrument. Information that requires special emphasis.

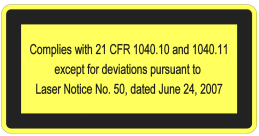
3.1.2 Precautionary labels

Read all labels and tags attached to the instrument. Personal injury or damage to the instrument could occur if not observed. A symbol on the instrument is referenced in the manual with a precautionary statement.

	Electrical equipment marked with this symbol may not be disposed of in European domestic or public disposal systems. Return old or end-of-life equipment to the manufacturer for disposal at no charge to the user.
	This symbol, if noted on the instrument, references the instruction manual for operation and/or safety information.
	This symbol indicates the need for protective eye wear.
	This symbol indicates a laser device is used in the equipment.
	This symbol identifies a risk of chemical harm and indicates that only individuals qualified and trained to work with chemicals should handle chemicals or perform maintenance on chemical delivery systems associated with the equipment.
	This symbol indicates radio waves.

3.1.3 Class 1 laser product

⚠ DANGER	
	Personal injury hazard. Never remove covers from the instrument. This is a laser-based instrument and the user risks injury if exposed to the laser.

	Class 1 laser product, IEC60825-1:2014, 850 nm, maximum 0.55 mW Location: Rear of the instrument.
	Conforms to U.S. regulations 21 CFR 1040.10 and 1040.11 in accordance with Laser Notice No. 50. Location: Rear of the instrument.

This instrument is a Class 1 Laser product. There is invisible laser radiation when the instrument is defective and when the instrument lid is open. This product complies with EN 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use" and with IEC/EN 60825-1, "Safety of Laser Products" and with 21 CFR 1040.10 in accordance with Laser Notice No. 50. Refer to the labels on the instrument that supply laser information.

3.1.4 RFID module

Instruments with the optional RFID module receive and transmit information and data. The RFID module operates with a frequency of 13.56 MHz.

RFID technology is a radio application. Radio applications are subject to national conditions of authorization. The use of instruments with the optional RFID module is currently permitted in the regions that follow:

EU (European Union) countries, EFTA (European Free Trade Association) countries, Turkey, Serbia, Macedonia, Australia, Canada, US, Chile, Ecuador, Venezuela, Mexico, Brazil, South Africa, India, Singapore, Argentina, Columbia, Peru and Panama

The use of instruments with the optional RFID module outside of the above-mentioned regions can violate national laws. The manufacturer reserves the right also to get authorization in other countries. In case of doubt, contact the manufacturer.

3.1.4.1 Safety information for RFID modules

⚠ WARNING

Multiple hazards. Do not disassemble the instrument for maintenance. If the internal components must be cleaned or repaired, contact the manufacturer.

⚠ WARNING

Electromagnetic radiation hazard. Do not use the instrument in dangerous environments.

NOTICE

This instrument is sensitive to electromagnetic and electromechanical interference. These interferences can have an effect on the analysis performance of this instrument. Do not put this instrument near equipment that can cause interference.

Obey the safety information that follows to operate the instrument in accordance with local, regional and national requirements.

- Do not operate the instrument in hospitals and equivalent establishments or near medical equipment, such as pace makers or hearing aids.
- Do not operate the instrument near highly flammable substances, such as fuels, highly flammable chemicals and explosives.

- Do not operate the instrument near combustible gases, vapors or dust.
- Keep the instrument away from strong vibration or shock.
- The instrument can cause interference in immediate proximity to televisions, radios and computers.
- The warranty does not cover improper use or wear.

3.1.4.2 FCC conformance for RFID

This instrument may contain a registered radio frequency identification device (RFID). Refer to Table 1 for the Federal Communications Commission (FCC) registration information.

Table 1 Registration information

Parameter	Value
FCC identification number (FCC ID)	YUH-QR15HL
IC	9278A-QR15HL
Frequency	13.56 MHz

3.1.5 Certification

⚠ CAUTION

This equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments.

Canadian Radio Interference-Causing Equipment Regulation, ICES-003, Class A:

Supporting test records reside with the manufacturer.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de classe A répond à toutes les exigences de la réglementation canadienne sur les équipements provoquant des interférences.

FCC Part 15, Class "A" Limits

Supporting test records reside with the manufacturer. The device complies with Part 15 of the FCC Rules. Operation is subject to the following conditions:

1. The equipment may not cause harmful interference.
2. The equipment must accept any interference received, including interference that may cause undesired operation.

Changes or modifications to this equipment not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment. This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at their expense. The following techniques can be used to reduce interference problems:

1. Disconnect the equipment from its power source to verify that it is or is not the source of the interference.
2. If the equipment is connected to the same outlet as the device experiencing interference, connect the equipment to a different outlet.
3. Move the equipment away from the device receiving the interference.
4. Reposition the receiving antenna for the device receiving the interference.
5. Try combinations of the above.

3.2 Product overview

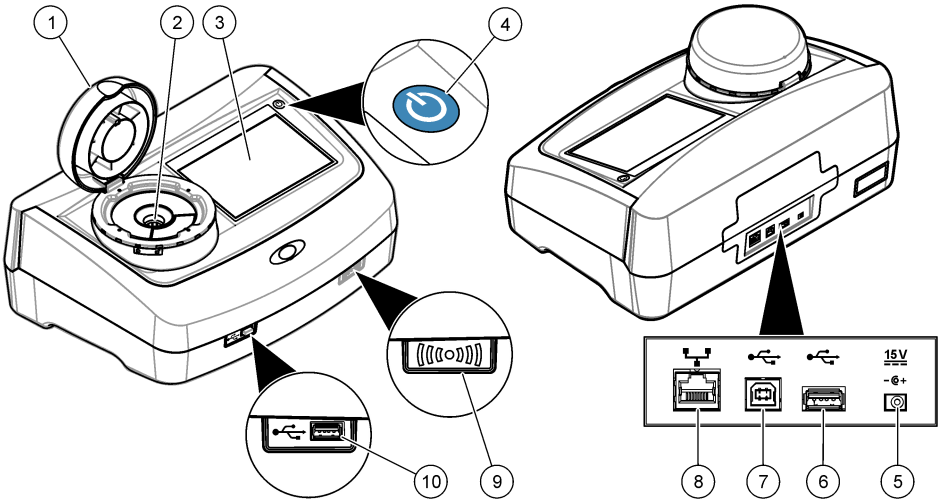
The TU5200 turbidimeter measures low turbidity mostly in finished drinking water applications. This laboratory instrument is factory calibrated and measures scattered light at an angle of 90° in a 360° radius around the axis of the incident light beam. Use the touch screen to operate the instrument. Refer to Figure 1.

An optional RFID module is available. Figure 1 shows the RFID module. The RFID module lets process and laboratory turbidity measurements be easily compared.

Instructional videos are available in the support section of the manufacturer's website.

For the accessories, refer to the expanded user manual on the manufacturer's website.

Figure 1 Product overview

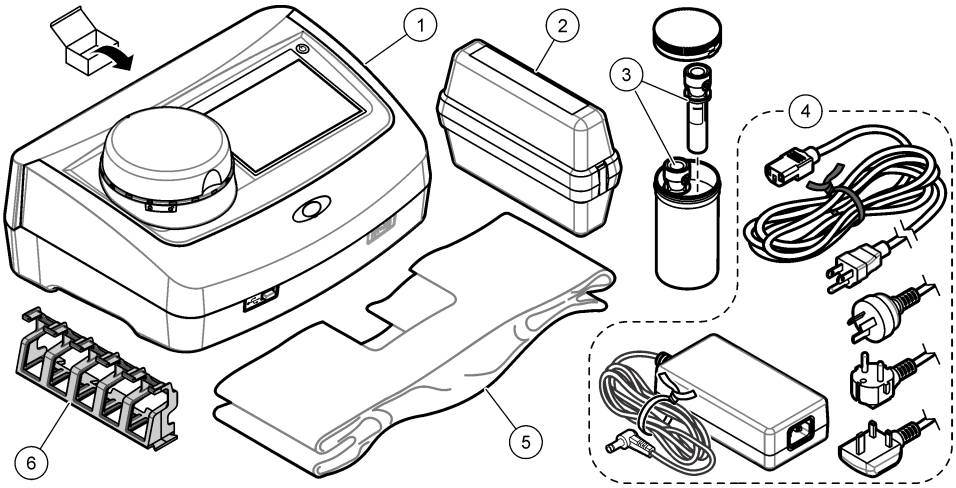


1 Lid	**6** USB port type A
2 Vial compartment	**7** USB port type B
3 Display	**8** Ethernet port for LAN connection
4 Power button	**9** RFID module indicator (optional)
5 Power supply connection	**10** USB port type A

3.3 Product components

Make sure that all components have been received. Refer to Figure 2. If any items are missing or damaged, contact the manufacturer or a sales representative immediately.

Figure 2 Product components



1 TU5200	**4** Power supply
2 StablCal kit, sealed vials with RFID (10, 20 and 600 NTU)	**5** Dust cover
3 Sample vials	**6** Vial stand

Section 4 Installation

⚠ CAUTION

Multiple hazards. Only qualified personnel must conduct the tasks described in this section of the document.

This instrument is rated for an altitude of 3100 m (10,710 ft) maximum. Use of this instrument at an altitude higher than 3100 m can slightly increase the potential for the electrical insulation to break down, which can result in an electric shock hazard. The manufacturer recommends that users with concerns contact technical support.

4.1 Installation guidelines

Install the instrument:

- On a level surface
- In a clean, dry, well ventilated, temperature controlled location
- In a location with minimum vibrations that has no direct exposure to sunlight
- In a location where there is sufficient clearance around it to make connections and to do maintenance tasks
- In a location where the power button and power cord are visible and easily accessible

4.2 Connect to external devices (optional)

NOTICE

Network and access point security is the responsibility of the customer that uses the wireless instrument. The manufacturer will not be liable for any damages, inclusive however not limited to indirect, special, consequential or incidental damages, that have been caused by a gap in, or breach of network security.

The instrument has three USB 1.1 ports and one Ethernet port. Refer to Figure 1 on page 8.

USB type A port—Connect to a printer, barcode handset scanner, USB flash drive, keyboard[2] or SIP 10 module.

USB type B port—Connect to a PC.

Ethernet port—Connect to a LAN with a shielded cable (e.g., STP, FTP, S/FTP). The maximum length of the shielded cable is 20 m (65.6 ft). To set up a LAN connection at the instrument, refer to the expanded user manual on the manufacturer's website .

Note: *USB cables must not be longer than 3 m (9.8 ft).*

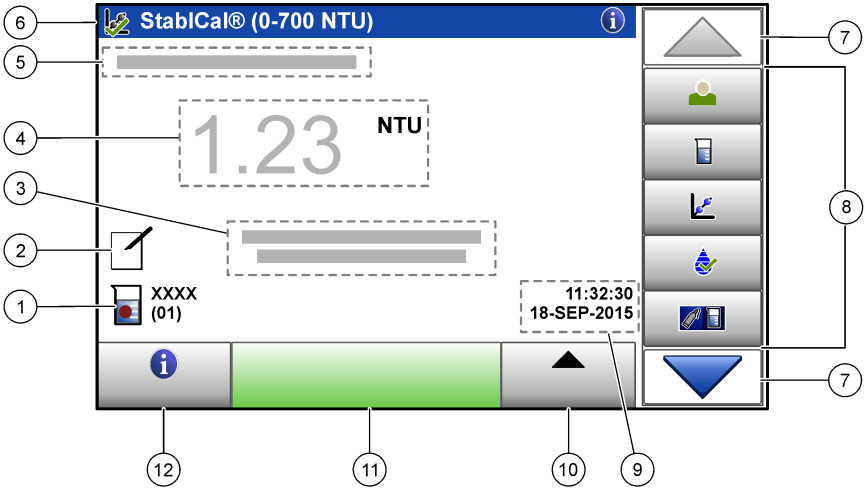
Section 5 User interface and navigation

The instrument display is a touch screen. Only use a clean, dry finger tip to navigate the functions of the touch screen. Do not use writing tips of pens or pencils or other sharp objects to make selections on the screen or damage to the screen will occur.

Refer to Figure 3 for an overview of the home screen.

[2] As an alternative to the touchscreen, use a keyboard to enter text into text boxes on the display (e.g., passwords and sample IDs).

Figure 3 Display overview



1	Sample ID and measurement number[3]	**7**	UP/DOWN navigation arrows
2	User comments	**8**	Sidebar menu (refer to Table 2)
3	Instructions	**9**	Time and date
4	Turbidity value, unit and reading mode	**10**	Options button
5	Warning or error message	**11**	Read button
6	Calibration status icon and calibration curve	**12**	Information (help) button

Table 2 Sidebar menu icons

Icon	Description
Login	Logs in or logs out an operator. To log in, select an operator ID and then push **Login**. To log out, push **Logout**. ***Note:*** *When an operator is logged in, the Login icon changes to the icon selected for the operator ID (e.g., fish, butterfly or soccer ball) and the text "Login" changes to the operator ID.*
Sample ID	Selects the sample ID.
Calibration	Starts a calibration.
Verification	Starts a verification.
Link2SC	Compares process and laboratory measurements.

[3] The measurement number increases by one each time a measurement is completed.

Table 2 Sidebar menu icons (continued)

Icon	Description
 Data Log	Shows the reading log, calibration log, verification log and compare log. Refer to Show the recorded data on page 20.
 Setup	Configures the instrument settings. Refer to Configure the instrument settings on page 13.
 Diagnostics	Shows the firmware information, instrument backup, instrument updates, signaling information and factory service data.
 Timer	Sets a timer.
	Goes to the manufacturer's website for the latest software versions and user manual when the instrument has a LAN connection.
 Documents	Shows the user manual and video(s) for the instrument.

Section 6 Startup

⚠ CAUTION

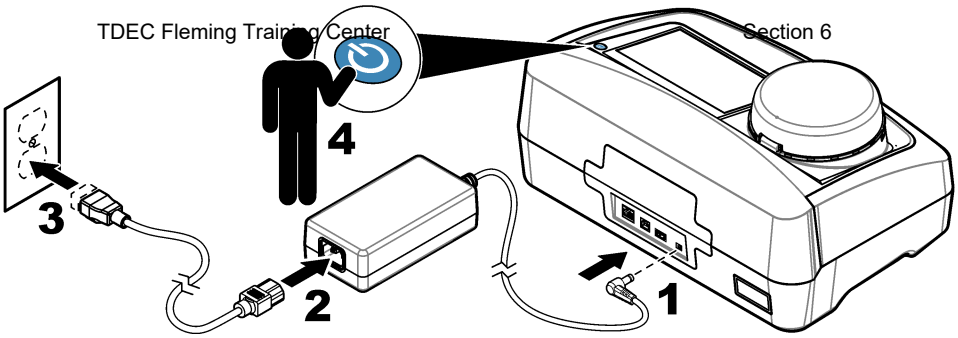
Personal injury hazard. Never remove covers from the instrument. This is a laser-based instrument and the user risks injury if exposed to the laser.

⚠ CAUTION

Personal injury hazard. Do not look into the vial compartment when the instrument is connected to power.

Refer to the illustrated steps that follow to connect power to the instrument and start the instrument. When the language menu shows, select the language and then push **OK**. The self-check will start.

Note: *To change the language after the initial startup, refer to* Change the language *on page 14.*



Section 7 Operation

7.1 Configuration

7.1.1 Configure the instrument settings

1. Push ▼ two times, then push **Setup**.
2. Select an option.

Option	Description
Location	Sets the location name of the instrument. The location is saved with measurements to the data log.
Date & Time	Sets the date format, the time format and the date and time. Enter the current date and time. **Date Format**—Sets the date format. Options: dd-mmm-yyyy (default), yyyy-mm-dd, dd-mm-yyyy or mm-dd-yyyy. **Time Format**—Sets the time format. Options: 12 or 24 hours (default).
Security	Enables or disables password protection for the settings and tasks in the security list. **Security Password**—Sets or changes the security (administrator) password (10 characters maximum). Passwords are case sensitive. **Security List**—Sets the security level for each setting and task in the security list. • **Off**—All operators can change the setting and or do the task. • **One key**—Only operators with a one-key or two-key security level can change the setting or do the task. Refer to Add operator IDs on page 14. • **Two keys**—Only operators with a two-key security level can change the setting or do the task. ***Note:*** *The Security setting is not set to on until Close is pushed.*
Sound Settings	Enables or disables the sound settings for individual events. Sets the sound volume for each event (1 to 10). To enable or disable all of the sound settings, select All and then push **Setup**.

Option	Description
Network & Peripherals	Shows the connection status of the devices that are directly connected to the instrument and connected to the instrument by LAN (local area network). • Printer—Local printer or network printer • Network—LAN connection • Controller—sc controller(s) • PC • USB Memory—USB flash drive • Keyboard
Power Management	Sets when the instrument is automatically set to sleep mode or off after a period of no activity. **Sleep Timer**—Sets when the instrument is set to sleep mode. Options: OFF, 30 minutes, 1 (default), 2 or 12 hours. **Power-Off Timer**—Sets when the instrument is set to off. Options: OFF, 2, 6, 12 (default) or 24 hours.

7.1.1.1 Change the language

NOTICE

Wait a minimum of 20 seconds after the power is set to off before the power is set to on again or damage to the instrument can occur.

To change the language after the initial startup, do the steps that follow.

1. Set the instrument to off.
2. Set the instrument to on.
3. During startup, touch the display until the language menu shows (approximately 45 seconds).
4. When the language menu shows, select the language and then push **OK**.

7.1.2 Add operator IDs

Add a unique operator ID for each person who will measure samples (30 maximum). Select an icon, operator password and security level for each operator ID.

1. Push **Login**.
2. Push **Options**>**New**.
3. Enter a new operator ID (characters maximum), then push **OK**.
4. Push the **LEFT** and **RIGHT** arrows to select the icon for the operator ID (e.g., fish, butterfly or soccer ball).
5. Push **Operator Password**, then enter a password for the operator ID.

   Note: *Passwords are case sensitive.*
6. Push **Security Level**, then select the security level for the operator ID.

   - **Off**—The operator cannot change the settings or do the tasks in the Security settings that have a security level of one key or two keys.
   - **One key**—The operator can change all the settings and do all the tasks in the Security settings that have a security level of off or one key.
   - **Two keys**—The operator can change all the settings and do all the tasks in the Security settings.

   Note: *Before a security level can be selected, the Security setting must be set to on. Refer to* Configure the instrument settings *on page 13.*
7. Push **OK**>**Close**.

8. To edit an operator ID, select the operator ID and then push **Options**>**Edit**.
9. To delete an operator ID, select the operator ID and then push **Options**>**Delete**>**OK**.

7.1.2.1 Configure an operator RFID tag (optional)

To use an operator RFID tag to log in to the instrument, save the applicable operator ID to an operator RFID tag as follows:

1. Push **Login**.
2. Select the operator ID, then push **Options**>**Initialize RFID Tag**.
3. Enter the password for the operator ID as necessary.
4. Complete the steps that show on the display.
5. Push **OK** to replace the operator ID on the RFID tag with a new operator ID if applicable.
6. Push **Close**.
7. Put the operator RFID tag in front of the RFID module to log in.

7.1.3 Add sample IDs

Add a unique sample ID for each sample (100 maximum). The sample ID identifies the sample location or other sample specific information.

As an alternative, import sample IDs from a spreadsheet file to the instrument. Refer to the expanded user manual on the manufacturer's website to import sample IDs.

Note: *When a sample bottle with a sample RFID sticker is put in front of the RFID module, the sample ID is automatically added to the instrument and selected on the instrument.*

1. Push **Sample ID**.
2. Push **Options**>**New**.
3. Enter a new sample ID (20 characters maximum).
4. If the sample bottle has a barcode that identifies the sample ID, read the barcode with a barcode handset scanner that is connected to the instrument. The barcode is added to the sample ID.
5. Push **OK**.
6. Select an option.

Option	Description
Add Date/Time	Adds the data and time that the sample was collected to the sample ID (optional). The date and time entered for each sample ID show on the Sample ID menu.
Add Number	Adds a measurement number to the sample ID (optional). Select the first number used for the measurement number (0 to 999). The measurement number shows in parenthesis after the sample ID on the home screen. Refer to Figure 3 on page 11.
Add Color	Adds a colored circle to the sample ID icon (optional). The sample ID icon shows before the sample ID on the home screen. Refer to Figure 3 on page 11.

7. Push **OK**>**Close**.
8. To edit a sample ID, select the sample ID and then push **Options**>**Edit**>**OK**.
9. To delete a sample ID, select the sample ID and then push **Options**>**Delete**>**OK**.

7.1.4 Configure the measurement settings

Select the reading mode, measurement units, data log settings, resolution and more.

1. At the main reading screen, push **Options**>**Reading Setup**.
2. Select an option.

Option	Description
Reading	Sets the reading mode to single, continuous or minimum mode. Default: Single. **Single**—The measurement stops when the reading is stable. **Continuous**—The measurement continues until the user pushes **Done**. **Minimum Mode**—Set to on when a process and laboratory measurement are compared and the process measurement is a lower NTU range. Removes the effect of non-representative particles in the grab sample. **Signal Avg**—The turbidity reading that shows on the display is an average of the values measured during the time interval selected. Options: For single measurement mode, 5 to 15 seconds. For continuous measurement mode, 5 to 90 seconds.
Unit	Selects the measurement units that show on the display and that are recorded to the data log. Options: NTU, FNU, TE/F, FTU, EBC, mNTU or mFNU. Default: FNU).
Data Log Setup	Sets the data log settings. **Auto Store**—Measurement data is automatically recorded in the reading log. Default: On. When not selected, push **Options**>**Store** to record the current measurement to the reading log as necessary. **Send Data Format**—Sets the output format of measurement data that is sent to external devices (CSV or XML). Default: XML. **Print Format**—Sets the output format of measurement data that is sent to a printer (Quick Print or Detailed Print (GLP)). **Comments**—Lets users add comments to log entries. **Auto Send**—Measurement data is automatically sent to all of the devices (e.g., printer, USB flash drive and FTP server) that are connected to the instrument after each measurement.
Resolution	Selects the number of decimal places that show on the display. Options: 0.001 (default) or 0.0001.
Bubble Reject	Sets the bubble reject to on (default) or off. When set to on, high turbidity readings caused by bubbles in the sample are not shown or saved to the data log.
Close lid to start reading	Enables or disables the instrument to start a measurement automatically when the lid is closed. Default: On. A measurement is only done when there is a sample vial in the instrument.

7.1.5 Set the acceptance range

Before process and laboratory measurements are compared on the instrument, set the acceptance range for the compare results. The acceptance range is the maximum difference permitted between the process and laboratory measurements.

1. Push **LINK2SC**.
2. Push **Options**>**Compare Setup**.
3. Push **Acceptance Range**>**Unit**.
4. Select an option.

Option	Description
%	Sets the acceptance range to a percentage (1 to 99%).
NTU	Sets the acceptance range to NTU units (0.015 to 100.00 NTU).

5. Push **Value**, then enter the acceptance range.

7.2 Measurement

7.2.1 Sample collection

- Collect samples in clean glass or plastic bottles with tight-fitting caps.
- Rinse the container a minimum of three times with the sample.
- When collecting a sample from a water tap in a distribution system or treatment plant, turn the water on for at least five minutes, then collect the sample. Do not adjust the flow because this can add particles.
- When collecting a sample from a body of water (e.g., a stream or storage tank), collect at least one liter (1 quart) and fully mix before taking an aliquot for measurement. If the quality of the sample source is not constant, collect samples at many locations at different depths as necessary. Then, mix the samples together to prepare one sample for measurement.
- Fill the container. Let the container overflow with the sample and then immediately put the cap on the sample container so that there is no headspace (air) above the sample.
- Write the sample information on the container.
- Start analysis as soon as possible to prevent temperature changes, bacteria growth and settling.

7.2.2 Prevent vial contamination

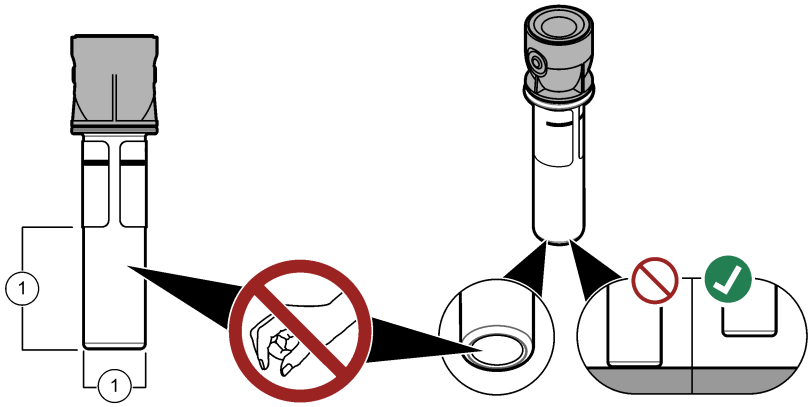
NOTICE

Do not to touch or scratch the glass of the sample vial. Contamination or scratches on the glass can cause measurement errors.

The glass must stay clean and have no scratches. Use a no-lint cloth to remove dirt, fingerprints or particles from the glass. Replace the sample vial when the glass has scratches.

Refer to Figure 4 to identify where not to touch the sample vial. Always keep the sample vials in the vial stand to prevent contamination on the bottom of the vial.

Figure 4 Sample vial overview



1 Measurement surface—Do not touch.

7.2.3 Prepare a sample vial

⚠ CAUTION

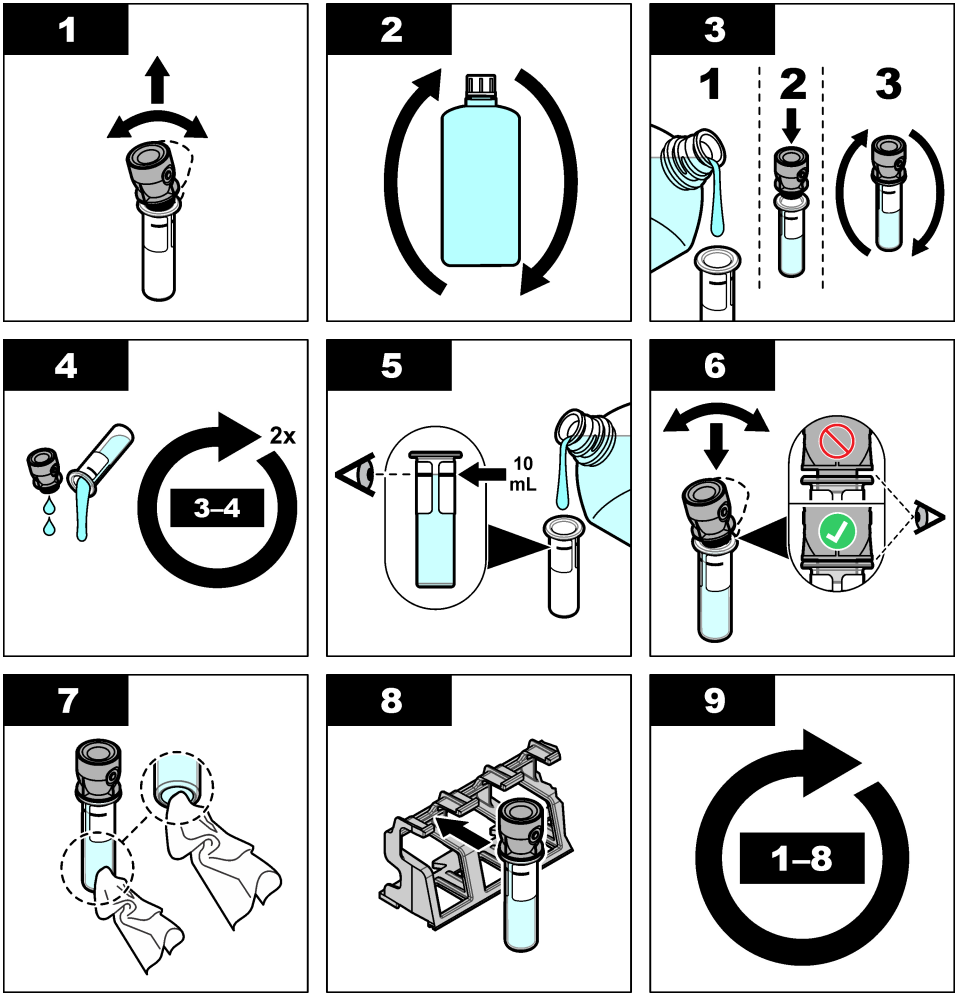
Chemical exposure hazard. Dispose of chemicals and wastes in accordance with local, regional and national regulations.

NOTICE

Always put a cap on the sample vial to prevent spills in the vial compartment.

Refer to the illustrated steps that follow to prepare a sample vial for measurement. Measure the sample immediately.

Note: *If there is contamination in the sample vial after it is rinsed with the sample, clean the sample vial. Refer to* Clean a sample vial *on page 22.*



7.2.4 Put the vial in the instrument

⚠ CAUTION

Personal injury hazard. Never remove covers from the instrument. This is a laser-based instrument and the user risks injury if exposed to the laser.

⚠ CAUTION

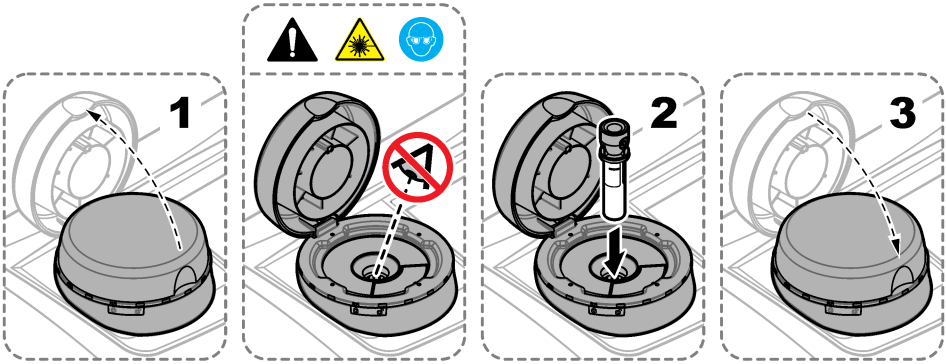
Personal injury hazard. Do not look into the vial compartment when the instrument is connected to power.

NOTICE

Keep the lid closed to keep contamination out of the vial compartment.

1. Log in to the instrument as follows:
   - Put an operator RFID tag in front of the RFID module or
   - Push **Login**. Select the applicable operator ID, then push **Select**.
2. Select the sample ID as follows:
   - Put the sample RFID sticker on the sample bottle in front of the RFID module or
   - Push **Sample ID**. Select the applicable sample ID, then push **Select**.

   Note: *To add sample IDs to the instrument, refer to* Add sample IDs *on page 15.*
3. Clean the sample vial with a no-lint cloth to remove contamination.
4. Dry the external surfaces of the vial with a no-lint cloth. Make sure to dry the bottom of the vial.
5. Put the sample vial in the vial compartment. Refer to the illustrated steps that follow.



7.2.5 Measure the sample

1. Push **Read** if a measurement does not start automatically when the lid is closed.
2. When the measurement is complete, push **Options**>**Store** to record the measurement to the reading log as necessary.

   Note: *If the Auto Save setting is set to on, "Data Stored" shows on the display and the measurement is automatically recorded to the reading log.*
3. To show the recorded measurements, push **Options**>**Reading Log**. Refer to Show the recorded data on page 20 for more options.
4. To send the measurement data to external devices that are connected to the instrument, push **Options**>**Send Data**. Refer to Show the recorded data on page 20 for more options.

   Note: *If the Auto Send settings is set to on, the measurement data is automatically sent to the external device(s) that is connected to the instrument.*

7.2.6 Compare process and laboratory measurements

Refer to the expanded user manual on www.hach.com to compare process and laboratory measurements.

Turbidity 221

7.3 Show the recorded data

All the recorded data is kept in the data log. The data log is divided into four logs:

- **Reading log**—Shows the recorded measurements.
- **Calibration log**—Shows the calibration history.
- **Verification log**—Shows the verification history.
- **Compare log**—Shows the recorded comparisons of process and laboratory measurements.

1. Push **Data Log** and select the applicable log to show.
2. To show the details of a log entry, select the log entry and then push **View Details**.
   Note: *To add a comment to the log entry, push the comments icon.*
3. To only show the log entries recorded during a time interval or with a specific operator ID or sample ID, do the steps that follow.
   a. Push **Filter**, then select On.
   b. Select an option.

Option	Description
Time Interval	Selects the time interval.
Operator ID	Selects the operator ID.
Sample ID	Selects the sample ID. This option only shows when Reading Log or Compare Log is selected.

4. To send log data to a device (e.g., printer or USB flash drive), delete a log entry or show a compare log or reading log entries in a graph, do the steps that follow.
   a. Push **Options**.

b. Select an option.

Option	Description
Delete	Removes one of the items that follow. • The selected log entry • The log entries for a time interval • The log entries with a specific operator ID • The log entries with a specific sample ID[4] • All the entries in the selected log
Send Data	Sends one of the items that follow to all the devices that are directly connected to the instrument (e.g., printer or USB flash drive) and connected to the instrument by LAN (network printer or FTP server). • The selected log entry • The log entries for a time interval • The log entries with a specific operator ID • The log entries with a specific sample ID[4] • All the entries in the selected log
View Graph	Shows the reading log entries that have the same sample ID in a graph. This option only shows when Compare Log or Reading Log is selected. To add the log entries for another sample ID to the graph, push **Options**>**Add Data**. Select a sample ID to add to the graph. To show the details of a data point, touch a data point on the display or push the **LEFT** and **RIGHT** arrows to select a data point. **Data points**—Selects the symbol used for the data points. **Control Limit**—Sets the minimum value and maximum value of the readings that show on the graph.

Section 8 Calibration

⚠ WARNING

Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Refer to the current safety data sheets (MSDS/SDS) for safety protocols.

The instrument is factory calibrated and the laser light source is stable. The manufacturer recommends that a calibration verification be done periodically to make sure that the system operates as intended. The manufacturer recommends calibration after repairs or comprehensive maintenance work.

Refer to the expanded user manual on the manufacturer's website to calibrate the instrument and do a calibration verification.

Section 9 Maintenance

⚠ CAUTION

Multiple hazards. Only qualified personnel must conduct the tasks described in this section of the document.

[4] This option only shows when Reading Log or Compare Log is selected.

⚠ CAUTION

Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Refer to the current safety data sheets (MSDS/SDS) for safety protocols.

⚠ CAUTION

Personal injury hazard. Never remove covers from the instrument. This is a laser-based instrument and the user risks injury if exposed to the laser.

NOTICE

Do not disassemble the instrument for maintenance. If the internal components must be cleaned or repaired, contact the manufacturer.

9.1 Clean spills

⚠ CAUTION

Chemical exposure hazard. Dispose of chemicals and wastes in accordance with local, regional and national regulations.

1. Obey all facility safety protocols for spill control.
2. Discard the waste according to applicable regulations.

9.2 Clean the instrument

Clean the exterior of the instrument with a moist cloth, and then wipe the instrument dry.

9.3 Clean a sample vial

⚠ CAUTION

Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Refer to the current safety data sheets (MSDS/SDS) for safety protocols.

Clean the sample vial when there is contamination in the sample vial after the sample vial is rinsed.

Items to collect:

- Hydrochloric acid (concentration 10%)
- Laboratory cleaning detergent for glass (concentration 0.1%)
- Distilled or deonized water
- Dilution water
- Vial wiper (optional)
- No-lint cloth

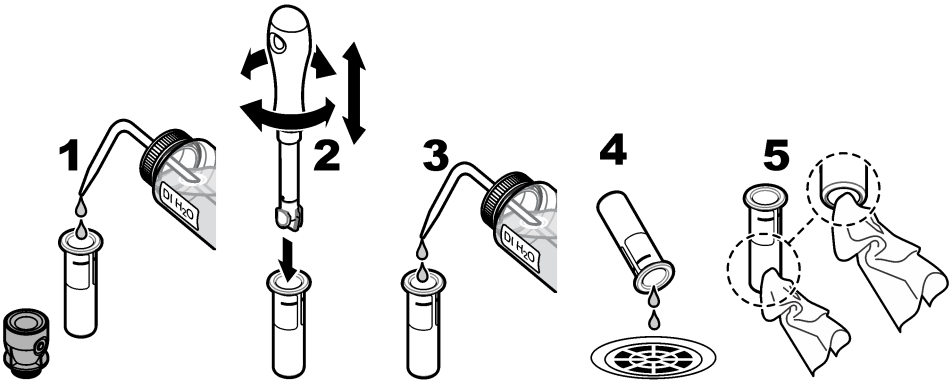
1. Put the exterior and interior surfaces of the sample vial and the cap in 10% hydrochloric acid for 15 minutes.
2. Clean the exterior and interior surfaces of the sample vial and the cap with laboratory cleaning detergent for glass (concentration 0.1%).
3. Fully rinse the sample vial three times with distilled or deionized water.

   Note: *If the sample vial is used to measure low range turbidity samples or dilution water, rinse with dilution water (not distilled or deionized water).*

4. For the best results, clean the sample vial with the optional vial wiper. Then fully rinse the sample vial again. Refer to Figure 5.
5. Dry the external surfaces of the sample cell with a soft, no-lint cloth. Do not let the sample vial air dry.
6. For storage, fill the sample vial with distilled or demineralized water.

   Note: *If the sample vial is used to measure low range turbidity samples or dilution water, fill the sample vial with dilution water (not distilled or deionized water).*
7. Immediately put the cap on the sample vial to keep the interior of the sample vial wet.

Figure 5 Clean the vial with the vial wiper (optional)



9.4 Clean the vial compartment

Table 3 Cleaning options

Contaminant	Options
Dust	Vial compartment wiper, micro fiber cloth, lint-free cloth
Liquid, oil	Cloth, water and cleaning agent

Section 10 Troubleshooting

Refer to the expanded user manual on www.hach.com for troubleshooting information.

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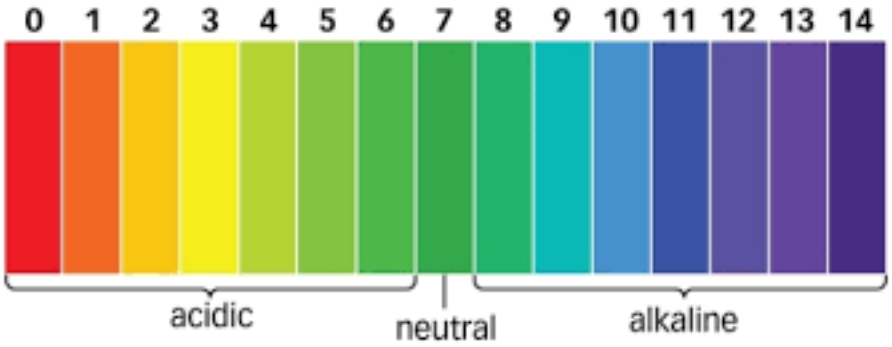
Section 7
pH



pH

1

pH

- One of the most important and frequently used tests in water chemistry
- A measure of the intensity of the acidic or alkaline character of a solution
- Logarithmic scale of ionic activity
  0 to 14 s.u.
- pH values cannot be averaged

2

pH Measurement

- pH is typically measured with a meter and probe
- This is an electrochemical method of analysis

3

pH Theory

- Acid
  - increases the hydrogen ion (H+) concentration in a solution
- Base
  - increases the hydroxide ion (OH-) concentration in a solution

4

pH Theory

- pH is defined as the negative log of the molar hydrogen ion concentration in aqueous solution

$$pH = -\log [H^+]$$

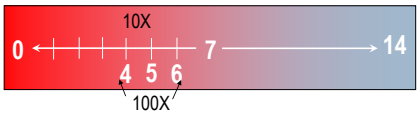
5

pH Scale

6

pH Scale

- pH is a negative logarithmic function
- Each decrease in pH unit = 10X increase in acidity
  - Solution at pH4 is 10X more acidic than solution at pH5
  - Solution at pH 4 is 100X more acidic than pH6 solution



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7

How Does a pH Probe Work?

- Probe measures hydrogen ion concentration
  - Two electrodes in probe:
    a) sensing half-cell,
    b) reference half-cell

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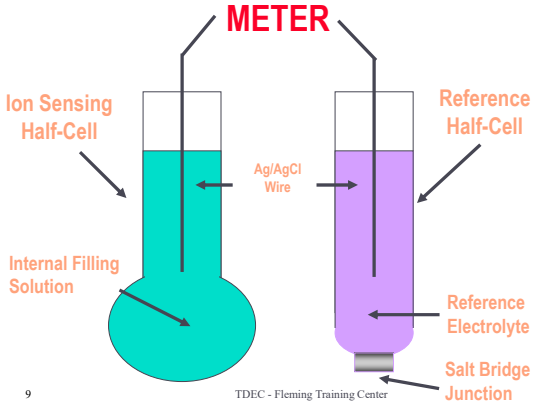
8



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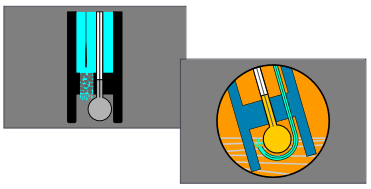
Reference Half-Cell

- Dispenses reference solution which completes circuit for meter



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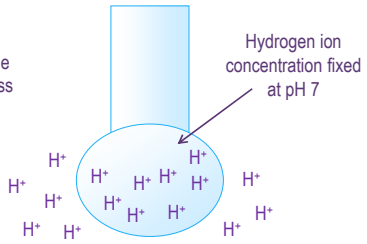
Sensing Half-Cell

pH 7 Solution

H^+ conc. the same both inside and outside glass bulb

*No potential develops



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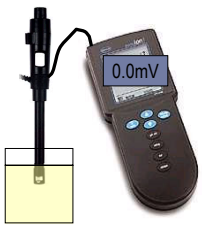
Sensing Half-Cell

pH 7 Solution

H^+ conc. the same both inside and outside glass bulb

*No potential develops



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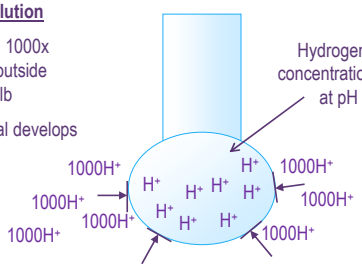
12

Sensing Half-Cell

pH 4 Solution

H^+ conc. 1000x greater outside glass bulb

*Potential develops



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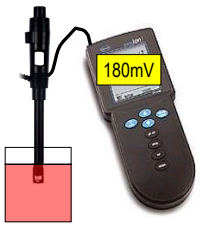
13

Sensing Half-Cell

pH 4 Solution

H^+ conc. 1000x greater outside glass bulb

*Potential develops



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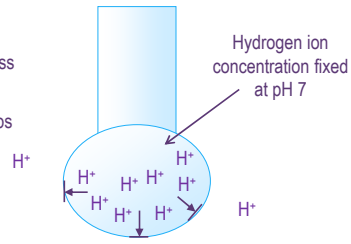
Sensing Half-Cell

pH 10 Solution

H^+ conc. 1000x greater inside glass bulb

*Potential develops



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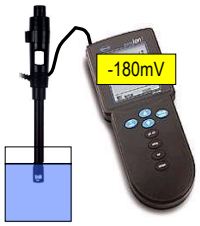
Sensing Half-Cell

pH 10 Solution

H^+ conc. 1000x greater inside glass bulb

*Potential develops



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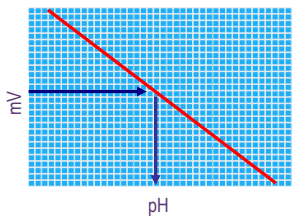
Calibration

- A calibration curve allows the meter to convert a measured millivolt potential into a pH reading.



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Calibration

- The optimal slope for pH is -58 ± 3 mV/decade.

What does this mean?

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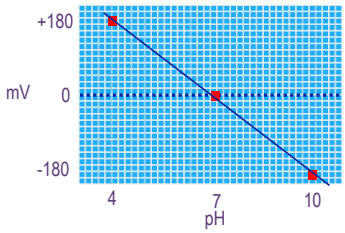
Calibration



The mV values from the previous demos (4, 7, and 10 buffers) are plotted on a graph of mV versus pH. A line is drawn between the points and the slope determined. Since slope is rise over run, it will be in units of mV per pH unit, or mV per decade (since the difference between pH units is 10X).

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Calibration

- -180mV difference measured between pH4 and pH7
- pH4 to pH7 (3 pH units) is 1000x concentration change
- Decade = 10-fold concentration change
  = 1pH unit
- -180/3 = -60 ≈ -58mV/decade

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pH calibration

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Importance of pH control

- Ammonia toxicity is influenced by pH
- pH plays an important role in the solubility of metal salts
- pH affects the rate at which chlorine reacts to form chloramines (which are less effective disinfectants)

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pH Sampling

- Holding time = 15 minutes
- Preservation = none
- Sample container = glass or plastic
- Grab sample
- Continuous monitoring possible

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pH Meter Calibration

- Follow manufacturers instructions
- Use fresh buffers (4, 7, & 10 s.u.)
- Stir buffers and samples at the same speed without a vortex
- Rinse and blot dry electrodes between samples and buffers
- Accurate and reproducible to within 0.1 s.u.

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pH Meter Calibration (cont.)

- Start with pH = 7.0 buffer (usually)
- Second buffer 3 s.u. different that brackets expected sample pH (4 or 10)
- Immerse in a third buffer - reading should be within 0.1 s.u.
- If response is accurate - read and record previous buffers as samples (pH and temperature)

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Common Deficiencies

- The pH meter was calibrated using one buffer or expired buffers
- The continuous pH meter was not calibrated on a regular basis
- Buffers were left open and being reused for a week

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Maintenance

- New probe
- Calibration
- Measurement/Storage
- Troubleshooting
- Cleaning

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New Probe

- Condition new pH probe in pH 7 buffer for approximately 30 minutes before initial use
- Probes must also be conditioned after cleaning or if it had been stored dry as long term storage

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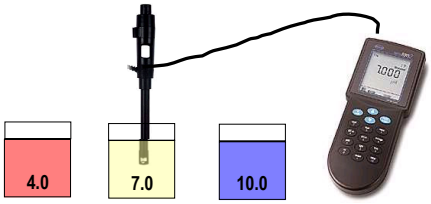
Calibrate

- Calibrate pH meters daily using two or three buffer solutions



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Measurement

- Place probe into sample, stir, and wait for readings to stabilize
- Rinse and **blot** dry between measurements
- Storage between measurements
  - As suggested by probe manufacturer
  - pH electrode storage solution *(best)*

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Troubleshooting

- mV reading in pH 7 buffer
  - Should read 0 ± 30 mV in pH 7 buffer
- Response time
  - May require cleaning if slow in buffered solution
- Slope
  - Optimal slope is -58 ± 3 mV/decade

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Cleaning

- Slow response may indicate need for cleaning
  - Alternate soaking in dilute hydrochloric acid and dilute sodium hydroxide
  - Rinse with deionized water
  - Condition in pH 7 buffer before use
  - Filler hole button was not removed
- Read probe manual for cleaning method recommended by manufacturer

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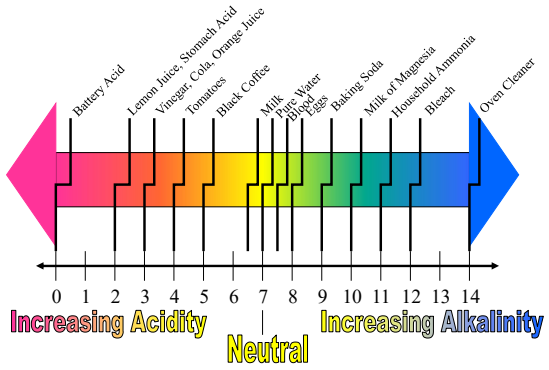

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pH SM4500-H⁺ B – 2000 Electrometric Method

- DOC
- Dup
- ICAL/CCV
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency

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pH SM4500-H⁺ B – 2000 Electrometric Measurement

- Read to 1/10th units only, 0.0 s.u.
- Demonstration of Capability (DOC)
  - Run buffer at least four times and compare to the limits listed in the method
  - Real people language: each operator running this test need to calibrate and analyze 4 buffers at a pH of 7
  - Documentation (signed form) that analyst has read and understands all appropriate SOPs and Methods.
  - Recommend backup analyst do this once a year.

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pH SM4500-H⁺ B – 2000 Electrometric Measurement

- Initial Calibration
  - Calibrate per manufactures instructions with fresh buffers daily (day of).
    - **2014 Update – Analyze a 7 buffer solution as a sample after calibration and before samples to verify initial calibration (ICV), should be within ± 0.2 s.u.**
- Calibration Verification
  - Read 7 buffer after analyzing samples daily

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pH SM4500-H^+ B – 2000 Electrometric Measurement

- Duplicates of the sample
  - Run on a 5% basis, one for every 20 samples
  - Within ± 0.2 s.u.
  - **2014 Update – For reporting purposes, all duplicates should be reported according to your permit limits. If your permit sets a minimum or maximum limit such as pH, then the minimum or maximum value should be reported even if falls outside your permit limit.**

37

pH – Review Questions

Laboratory portion:

1. How many standards did we use to calibrate our pH meters? How many are required for wastewater analysis?

2. pH measurements are always affected by _____, for this reason, you must always report the temperature at which the sample is being measured. Your probe should be equipped with an ATC, which stands for _____.

3. pH probes are stored in a storage solution when not in use. What is the preferred storage solution for combination electrodes?

4. During calibration, you must stir the sample gently, without a vortex, to minimize what?

5. Give a few examples of wastewater tests that require pH adjustment for preservation (if they will not be analyzed immediately).

6. We lowered the pH of our sample to _____ using _____.

7. After lowering the pH, we then brought it up using TUMS. What is the active ingredient in TUMS?

8. Is the ingredient answered in #4 an acid or a base? Explain how the ingredient works to bring the pH up.

Classroom portion:

9. What is the holding time for a pH sample?

10. What is the approved methodology (according to 40 CFR 136) for Hydrogen ion (pH) analysis?

11. How often should your pH meter be calibrated?

12. Standard Methods requires that you always use a fresh buffer with each calibration. Why?

13. pH is described as the *intensity* of what?

14. An acid increases the _____ ion (H+) concentration in a solution.

15. A base increases the _____ion (OH-) concentration in a solution.

16. Each decrease in pH unit = _____ in acidity.

17. A solution with a pH at 4 is how many times more acidic than a solution with a pH of 8?

18. Name the two electrodes found within a pH probe and describe the function of each.

19. Does an electrical potential develop when a pH probe is immersed in pH 7 buffer? Why or why not?

20. pH meters read in pH standard units and also _____.

21. Define what a “decade” means in relation to pH.

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Method: SM 4500-H^+B-2011
pH Calibration Record

Date	Time	Temp of Buffers	Slope	Buffers Used Lot#s			ICV	CCV	Analyst Initials	Remarks
				4	7	10				

Date	Time	Sample Name	Temp. Solution	Date of Last Calibration	Measured pH	Analyst Initials
		Vinegar				
		Coca Cola				
		Lemon Juice				
		Ammonia Cleaner				
		Alkaline Water				

pH VALUE 4500 – H^+ B. Electrometric Method

Reference: Standard Method 4500-H+ 22ND Edition, 2000, Editorial revisions, 2011.

Principles:

- Measurement of pH is one of the most important and frequently used tests in water chemistry. Practically every phase of water supply and wastewater treatment (acid-base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control) is pH-dependent.
- At a given temperature the *intensity* of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity.
- Buffer capacity is the amount of strong acid or base, usually expressed in moles per liter, needed to change the pH value of a 1 Liter sample by 1 unit. pH is defined as –log[H+]; it is the "intensity" factor of acidity.
- Natural waters usually have pH values in the range of 4 to 9, and most are slightly basic because of the presence of bicarbonates and carbonates of the alkali and alkaline earth metals.

General Discussion:

- Choose buffers to bracket the sample
- pH measurements are affected by temperature: always report temperature at which pH is measured.
- For routine work, use a pH meter accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature compensation adjustment. (also called "ATC")

Procedure:

Instrument calibration: Follow manufacturer's instructions for pH meter and for storage and preparation of electrodes.

1. Before use, remove electrodes from storage solution, rinse with distilled water, blot dry with a soft tissue, place in pH buffer 7, and read.
2. Choose a second buffer within 2 pH units of expected sample pH (either 4 or 10). Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Read and record temperature of measurement.
    - Saturated KCl is the preferred storage solution for a combination electrode.
    - Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

- Some pH meters may be calibrated using three or more buffers.
- The meter should be calibrated daily and each calibration should use fresh buffer solutions. (Buffer solutions may deteriorate as a result of mold growth or contamination, so prepare fresh as needed. For routine analysis, use commercially available buffer solutions of tested quality.)

Sample analysis:

1. Stir sample to ensure homogeneity during analysis; stir gently to minimize carbon dioxide entrainment.
2. Rinse electrode with distilled water, blot dry, immerse in a fresh portion of the sample, and read pH.

QA/QC:

*Note: Frequency depends on NPDES permit

1. Demonstration of Capability (DOC)
    - Each analyst should keep a file with documentation that they have calibrated and analyzed a reagent blank and at least 4 standards (LFBs).
    - Ensure that precision (duplicates) and accuracy (percent recovery) calculated for standards (LFBs) are within acceptable criteria.
    - Include (signed) documentation that analyst has read and understands all appropriate SOPs and methods.
2. Duplicate (Dup)
    - Analyze the same sample twice.
3. Initial Calibration Verification/Continuing Calibration Verification
    - Calibrate the meter.
    - Verify the calibration (especially if preset by manufacturer) at beginning of day and/or after every 10 readings, whichever comes first.
4. Corrective Action
    - Have a Corrective Action plan in SOP for each method explaining what to do if QC tests fail or are out of range. (Ex: if standards fail, recalibrate and read again.)
5. QC Acceptance
    - Have in SOP the acceptable ranges for standards, duplicates, spikes, etc. and make sure they match the method requirements.
6. Batch size 20
    - Each batch could be daily, every 10 samples, or every 20 samples. Check method.

Section 8
Bacteriological Analyses

Add Colilert to sample and shake to dissolve

Pour mixture into a Quanti-Tray

Bacteriological Analysis

Wastewater Laboratory Class

1

1

Coliform Bacteria

- MPN of coliform bacteria are estimated to indicate the presence of bacteria originating from the intestines of warm-blooded animals
- Coliform bacteria are generally considered harmless
  - But their presence may indicate the presence of pathogenic organisms

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Coliform Bacteria

- Comprised of all the aerobic and facultative anaerobic gram negative, nonspore-forming, rod-shaped bacteria that ferment lactose within 48 hours at ~35°C
- Coliform bacteria can be split into fecal and non-fecal groups
  - The fecal group can grow at higher temperatures (~45°C) than the non-fecal coliforms

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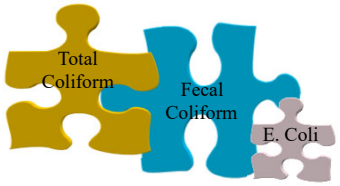
Family Portrait

- Indicators of water contamination



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Sampling

- Clean, sterilized borosilicate glass or plastic bottles or sterile plastic bags.
- Leave ample air space for mixing.
- Collect samples representative of wastewater tested.
- Use aseptic techniques; avoid sample contamination.
- Test samples as soon as possible.

5

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Safety

- Follow standard safety practices appropriate to microbiological laboratories.
- Materials suspected of containing viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding.

6

6

Approved Methods

- Coliform (fecal)
  - Number per 100 mL
    - Membrane filtration
- E. coli
  - Number per 100 mL
    - Multiple tub/multiple well (Colilert®)
    - Membrane filtration
      - m-ColiBlue24®
      - Modified mTEC agar

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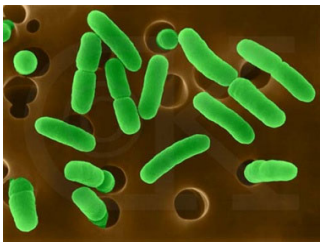

Fecal Coliform

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Summary of Method

- A 100 mL volume of sample is filtered through a 47-mm membrane filter using standard techniques.
- Filter is transferred to a 50-mm petri plate containing an absorbent pad saturated with mFC Broth.
- Invert filter and incubate at **44.5±0.2°C** for 24 hrs.
- Count **blue** colonies.

9

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Interferences

- No interferences
- Excess particulates may cause colonies to grow together on a crowded filter or slow the sample filtration process.

10

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Equipment

- Water bath or air incubator operating at 44.5±0.2°C
- Vacuum pump
- UV sterilizer or boiling water bath
- 10-15 X dissecting microscope; should have fluorescent illuminator
- Alcohol burner

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11

Supplies and Glassware

- Sterile graduated cylinder
- Sterile pipets
- Sterile MF filtration flask
- Sterile dilution water
- Sterile sample vessels
- Samples containing chlorine must be treated with 3% sodium thiosulfate solution
- mFC Broth

12

12

Sampling

- Maximum hold time is 8 hrs at < 10°C
- Ideal sample volume yields 20-60 colonies
- Samples <20 mL, add 10 mL sterile dilution water to filter funnel before applying vacuum.
- Sterilize funnel between samples.

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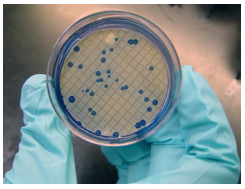
Fecal Data Analysis

- Visually determine colony counts on membrane filters.
- Verify using 10-15 X binocular wide-field microscope.
- Fecal coliforms appear blue.



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Fecal Data Interpretation

- Incubation time is 24 ± 2 hrs.
- Fecal coliform density reported as number of colonies per 100 mL of sample.
- NPDES permit limit: monthly average of 200/100 mL; daily maximum of 1000/100 mL.

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Appendix C: Pathogen Reduction Alternatives for Class B Biosolids

- Class B—Alternative 1
  - (i) Seven representative samples of the biosolids that are applied to the land shall be collected.
  - (ii) The geometric mean of the density of fecal coliform in the samples collected in subpart (i) of this part shall be less than either 2,000,000 Most Probable Number per gram of total solids (dry weight basis) or 2,000,000 Colony Forming Units per gram of total solids (dry weight basis).

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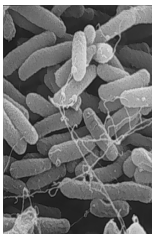

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Escherichia coli (E.coli)

Colilert

17

Colilert® & Colilert-18® SM 9223B-2016

- Enzyme substrate tests use hydrolysable chromogenic fluorogenic substrates to simultaneously detect enzymes produced by total coliforms and *Escherichia coli* (*E.coli*)
- Total coliform bacteria produce ß-D-galactosidase
  - Cleaves the chromogenic substrate in the medium to release chromogen
- *E. coli* strains produce the enzyme B-glucuronidase
  - Cleaves a fluorogenic substrate in the medium to release fluorogen

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18

COLILERT® & COLILERT-18®
SM 9223B-2016

MPN Method

- Add substrate to a 100 mL sample
- If making dilutions, use sterile DI water, not sterile buffered water.

19

19

COLILERT® & COLILERT-18®

- Shake sample vigorously. Wait for bubbles to dissipate.
- Pour into QuantiTray.

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COLILERT® & COLILERT-18®

- Seal sample in Quanti-Tray
- Incubate at **35±0.5**°C for 18 hrs (Colilert-18) OR 24 hrs (Colilert)

21

21

COLILERT® & COLILERT-18®

- Examine tray for appropriate color change
- Yellow is an indicator of total coliforms

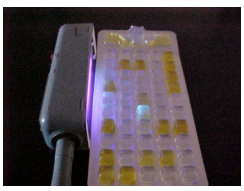
Left: The 97 well QuantiTray 2000 will count up to 2419 cfu without dilution.
Right: The 51 well QuantiTray will count up to 200 cfu without dilution.

22

22

COLILERT® & COLILERT-18®

- Examine positive total coliform for fluorescence using a UV light in a dark environment
- Fluorescence is a positive indicator for E. coli
- Calculate MPN value according to the table provided with the QuantiTray



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COLILERT® & COLILERT-18®

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.3	13.3	14.3	15.4	16.4	17.5	18.5	19.6	20.6	21.7	22.8	23.8	24.9	25.9	27.0	28.0
4	4.1	5.2	6.2	7.2	8.2	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.6	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.8	15.9	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.1	29.2	30.3	31.5	32.7
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.9	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.6	23.7	24.9	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.4	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.4	40.6	41.8
13	14.6	15.8	17.0	18.2	19.4	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.4	38.6	39.8	41.0	42.2	43.4
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.8	44.0	45.3
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.8	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.8	42.1	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.8	29.2	30.5	31.9	33.2	34.6	35.9	37.3	38.7	40.0	41.4	42.9	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	30.0	31.4	32.8	34.2	35.6	37.0	38.4	39.9	41.3	42.8	44.2	45.7	47.2	48.6	50.1	51.6	53.1	54.6	56.1	57.6	59.1	60.6	62.1	63.7	65.2
24	31.7	33.1	34.6	36.0	37.5	38.9	40.4	41.9	43.3	44.8	46.3	47.8	49.3	50.8	52.3	53.8	55.3	56.8	58.3	59.8	61.4	62.9	64.4	65.9	67.5
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.9	44.4	45.9	47.4	48.9	50.5	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.8	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	44.0	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.9	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	54.0	55.8	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.9	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.9	58.7	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.4	76.5	78.6	80.7	82.9	85.1	87.3	89.5	91.8	94.1	96.4	98.7	101.0	103.3	105.7	107.9
36	60.0	61.8	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.2	82.4	84.6	86.8	89.1	91.4	93.7	96.0	98.4	100.8	103.1	105.6	108.1	110.7	113.2
37	63.4	65.3	67.3	69.3	71.3	73.4	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	116.0	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.6	88.2	90.8	93.5	96.2	98.9	101.7	104.5	107.4	110.3	113.3	116.3	119.4	122.5	125.7	128.9	132.2	135.4	138.8	142.3	145.8	149.3
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.8	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.6	149.7	154.1	158.6	163.1	167.8	172.7	177.7	182.8	188.2
45	99.1	102.3	105.6	108.9	112.4	116.0	119.7	123.5	127.4	131.5	135.7	140.0	144.5	149.1	153.9	158.8	163.9	169.2	174.6	180.2	186.0	192.0	198.2	204.6	211.3
46	106.3	109.9	113.6	117.3	121.3	125.3	129.5	133.9	138.4	143.1	148.0	153.1	158.4	163.9	169.6	175.6	181.8	188.2	194.9	201.8	209.0	216.5	224.3	232.4	240.9
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.4	128.4	133.1	137.9	143.0	148.3	153.8	159.7	165.8	172.2	178.8	185.8	193.0	200.6	208.6	216.7	225.2	234.2	243.5	253.3	263.5	274.3	285.1	296.7	313.8
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.8	325.5	344.8	365.4	387.3	410.8	435.2

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COLILERT® & COLILERT-18®

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																						
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.5	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.8	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.3	45.3	46.4	47.6	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.8	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.8	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.4	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.3	60.6	61.8	63.1	64.4	65.7	66.9	68.2
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.2	57.4	58.7	60.0	61.2	62.5	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.1	60.5	62.0	63.4	64.9	66.4	67.9	69.4	70.9	72.4	73.9	75.4	77.0	78.5	80.1	81.6	83.2	84.8	86.4	88.0	89.6	91.2	92.8	94.4
21	61.5	62.9	64.5	65.9	67.5	69.0	70.5	72.0	73.6	75.1	76.7	78.3	79.8	81.4	83.0	84.6	86.3	87.9	89.5	91.2	92.8	94.5	96.2	97.8
22	63.9	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.9	76.5	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.8	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.8	79.4	81.1	82.8	84.6	86.4	88.2	90.0	91.8	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.9	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.7	93.7	95.7	97.7	99.8	101.9	104.0	106.1	108.3	110.5	112.7	114.9	117.2	119.5	121.8	124.1	126.5	128.9	131.3	133.8	136.3	138.8	141.4	144.0
32	96.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.2	124.6	127.0	129.4	131.9	134.4	136.9	139.5	142.1	144.8	147.5	150.2
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.8	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	108.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.8	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	113.2	117.9	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.5	160.5	163.8	166.9	170.2	173.5	176.8	179.9	183.7
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.8	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.9	168.9	173.0	177.2	181.5	185.9	190.3	194.8	199.4	204.2	208.9	213.8	218.8	223.9	229.1	234.4	239.8	245.3	250.9	256.7	262.6
42	163.3	167.5	171.8	176.2	180.7	185.3	190.0	194.8	199.7	204.8	210.0	215.3	220.7	226.3	232.0	237.8	243.8	249.9	256.2	262.6	269.2	275.9	282.8	289.9
43	173.5	178.0	182.5	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4
44	190.6	196.0	201.5	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.8	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.8	312.5	322.5	332.9	343.6	354.6	366.0	377.7	389.8	402.2	414.9	428.1	441.6
46	241.5	250.0	258.8	268.0	277.6	287.6	298.1	308.9	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.6	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.8	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	481.1	498.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.6	980.4	1046.2	1118.4	1203.3	1306.7	1413.4	1553.1	1732.9	1980.3	2418.6	12418.0

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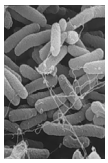

ESCHERICHIA COLI (E.COLI)

Modified mTEC Agar with Membrane Filtration

26

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EPA METHOD 1603

- Membrane Filter – modified mTEC agar
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)
- Place sample in a petri dish with modified mTEC agar
- Invert dish and incubate for **35± 0.5°C for 2 hours**
  - Resuscitates injured or stressed bacteria
- Then incubate at **44.5± 0.2°C for 22 hours**
- After incubation, remove the plate from the water bath or dry air incubator
- Daily QC adds quite a bit to this test

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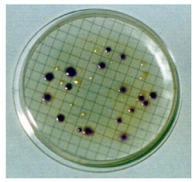
METHOD 1603

- Count and record the number of **red or magenta** colonies (verify with stereoscopic microscope)
- See the USEPA microbiology methods manual, Part II, Section C, 3.5, for general counting rules



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METHOD 1603

- QC Tests:
  - Initial precision and recovery
  - Ongoing precision and recovery
  - Matrix spike
  - Negative control
  - Positive control
  - Filter sterility check
  - Method blank
  - Filtration blank
  - Media sterility check

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METHOD 1603

- Initial precision and recovery
  - Should be performed by each lab before the method is used for monitoring field samples
- Ongoing precision and recovery
  - Run after every 20 field and matrix spike samples or one per week that samples are analyzed
- Matrix spike
  - Run 1 per 20 samples

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METHOD 1603

- Negative control
  - Should be analyzed whenever a new batch of media or reagents is used
- Positive control
  - Should be analyzed whenever a new batch of media or reagents is used
- Filter sterility check
  - Place at least one membrane filter per lot of filters on a tryptic soy agar (TSA) plate and incubate for 24 ± 2 hours at 35°C ± 0.5°C .
  - Absence of growth indicates sterility of the filter.
  - Run ***daily***.

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METHOD 1603

- Method blank
  - Filter a 50-mL volume of sterile buffered dilution water and place on a modified mTEC agar plate and incubate.
  - Absence of growth indicates freedom of contamination from the target organism.
  - Run ***daily***.
- Filtration blank
  - Filter a 50-mL volume of sterile buffered dilution water and place on a TSA plate and incubate at just at 35°C ± 0.5°C for 24 ± 2 hours .
  - Absence of growth indicates sterility of the buffer and filtration assembly.
  - Run ***daily***.

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METHOD 1603

- Media sterility check
  - The lab should test media sterility by incubating one unit (tube or plate) from each batch of medium (TSA, modified mTEC and verification media) as appropriate and observing for growth.
  - Absence of growth indicates media sterility.
  - Run ***daily***.

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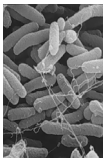

ESCHERICHIA COLI (E.COLI)

m-ColiBlue24® with Membrane Filtration

34

34

M-COLIBLUE24®

- Membrane Filter
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)

35

M-COLIBLUE24®

- Place sample in a petri dish with absorbent pad containing 2 mL mColiBlue 24 broth
- Invert dish and incubate at **35± 0.5°C for 24 ±2 hours**

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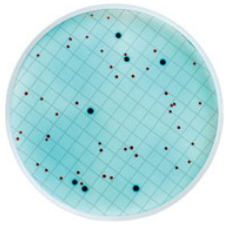
M-ColiBlue24®

- After incubation, remove the plate from the water bath or dry air incubator
- Count and record the number of **blue** colonies (verify with stereoscopic microscope)
- See the USEPA microbiology methods manual, Part II, Section C, 3.5, for general counting rules



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E. coli Data Analysis

- Maximum sample hold time: 6 hrs
- Samples and equipment known or suspected to have viable E. coli attached or contained must be sterilized prior to disposal.

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E. coli Data Interpretation

- Permit limit: 126 colonies/100 mL monthly average; or daily max of 487 or 941/100 mL depending on permit
- For MF method:
  - Select sample volumes to produce 20-80 colonies on the membranes.
  - Run minimum of 3 dilutions.
  - Must use sterile buffered water for dilutions and to rinse filtration unit.

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Expected Reactions of Various Microorganisms

- Total coliforms will produce a red colony
  - Enterobacter species
    - *E. cloacae*
    - *E. aerogenes*
  - Klebsiella species
    - *K. pneumoniae*
  - Citrobacter species
    - *C. freundii*
- *Escherichia coli* will produce a blue colony
  - *E. coli* O157:H7 will not produce a blue colony, but will grow as a red colony

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Expected Reactions of Various Microorganisms

- Known negative reaction (no growth) after 24-25 hours
  - *Pseudomonas aeruginosa*
    - *Variable reaction may be positive for total coliform when incubated longer than 25 hours*
  - *Proteus vulgaris*
  - *Aeromonas hydrophila*

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Expected Reactions of Various Microorganisms

- Some strains of the following microorganisms are known to produce a false-positive total coliform reaction (a red colony, but not a true total coliform)

•Serratia species	*•Yersinia enterocolitica*
•Hafnia alvei	*•Leclercia adecarboxylata*
•Vibrio fluvialis	*•Ewingella americana*
•Aeromonas species	*•Staphylococcus species*
•Proteus vulgaris	*•Proteus mirabilis*
•Providencia stuartii	

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M-ColiBlue24® Trouble-Shooting Guide, Hach Company, www.Hach.com

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E. COLI INFORMATION

- For Colilert ®: IDEXX Laboratories, www.idexx.com
- For mTEC Agar and mColiBlue-24® media: Hach Company, www.Hach.com
- EPA Method 1603: E.coli In Water By Membrane Filtration Using Modified-Thermotolerant Escherichia coli Agar (Modified mTEC), September 2002, EPA-821-R-02-023

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E. COLI

- Two Approved Methods
  - SM 9223 B – 2016 IDEXX Colilert Quanti-Tray
  - Hach Method 10029 – m -ColiBlue24® -

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SM 9020 B. QC GUIDELINES

- General Considerations
  - The program must be practical and require only a reasonable amount of time or it will be bypassed.
- Facilities
  - Provide a dust and draft free lab that has a stable temperature that does not have extreme temperature variations.
  - Minimize through traffic and visitors
  - Provide adequate space for conducting the analysis
  - Keeps work area clean and disinfected

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SM 9020 B. QC GUIDELINES

- Lab Equipment
  - Verify thermometer accuracy annually. (~LabtronX or other certification vendor) 9020 B.4.a
  - UV lamps – 9020 B.4.l (if used)
    - Clean monthly with soft cloth moistened with ethanol
    - Recommend replacing bulbs annually
  - Incubators – 9020 B.4.o
    - Verify thermometers ~ annually
    - Record temperature twice daily (day of), at least 4 hours apart
    - Verify that cold samples incubate for specified time. May need to warm samples in very cold weather
    - Protect incubator from extreme room temperatures. Ideal is 60-80°F

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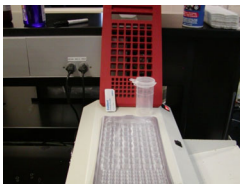
SM 9020 B. QC GUIDELINES

- Lab Equipment - continued
  - Media
    - Check reagent media appearance with each use and discard if there is a color change.
    - Protect reagent media from light
  - Refrigerators – 9020 B.4.i
    - Maintain temperature at 2-8°C
    - Check and record temps daily (day of)



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SM 9020 B. QC GUIDELINES

- Lab Equipment – continued
  - Membrane Filtration Equipment (if MF procedure is used) – 9020 B.4.k
    - Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize
    - UV sterilize or boil funnel apparatus between samples
      - If using boiling water, make sure membrane filtration equipment is cool before adding next sample

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SM 9020 B. QC Guidelines

- Lab Equipment – continued
  - Autoclave – 9020 B.4.h
    - For routine use, verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached
    - Test **monthly** for sterilization efficacy (with *Geobacillus stearothermophilus*)
    - If any media, bottles, filters or other equipment that comes into contact with the samples are sterilized in the autoclave, the sterilization efficacy test must be performed **monthly**
    - If you are only using your autoclave to sterilize waste, you just need an MRT (maximum registering thermometer)

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SM 9020 B. QC Guidelines

- Lab Equipment – continued
  - Membrane filters and pads (if MF procedure is used) – 9020 B.5.i.3
    - Check filters for brittleness if lot is held for one or more years

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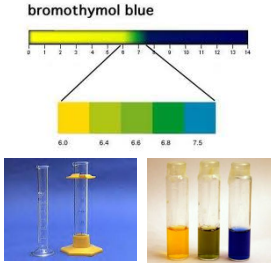
SM 9020 B. QC Guidelines

- Lab Supplies
  - Glassware – 9020 5.1
    - pH check to test clean glassware for alkaline or acid residue, add a few drops of 0.04% bromothymol blue (BTB) or other pH indicator and observe the color reaction.
      - BTB should be blue-green in the acceptable neutral range



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SM 9020 B. QC Guidelines

- Lab Supplies – continued
  - Dilution water bottles – 9020 B.5.c
    - Dilution waters available commercially are acceptable.
    - Check one per lot for pH and volume (99 ± 2 mL) and examine bottles for a precipitate
    - Discard by expiration date
    - Before use of each batch conduct sterility (one bottle per batch) – More information on slide #55

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SM 9020 B. QC Guidelines

- Lab Supplies – continued
  - Dilution water bottles sterility check - continued
    - Sterility Checks – 9020B.9.d
      - Check each new batch (or lot) of buffered water for sterility before first use by adding 50 mL of water to 50 mL of a double-strength broth (e.g. tryptic soy, trypticase soy or tryptose broth).
      - Alternatively, aseptically pass 100 mL of dilution water through a membrane filter and place filter on nonselective medium.
      - Incubate at 35±0.5°C for 24 hours and observe for growth.
      - For membrane filter tests, check the sterility of the entire process by using sterile reagent or dilution water as the sample at the beginning and end of each filtration series of samples and test for growth

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SM 9020 B. QC Guidelines

- Lab Supplies – continued
  - Sample bottles – 9020 B.5.d.
    - Minimally test for sterility one sample bottle per batch sterilized in the lab. Document results. – More information on slide #55
    - Check accuracy of 100 mL mark, one per lot and record results.

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SM 9020 B. QC Guidelines

- Lab Supplies – continued
  - Multi-well trays and sealers – 9020 B.5.e
    - Analyze a method blank once per lot (of sterile water, media, bottles and trays) or once per quarter, whichever is more frequent, to demonstrate sterility.
    - Check one per lot for sterility beforehand by aseptically adding 100 mL of sterile trypic soy broth or other non-selective medium, sealing, and incubating at 35 ± 0.5 °C

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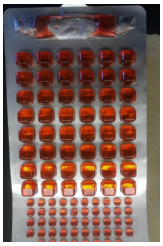
SM 9020 B. QC Guidelines

- Lab Supplies – continued
  - Multi-well trays and sealers – 9020 B.5.e
    - Evaluate sealing performance of heat sealer unit **monthly** by adding one to two drops of food-color dye to 100 mL deionized water sample, run through sealer, and visually check each well for leakage.
    - As a monthly check of a sealer efficacy, perform and document a visual check that trays are properly sealed. If all sample wells are positive for total coliform and sufficient contrast, visually examine the tray cells for leakage and document the check. If insufficient color contrast is present us food-color dye as previously recommended by method.
    - Perform cleaning and maintenance on sealer annually or more frequently, if needed.



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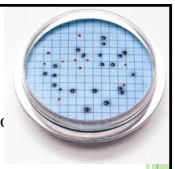
SM 9020 B. QC Guidelines



- Coliforms – Total and E. coli Hach Meth – m-ColiBlue24®
  - Blank – daily (day of)
    - Run at least one membrane filter blank at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter, placing in a petri dish with mColiBlue broth and testing for growth.
  - Positive and Negative Controls – Check certified control cultures with each lot of media and petri dishes with pads OR once a quarter, whichever is more frequent.
    - Pseudomonas aeruginosa is recommended as a negative control and Escherichia coli as a positive control.
  - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.

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SM 9020 B. QC Guidelines

- Enzyme Substrate Test SM 9223 B, 23nd Edition (2016) – Colilert Method
  - Quality Control
    - Verify its performance via positive and negative control organisms.
      - Inoculate medium with 3 control bacteria
    - Also add a sterile water control. If a sterile water control exhibits faint fluorescence or faint positive coliform, discard use and use a new batch of substrate.
    - Incubate these controls at 35±0.5°C as indicated above.
  - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.

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SM 9020 B. QC Guidelines

- Reporting Duplicates
  - Both results should be documented on bench sheet.
  - All duplicates should be reported according to your permit limits. If your permit sets a maximum limit, then the maximum value should be reported even if falls outside your permit limit.
  - If both values fall below your daily max, average (arithmetically) the daily duplicates to get one result and then using that averaged result as part of the monthly geometric mean calculation.

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Membrane Filter Counts

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Within Acceptable Limits

$$\text{Count per 100 mL} = \frac{\text{Number of colonies}}{\text{Vol. of sample filtered (mL)}} \times 100$$

- Assume that filtration of volumes 50, 15, 5, 1.5 and 0.5 mL produced colony counts of 200, 110, 40, 10 and 5 respectively
- You do not need to count the colonies on all the filters. Select the membrane filters (MF's) with 20-60 Fecal Coliforms and 20-80 E. coli colonies

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Within Acceptable Limits

$$\text{Count per 100 mL} = \frac{\text{Number of colonies}}{\text{Vol. of sample filtered (mL)}} \times 100$$

- After selecting the best MF's with a 40 colony count, you apply the general formula as follows

$$\text{Count per 100 mL} = \frac{40}{5} \times 100 = 800/100 \text{ mL}$$

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More Than One Acceptable Count

- If there are acceptable counts on replicate plates, carry counts independently to final reporting units, then calculate the arithmetic mean of these counts to obtain the final reported value

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More Than One Acceptable Count

- For example, 1 mL volumes produce coliform counts of 26 and 36 or counts of 2600 and 3600/100 mL

$$\frac{2600 + 3600}{2} = 3100/100 \text{ mL}$$

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More Than One Acceptable Count

- If more than one dilution, independently carry counts to final reporting units, then average for final reported value

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More Than One Acceptable Count

- For example, assume that volumes of 0.3, 0.05, 0.03 and 0.01 mL produced coliform colony counts of TNTC (Too Numerous To Count), 55, 30 and 8 respectively.
- In this example, two volumes, 0.05 and 0.03 produce colonies in the acceptable counting range

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More Than One Acceptable Count

- Independently carry each MF count to a count per 100 mL

$\frac{55}{0.05} \times 100 = 110{,}000/100\ mL$ $\frac{30}{0.03} \times 100 = 100{,}000/100\ mL$

- Then calculate the arithmetic mean of these counts to obtain the final reported value

$$\frac{110{,}000 + 100{,}000}{2} = 105{,}000/100\ mL$$

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If All MF Counts are Below the Lower Limit

- Select the most nearly acceptable count
- For example, assume a count in which sample volumes of 1, 0.3 and 0.01 mL produced colony counts of 14, 3 and 0 respectively
- Here, no colony count falls within the recommended limits.
  - Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark

$\frac{14}{1.0} \times 100 = 1400/100\ mL$

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If All MF Counts are Below the Lower Limit

- Here, no colony count falls within the recommended limits.
  - Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark

$\frac{14}{1.0} \times 100 = 1400$ **Report as: estimated 1400/100mL**

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If Counts from All MF are Zero

- Calculate using count from largest filtration volume
- For example, sample volumes of 25, 10 and 2 mL produced colony counts of 0, 0 and 0 respectively and no actual calculation is possible, even as an estimated report.

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If Counts from All MF are Zero

- Calculate the number of colonies per 100 mL that would have been reported if there had been one colony on the filter representing the largest filtration volume

$\frac{1}{25} \times 100 = 4$ **Report as: < (Less than) 4/100mL**

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If All Membrane Counts are Above the Upper Limit

- Calculate the count with the smallest volume filtered
- For example, assume that the volumes 1, 0.3 and 0.01 mL produced colony counts of TNTC, 150 and 110.

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If All Membrane Counts are Above the Upper Limit

- Since all colony counts are above the recommended limit, use the colony count from the smallest sample volume filtered and estimate the count as

$$\frac{110}{0.01} \times 100 = 1{,}100{,}000$$ **Report as: estimated 1,100,000/100 mL**

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If Colonies are Too Numerous To Count

- Use upper limit with smallest filtration volume
- For example, assume that the volumes 1.0, 0.3 and 0.01 mL all produced too many colonies to show separated colonies and that the laboratory bench records showed TNTC

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If Colonies are Too Numerous To Count

- Use 60 colonies for Fecals and 80 for E. coli as the basis of calculation with the smallest filtration volume

$$\frac{60}{0.01} \times 100 = 600{,}000$$ **Report as: > (Greater Than) 600,000/100 mL**

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Calculating Geometric Mean

- When there are individual sample results that are reported as <, > or estimated
  - If any individual sample result is reported as an estimate, drop the estimate when calculating the geometric mean

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Calculating Geometric Mean

- When there are individual sample results that are reported as <, > or estimated
  - If there are any individual samples reported as <, drop the < signs when calculating the geometric mean
    - However, report the geometric mean as a < value

77

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Calculating Geometric Mean

- When there are individual sample results that are reported as <, > or estimated
  - If there are any individual samples reported as >, drop the > signs when calculating the geometric mean
    - However, report the geometric mean as a > value

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Calculating Geometric Mean

- When there are individual sample results that are reported as <, > or estimated
  - If there are samples reported as < and one or more samples reported as >, drop the < and > signs when calculating the geometric mean
    - However, report the geometric mean as a > value

EPA Microbiological Methods for Monitoring the Environment Water and Wastes, EPA-600/8-78-017, December 1978

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Any Questions?

TDEC - Fleming Training Center

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Bacteriological Analysis – Review Questions

Laboratory Portion:

1. What bacteriological tests did we conduct (include method numbers)? And what media is associated with each of these tests?

2. Was there a difference in the type of reagent water we used in the 2 separate tests? Explain your answer.

3. The sample we tested was river water. What factors did we use to determine the dilutions that were used in class?

4. During the membrane filtration procedure, we had to place the membrane filtration apparatus into the UV sterilization box in between each sample/dilution for how long? What is the purpose of this step? And what control did we use to test the effectiveness of the UV box?

5. *E.coli* was indicated by what color of bacterial colony in the mColiBlue-24 test?

6. In the Colilert test, yellow coloration indicates what? What does fluorescence indicate?

7. What was the final step in today's laboratory experiment?

Classroom portion:

8. What was used to sterilize the reagent water used in our *E. coli* tests?

9. What does MPN stand for?

10. Materials suspected of containing viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding. True or False?

11. The Fecal Coliform test uses a water bath or an air incubator set at what temperature?

12. What is the maximum holding time and preservation requirements for Total Coliform, Fecal Coliform and E. coli tests?

13. What is the purpose of Sodium Thiosulfate in bacterial tests? What is the chemical formula?

14. How does the Fecal Coliform test relate to 40 CFR 503 Pathogen Reduction Alternatives for Class B Biosolids?

15. The Colilert samples were incubated for how long and at what temperature? The results are definitive for how long?

16. The mColiBlue-24 samples were incubated for how long and at what temperature?

17. If you do not have access to a UV Sterilization box, how would you sterilize your membrane filtration apparatus in between samples? Name some important things to keep in mind when using this technique.

18. What is the NPDES permit limit for *E.coli*?

19. When choosing which dilution to count for *E. coli* calculations, choose the filters that have what number range of colonies?

20. E.coli results are reported on the DMR as geometric mean. True or False?

Coliforms—Total and *E. coli*

USEPA Membrane Filtration Method

Method 10029[1]

m-ColiBlue24®

Scope and Application: For potable water, nonpotable water, recreation water and wastewater.

[1] USEPA approved.

Test preparation

Before starting the test:

When the sample is less than 20 mL (diluted or undiluted), add 10 mL of sterile dilution water to the filter funnel before applying the vacuum. This aids in distributing the bacteria evenly across the entire filter surface.
The volume of sample to be filtered will vary with the sample type. Select a maximum sample size to give 20 to 200 colony-forming units (CFU) per filter. The ideal sample volume of nonpotable water or wastewater for coliform testing yields 20–80 coliform colonies per filter. Generally, for finished, potable water, the volume to be filtered will be 100 mL.
If using PourRite™ ampules, allow the media to warm to room temperature before opening.
Disinfect the work bench with a germicidal cloth, dilute bleach solution, bactericidal spray or dilute iodine solution. Wash hands thoroughly with soap and water.

m-ColiBlue24 Broth PourRite Ampules

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages; surface, well, and groundwater, waste water, recreational waters and process water for ultrapure, chemical processing and pharmaceutical applications.

Simultaneous total coliform and *E. coli* screening, method 10029

1. Use sterilized forceps to place a sterile, absorbent pad in a sterile petri dish. Replace the lid on the dish.

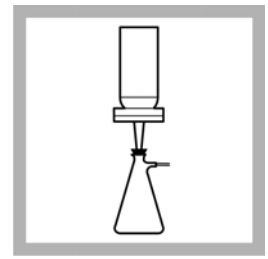
Do not touch the pad or the inside of the petri dish.

To sterilize the forceps, dip them in alcohol and flame in an alcohol or Bunsen burner. Let the forceps cool before use.

2. Invert ampules two or three times to mix broth. Break open an ampule of m-ColiBlue24 Broth using an ampule breaker. Pour the contents evenly over the absorbent pad. Replace the petri dish lid.

3. Set up the Membrane Filter Apparatus. With sterile forceps, place a membrane filter, grid side up, into the assembly.

Alternatively, a sterile disposable filter unit may be used.

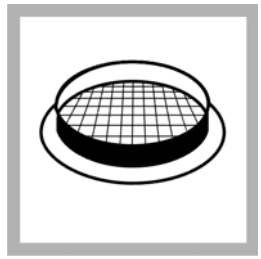


4. Invert the sample for 30 seconds to mix. Pour 100 mL of sample or diluted sample into the funnel. Apply vacuum and filter the sample. Rinse the funnel walls with 20 to 30 mL of sterile buffered dilution water. Apply vacuum. Rinse again two more times.

Release the vacuum when the filter is dry to prevent damage to the filter.

Simultaneous total coliform and *E. coli* screening, method 10029 (continued)

5. Turn off the vacuum and lift off the funnel top. Using sterile forceps, transfer the filter to the previously prepared petri dish.



6. With a slight rolling motion, place the filter, grid side up, on the absorbent pad. Check for trapped air under the filter and make sure the filter touches the entire pad. Replace the petri dish lid.

7. Invert the petri dish and incubate at 35 ± 0.5 °C for 24 hours.

8. Remove the petri dish from the incubator and examine the filters for colony growth. Colonies are typically readily visible; however, a microscope or other 10–15X magnifier may be useful. Red and blue colonies indicate total coliforms and blue colonies specifically indicate *E. coli*.

Sometimes only the center of a colony will show color. Therefore, a colony with any amount of red color should be counted as red and a colony with any amount of blue should be counted as a blue colony. Red colonies may vary in color intensity. Blue colonies may appear blue to purple. Count all the red and blue colonies as total coliforms. Count all the blue to purple colonies as *E. coli.*

Optional testing of red colonies

The m-ColiBlue24 Broth is formulated so that coliforms other than *E. coli* grow as red colonies. The percentage of red colonies that are false positives (non-coliforms) is comparable to the percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms); therefore, confirmation is not required.

A few varieties of the non-coliform bacteria *Pseudomonas, Vibrio,* and *Aeromonas* spp. may grow on m-ColiBlue24 Broth and form red colonies. Such bacteria can be readily distinguished from total coliforms by the oxidase test. *Pseudomonas, Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If your sample contains high levels of interfering bacteria, you can perform an oxidase test to confirm which red colonies are total coliforms.

Two oxidase procedures are provided. Count the red and blue colonies on the m-ColiBlue24 Broth membrane filter before starting the oxidase test.

Oxidase method 1
This method enables you to conveniently and rapidly evaluate membrane filters that have numerous colonies. Use this method after 24 hours of incubation on m-ColiBlue24 Broth.

Research* shows that the oxidase test cannot be performed on media that undergoes acidification during bacterial growth. The m-ColiBlue24 Broth is formulated so that the medium does not undergo such acidification. Consequently, many colonies can be simultaneously tested for their oxidase reaction using the following procedure.

1. Remove the lid from the petri dish containing the m-ColiBlue24 Broth membrane filter, invert the lid, and place it on the bench top.

   Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive controls and *Escherichia coli* for negative controls. Use Aqua QC-Stiks™ for quality control procedures.

2. Drop approximately 0.5 mL of Difco SpotTest™ Oxidase Reagent into the center of the inverted lid.

3. Using sterile forceps, transfer the membrane filter from the pad and place the filter upright in the inverted lid.

4. Within 10 to 15 seconds, the oxidase reagent will soak into the filter and cause the oxidase-positive colonies to turn purple. This purple color may be visible in the colony itself or adjacent to the colony. Oxidase-negative colonies will retain the red color they developed when incubated on m-ColiBlue24 Broth.

5. After the initial 10 to 15 second reaction time, start counting the red colonies that turn purple. Count individual colonies by using a microscope with 10–15X magnification

   Note: *To simplify colony counting place a spare lid on the lid containing the oxidase reagent and membrane filter. Use a felt-tip pen to mark the lid as you identify the purple colonies. After 30 seconds, you can count marks that indicate purple (oxidase-positive) colonies.*

6. Stop counting 30 seconds after initial 10 to 15 second reaction time, because oxidase-negative colonies will start to develop a purple color.

   Note: *Bacteria are not killed with this procedure, so colonies may be selected for streaking and for additional testing.*

Colonies that are blue after the initial 24-hour incubation on m-ColiBlue24 Broth are almost always *E. coli* and do not need confirmation with the oxidase procedure.

Oxidase method 2
This method is the official oxidase test described in *Standard Methods for the Examination of Water and Wastewater,* 18th edition, 1992.

1. Select red colonies from an m-ColiBlue24 Broth membrane filter and streak onto Tryptic Soy Agar.

2. Incubate Tryptic Soy Agar plates at 35 °C (95 °F) for 18–24 hours or until isolated colonies are obtained.

* A.H. Havelaar et al. 1980. False-negative oxidase reaction as a result of medium acidification. *Antonie van Leeuwenhoek*. 46, 301-312. L.K. Hunt et al. 1981. Role of pH in oxidase variability of *Aeromonas hydrophila. Journal of Clinical Microbiology*. 13: 1054-1059.

Controls: Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended for positive and *Escherichia coli* for negative controls. Use Aqua QC-Stiks™* for quality control procedures.

3. Saturate a piece of filter paper with Difco SpotTest Oxidase Reagent. (This reagent contains a stabilized solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride.)

   Note: *Alternatively, oxidase reagent can be dropped directly onto colonies growing on Tryptic Soy Agar. Oxidase-positive colonies will turn from pink to purple.*

4. Using a sterile nichrome inoculating needle, transfer cellular material from an isolated Tryptic Soy Agar colony to the moist filter paper.

   Note: *Do not use iron or other reactive needles for inoculation, because they will cause false-positive results. Wooden applicator sticks work well.*

5. Oxidase-negative colonies will not react with the reagent, but oxidase-positive colonies will cause the reagent to turn dark purple within 10 seconds.

6. Oxidase-negative colonies should be counted as total coliform bacteria.

Interpreting and reporting results

Report coliform density as the number of colonies per 100 mL of sample. For total coliforms, use samples that produce 20 to 80 coliform colonies, and not more than 200 colonies of all types, per membrane to compute coliform density. For fecal coliform testing, samples should produce 20 to 60 fecal coliform colonies.

Use ***Equation A*** to calculate coliform density. Note that "mL sample" refers to actual sample volume, and not volume of the dilution.

Equation A—Coliform density on a single membrane filter

$$\text{Coliform colonies per 100 mL} = \frac{\text{Coliform colonies counted}}{\text{mL of sample filtered}} \times 100$$

- If growth covers the entire filtration area of the membrane, or a portion of it, and colonies are not discrete, report results as "Confluent Growth With or Without Coliforms."
- If the total number of colonies (coliforms plus non-coliforms) exceeds 200 per membrane or the colonies are too indistinct for accurate counting, report the results as "Too Numerous To Count" (TNTC).

In either case, run a new sample using a dilution that will give about 50 coliform colonies and not more than 200 colonies of all types.

When testing nonpotable water, if no filter meets the desired minimum colony count, calculate the average coliform density with Equation B.

Equation B—Average coliform density for 1) duplicates, 2) multiple dilutions, or 3) more than one filter/sample

$$\text{Coliform colonies per 100 mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

Controls:
Positive and negative controls are important. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control. Use the AQUA QC-STIK™ Device for quality control procedures. Instructions for use come with each AQUA QC-STIK Device.

Potable water samples from municipal treatment facilities should be negative for total coliforms and fecal coliforms.

* Aqua QC-Stiks is a trademark of MicroBiologics.

Consumables and replacement items

Required media and reagents

Description	Unit	Catalog number
m-ColiBlue24® Broth Ampules, glass	20/pkg	2608420
m-ColiBlue24® Broth Ampules, plastic	50/pkg	2608450
m-ColiBlue24® prepared agar plates	15/pkg	2805215

Required apparatus

Description	Unit	Catalog number
Ampule Breaker, PourRite™	each	2484600
Bags, Whirl-Pak®, with dechlorinating agent, 180 mL	100/pkg	2075333
Counter, hand tally	each	1469600
Dilution Water, buffered, sterile, 99 mL	25/pkg	1430598
Dish, Petri, with pad, 47-mm, sterile, disposable, Gelman	100/pkg	1471799
Dish, Petri, with pad, 47-mm, sterile, disposable, Millipore	150/pkg	2936300
Filter Holder, magnetic coupling (use with 24861-00)	each	1352900
Filter Funnel Manifold, aluminum, 3-place (use with 13529-00)	each	2486100
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Gelman	200/pkg	1353001
Filters, Membrane, 47-mm, 0.45-µm, gridded, sterile, Millipore	150/pkg	2936100
Filtering Flask, 1000-mL	each	54653
Forceps, stainless steel	each	2141100
Incubator, Culture, 120 VAC, 50/60 Hz	each	2619200
Incubator, Culture, 220 VAC, 50/60 Hz	each	2619202
Microscope, Compound	each	2942500
Pump, vacuum/pressure, portable, 115 VAC, 60 Hz	each	2824800
Pump, vacuum/pressure, portable, 220 VAC, 50 Hz	each	2824802
Stopper, rubber, one hole, No. 8	6/pkg	211908
Tubing, rubber, 0.8 cm ($^{5}/_{16}$ in.) ID	3.7 m (12 ft)	56019

Optional media, reagents and apparatus

Description	Unit	Catalog number
Adapter for rechargeable battery pack, 230 VAC (for 2580300)	each	2595902
Alcohol Burner	1	2087742
Aspirator, water	each	213102
Autoclave, 120 VAC, 50/60 Hz	each	2898600
Bag, for contaminated items	200/pkg	2463300
Bags, Whirl-Pak®, without dechlorinating agent, 207 mL	100/pkg	2233199
Bags, Whirl-Pak®, without dechlorinating agent, 720 mL	10/pkg	1437297
Battery eliminator	each	2580400
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	12/pkg	2599112

Optional media, reagents and apparatus (continued)

Description	Unit	Catalog number
Bottle, sample, sterilized, 100-mL, disposable with dechlorinating agent	50/pkg	2599150
Bottle, sample, sterilized, 100-mL, disposable	12/pkg	2495012
Bottle, sample, sterilized, 100-mL, disposable	50/pkg	2495050
Bunsen burner with tubing	each	2162700
Dechlorinating Reagent Powder Pillows	100/pkg	1436369
Dish, Petri, 47-mm, sterile, disposable	100/pkg	1485299
Dish, Petri, 47-mm, sterile, disposable	500/pkg	1485200
Filter Funnel Manifold, aluminum, 3-place (use with 1352900)	each	2486100
Filter Unit, sterile, disposable with gridded membrane (use with 2656700)	12/pkg	2656600
Filtration Support (for field use), stainless steel	each	2586200
Funnels, Push-Fit and membrane filters (use with 2586200)	72/pkg	2586300
Germicidal Cloths	50/pkg	2463200
Incubator, portable, 12 VDC	each	2569900
Incubator, water bath, 120 VAC, 50/60 Hz	each	2616300
Isopropyl alcohol	500 mL	1445949
m-ColiBlue24® Broth, 100 mL glass bottle	1 each	2608442
Pad, absorbent, with dispenser	1000/pkg	1491800
Powder Pillows for buffered dilution water (25 of each)[1]	50/pkg	2143166
Pump, hand vacuum	each	1428300
Sterilization Indicator, Sterikon®	15/pkg	2811115
Sterilization Indicator, Sterikon®	100/pkg	2811199
Syringe, 140-mL, polypropylene (use with 2586200)	each	2586100
Wicks, replacement, for alcohol burner 2087742	—	2097810

[1] Add the contents of one potassium dihydrogen phosphate and one magnesium chloride powder pillow to one liter of distilled water and autoclave (sterilize) to prepare American Public Health Association buffered dilution water.

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
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HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

Simultaneous Detection and Enumeration of Total Coliforms and Escherichia coli using m-ColiBlue24 Membrane Filtration Medium

Hach Company Method 10029
Rev. 2, 1999

1.0 Scope and Application

1.1 This test method describes a sensitive and differential membrane filter (MF) medium, using m-ColiBlue24 agar or broth, for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities and selective dye. m-ColiBlue24 is a nutritive, lactose-based medium, containing inhibitors to selectively eliminate growth of non-coliforms. It is analogous to an improved version of m-Endo. Total coliform colonies growing on the medium are highlighted by a non-selective dye, 2,3,5-Triphenoltetrazolium Chloride (TTC), which produces red colored colonies. Among the total coliform colonies, which grow up on the medium, any *E. coli* colonies are distinguishable by a selective blue color, resulting from the action of b-glucuronidase enzyme on 5-Bromo-4-Chloro-3-Indolyl-Beta-D-glucuronide (BCIG).

1.2 Total coliforms include species that may inhabit the intestines of warm-blooded animals or occur naturally in soil, vegetation, and water. They are usually found in fecal-polluted water and are often associated with disease outbreaks. Although they are not usually pathogenic themselves, their presence in drinking water indicates the possible presence of pathogens. *E. coli*, one species of the coliform group, is always found in feces and is, therefore, a more direct indicator of fecal contamination and the possible presence of enteric pathogens. In addition, some strains of *E. col*i are pathogenic (Reference 16.12).

1.3 This method, which has been validated for use with drinking water, source water, and wastewater in single-lab and multi-lab studies (References 16.8 - 16.10).

1.4 Since a wide range of sample volumes or dilutions can be analyzed by the MF technique, a wide range of *E. coli* and TC levels in water can be detected and enumerated.

2.0 Summary of Method

2.1 An appropriate volume of a water sample (100 mL for drinking water) is filtered through a 47-mm, 0.45-µm pore size cellulose ester that retains the bacteria present in the sample. The filter is then transferred to a 50-mm Petri plate containing an absorbent pad saturated with m-ColiBlue24 broth or m-ColiBlue24 agar plate and incubated at 35^{o} C for up to 24 hours. Both red and

blue colonies may appear; the blue colonies are specific to the presence of *E. coli* while the red colonies are specific to non-*E. coli* coliforms.

3.0 Definitions

3.1 Total Coliform Bacteria – Bacteria belonging to the genera *Klebsiella* sp., *Enterobacter* sp., *Cirobacter* sp., or *Escherichia* sp.

3.2 Coliform Positive Colony – A red or blue colony.

3.3 Coliform Negative Colony – A clear or white colony.

3.4 *Escherichia coli* or *E. coli* Bacteria – A genus within the total coliform group typified by possession of the enzyme *b*-Glucuronidase, ability to grow at 44.5^{o} C, and form indole from tryptophan.

3.5 *E. coli* Positive Colony – A blue colony.

3.6 *E. coli* Negative Colony - A non-blue colony.

4.0 Interferences

4.1 No interferences to the colony color development have been found in drinking water, source water, and wastewater samples. Similarly, particulates in water samples do not alter the efficacy of the medium, although excess particulates may cause colonies to grow together on crowed filters or slow the sample filtration process.

5.0 Safety

5.1 Standard safety practices appropriate to microbiology laboratories should be followed.

5.2 Solid and liquid waste materials containing or suspected to contain viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding.

5.3 Refer to the appropriate Material Safety Data Sheets supplied for each reagent for comprehensive safety data essential to proper use.

6.0 Equipment and Supplies

6.1 Equipment

6.1.1 Air Incubator – Capable or operating at 35^{o} C ± 0.5^{o} C.

6.1.2 Vacuum pump.

6.1.3 Membrane filtration-funnel unit and flask.

6.1.4 Dissecting microscope, capable of 10-15X magnification. The microscope should be equipped with a fluorescent illuminator.

6.2 Supplies/Glassware – Cleanse all glassware thoroughly with a suitable detergent and hot water, rinse with hot water to removes traces of detergent residual, and rinse again with laboratory-pure water. Sterilize all glassware by autoclaving 15 min. at 121^{o} C or by heating in an oven for at least 1 hour at 170^{o} C.

6.2.1 Pre-sterilized 50-mm MF Petri plates with pads

6.2.2 45-mm pre-sterilized membrane filters.

6.2.3 Sterile forceps.

6.2.4 Sterile glass or plastic sample collection containers.

6.2.5 Sterile graduated cylinders.

6.2.6 Sterile pipettes.

6.2.7 Sterile MF filtration unit.

6.2.8 Side-arm flask.

6.2.9 Biohazard bag.

7.0 Reagents and Standards

7.1 Growth Medium

7.1.1 m-ColiBlue24 broth (Hach Number 2608420, 2608442, or 2608450) or m-ColiBlue24 agar plates (Hach Number 2805215).

7.2 Dechlorinating Reagent

7.2.1 Hach dechlorinating reagent Powder Pillow (1436369) containing sodium thiosulfate and sodium sulfate.

7.2.2 Prepare a 3% sodium sulfate solution by adding 44.18 g of sodium thiosulfate pentahydrate to approximately 500 mL of deionized water, then dilute to 1 L with deionized water.

7.3 Buffered Dilution Water

7.3.1 Magnesium Chloride and Potassium Dihydrogen Phosphate Buffer, 99mL per dilution bottle (Hach Number 1430598).

8.0 Sample Collection, Dechlorination, Preservation, Shipment and Storage

8.1 Water Sample Collection

8.1.1 Sample Collection Containers – Samples should be collected in sterile, clean glass or heat-resistant bottles. Pre-sterilized Whirl-Pak® Bags may also be used.

8.1.2 Sample Procedure – Potable water samples are taken by first flushing the tap 2-3 minutes to clear the service line. Collect samples using aseptic techniques to avoid contamination. For other samples, aseptically collect water representative of the source.

8.2 Dechlorination – Water containing chlorine or other halogens must be treated with sodium thiosulfate to allow accurate evaluation of microbial content. Add one dechlorinating reagent Powder Pillow (1436369) by aseptically cutting of the tip of an alcohol-rinsed pillow and pouring the contents into 100 mL of the chlorinated water sample. Alternatively, pipette 0.1 mL of a 3% sodium thiosulfate solution into 100 mL of the chlorinated sample. Pre-sterilized Whirl-Pak Bags contain sufficient sodium thiosulfate powder to neutralize a 100 mL chlorinated water sample.

8.3 Preservation, Shipment, Storage – Samples should be tested as soon as possible. If analysis cannot be done within 1 hr of collection, water samples should be held on ice or refrigerated to 2-8^{o} C for a maximum holding time of 8 hours from sampling.

9.0 Quality Control

9.1 m-ColiBlue24 undergoes quality control (QC) testing at the time of manufacture. A Certificate of Analysis is include with every m-ColiBlue24 shipment stating the m-ColiBlue24, as received by the analysis, is ready for use in analyzing water samples by the membrane filtration procedure[1]. It is recommended that the

[1] **Note:** Hach Company has verified the performance of Method 10029 (m-ColiBlue24) in source water, finished drinking, and wastewater using ancillary supplies and source of test organisms listed below. Brand

laboratory perform a QC check for detection and enumeration with each ne w lot of membrane filters and pads using test organisms derived from pre-chlorinated primary treated effluent or from an ATCC strain of organisms known compatibility with the medium.

10.0 Procedure

10.1 Test Procedure

10.1.1 If using broth medium, aseptically open an ampoule containing m-ColiBlue24 and pour the broth onto the pad in a 50-mm MF Petri plate.

10.1.2 Place a sterile filter onto a sterile filter holder. Using a sterile graduated cylinders and pipettes, measure an appropriate sample volume. Pour water sample into the reservoir funnel and draw the water through the filter using a vacuum pump. Rinse the funnel with several 20-30 mL volume of sterile rinse water. With sterile forceps, transfer the filter to a Petri plate containing a pad saturated with m-ColiBlue24 or an agar plate of m-ColiBlue24. Invert the plate and incubate at 35^{o} C ± 0.5^{o} C for 24 hours.

10.2 Interpretation

10.2.1 If no blue or red colonies are present after 24 hours, the sample is free from total coliforms and *E. Coli*.

10.2.2 Examine the filters for colony growth. Colonies are typically readily visible, but a microscope may prove useful.

10.2.3 Presence /Absence detection for Drinking Water – A red colony is a total coliform positive result. A clear or white colony is a total coliform negative result. A blue colony is an *E. coli* positive result. A non-blue colony is an *E. coli* negative result.

10.2.4 Enumeration for Source Water and Wastewater – Refer to Standard Methods 9222B for appropriate dilutions of the sample to filter so that 20-

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names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent method performance may be achieved using materials and sources of organisms other than those specified here, but demonstration of equivalent performance that meets the requirements of this method and its use for regulatory compliance reporting purposes is the sole responsibility of the laboratory.

Membrane filters: Millipore 0.45µ membrane filters; Part Number XXXX
Pads and Petri Dishes: Pall/Gilman or Sartorius pre-sterilized, cellulose or glass pads with glass or plastic Petri dishes; Part Numbers XXXXX and XXXXXXX

80 coliform forming units (CFU) is present after incubation. Calculate the number of blue *E. coli* colonies and red non-E. coli coliform colonies according to Standard Methods 9222B. Total coliforms are counted as the sum of blue and red colonies.

11.0 Method Performance Characteristics

11.1 Drinking Water

11.1.1 The specificity of m-ColiBlue24 for recovery of total coliforms and *E. coli* following the EPA Protocol of June 30, 1992 is the following: *E. coli* false positive error – 2.5%; *E. coli* false negative error – 0%. Overall agreement between m-ColiBlue24 and the EPA reference method (m-Endo) – 98.8%. Total coliform false positive error – 26.8%; Total coliform false negative error – 1.6%.

11.2 Source Water[2]

11.2.1 *E. coli* false positive error – 2.3%; *E. coli* false negative error – 0%.

11.3 Wastewater[3]

11.3.1 The specificity of m-ColiBlue24 for recovery of total coliforms and *E. coli* following the EPA Protocol of March 2003 is the following: *E. coli* false positive error – 2.3%; *E. coli* false negative error – 4.9%.

12.0 Pollution Prevention

12.1 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better; Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C., 20036.

13.0 Waste Management

13.1 It's the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and

[2] Grant, M.A. 1997. "A New membrane Filtration Medium for Simultaneous Detection and Enumeration of Escherichia coli and Total Coliforms." Applied and Environmental Microbiology, 63:3526-3530.

[3] "Guidelines Establishing Test Procedures for the Analysis of Pollutants; Analytical Methods for Biological Pollutants in Wastewater" (Federal Register / Vol. 66, No. 169 / Thursday, August 30, 2001 / Proposed Rules)

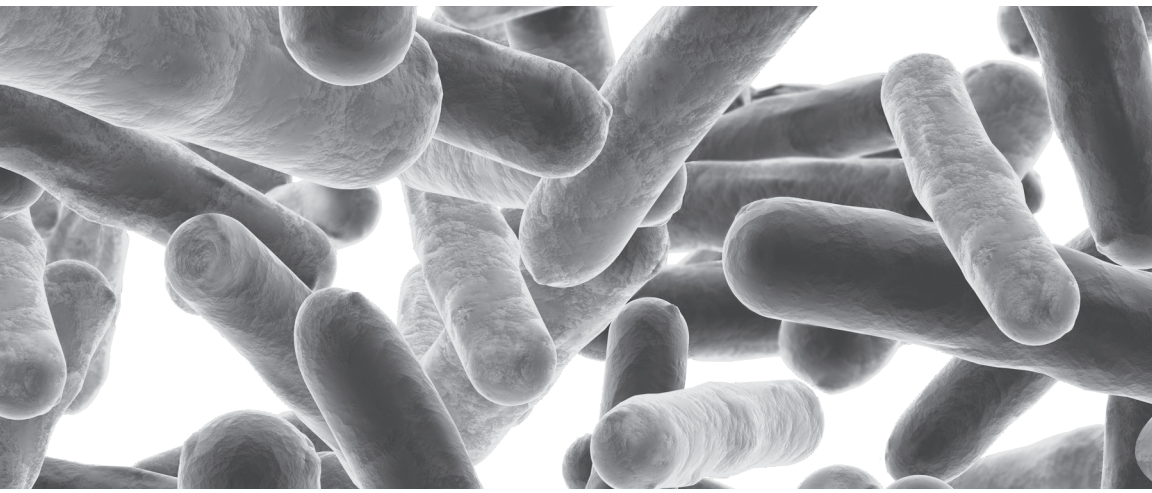
Simultaneous Detection and Enumeration of Total Coliforms and Escherichia coli using m-ColiBlue24 Membrane Filtration Medium

land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

13.2 See the MSDS for product composition information and further guidance on waste disposal.

13.3 For more information on laboratory waste management, consult Waste Management Manual for Laboratory Personnel, available from the American Chemical Society's department of Government Regulations and Science Policy, 1155 16th Street N.W., Washington D.C., 20036.

Colilert*



06-12999-08

IDEXX

For Technical Support, please call:

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IDEXX

IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092 USA

idexx.com/water

Colilert* Test Kit

Introduction

Colilert* simultaneously detects total coliforms and *E. coli* in water. It is based on IDEXX's proprietary Defined Substrate Technology*. When total coliforms metabolize Colilert's DST* nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert's DST* nutrient-indicator, MUG, the sample also fluoresces. Colilert can simultaneously detect these bacteria at 1 cfu/100 mL within 24 hours even with as many as 2 million heterotrophic bacteria per 100 mL present.

Storage

Store at 2–30°C away from light.

Presence/Absence (P/A) Procedure

1. Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. Incubate at 35±0.5°C for 24 hours.
4. Read results according to Result Interpretation table below.

Quanti-Tray* Enumeration Procedure

1. Add contents of one pack to a 100 mL water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray* or Quanti-Tray*/2000 and seal in an IDEXX Quanti-Tray* Sealer.
4. Place the sealed tray in a 35±0.5°C incubator for 24 hours.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Less yellow than the comparator[1]	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for *E. coli*

- Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
- Colilert results are to be read after 24 hours of incubation.
- However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 28 hours total) to allow the color and/or fluorescence to intensify.
- Positives for both total coliforms and *E. coli* observed before 24 hours and negatives observed after 28 hours are also valid.
- In addition, laboratories may incubate samples for additional time (up to 28 hours total) for their convenience.

Procedural Notes

- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures. For example, samples run in other countries are incubated at 36±2°C for 24–28 hours.
- Colilert can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*[2] MPN tables should be used to find Most Probable Numbers (MPNs).
- If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.
- If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert. If this is seen, consider sample invalid and discontinue testing.
- Aseptic technique should always be followed when using Colilert. Dispose of in accordance with Good Laboratory Practices.

Quality Control Procedures

1. One of the following quality control procedures is recommended for each lot of Colilert:
   A. IDEXX-QC Coliform and *E.coli* [3]: *Escherichia coli*, *Klebsiella variicola*‡, and *Pseudomonas aeruginosa*
   B. Quanti-Cult*[4]: *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.
   C. Fill three sterile vessels with 100 mL of sterile nonbuffered oxidant-free water and inoculate with a sterile loop of ATCC[5] strains, *Escherichia coli* ATCC 25922/WDCM 00013 or ATCC 11775/WDCM 00090, *Klebsiella variicola*‡ ATCC 31488/ WDCM 00206 and *Pseudomonas aeruginosa* ATCC 10145/WDCM 00024 or ATCC 27853.
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

NOTE: IDEXX internal quality control testing is performed in accordance with ISO 11133:2014. Quality Control Certificates are available at idexx.com/water.

1. IDEXX P/A Comparator, catalog #WP104; Quanti-Tray Comparator #WQTC, or Quanti-Tray/2000 Comparator #WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
3. IDEXX-QC Coliform and *E. coli*—IDEXX Catalog #UN3373-WQC-TCEC
4. Quanti-Cult cultures—IDEXX catalog # WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org

‡. *Klebsiella pneumoniae* (ATCC 31488/WDCM 00206) has been renamed to *Klebsiella variicola*.

9223 ENZYME SUBSTRATE COLIFORM TEST*

9223 A. Introduction

Enzyme substrate tests use hydrolyzable chromogenic and fluorogenic substrates to simultaneously detect enzymes produced by total coliforms and *Escherichia coli* (*E. coli*). In this method, total coliform bacteria produce the enzyme β-D-galactosidase, which cleaves the chromogenic substrate in the medium to release chromogen. Most *E. coli* strains produce the enzyme β-glucuronidase, which cleaves a fluorogenic substrate in the medium to release fluorogen. The release of chromogen indicates that coliform bacteria are present, and the release of fluorogen indicates that *E. coli* are present.

Multiple-tube, multi-well, or presence–absence (single 100-mL sample) formats are available for use with these enzyme substrate tests.

1. Principle

a. Total coliform bacteria: Colilert®, Colilert-18®, and Colisure® media use the chromogenic substrates ortho-nitrophenyl-β-D-galactopyranoside (ONPG) and chlorophenol red-β-D-galactopyranoside (CPRG), respectively, to detect the enzyme β-D-galactosidase, which is produced by total coliform bacteria. The β-D-galactosidase enzyme hydrolyzes the chromogenic substrate that produces a color change, thereby indicating the presence of total coliforms without additional procedures.

Although non-coliform bacteria (e.g., *Aeromonas, Flavobacterium,* and *Pseudomonas* species) may produce small amounts of the enzyme β-D-galactosidase, the growth of these organisms is suppressed so they generally will not produce a false-positive result unless $>10^6$ CFU/100 mL are present.

b. Escherichia coli: The fluorogenic substrate 4-methyl-umbelliferyl-β-D-glucuronide (MUG) is used to detect the enzyme β-D-glucuronidase, which is produced by most strains of *E. coli.* The β-D-glucuronidase enzyme hydrolyzes the fluorogenic substrate that produces bluish fluorescence when viewed under long-wavelength (365–366 nm) ultraviolet (UV) light. Together, the color change (due to β-D-galactosidase) and the fluorescence (due to β-D-glucuronidase) indicate that a sample contains *E. coli.*

Large numbers of some bacteria or strains of bacteria (e.g., some strains of *Shigella* and *Salmonella* spp.) may cause a sample to fluoresce but will not change its color because they lack β-D-galactosidase. Such samples would be considered negative for *E. coli*.

2. Applications

These enzyme substrate coliform tests are recommended for the analysis of drinking water, source water, groundwater, and wastewater samples. If a laboratory has not used this method before, it is desirable to conduct parallel testing (including seasonal variations) with the existing method to assess site-specific effectiveness and to compare results. The results of many method-performance studies are available in the literature and the rates of false-positive and -negative results differ among various media. Users should carefully select the medium and procedure that best fits their needs. See Section 9020B.11 for guidance on validating new methods.

Water samples containing humic or other material may be colored. If there is a natural background color, note what it is. If the water is yellow enough to be misinterpreted as a weak positive after incubation, use a medium that does not turn yellow (e.g., Colisure). Some waters' high calcium-salt content can cause precipitation, but this should not affect the reaction. In samples with excessive chlorine, a blue flash may be seen while adding Colilert or Colilert-18 media. If this occurs, consider sample invalid and discontinue testing.

Do not use the enzyme substrate test to verify presumptive coliform cultures or membrane-filter colonies, because the substrate may be overloaded by the heavy inoculum of weak β-D-galactosidase-producing noncoliforms, causing false-positive results.

* Approved by Standard Methods Committee, 2016.
Joint Task Group: Jennifer Best (chair), Bennie L. Cockerel, Jr., Gil Dichter, Nancy H. Hall, William W. Northeimer, Viola Reynolds, Helena Solo-Gabriele.

9223 B. Enzyme Substrate Test

1. Samples

Collect samples as directed in Section 9060A, using sample containers specified in Section 9030B.19. When collecting chlorinated water samples, use sodium thiosulfate as described in Section 9060A.2. Follow the quality control (QC) guidelines for sample bottles described in Section 9020B.5*d*. Adhere to sample holding times and conditions as described in Section 9060B or required by regulations. Take care to ensure that samples are held at the appropriate temperature and analyzed as soon as possible after sample collection because failure to do so could compromise results. Ensure that samples meet laboratory-acceptance criteria upon receipt.

2. Quality Control

Method users must adhere to the quality assurance (QA)/QC guidelines in Section 9020, including, but not limited to, analytical QC (Section 9020B.9), instrumentation/equipment (Sections 9020B.4 and 9030B), and supplies (Section 9020B.5). Refer to Table 9020:I for key QC procedures.

Before using each lot of new medium, verify its performance via positive and negative control organisms. To conduct culture controls, inoculate medium with three control bacteria: *E. coli*, a total coliform strain other than *E. coli* (e.g., *Enterobacter cloacae*), and a noncoliform (see Table 9020:VI). An uninoculated negative control should also be analyzed. In addition, test me-

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dium and vessels (bottles, multi-well trays, tubes) to confirm sterility and lack of autofluorescence.

3. Substrate Media

Colilert, Colilert-18, and Colisure media are available commercially* in premeasured packets for presence–absence testing or in disposable tubes for use in a multiple-tube format. The Quanti-Tray® and Quanti-Tray/2000* are multi-well formats that may be used with the premeasured packets to quantitate the coliform bacteria present in a sample.

Store media according to directions and use before expiration date. Avoid prolonged exposure of media to direct sunlight. Discard media that have changed color, appearance, and/or texture (media are hygroscopic and will clump and darken if exposed to moisture).

4. Procedure

Begin analysis by mixing the sample properly to promote even distribution of bacteria. For proper mixing to occur, samples should have ≥1-in. headspace and be shaken vigorously for 7 s (back and forth 1 ft approximately 25 times).

Failure to properly mix sample can lead to erroneous results, as bacteria are known to clump together and are therefore not homogeneously distributed throughout sample. For instance, most probable number (MPN) results are based on a Poisson (random) distribution of cells in the sample; failure to properly mix sample before analysis will result in an MPN value that underestimates actual bacterial density. Removing a portion of sample without proper mixing—such as when performing presence–absence analyses with a single bottle (one bottle used to both collect and analyze sample)—may result in false negative results if the target organisms were clumped together and removed from the bottle without being homogenized.

If the bottle lacks enough headspace for adequate mixing, pour sample into a larger sterile vessel so it can be mixed properly. Measure out desired sample volume and proceed with analysis.

For each medium or format used, tests should be placed in the incubator within 30 min after medium is added to sample. No matter which format is used, all media must be incubated at 35 ± 0.5°C. Colilert medium must be incubated for ≥24 h, Colilert-18 medium must be incubated for ≥18 h, and Colisure medium must be incubated for ≥24 h.

The coliform tests described here have been developed to obtain optimal bacterial growth at the indicated incubation temperatures. Failure to maintain this temperature throughout incubation could result in false negative results, especially with the shorter incubation times for Colilert-18. To ensure that samples are at proper temperature for the entire incubation period, laboratories should pre-warm samples after adding medium but before placing them in the incubator.

To pre-warm a test sample, place it in a 35 ± 0.5°C water bath for 20 min or in a 44.5 ± 0.2°C waterbath for 7 to 10 min to bring it to incubation temperature. The laboratory may need to conduct load studies to determine how long samples need to be incubated for effective pre-warming (depends on number of samples being incubated). Pre-warming is unnecessary if the Quanti-Tray format is used.

a. Presence–absence procedure (P/A): Aseptically add contents of packet containing premeasured medium to a 100-mL sample in a sterile, transparent, non-fluorescent borosilicate glass or equivalent bottle or container. Aseptically cap and shake vigorously to dissolve medium. Some medium may remain undissolved, but this will not affect test performance.

b. Multiple-tube procedure:

1) Multiple-tube procedure using a 5- or 10-tube MPN test—A 5-tube series (20 mL sample per tube) or 10-tube series (10 mL sample per tube) can be used when bacteria levels are anticipated to be fairly low or a fixed 100-mL sample volume must be analyzed (e.g., for regulatory compliance).

Add a premeasured packet of medium to a well-mixed 100-mL water sample in a container and shake vigorously to dissolve medium. Arrange tubes in rows of 5 or 10 in a test tube rack, and label each set of tubes. Aseptically dispense 20 mL sample into each of 5 sterile tubes or 10 mL into each of 10 sterile tubes, cap tightly, and mix vigorously to dissolve medium. If using 10 tubes already containing premeasured medium (available from manufacturer), aseptically dispense 10 mL sample into each tube.

Some medium particles may remain undissolved; this will not affect test performance.

After incubation, refer to Tables 9221:II and III to determine the MPN of total coliforms and *E. coli* present.

2) Multiple-tube procedure using 15-tube MPN test—A 15-tube test typically involves three serial dilutions of a sample, with each dilution inoculated into 5 tubes. Typically, 5 tubes contain undiluted sample, 5 contain a 1:10 dilution, and 5 contain a 1:100 dilution.

Use this technique when a water sample may contain higher bacteria levels and there is no requirement to analyze a fixed volume (e.g., when analyzing nonpotable waters). The number of tubes and sample volumes selected depend on the quality and characteristics of the water to be examined. To preclude any unwanted interaction with the medium, use only sterile, non-buffered, oxidant-free water (e.g., deionized or distilled water) to prepare dilutions.

When working with diluted samples, best laboratory practice is to ensure that all tubes are in place and labeled before analysis begins. Additionally, use clean, sterile pipets to pipet each dilution because bacterial carryover from dirty pipets will make test results inaccurate.

a) Using disposable tubes containing premeasured medium (available from manufacturer)

i) Preparing sample for the undiluted series—Aseptically pipet 10 mL of well-mixed sample into each of 5 tubes containing predispensed medium. Cap tubes and mix vigorously to dissolve medium.

ii) Preparing 1:10 dilution—Aseptically pipet 10 mL of well-mixed sample into a sterile vessel containing 90 mL of sterile, non-buffered, oxidant-free water (e.g., deionized or distilled water). Mix well. Aseptically pipet 10 mL of this dilution into each of 5 tubes containing pre-dispensed medium. Cap tubes and mix vigorously to dissolve medium.

iii) Preparing 1:100 dilution—Aseptically pipet 10 mL of well-mixed sample from the 1:10 dilution into a sterile vessel containing 90 mL of sterile, non-buffered, oxidant-free water

* Available from IDEXX Laboratories, Inc., Westbrook, ME.

(e.g., deionized or distilled water). Mix well. Aseptically pipet 10 mL of this dilution into each of 5 tubes containing predispensed medium. Cap tubes and mix vigorously to dissolve medium.

b) Using packets of premeasured medium

i) Preparing sample for the undiluted series—Add one packet of premeasured medium to a sterile vessel containing 100 mL of well-mixed sample, and mix vigorously to dissolve medium. Aseptically pipet 10 mL of sample/medium mixture into each of 5 sterile, non-fluorescing tubes.

ii) Preparing 1:10 and 1:100 dilutions—Add one packet of premeasured medium to 100 mL sterile, non-buffered, oxidant-free water (e.g., deionized or distilled water) in a sterile container, and mix vigorously to dissolve medium. Aseptically pipet 9 mL of prepared medium into 10 sterile, non-fluorescing tubes. This preparation of enzyme substrate medium must be completed ≤1 h of adding sample to prepared medium.

iii) Inoculating tubes for 1:10 dilution—Aseptically pipet 1 mL of well-mixed sample into each of 5 tubes containing 9 mL of prepared medium. Cap and mix well.

iv) Inoculating tubes for 1:100 dilution—Pipet 10 mL of well-mixed sample into a vessel containing 90 mL sterile, non-buffered, oxidant-free water (e.g., deionized or distilled water). Close and mix well to dissolve medium. Aseptically pipet 1.0 mL of this diluted sample into 5 tubes containing 9 mL of prepared medium. Cap and mix well.

For any additional dilutions needed, continue with the dilution process as described above.

After incubation, use Table 9221:IV to determine the MPN for both total coliforms and *E.coli*. If further dilutions were performed, the MPN value must be multiplied by the dilution factor to obtain the proper quantitative results.

c. Multi-well procedure: This procedure is performed with sterilized disposable multi-well trays [either the Quanti-Tray (51 well) or Quanti-Tray/2000]. Aseptically add premeasured medium from packet to a 100-mL water sample in a container and shake vigorously to dissolve medium. To open Quanti-Tray, use one hand to hold unit upright (with the well side facing the palm) and squeeze the upper part of the tray so it bends toward the palm. Gently pull foil tab to separate foil from tray, being careful not to touch the inside of either foil or tray. Add reagent–water sample mixture directly into tray, avoiding contact with foil tab. Gently tap the small wells (Quanti-Tray/2000) 2 to 3 times to release any air bubbles that may be trapped. Allow foam to settle, although some foam is acceptable. Place tray into the appropriate rubber insert with the well (plastic) side facing down, and feed it into the Quanti-Tray sealer. The sealer disperses the sample into the wells and seals the package.

5. Interpretation

a. Total coliform bacteria: The bacterial enzyme β-D-galactosidase hydrolyzes ONPG (Colilert and Colilert-18) to yield a yellow color and hydrolyzes CPRG (Colisure) to yield a red or magenta color. After the minimum incubation period, examine for the appropriate color change (Table 9223:I). If color response is not uniform throughout sample, mix by inversion before reading.

Use an unexpired color comparator (available from manufacturer) to ensure that Colilert and Colilert-18 test results are read accurately. The comparator used must have the same volume in the same type of container as the sample.

Table 9223:I. Color Changes for Various Media

Substrate	Total Coliform Positive	*E. coli* Positive	Negative Result
Colilert® Colilert-18®	Yellow	Blue fluorescence	Colorless or color lighter than the comparator/no fluorescence
Colisure®	Red or magenta	Blue fluorescence	Yellow, pink, or orange/no fluorescence

1) Colilert—If sample color is as yellow as or darker yellow than the comparator, then it is positive for total coliforms. If not, then the sample is negative for total coliforms.

However, if the chromogenic response is ambiguous (color cannot be discerned) after 24 h, incubate sample for up to 4 h longer to allow test color to intensify. If the color does become as yellow as or darker than that of the comparator within this period, then the sample is positive for total coliforms. If not, then the sample is negative for total coliforms.

Colilert can be incubated for ≤28 h. After 28 h, negative test results are still considered valid, but positive results are not.

2) Colilert-18—If sample color is as yellow as or darker yellow than the comparator, then it is positive for total coliforms. If not, then it is negative for total coliforms.

However, if the chromogenic response is ambiguous (color cannot be discerned) after 18 h, incubate sample for up to 4 h longer to allow the test color to intensify. If the color does become as yellow as or darker than that of the comparator within this period, then the sample is positive for total coliforms. If not, then the sample is negative for total coliforms.

Colilert-18 can be incubated for ≤22 h. After 22 h, negative test results are still considered valid, but positive results are not.

3) Colisure—If the sample has a red or magenta color, it is positive for total coliforms. If the chromogenic response is questionable (color may be orange or pink) after 24 h, incubate sample for up to 24 h longer to allow test color to intensify. If color does become red or magenta within this period, then the sample is positive for total coliforms.

Colisure tests turn yellow after medium is added; if color does not change to red or magenta after incubation, then the sample is negative for total coliforms.

Colisure can be incubated for ≤48 h. After 48 h, results are not valid.

Sometimes a sample's high calcium-salt content can cause precipitation, but this will not affect the reaction. However, if the test medium turns an inappropriate color (e.g., green or black) that interferes with test-result reading, another method must be used.

b. Escherichia coli: The fluorogenic substrate MUG is hydrolyzed by the bacterial enzyme ß-D-glucuronidase to yield a bluish fluorescence when viewed under long-wavelength (365–366 nm) UV light. The color change (indicating β-D-galactosidase is active) and fluorescence (indicating β-D-glucuronidase is active) together show that *E. coli* is present.

After the minimum incubation period, examine positive total coliform tests for a bluish fluorescence; use a long-wavelength (365–366 nm) UV lamp with a 6-W bulb and hold it within 5 in. of sample in a dark environment. Use a color comparator (available

from the manufacturer) before its expiration date to ensure that test results are read accurately. The comparator used must have the same volume in the same type of container as the sample.

1) Colilert—If the sample has a bluish fluorescence equal to or greater than that of a total-coliform-positive comparator, then it is positive for *E. coli*. If the fluorescence is ambiguous (cannot be discerned) after 24 h, the sample may be incubated for up to 4 h longer to allow the fluorescence to intensify. If sample fluorescence does intensify to equal to or greater than that of the comparator within this period, then the sample is positive for *E. coli*.

If sample florescence remains less than that of the comparator after 28 h of incubation, then it is negative for *E. coli*. Samples that are negative for total coliform bacteria are also negative for *E. coli*.

2) Colilert-18—If the sample has a bluish fluorescence equal to or greater than that of a total-coliform-positive comparator, then it is positive for *E. coli*. If the fluorescence is ambiguous (cannot be discerned), the sample may be incubated for up to 4 h longer to allow the fluorescence to intensify. If sample fluorescence does intensify to equal to or greater than that of the comparator within this period, then the sample is positive for *E. coli*.

If sample florescence remains less than that of the comparator after 22 h of incubation, then it is negative for *E. coli*. Samples that are negative for total coliform bacteria are also negative for *E. coli*.

3) Colisure—If a total-coliform-positive sample fluoresces, then it is positive for *E. coli*. If the fluorescence is ambiguous (cannot be discerned), the sample should be incubated for up to 24 h longer to allow the fluorescence to intensify. If the sample clearly fluoresces within this period, then it is positive for *E. coli*.

If sample does not fluoresce after 48 h of incubation, then it is negative for *E. coli*. Samples that are negative for total coliform bacteria are also negative for *E. coli*.

6. Reporting

For the presence–absence procedure, report results as total coliforms and *E. coli* present or absent in a 100-mL sample.

For the multiple-tube procedure, calculate the MPN value for total coliforms and *E. coli* from the number of positive tubes, as described in Section 9221C.

For the multi-well procedure, determine the MPN from the appropriate MPN tables obtained from the tray manufacturer.

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Membrane Filtration Method
E. coli
Calculation of Results

Select the membrane filter (MF) with the number of colonies in the 20 to 80 range and calculate count per 100 mL according to the general formula:

$$\text{count per 100 mL} = \frac{\text{No. of colonies counted}}{\text{Volume of sample filtered, in mL}} \times 100$$

Counts Within the Acceptable Limits

A. Assume that filtration of volumes of 50, 15, 5, 1.5, and 0.5 mL produced colony counts of 200, 110, 40, 10, and 5, respectively.

B. You do not need to actually count the colonies on all filters. By inspection you should select the MFs with 20-80 coliform colonies and then limit the actual counting to such membranes.

C. After selecting the best MFs for counting, in this example the MF with a 40 colony count, you apply the general formula as follows:

$$\text{Colonies per 100 mL} = \frac{40}{5} \times 100 = 800/100 \text{ mL}$$

More Than One Acceptable Count

A. If there are acceptable counts on replicate plates, carry counts independently to final reporting units, then calculate the arithmetic mean of these counts to obtain the final reported value.

   For example, 1 mL volumes produce coliform counts of 26 and 36 or counts of 2600 and 3600/100 mL:

$$\frac{2600 + 3600}{2} = 3100/100 \text{ mL}$$

B. If more than one dilution, independently carry counts to final reporting units, then average for final reported value.

For example, assume that volumes of 0.3, 0.05, 0.03, and 0.01 mL produced coliform colony counts of TNTC (Too Numerous To Count), 55, 30, and 8, respectively, In this example, two volumes, 0.05 and 0.03 produce colonies in the acceptable counting range.

Independently carry each MF count to a count per 100 mL:

$\frac{55}{0.05} \times 100 = 110{,}000/100\text{ mL}$ and $\frac{30}{0.03} \times 100 = 100{,}000/100\text{ mL}$

Then calculate the arithmetic mean of these counts to obtain the final reported value:

$$\frac{110{,}000 + 100{,}000}{2} = 105{,}000\text{ mL}$$

If All MF Counts are Below the Lower Limit

Select the Most Nearly Acceptable Count

For example, assume a count in which sample volumes of 1, 0.3, and 0.01 mL produced colony counts of 14, 3, and 0, respectively.

Here, no colony count falls within recommended limits. Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark:

$\frac{14}{1.0} \times 100 = 1400$ Report as: estimated 1400/100mL

If Counts from All Membranes are Zero

Calculate Using Count from Largest Filtration Volume

For example, sample volumes of 25, 10, and 2 mL produced colony counts of 0, 0, and 0, respectively, and no actual calculation is possible, even as an estimated report. Calculate the number of colonies per 100 mL that would have been reported if there had been one colony on the filter representing the largest filtration volume, thus:

$\frac{1}{25} \times 100 = 4$ Report as: < (Less than) 4/100 mL

If All Membrane Counts are Above the Upper Limit

Calculate Count with Smallest Volume Filtered

For example, assume that the volumes 1, 0.3, and 0.01 mL produced colony counts of TNTC, 150, and 110 colonies. Since all colony counts are above the recommended limit, use the colony count from the smallest sample volume filtered and estimate the count as:

$\frac{110}{0.01} \times 100 = 1{,}100{,}000/100\ mL$ Report as estimated 1,100,000/100 mL

If Colonies are Too Numerous To Count

Use Upper Limit with Smallest Filtration Volume

For example, assume that the volumes 1.0, 0.3, and 0.01 mL, all produced too many colonies to show separated colonies, and that the laboratory bench record showed TNTC.

Use 80 colonies (upper limit count for E. Coli) as the basis of calculation with the smallest filtration volume, thus:

$\frac{80}{0.01} \times 100 = 800{,}000$ Report as: > (Greater than) 800,000/100 mL

Calculating Geometric Mean when there are Individual Sample Results that are Reported as <, >, or Est.

A. If any individual sample result is reported as an estimate, drop the est. when calculating the geometric mean.

B. If there are any individual samples reported as <, drop the < signs when calculating the geometric mean. However, report the geometric mean as a < value.

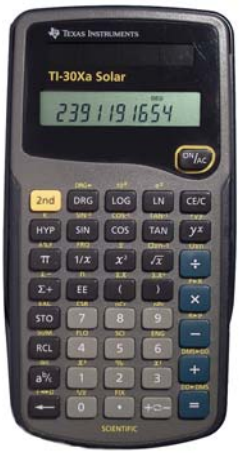
C. If there are any individual samples reported as >, drop the > signs when calculating the geometric mean. However, report the geometric mean as a > values.

D. If there are samples reported as < and one or more samples reported as >, drop the < and > signs when calculating the geometric mean. However, report the geometric mean as a > value.

Source of information
Standard Methods 9222B

Applied Math for Wastewater Treatment
Geometric Mean

Geometric Mean Using a Texas Instrument TI-30Xa



Example:
60 100 0 0

Geometric Mean – $(X_1)(X_2)(X_3)\ldots(X_n)^{1/n}$

Step 1: 1/n → 1 divided by the number of test results. For our example above, there are four test results.

- 1 ÷ 4 = 0.25 (write this number down, you will use it in Step 3)

Step 2: Multiply all of the test results together and punch the = button on the calculator. Remember to count 0 as a 1.

- 60 x 100 x 1 x 1 = 6000 (Do Not clear out your calculator)

Step 3: Punch the y^x button and then type in the number from Step 1, then punch =.

- 6000 y^x 0.25 = 8.8011

Geometric Mean Using a Texas Instrument TI-30XIIB

Example:
60 100 0 0

Geometric Mean – $(X_1)(X_2)(X_3)\ldots(X_n)^{1/n}$

Step 1: 1/n → 1 divided by the number of test results. For our example above, there are four test results.

- 1 ÷ 4 = 0.25 (write this number down, you will use it in Step 3)

Step 2: Multiply all of the test results together and punch the = button on the calculator. Remember to count 0 as a 1.

- 60 x 100 x 1 x 1 = 6000 (Do Not clear out your calculator)

Step 3: Punch the ^ button, then type in the number from Step 1, & then punch =.

- 6000 y^x 0.25 = 8.8011

Quality Assurance for E. coli Analysis

Laboratory Equipment and Instrumentation

- Thermometers – 9020B.4.a
  - Annually check accuracy of all working temperature-sensing devices… against a certified NIST thermometer or one traceable to NIST and conforming to NIST specifications.
  - Record calibration results, along with the date and the technician's signature, in a quality control logbook.
  - Mark the necessary calibration correction factor on each temperature measuring device so that only calibrated-corrected temperature values are recorded.
  - Verify accuracy of the reference certified thermometer as specified on the certificate of calibration or at least every 5 years.
  - For general purposes use thermometers graduated in increments of 0.5°C or less.
- Autoclave – 9020B.4.h
  - For routine use, verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached.
  - Test monthly for sterilization efficacy (with *Geobacillus stearothermophilus*)
  - Verify the autoclave temperature weekly by using a maximum registering thermometer (MRT) to confirm that 121°C has been reached.
- Refrigerator – 9020B.4.i
  - Maintain temperature at 2-8°C
  - Check and record temperature daily
- Membrane filtration equipment (if MF procedure is used) – 9020B.4.k
  - Wash and rinse filtration assemblies thoroughly after use, wrap in nontoxic paper or foil, and sterilize.
  - UV sterilize or boil funnels between samples
    - If using boiling water, make sure membrane filtration equipment is cool before adding next sample
- Membrane filters and pads (if MF procedure is used) – 9020B.5.i.3
  - Check filters for brittleness if lot is held for one or more years
- Ultraviolet lamps (if used) – 9020B.4.l
  - When used, disconnect lamps monthly and clean bulbs with a soft cloth moistened with ethanol
- Incubator – 9020 B.4.o
  - During usage periods check and record calibration-corrected temperature twice daily (morning and afternoon, separated by at least 4 hours) on each shelf in use to ensure temperature consistency throughout unit.

Laboratory Supplies

- Glassware – 9020 B.5.a
  - 1) pH check - To test clean glassware for alkaline or acid residue add a few drops of 0.04% bromthymol blue (BTB) or other pH indicator and observe the color reaction.
  - BTB should be blue-green (in the acceptable neutral range).
- Dilution water bottles – 9020 B.5.c
  - Dilution waters available commercially are acceptable.

- Check one per lot for pH and volume (99 ± 2 mL) and examine bottles for a precipitate
- Discard by expiration date
- Before use of each batch or lot conduct sterility (one bottle per lot or quarter with that same lot number, whichever is more frequent)
  - Sterility Checks – 9020B.9.d
    - Check each new batch (or lot) of buffered water for sterility before first use by adding 50 mL of water to 50 mL of a double-strength broth (e.g. tryptic soy, trypticase soy or tryptose broth).
    - Alternatively, aseptically pass 100 mL of dilution water through a membrane filter and place filter on nonselective medium.
    - Incubate at 35±0.5°C for 24 hours and observe for growth.
    - For membrane filter tests, check the sterility of the entire process by using sterile reagent or dilution water as the sample at the beginning and end of each filtration series of samples and test for growth

- Sample bottles – 9020 B.5.d.
  - Check accuracy of 100 mL mark, one per lot and record results.
- Multi-well trays and sealers – 9020 B.5.e
  - Evaluate sealing performance of heat sealer unit monthly by adding one to two drops of food-color dye to 100 mL deionized water sample, run through sealer and visually check each well for leakage.
  - **Real people language – analyze a method blank once per lot (of sterile water, media, bottles and trays) or once per quarter, whichever is more frequent, to demonstrate sterility.**
  - **As a monthly check of a sealer efficacy, perform and document a visual check that trays are properly sealed. If all sample wells are positive for total coliform and sufficient contrast, visually examine the tray cells for leakage and document the check. If insufficient color contrast is present us food-color dye as previously recommended by method.**

General QC Requirements

- Coliforms – Total and E. coli Hach Method 10029 – m-ColiBlue24®
  - Blank – daily
    - Run at least one membrane filter blank at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter, placing in a petri dish with mColiBlue broth and testing for growth.
  - Positive and Negative Controls – Check certified control cultures with each lot of media ***and*** petri dishes with pads OR once a quarter, whichever is more frequent.
    - *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* as a positive control.
  - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.
- Enzyme Substrate Test SM 9223 B, 22nd Edition (2004) – Colilert Method
  - Quality Control

- Test each lot of media or quarterly (whichever is more frequent) purchased for performance by inoculation with two certified control bacteria: *Escherichia coli* and a noncoliform.
- Also add a sterile water control. If a sterile water control exhibits faint fluorescence or faint positive coliform, discard use and use a new batch of substrate.
- Incubate these controls at 35±0.5°C as indicated above.

o Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.

Bibliography

American Public Health Association (APHA), American Waterworks Association (AWWA), and Water Environment Federation (WEF). 2012. *Standard Methods for the Examination of Water and Wastewater*. 22nd ed. American Public Health Association, Washington, D.C.

Expected reactions of various microorganisms with m-ColiBlue24®

Total Coliforms will produce a **red colony.** Specifically Enterobacter, Klebsiella and Citrobacter species are expected to produce a red colony. Not every strain of the above species has been tested. The following strains have been tested and may be recommended for use in quality control testing.

Enterobacter species - *E. cloacae* ATCC #13047
E. cloacae ATCC #23355
E. aerogenes ATCC #13048

Klebsiella species - *K. pneumoniae* ATCC #13883

Citrobacter species - *C. freundii* ATCC #8090

Escherichia coli will produce a **blue colony**. Not every strain of *E. coli* has been tested; however, the following strains are known to give a positive reaction (blue colony).

E. coli ATCC #25922
E. coli ATCC #11775

E. coli O157:H7 is beta-glucuronidase negative and will not produce a blue colony, but will grow as a red colony.

Known **Negative reaction**/No growth after 24-25 hours:

Pseudomonas aeruginosa ATCC #27853
Proteus vulgaris ATCC #13315
Aeromonas hydrophila ATCC #7965
Aeromonas hydrophila ATCC #35654
Aeromonas hydrophila ATCC #49140

Some strains of the following microorganisms are known to produce a **false-positive** total coliform reaction (i.e. a red colony, but not a true total coliform):

Serratia species
Hafnia alvei
Vibrio fluvialis
Aeromonas species
Proteus vulgaris ATCC #6380
Providencia stuartii
Yersinia enterocolitica
Leclercia adecarboxylata
Ewingella americana
Staphylococcus species
Proteus mirabilis ATCC #25933
Pseudomonas aeruginosa ATCC #9027 - variable reaction may be positive when incubated longer than 25 hours.

PLEASE NOTE: Many of the bacteria listed above which cause false-positive reactions share key physiological similarities with the 4 traditional coliform* genera (Escherichia, Enterobacter, Klebsiella, and Citrobacter). In addition, m-ColiBlue24® was developed to provide optimal growth conditions for all of the strains of coliform bacteria, especially those stressed due to treatment methods for drinking

water. As a result, it is difficult to insure maximum recovery of these 4 target groups without allowing the growth of **some nontarget** microorganisms. The interference caused by these bacteria can be corrected by use of the standard cytochrome oxidase test. Total coliforms, including *E. coli,* are oxidase negative while some interfering bacteria are oxidase positive. The oxidase test is described in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, Section 9225 (p. 9-70). Additionally, two methods of oxidase confirmation are described in the Hach Analytical Procedure. The presence of total coliforms can also be confirmed using the ONPG test. Total coliforms will cleave ONPG producing a positive result, or yellow color. The ONPG test is described in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, Section 9020B (p. 9-10).

Results from the Specificity Study, conducted as a part of the USEPA approval process, show that a false-positive reaction is rare (only 2.5%) with *E. coli* (blue colonies), because the formation of blue colonies results from a very specific enzymatic reaction. There are some strains of bacteria, other than *E. coli*, that produce beta-glucuronidase, but these strains are not typically found in water.

Method Performance Characteristics (taken from the studies conducted for USEPA approval)

For *E. coli* false positive error 2.5%
false negative error 0.0%
Agreement between m-ColiBlue24® and reference methods was 98.8%.

For total coliform false positive error 26.8%
false negative error 1.6%
Agreement between m-ColiBlue24® and reference method m-Endo, was 86.2%.
m-Endo method has a false positive error of 29.6% and a false negative error of 3.4%.

*The definition of what constitutes a true coliform varies depending on regulatory agency. Certain agencies list true coliforms as Escherichia, Enterobacter, Klebsiella, Citrobacter, Serratia and Proteus. Some European water companies indicate true coliforms are Escherichia, Enterobacter, Klebsiella, Citrobacter, Serratia, Hafnia and Yersinia. Please contact your local regulatory agency for a their description of a true coliform.

Section 9
QA/QC

QA/QC

Wastewater Laboratory Week 1

Sarah Snyder

Fleming Training Center

TN Department of Environment & Conservation

1

Terminology

- Accuracy – estimate of how close a measured value is to the true value; includes expressions for bias and precision
- Analyte – the element, compound, or component being analyzed
- Bias – consistent deviation of measured values from the true value, caused by systematic errors in a procedure
- Calibration check standard – standard used to determine an instrument's accuracy between recalibrations
- Confidence coefficient – the probability (%) that a measurement will lie within the confidence interval between confidence limits

TDEC-Fleming Training Center

2

Terminology

- Confidence interval – set of possible values with in which the true value will lie with a specified level of probability
- Control Chart – a graphical representation of the data and variances from the mean is constructed
- Instrument detection level (IDL) – the constituent concentration that produces a signal greater than five times the instrument's signal: noise ratio. The IDL is similar to the critical level and criterion of detection, which Is 1.645 times the s of blank analyses (s is the estimate of standard deviation)

TDEC-Fleming Training Center

3

Terminology

- Method detection level (MDL) – the constituent concentration that, when processed through the entire method, produces a signal that has 99% probability of being different from the blank. For seven replicates of the sample, the mean must be 3.14s above the blank result (s is the standard deviation of seven replicates). Compute MDL from replicate measurements of samples spiked with analyte at concentrations more than 5X the estimated MDL. The MDL will be larger than the LLD because typically 7 or less replicates are used. Additionally, the MDL will vary with matrix

TDEC-Fleming Training Center

4

Terminology

- Reporting Level (RL) – the lowest quantified level within an analytical method's operational range deemed reliable enough, and therefore appropriate, for reporting by the laboratory. RLs may be established by regulatory mandate or client specifications, or arbitrarily chosen based on a preferred level of acceptable reliability.
  - Minimum reporting level (MRL) – the minimum concentration that can be reported as a quantified value for a target analyte in a sample. This defined concentration that can be reported as a quantified value for a target analyte in a sample. This defined concentration is no lower than the concentration of the lowest calibration standard for the analyte and can only be used if acceptable QC criteria for this standard are met.

TDEC-Fleming Training Center

5

Terminology

- Duplicate – 1) smallest number of replicates (two) 2) duplicate samples (i.e. two samples taken at the same time from one location) or replicate of laboratory analyzed sample
- Fortification (Spike) – adding a known quantity of analyte to a sample or blank to increase the analyte concentration, usually for the purpose of comparing to test result on the unfortified sample and estimating percent recovery or matrix effects on the test to assess accuracy
- Laboratory control standard – a standard usually certified by an outside agency that is used to measure the bias in a procedure. For certain constituents and matrices.

TDEC-Fleming Training Center

6

Terminology

- Mean – the arithmetic average (the sum of measurements divided by the number of items being summed) of a data set
- Median – the middle value (odd count) or mean of the two middle values (even count) of a data set
- Precision (usually expressed as standard deviation) – a measure of the degree of agreement among replicate analyses of a sample
- Quality assessment – procedure for determining the quality of laboratory measurements via data from internal and external quality control measures

7

Terminology

- Quality assurance – a definitive plan for laboratory operations that specifies the measures used to produce data with known precision and bias
- Quality control – set of measures used during an analytical method to ensure that the process is within specified control parameters
- Standard Deviation – statistical calculation which is done to check precision, "variance from the mean"

8

Importance of Quality Control

- To be valuable or useful, data must:
  - Be representative
  - Accurately describe the characteristics and concentrations of constituents in the samples
  - Be reliable
- Approximate or incorrect results are worse than no result at all
  - Lead to faulty interpretations

9

Good Lab Data

- Lab data does what?
  - Ambient water quality standards (pH, DO, heavy metals, pesticides) establish satisfactory conditions for drinking water, fishing, irrigation, power generation, etc.
  - Wastewater:
    - Identifies characteristics of plant influent
    - Identifies characteristics of final load imposed on receiving waters
    - Indicates effectiveness of treatment steps

10

Good Lab Data

- Lab data factors into:
  - Decisions on process changes
  - Plant modifications
  - Construction of new facilities/Plant upgrades
  - Effective research in water pollution control
  - The extent of compliance
- Lab analyst has considerable responsibility
  - Results could be challenged (perhaps in court)

11

What do you do with data?

- Data reported to State
- Data collected for Process Control
  - Improve operations
    - Right now and also in the future
    - Example of "right now" = COD test
    - Example of "future" = historical trends, plant modifications

12

Standard Methods

- 1880's movement for "securing the adoption of more uniform and efficient methods of water analysis"
- Drinking water only until 1925
- 1933 joint publication
  - Standard Methods of the Examination of Water and Sewage

13

Standard Methods

- Methods believed to be best available
- Recommendations of specialists, ratified by large number of analysts and other experts
- Truly consensus standards
- Offers valid and recognized basis for control and evaluation

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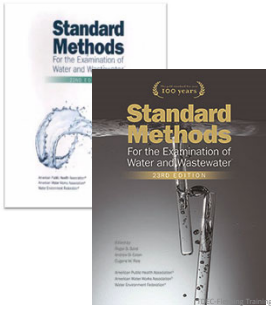
Standard Methods



- Standard Methods for the Examination of Water and Wastewater
- Often a lag time between most updated SM and 40 CFR 136 approved methods
- Go by the Editorial Revision date on method

15

Code of Federal Regulations (CFR)

- The purpose of the CFR is to present the official and complete text of agency regulations in one organized publication and to provide a comprehensive and convenient reference for all those who may need to know the text of general and permanent Federal regulations
- CFR can supersede Standard Methods

16

Code of Federal Regulations (CFR)

- The CFR is divided into 50 titles representing broad areas subject to Federal regulation
  - Each title divided into chapters
  - Each chapter divided into parts
  - Each part divided into sections

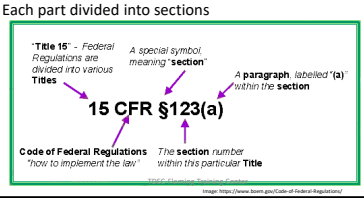


Image: https://www.loom.gov/Code-of-Federal-Regulations/

17

Code of Federal Regulations (CFR)

- CFR will list approved methods for testing
- Includes:
  - Standard Methods
  - EPA methods
  - Hach methods
- **Wastewater: 40 CFR 136**
- Always check to make sure you are using an approved method!

18

Method Updates in Part 136

- New 2021 update
- SM 9223 B – 2016
  - Now includes Colilert
  - SM2540 Series – 2015
  - SM 4500-NO3 – 2016
  - SM 4500 0 (B-F, and G) –
  - SM 5210 B – 2016 Biochemical Oxygen Demand

New versions of approved ASTM methods
New USGS inorganic methods based on previously approved technologies

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Section 136.7 Lab QA

- …suitable QA/QC procedures…
- …QA/QC procedures are generally included in the method or may be found in the methods compendium… (Standard Methods)
- "The permittee/lab shall follow these QA/QC procedures, as described in the method or methods compendium. (Standard Methods)
- If the method lacks QA/QC…

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Three QA Options

- A. …follow equivalent EPA procedures
- B. Refer to QA/QC in consensus organization compendium (Ex: Follow Standard Methods) Didn't we have that on the previous slide?
- **C. Follow the 12 Steps where applicable**

21

Quality Control Elements

1. DOC – demonstration of capability
2. MDL – method detection level
3. LRB/MB – method blank
4. LFB – laboratory fortified blank (standard)
5. LFM/LFMD – laboratory fortified matrix/duplicate (spike)
6. Internal standards, surrogate standards or tracer – ***in general, only applies to organic and metals analysis, and radiochemistry***
7. Calibration- initial and continuing
8. Control charts or other trend analysis
9. Corrective action – root cause analysis
10. QC acceptance criteria
11. Definition of a batch (preparation and analytical)
12. Minimum frequency for conducting all QC elements
13. SOP – Now required by 40 CFR 136

Not all of these items apply to all tests, there are many exceptions!

22

SM 1020 A

Quality Assurance

23

Introduction

- Laboratory operations program that specifies the measures required to produce defensible data with known precision and accuracy
  - Defined in a QA manual
  - Written procedures
  - Work instructions
  - Records
- QA Manual
  - Policy defining the stating the statistical level of confidence used to express data precision and bias
  - Method detection levels (MDLs)
  - Minimum reporting levels (MRLs)
  - All QA policies and quality control processes to demonstrate the laboratory's competence and ensure and document the quality of its analytical data

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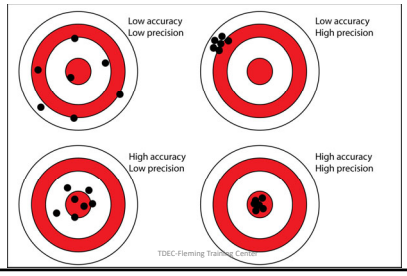
Accuracy vs. Precision



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Standard Operating Procedures

- Describe the analytical methods to be used in the laboratory in sufficient detail that a competent analyst unfamiliar with a method can conduct a reliable review and/or obtain acceptable results
- Should include:
  - Title of referenced,
  - Consensus test method
  - Sample matrix or matrices
  - MDL
  - Scope and application
  - Summary of SOP
  - Definitions
  - Interferences
  - Safety considerations
  - Waste management
  - Apparatus, equipment, and supplies
  - Reagents and standards
  - Sample collection, preservation, shipment and storage requirements
  - Specific QC practices, frequency, acceptance criteria, and required corrective action if acceptance criteria are not met
  - Calibration and standardization
  - Details on actual test procedure
  - Calculations
  - Qualifications and performance requirements for analysts
  - Data assessment/data management
  - References

26

SM 1020 B.

Calibration Curves

27

Calibration Curves

- Determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration
- Instrument calibration:
  - Perform instrument maintenance and calibration according to method or instrument manual instructions
  - Conduct instrument performance according to method or SOP instructions

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Calibration Curves

- Initial calibration:
  - Use at least 3 concentrations of standards for linear curves, or as specified by method of choice
  - Lowest concentration at reporting limit
  - Highest concentration defines the upper end of the calibration range
  - Ensure that the calibration range encompasses the analytical concentration values expected in samples or dilutions
- Choose calibration standard concentrations with no more than one order of magnitude between concentrations
  - Calibration of a pH probe you are always within one order of magnitude of your calibration concentrations
- Linear regression, use the minimum correlation coefficient in the method, if not mention use a minimum value of 0.995

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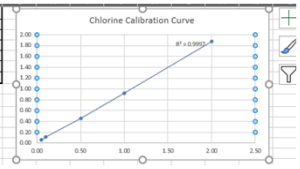
Calibration Curves

- Calibration Verification:
- Analysts periodically use a calibration standard to confirm that instrument performance has not changed significantly since initial calibration
  - Based on time or number of samples (every 10 samples)
  - Verify calibration by analyzing one standard at a concentration near or at the midpoint of the calibration range

30

Subject	true value (x)	value read (y)	(x)(y)	x^2	y^2
1	2.00	1.88	3.7600	4.0000	3.5344
2	1.00	0.92	0.9200	1.0000	0.8464
3	0.50	0.46	0.2300	0.2500	0.2116
4	0.10	0.11	0.0110	0.0100	0.0121
5	0.05	0.06	0.0030	0.0025	0.0036
Sum (Σ)	3.65	3.43	4.9240	5.2625	4.6081

R^2 = 0.99967267



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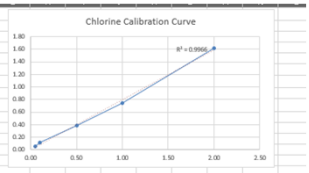
Subject	true value (x)	value read (y)	(x)(y)	x^2	y^2
1	2.00	1.62	3.2400	4.0000	2.6244
2	1.00	0.74	0.7400	1.0000	0.5476
3	0.50	0.39	0.1950	0.2500	0.1521
4	0.10	0.12	0.0120	0.0100	0.0144
5	0.05	0.05	0.0025	0.0025	0.0025
Sum (Σ)	3.65	2.92	4.1895	5.2625	3.3410

R^2 = 0.99655305



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Control Charts

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Control Charts

- A graphical record of quality by displaying QC results over time to demonstrate statistical control of an analytical process and to detect apparent changes in the analytical process that may erode such control
- Use accuracy and precision QC measures
- Two types of control charts are commonly used in laboratories are:
  - Accuracy (means) charts for QC samples
  - Precision (range) charts for replicate or duplicate analysis

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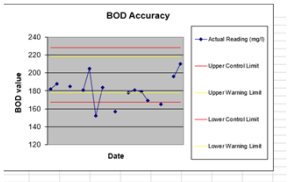
Accuracy Chart

- QC samples is constructed from the average and standard deviation of a specified number of measurements of the analyte of interest
  - Reagent blanks, calibration check standards, LFBs, LFMs
- Upper and lower warning levels (WLs)
  - ±2*s*
- Upper and lower control levels (CLs)
  - ±3*s*
- *s* = Standard deviation
  - Standard deviation is how dispersed the data is in relation to the mean



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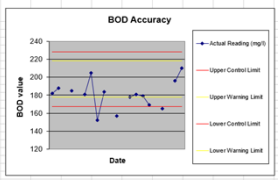
Accuracy Chart

BOD Accuracy

Date	Actual Reading (mg/l)	Standard Value	Difference	*Upper Limit	*Lower Limit	*2/3 Upper	*2/3 Lower
8/22	182	198	-16	228.5	167.5	218.33	177.67
8/23	188	198	-10	228.5	167.5	218.33	177.67
8/24		198		228.5	167.5	218.33	177.67
8/25	185	198	-13	228.5	167.5	218.33	177.67
8/26		198		228.5	167.5	218.33	177.67
8/27	181	198	-17	228.5	167.5	218.33	177.67
8/28	205	198	7	228.5	167.5	218.33	177.67
8/29	152	198	-46	228.5	167.5	218.33	177.67
8/30	184	198	-14	228.5	167.5	218.33	177.67
8/31		198		228.5	167.5	218.33	177.67
9/1	157	198	-41	228.5	167.5	218.33	177.67
9/2		198		228.5	167.5	218.33	177.67
9/3	178	198	-20	228.5	167.5	218.33	177.67
9/4	181	198	-17	228.5	167.5	218.33	177.67
9/5	179	198	-19	228.5	167.5	218.33	177.67
9/6	169	198	-29	228.5	167.5	218.33	177.67
9/7		198*	12	228.5	167.5	218.33	177.67
9/8	165	198	-33	228.5	167.5	218.33	177.67
9/9		198		228.5	167.5	218.33	177.67
9/10	196	198	-2	228.5	167.5	218.33	177.67
9/11	210	198	12	228.5	167.5	218.33	177.67

* Control and warning limits are based on +/- 30.5 mg/l, the standard value of the glucose solution.



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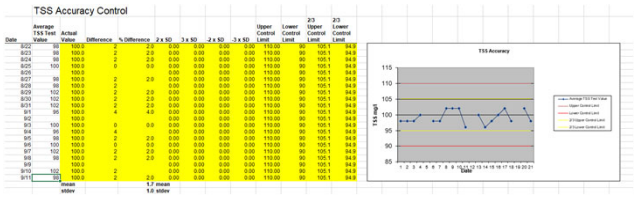
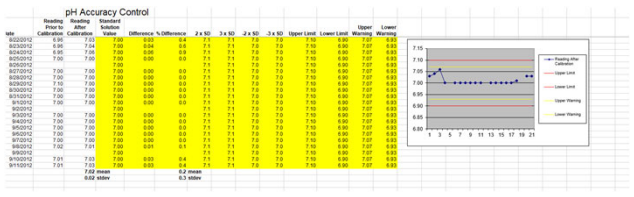
Accuracy Chart



37

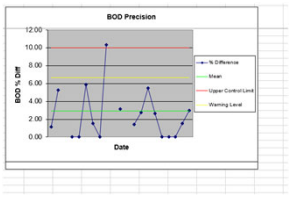
Accuracy Chart



38

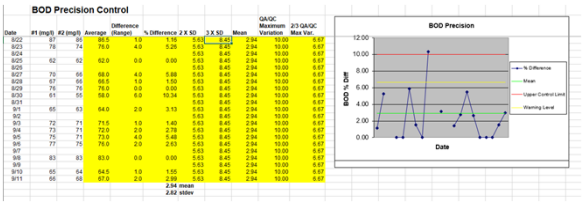
Precision Chart

- Constructed from the average and standard deviation of a specified number of measurements
- Used for replicate or duplicate analyses of the analyte of interest
- Only upper WLs and upper CLs are meaningful
- If replicates in perfect agreement than difference is zero



39

Precision Chart



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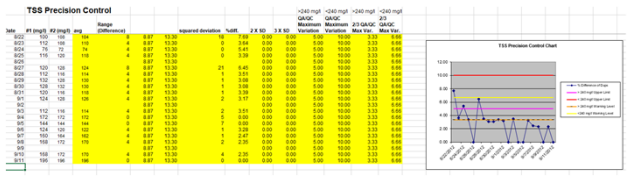
Precision Chart



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Precision Chart

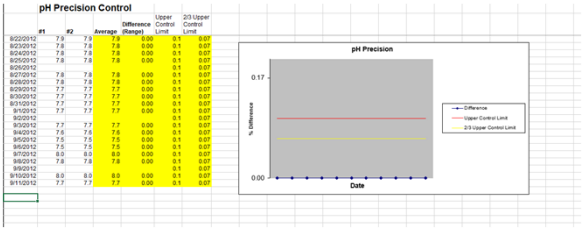

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Chart Analyses

- WLs are at the 95% confidence level, average of 1 out of 20 samples would exceed the limit
- 1 out of 100 will exceed the CLs
- The choice of rules to evaluate control charts should balance the risk between false positives and false negatives in method performance

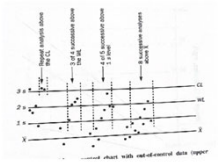

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Chart Analyses

- Control limit – if one measurement exceeds a CL, repeat the analysis immediately
  - If within CL, continue analyses
  - If exceed CL, discontinue analyses and correct the problem
- Warning limit – if two out of three successive points exceed a WL, analyze another sample
  - If within WL, continue analyses
  - If exceeds WL, evaluate potential bias and correct the problem

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Chart Analyses

- Standard deviation – if 4 out of 5 exceed 1 *s* or are in decreasing or increasing order, analyze another sample
  - If less than 1 *s* or changes the order, continue analysis
  - if not, discontinue and correct the problem
- Trending – if 7 successive samples are on the same side of the central line, discontinue analyses and correct the problem

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SM 1020 B.

Quality Control

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Quality Control

- Initial/ongoing Demonstration of capability
- MDL determination
- Reagent blank (method blank)
- Laboratory-fortified blank (LFB)
- Laboratory-fortified matrix/duplicate
- duplicate
- Calibration
- Control charts
- Corrective action
- Frequency of QC indicators
- QC acceptance criteria

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Initial Demonstration of Capability

- Each analyst should conduct at least once before analyzing any sample to demonstrate proficiency in performing the method and obtaining acceptable results for each analytes
- Also used to demonstrate that the laboratory's modifications to a method will produce as precise and accurate as those produced by the reference method
- At a minimum, include a reagent blank, and at least four LFBs at a concentration between 10x the MDL and the midpoint of the calibration curve
- Run after calibration
- Ensure that precision (precent relative standard deviation) and accuracy (percent recovery) calculated for LFBs are within the acceptance criteria listed in the method

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Method Detection Limit (MDL)

- Minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank
- straightforward technique for estimation of the detection limit for a broad variety of physical and chemical methods
- Procedure requires a complete, specific, and well-defined analytical method
- It is essential that all sample processing steps used by the laboratory be included in the determination of the method detection limit

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Method Detection Limit (MDL) Procedure

- Estimate initial MDL by one or more of the following:
  - Mean determined concentration plus 3 times the standard deviation of a set of method blanks
  - Concentration value that corresponds to an instrument signal-to-noise ratio in the range of 3 to 5
- The concentration equivalent to 3 x the standard deviation of replicate instrumental measurements of spiked blanks
- The region of the calibration where there is a significant change in sensitivity i.e., a break in the slope of the calibration
- Instrumental limitations
- Previously determined MDL

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Method Detection Limit (MDL) Procedure

- Determine the initial MDL
  - Select a spiking level, typically 2-10 times the estimated MDL in first section
- Process a minimum or seven spiked samples and seven method blank samples through all steps of the method. The samples used for the MDL must be prepared in at least three batches on three separate calendar dates (preparation and analysis may be on the same day)

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Method Detection Limit (MDL) Procedure

- Existing data may be used if compliant with requirements for at least three batches, and generated within last 24 months
- Most recent available data for method blanks and spiked samples must be used
- Statistical outlier removal procedures should not be used to remove data for the initial MDL determination, since the total number of observations is small, and the purpose of the MDL procedure is to capture routine method variability.

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Method Detection Limit (MDL) Procedure

- Gross failures may be excluded from the calculations, provided that at least seven spiked samples and seven method blanks are available
- Multiple instruments assigned same MDL, sample analyses must be distributed across all instruments
- Minimum of two spiked samples and two method blank samples prepared and analyzed on different calendar dates is required for each instrument

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Method Detection Limit (MDL) Procedure

- Evaluate the spiking level: if any result for any individual analyte from the spiked samples does not meet the method qualitative identification criteria or does not provide a numerical result greater than zero, then repeat the spiked samples at a higher concentration
- Compute MDL_s and MDL_b
  - Fleming Training Center Website

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Method Detection Limit (MDL) Procedure

- Ongoing Data Collection
  - During any quarter in which samples are being analyzed, prepare and analyze a minimum of two spiked samples on each instrument, in separate batches, using the same spiking concentration
    - Analytes repeatedly not detected in quarterly spiked sample analyses, or do not meet the qualitative identification criteria of the method then this is an indication that the spiking level is not high enough and should be adjusted
    - Not necessary to run separate method blanks than what is already routinely analyzed

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Method Detection Limit (MDL) Procedure

- Ensure that at least seven spiked samples and seven method blanks are completed for the annual verification. IF only one instrument is in use, a minimum of seven spikes are still required, but they may be drawn from the last two years of data collection.
- At least once per year, re-evaluate the spiking level.
  - If more than 5% of spikes do not return positive numerical results that meet all method qualitative identification criteria, then the spiking level must be increased, and the initial MDL re-determined

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Method Detection Limit (MDL) Procedure

- Ongoing Annual Verification
  - At least once of every thirteen months, re-calculate MDL_s and MDL_b from the collected spiked samples and method blank results
  - Include data generated within the last 24 months, but only data with spiking level.
  - Ideally, used all method blank results from the last 24 months
- Only use data associated with acceptable calibrations and batch QC. Include all routine data, except for batches that are rejected, and the associated samples reanalyzed. IF the method has been altered in a way that can be reasonable expected to change sensitivity, then use only data collected after the change
- Verified MDL is greater of the MDL_s or MDL_b

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Reagent Blank

- Method blank
- Reagent water and all reagents (including preservatives) that normally are in contact with a sample during the entire analytical procedure
- Determine whether, and how much, reats and the preparative analytical steps contribute to the measurement uncertainty
- Include one reagent blank with each sample set or on a 5% basis, which ever is more frequent
- If a carryover is suspected analyze a blank after daily calibration standard or after highly contaminated samples
- Evaluate results for contamination
  - If present, identify and eliminate the source
  - Samples analyzed with a contaminated blank must be re-prepared and re-analyzed

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Laboratory – Fortified Blank (LFB)

- Standard
- A reagent water sample to which a known concentration of analyte of interest has been added
- Evaluate laboratory performance and analyte recovery in a blank matrix
- High enough to be measured precisely, but not high enough to be irrelevant to measured environmental concentrations
  - At least 10x the MDL les than or equal to the midpoint of the calibration curve
- Include one LFB with each batch or on a 5% basis
- Process standard through all sample preparation analysis steps
- Low-level LFB at 2 to 5 times the MDL can be used as a check for false negatives and for MDL verification
- Use LFB results to evaluate batch performance, calculate recovery limits, and plot control charts

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Laboratory – Fortified Matrix (LFM)

- An additional portion of a sample to which a known amount of the analyte(s) of interest is added before sample preparation
  - Not relevant for all tests
- Evaluate analyte recovery in a sample matrix
- Include at least one with every batch or on a 5% basis
- Add a concentration that is at least 10 times the MDL, less than or equal to the midpoint of the calibration curve, or method-specified level to the selected sample.
- Preferably use the same concentration and source as for the LFB to allow analysts to separate the matrix's effect from laboratory performance
- Make the addition such that sample background levels do not adversely affect recovery
  - If the sample contains the analyte of interest, then add approximately as much analyte to the LFM sample as the concentration found in the sample
- Evaluate results for LFMs for accuracy or percent recovery

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Duplicate Sample/ LFM Duplicate

- Samples analyzed randomly to assess precision on an ongoing basis
- If analyte is rarely detected in a matrix use an LFM duplicate
  - Is a second portion of the sample to which a known amount of the analyte of interest is added before sample preparation
  - Processed the same as the LFM
- Include on a 5% basis

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Corrective Action

- Standard Methods 1020 B.15
  - QC data that are outside the acceptance limits or exhibit a trend are evidence of unacceptable error in the analytical process.
  - Take corrective action promptly to determine and eliminate the source of error.
  - Do not report data until the cause of the problem is identified and either corrected or qualified (see Table 1020:II)

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Corrective Action

- The corrective action plan needs to be in your SOP for each method on what to do if your QC tests fail or are out of range
- If you have a "boo boo", write down how you fixed it
- Any issues should be recorded and a sentence on how it can be prevented, if possible, in the future
- Common problems and their corrections should be covered in your Standard Operating Procedures (SOP)
  - If you see things frequently, you can give them qualifiers that are noted in your SOP;
    - R = rain event
    - D = bad dilution, etc.

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QC Acceptance

- Have in SOP for each method the acceptance ranges for standards, duplicates, spikes, etc. and make sure they match the method requirements.
- If not mentioned in method, these are the accepted criteria for QC:
  - Blank < reporting limit
  - LFB ± 15%
  - MS/MSD ± 20%
  - ICV/CCV ± 10%
  - RPD < 20%
  - Reporting limit = MDL

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Batch Size

- Each "Batch" could be daily (day of), every 10 samples or every 20 samples.
- Check method
- *Influent and Effluent are 2 different samples*
- If you sample only once a month, need to run QC each time.
  - Once per month is minimum requirement

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Batch Size

- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
  - If a permit stated that 3 analyses per week, how many samples would that be a week?
    - TSS and BOD would be 6 (Influent and Effluent), Cl_2 would be 3
  - If a permit stated 5 analyses per week, how many samples would that be a week?
    - TSS and BOD would be 10 (Influent and Effluent), Cl_2 would be 5

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QC Frequency

- Usually lumped in with the definition of a "batch" and should be in the SOP of some kind

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Documentation

- Review of log books
  - ❖Instrument calibration (daily)
  - ❖Temperature
  - ❖Maintenance
  - ❖Sampler
  - ❖Standard preparation
  - ❖Calibration
- Lab instruments - yearly maintenance check (or more frequently)
  - Including thermometers and weights
- Flow measurement devices – yearly maintenance check

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Bench Sheets

- Where the analyst records the test results
- Even though data is transferred to the DMR, bench sheets are still an official record
- At a minimum, it should include:
  1. Date
  2. Time
  3. Analyst's initials
  4. Name of test/Method #
  5. Sample results
  6. Lot #s

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QA/QC - Review Questions

1. In order to be useful, data must be _____.

2. Explain the difference between Precision and Accuracy. Which QA/QC procedure is associated with determining precision? Which QA/QC procedure is associated with determining accuracy?

3. Your NPDES permit spells out the exact QA/QC program and procedure that you should implement in your lab.
    a. True
    b. False

4. When determining if you are using an approved Standard Method, what date do you need to check for? Where do you locate this date?

5. Code of Federal Regulations (CFR) can supersede Standard Methods.
    a. True
    b. False

6. Which section of the CFR applies to municipal wastewater treatment plants?

7. Write out the full names of the following acronyms:
    a. DOC
    b. MDL
    c. LRB
    d. LFB
    e. LFM/LFMD
    f. “Dup”
    g. ICV
    h. CCV

8. This QC procedure is needed to show that an operator has received adequate training and knows how to properly conduct a test procedure and is capable of producing data that meets QC requirements.

9. This QC procedure will give the lowest concentration that a lab can detect an analyte with 99% confidence that it is distinguishable from a method blank.

10. Explain how often data must be obtained and entered into the MDL calculator as part of the "ongoing data collection" requirement for the MDL procedure.

11. What is the QC name for a reagent water sample to which a known concentration of the analyte of interest has been added?

12. What is the purpose in running a Laboratory Fortified Matrix (LFM)?

13. When does the Method Update Rule require a plant to create and maintain control charts?

14. When should an operator conduct a corrective action?

15. If a laboratory analysis produced data that did not meet the QC acceptance limits, the only action that an operator needs to take is to fill out a corrective action form.
    a. True
    b. False

16. What should be included in the annual review of your SOPs?

17. Even though bench sheets are not submitted to the State each month, they are considered official records.
    a. True
    b. False

Section 10
40 CFR 136 Excerpt

Title 40 - Protection of Environment
Chapter I - Environmental Protection Agency
Subchapter D - Water Programs
Part 136 - Guidelines Establishing Test Procedures for the Analysis of Pollutants

§ 136.3 Identification of test procedures.

(a) Parameters or pollutants, for which methods are approved, are listed together with test procedure descriptions and references in Tables IA, IB, IC, ID, IE, IF, IG, and IH of this section. The methods listed in Tables IA, IB, IC, ID, IE, IF, IG, and IH are incorporated by reference, see paragraph (b) of this section, with the exception of EPA Methods 200.7, 601-613, 624.1, 625.1, 1613, 1624, and 1625. The full texts of Methods 601-613, 624.1, 625.1, 1613, 1624, and 1625 are printed in appendix A of this part, and the full text of Method 200.7 is printed in appendix C of this part. The full text for determining the method detection limit when using the test procedures is given in appendix B of this part. In the event of a conflict between the reporting requirements of 40 CFR parts 122 and 125 and any reporting requirements associated with the methods listed in these tables, the provisions of 40 CFR parts 122 and 125 are controlling and will determine a permittee's reporting requirements. The full texts of the referenced test procedures are incorporated by reference into Tables IA, IB, IC, ID, IE, IF, IG, and IH. The date after the method number indicates the latest editorial change of the method. The discharge parameter values for which reports are required must be determined by one of the standard analytical test procedures incorporated by reference and described in Tables IA, IB, IC, ID, IE, IF, IG, and IH or by any alternate test procedure which has been approved by the Administrator under the provisions of paragraph (d) of this section and §§ 136.4 and 136.5. Under certain circumstances (paragraph (c) of this section, § 136.5(a) through (d) or 40 CFR 401.13,) other additional or alternate test procedures may be used.

Table IA - List of Approved Biological Methods for Wastewater and Sewage Sludge

Parameter and units	Method[1]	EPA	Standard methods	AOAC, ASTM, USGS	Other
Bacteria					
1. Coliform (fecal), number per 100 mL or number per gram dry weight	Most Probable Number (MPN), 5 tube, 3 dilution, or	p. 132,[3] 1680,[11] [15] 1681 [11] [20]	9221 E-2014		
	Membrane filter (MF) [2] [5], single step	p. 124 [3]	9222 D-2015 [29]	B-0050-85 [4]	
2. Coliform (fecal), number per 100 mL	MPN, 5 tube, 3 dilution, or	p. 132 [3]	9221 E-2014; 9221 F-2014 [33]		
	Multiple tube/multiple well, or				Colilert-18®.[13] [18] [28]
	MF [2] [5], single step [5]	p. 124 [3]	9222 D-2015 [29]		
3. Coliform (total), number per 100 mL	MPN, 5 tube, 3 dilution, or	p. 114 [3]	9221 B-2014		
	MF [2] [5], single step or two step	p. 108 [3]	9222 B-2015 [30]	B-0025-85 [4]	

Parameter and units	Method[1]	EPA	Standard methods	AOAC, ASTM, USGS	Other
	MF [2 5], with enrichment	p. 111 [3]	9222 B-2015 [30]		
4. *E. coli,* number per 100 mL	MPN [6 8 16] multiple tube, or		9221 B2014/9221 F-2014 [12 14 33]		
	multiple tube/multiple well, or		9223 B-2016 [13]	991.15 [10]	Colilert® [13 18] Colilert-18® [13 17 18]
	MF [2 5 6 7 8], two step, or		9222 B-2015/9222 I-2015 [31]		
	Single step	1603 [21]			m-ColiBlue24®.[19]
5. Fecal streptococci, number per 100 mL	MPN, 5 tube, 3 dilution, or	p. 139 [3]	9230 B-2013		
	MF [2], or	p. 136 [3]	9230 C-2013 [32]	B-0055-85 [4]	
	Plate count	p. 143 [3]			
6. Enterococci, number per 100 mL	MPN, 5 tube, 3 dilution, or	p. 139 [3]	9230 B-2013		
	MPN [6 8], multiple tube/multiple well, or		9230 D-2013	D6503-99 [9]	Enterolert®.[13 23]
	MF [2 5 6 7 8] single step or	1600 [24]	9230 C-2013 [32]		
	Plate count	p. 143 [3]			
7. *Salmonella,* number per gram dry weight [11]	MPN multiple tube	1682 [22]			
Aquatic Toxicity					
8. Toxicity, acute, fresh water organisms, LC_{50}, percent effluent	Water flea, Cladoceran, *Ceriodaphnia dubia* acute	2002.0 [25]			
	Water fleas, Cladocerans, *Daphnia pulex* and *Daphnia magna* acute	2021.0 [25]			

Parameter and units	Method[1]	EPA	Standard methods	AOAC, ASTM, USGS	Other
	Fish, Fathead minnow, *Pimephales promelas,* and Bannerfin shiner, *Cyprinella leedsi,* acute	2000.0 [25]			
	Fish, Rainbow trout, *Oncorhynchus mykiss,* and brook trout, *Salvelinus fontinalis,* acute	2019.0 [25]			
9. Toxicity, acute, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, LC_{50}, percent effluent	Mysid, *Mysidopsis bahia,* acute	2007.0 [25].			
	Fish, Sheepshead minnow, *Cyprinodon variegatus,* acute	2004.0 [25]			
	Fish, Silverside, *Menidia beryllina, Menidia menidia,* and *Menidia peninsulae,* acute.	2006.0 [25].			
10. Toxicity, chronic, fresh water organisms, NOEC or IC_{25}, percent effluent	Fish, Fathead minnow, *Pimephales promelas,* larval survival and growth	1000.0 [26]			
	Fish, Fathead minnow, *Pimephales promelas,* embryo-larval survival and teratogenicity	1001.0 [26]			
	Water flea, Cladoceran, *Ceriodaphnia dubia,* survival and reproduction	1002.0 [26]			
	Green alga, *Selenastrum capricornutum,* growth	1003.0 [26]			

Parameter and units	Method[1]	EPA	Standard methods	AOAC, ASTM, USGS	Other
11. Toxicity, chronic, estuarine and marine organisms of the Atlantic Ocean and Gulf of Mexico, NOEC or IC_{25}, percent effluent	Fish, Sheepshead minnow, *Cyprinodon variegatus,* larval survival and growth	1004.0 [27].			
	Fish, Sheepshead minnow, *Cyprinodon variegatus,* embryo-larval survival and teratogenicity	1005.0 [27]			
	Fish, Inland silverside, *Menidia beryllina,* larval survival and growth	1006.0 [27]			
	Mysid, *Mysidopsis bahia,* survival, growth, and fecundity	1007.0 [27]			
	Sea urchin, *Arbacia punctulata,* fertilization	1008.0 [27]			

Table IA notes:

[1] The method must be specified when results are reported.

[2] A 0.45-µm membrane filter (MF) or other pore size certified by the manufacturer to fully retain organisms to be cultivated and to be free of extractables which could interfere with their growth.

[3] Microbiological Methods for Monitoring the Environment, Water and Wastes, EPA/600/8-78/017. 1978. U.S. EPA.

[4] U.S. Geological Survey Techniques of Water-Resource Investigations, Book 5, Laboratory Analysis, Chapter A4, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples. 1989. USGS.

[5] Because the MF technique usually yields low and variable recovery from chlorinated wastewaters, the Most Probable Number method will be required to resolve any controversies.

[6] Tests must be conducted to provide organism enumeration (density). Select the appropriate configuration of tubes/filtrations and dilutions/volumes to account for the quality, character, consistency, and anticipated organism density of the water sample.

[7] When the MF method has been used previously to test waters with high turbidity, large numbers of noncoliform bacteria, or samples that may contain organisms stressed by chlorine, a parallel test should be conducted with a multiple-tube technique to demonstrate applicability and comparability of results.

[8] To assess the comparability of results obtained with individual methods, it is suggested that side-by-side tests be conducted across seasons of the year with the water samples routinely tested in accordance with the most current *Standard Methods for the Examination of Water and Wastewater* or EPA alternate test procedure (ATP) guidelines.

[9] Annual Book of ASTM Standards-Water and Environmental Technology, Section 11.02. 2000, 1999, 1996. ASTM International.

[10] Official Methods of Analysis of AOAC International. 16th Edition, 4th Revision, 1998. AOAC International.

[11] Recommended for enumeration of target organism in sewage sludge.

[12] The multiple-tube fermentation test is used in 9221B.2-2014. Lactose broth may be used in lieu of lauryl tryptose broth (LTB), if at least 25 parallel tests are conducted between this broth and LTB using the water samples normally tested, and this comparison demonstrates that the false-positive rate and false-negative rate for total coliform using lactose broth is less than 10 percent. No requirement exists to run the completed phase on 10 percent of all total coliform-positive tubes on a seasonal basis.

[13] These tests are collectively known as defined enzyme substrate tests.

[14] After prior enrichment in a presumptive medium for total coliform using 9221B.2-2014, all presumptive tubes or bottles showing any amount of gas, growth or acidity within 48 h ± 3 h of incubation shall be submitted to 9221F-2014. Commercially available EC-MUG media or EC media supplemented in the laboratory with 50 µg/mL of MUG may be used.

[15] Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation Using Lauryl-Tryptose Broth (LTB) and EC Medium, EPA-821-R-14-009. September 2014. U.S. EPA.

[16] Samples shall be enumerated by the multiple-tube or multiple-well procedure. Using multiple-tube procedures, employ an appropriate tube and dilution configuration of the sample as needed and report the Most Probable Number (MPN). Samples tested with Colilert® may be enumerated with the multiple-well procedures, Quanti-Tray® or Quanti-Tray®/2000 and the MPN calculated from the table provided by the manufacturer.

[17] Colilert-18® is an optimized formulation of the Colilert® for the determination of total coliforms and *E. coli* that provides results within 18 h of incubation at 35 °C rather than the 24 h required for the Colilert® test and is recommended for marine water samples.

[18] Descriptions of the Colilert®, Colilert-18®, Quanti-Tray®, and Quanti-Tray®/2000 may be obtained from IDEXX Laboratories, Inc.

[19] A description of the mColiBlue24® test is available from Hach Company.

[20] Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation Using A-1 Medium, EPA-821-R-06-013. July 2006. U.S. EPA.

[21] Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified Membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC), EPA-821-R-14-010. September 2014. U.S. EPA.

[22] Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, EPA-821-R-14-012. September 2014. U.S. EPA.

[23] A description of the Enterolert® test may be obtained from IDEXX Laboratories Inc.

[24] Method 1600: Enterococci in Water by Membrane Filtration Using Membrane-Enterococcus Indoxyl-β-D-Glucoside Agar (mEI), EPA-821-R-14-011. September 2014. U.S. EPA.

[25] Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, EPA-821-R-02-012. Fifth Edition, October 2002. U.S. EPA; and U.S. EPA Whole Effluent Toxicity Methods Errata Sheet, EPA 821-R-02-012-ES. December 2016.

[26] Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013. Fourth Edition, October 2002. U.S. EPA; and U.S. EPA Whole Effluent Toxicity Methods Errata Sheet, EPA 821-R-02-012-ES. December 2016.

[27] Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, EPA-821-R-02-014. Third Edition, October 2002. U.S. EPA; and U.S. EPA Whole Effluent Toxicity Methods Errata Sheet, EPA 821-R-02-012-ES. December 2016.

[28] To use Colilert-18® to assay for fecal coliforms, the incubation temperature is 44.5 ± 0.2 °C, and a water bath incubator is used.

[29] On a monthly basis, at least ten blue colonies from positive samples must be verified using Lauryl Tryptose Broth and EC broth, followed by count adjustment based on these results; and representative non-blue colonies should be verified using Lauryl Tryptose Broth. Where possible, verifications should be done from randomized sample sources.

[30] On a monthly basis, at least ten sheen colonies from positive samples must be verified using lauryl tryptose broth and brilliant green lactose bile broth, followed by count adjustment based on these results; and representative non-sheen colonies should be verified using lauryl tryptose broth. Where possible, verifications should be done from randomized sample sources.

[31] Subject coliform positive samples determined by 9222 B-2015 or other membrane filter procedure to 9222 I-2015 using NA-MUG media.

[32] Verification of colonies by incubation of BHI agar at 10 ± 0.5 °C for 48 ± 3 h is optional. As per the Errata to the 23rd Edition of *Standard Methods for the Examination of Water and Wastewater* "Growth on a BHI agar plate incubated at 10 ± 0.5 °C for 48 ± 3 h is further verification that the colony belongs to the genus *Enterococcus.*"

[33] 9221 F.2-2014 allows for simultaneous detection of *E. coli* and thermotolerant fecal coliforms by adding inverted vials to EC-MUG; the inverted vials collect gas produced by thermotolerant fecal coliforms.

Table IB - List of Approved Inorganic Test Procedures

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
1. Acidity, as $CaCO_3$, mg/L	Electrometric endpoint or phenolphthalein endpoint		2310 B-2011	D1067-16	I-1020-85.[2]
2. Alkalinity, as $CaCO_3$, mg/L	Electrometric or Colorimetric titration to pH 4.5, Manual		2320 B-2011	D1067-16	973.43,[3] I-1030-85.[2]
	Automatic	310.2 (Rev. 1974)[1]			I-2030-85.[2]
3. Aluminum - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 D-2011 or 3111 E-2011		I-3051-85.[2]
	AA furnace		3113 B-2010.		
	STGFAA	200.9, Rev. 2.2 (1994)/			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Direct Current Plasma (DCP)[36]			D4190-15	See footnote.[34]
	Colorimetric (Eriochrome cyanine R)		3500-Al B-2011.		
4. Ammonia (as N), mg/L	Manual distillation[6] or gas diffusion (pH > 11), followed by any of the following:	350.1, Rev. 2.0 (1993)	4500-NH_3 B-2011		973.49.[3]
	Nesslerization			D1426-15 (A)	973.49,[3] I-3520-85.[2]
	Titration		4500-NH_3 C-2011.		
	Electrode		4500-NH_3 D-2011 or E-2011	D1426-15 (B).	
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction-based methods		4500-NH_3 F-2011		See footnote.[60]
	Automated phenate, salicylate, or other substituted phenols in Berthelot reaction-based methods	350.1,[30] Rev. 2.0 (1993)	4500-NH_3 G-2011 4500-NH_3 H-2011		I-4523-85,[2] I-2522-90.[80]
	Automated electrode				See footnote.[7]
	Ion Chromatography			D6919-17.	
	Automated gas diffusion, followed by conductivity cell analysis				Timberline Ammonia-001.[74]
	Automated gas diffusion followed by fluorescence detector analysis				FIAlab100.[82]
5. Antimony - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011.		
	AA furnace		3113 B-2010.		

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12.	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]
6. Arsenic-Total,[4] mg/L	Digestion,[4] followed by any of the following:	206.5 (Issued 1978)[1].			
	AA gaseous hydride		3114 B-2011 or 3114 C-2011	D2972-15 (B)	I-3062-85.[2]
	AA furnace		3113 B-2010	D2972-15 (C)	I-4063-98.[49]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12.	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05.[70]
	Colorimetric (SDDC)		3500-As B-2011	D2972-15 (A)	I-3060-85.[2]
7. Barium-Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 D-2011		I-3084-85.[2]
	AA furnace		3113 B-2010	D4382-18.	

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	ICP/AES[36]	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011		I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]
	DCP[36]				See footnote.[34]
8. Beryllium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 D-2011 or 3111 E-2011	D3645-15 (A)	I-3095-85.[2]
	AA furnace		3113 B-2010	D3645-15 (B).	
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]
	DCP			D4190-15	See footnote.[34]
	Colorimetric (aluminon)		See footnote.[61].		
9. Biochemical oxygen demand (BOD_5), mg/L	Dissolved Oxygen Depletion		5210 B-2016[85]		973.44,[3] p. 17,[9] I-1578-78,[8] See footnote.[10, 63]
10. Boron - Total,[37] mg/L	Colorimetric (curcumin)		4500-B B-2011		I-3112-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	ICP/AES	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	DCP			D4190-15	S7ee footnote.[34]
11. Bromide, mg/L	Electrode			D1246-16	I-1125-85.[2]
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997)	4110 B-2011, C-2011, D-2011	D4327-17	993.30,[3] I-2057-85.[79]
	CIE/UV		4140 B-2011	D6508-15	D6508, Rev. 2.[54]
12. Cadmium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011 or 3111 C-2011	D3557-17 (A or B)	974.27,[3] p. 37,[9] I-3135-85[2] or I-3136-85.[2]
	AA furnace		3113 B-2010	D3557-17 (D)	I-4138-89.[51]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-1472-85[2] or I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]
	DCP[36]			D4190-15	See footnote.[34]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Voltammetry[11]			D3557-17 (C).	
	Colorimetric (Dithizone)		3500-Cd-D-1990.		
13. Calcium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011	D511-14 (B)	I-3152-85.[2]
	ICP/AES	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011		I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	DCP				See footnote.[34]
	Titrimetric (EDTA)		3500-Ca B-2011	D511-14 (A).	
	Ion Chromatography			D6919-17.	
14. Carbonaceous biochemical oxygen demand ($CBOD_5$), mg/L[12]	Dissolved Oxygen Depletion with nitrification inhibitor		5210 B-2016[85]		See footnote.[35 63]
15. Chemical oxygen demand (COD), mg/L	Titrimetric	410.3 (Rev. 1978)[1]	5220 B-2011 or C-2011	D1252-06(12) (A)	973.46,[3] p. 17,[9] I-3560-85.[2]
	Spectrophotometric, manual or automatic	410.4, Rev. 2.0 (1993)	5220 D-2011	D1252-06(12) (B)	See footnotes.[13 14 83], I-3561-85.[2]
16. Chloride, mg/L	Titrimetric: (silver nitrate)		4500-Cl^- B-2011	D512-12 (B)	I-1183-85.[2]
	(Mercuric nitrate)		4500-Cl^- C-2011	D512-12 (A)	973.51,[3] I-1184-85.[2]
	Colorimetric: manual				I-1187-85.[2]
	Automated (ferricyanide)		4500-Cl^- E-2011		I-2187-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Potentiometric Titration		4500-Cl^- D-2011.		
	Ion Selective Electrode			D512-12 (C).	
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997)	4110 B-2011 or 4110 C-2011	D4327-17	993.30,[3] I-2057-90.[51]
	CIE/UV		4140 B-2011	D6508-15	D6508, Rev. 2.[54]
17. Chlorine-Total residual, mg/L	Amperometric direct		4500-Cl D-2011	D1253-14.	
	Amperometric direct (low level)		4500-Cl E-2011.		
	Iodometric direct		4500-Cl B-2011.		
	Back titration ether end-point[15]		4500-Cl C-2011.		
	DPD-FAS		4500-Cl F-2011.		
	Spectrophotometric, DPD		4500-Cl G-2011.		
	Electrode				See footnote.[16]
17A. Chlorine-Free Available, mg/L	Amperometric direct		4500-Cl D-2011	D1253-14.	
	Amperometric direct (low level)		4500-Cl E-2011.		
	DPD-FAS		4500-Cl F-2011.		
	Spectrophotometric, DPD		4500-Cl G-2011.		
18. Chromium VI dissolved, mg/L	0.45-micron filtration followed by any of the following:				
	AA chelation-extraction		3111 C-2011		I-1232-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Ion Chromatography	218.6, Rev. 3.3 (1994)	3500-Cr C-2011	D5257-17	993.23.[3]
	Colorimetric (diphenyl-carbazide)		3500-Cr B-2011	D1687-17 (A)	I-1230-85.[2]
19. Chromium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011	D1687-17 (B)	974.27,[3] I-3236-85.[2]
	AA chelation-extraction		3111 C-2011.		
	AA furnace		3113 B-2010	D1687-17 (C)	I-3233-93.[46]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003),[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12.	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05[70] I-4472-97.[81]
	DCP[36]			D4190-15	See footnote.[34]
	Colorimetric (diphenyl-carbazide)		3500-Cr B-2011.		
20. Cobalt - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011 or 3111 C-2011	D3558-15 (A or B)	p. 37,[9] I-3239-85.[2]
	AA furnace		3113 B-2010	D3558-15 (C)	I-4243-89.[51]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES	200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05[70] I-4472-97.[81]
	DCP			D4190-15	See footnote.[34]
21. Color, platinum cobalt units or dominant wavelength, hue, luminance purity	Colorimetric (ADMI)		2120 F-2011[78].		
	Platinum cobalt visual comparison		2120 B-2011		I-1250-85.[2]
	Spectrophotometric				See footnote[18]
22. Copper - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011 or 3111 C-2011	D1688-17 (A or B)	974.27,[3] p. 37,[9] I-3270-85[2] or I-3271-85.[2]
	AA furnace		3113 B-2010	D1688-17 (C)	I-4274-89.[51]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05,[70], I-4472-97.[81]
	DCP[36]			D4190-15	See footnote.[34]
	Colorimetric (Neocuproine)		3500-Cu B-2011.		
	Colorimetric (Bathocuproine)		3500-Cu C-2011		See footnote.[19]
23. Cyanide - Total, mg/L	Automated UV digestion/distillation and Colorimetry				Kelada-01.[55]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Segmented Flow Injection, In-Line Ultraviolet Digestion, followed by gas diffusion amperometry			D7511-12(17).	
	Manual distillation with $MgCl_2$, followed by any of the following:	335.4, Rev. 1.0 (1993)[57]	4500-CN^- B-2016 and C-2016	D2036-09(15) (A), D7284-13(17)	10-204-00-1-X.[56]
	Flow Injection, gas diffusion amperometry			D2036-09(15) (A) D7284-13(17).	
	Titrimetric		4500-CN^- D-2016	D2036-09(15) (A)	p. 22.[9]
	Spectrophotometric, manual		4500-CN^- E-2016	D2036-09(15) (A)	I-3300-85.[2]
	Semi-Automated[20]	335.4, Rev. 1.0 (1993)[57]	4500-CN^- N-2016		10-204-00-1-X,[56] I-4302-85.[2]
	Ion Chromatography			D2036-09(15) (A).	
	Ion Selective Electrode		4500-CN^- F-2016	D2036-09(15) (A).	
24. Cyanide-Available, mg/L	Cyanide Amenable to Chlorination (CATC); Manual distillation with $MgCl_2$, followed by Titrimetric or Spectrophotometric		4500-CN^- G-2016	D2036-09(15) (B).	
	Flow injection and ligand exchange, followed by gas diffusion amperometry[59]			D6888-16	OIA-1677-09.[44]
	Automated Distillation and Colorimetry (no UV digestion)				Kelada-01.[55]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
24.A Cyanide-Free, mg/L	Flow Injection, followed by gas diffusion amperometry			D7237-15 (A)	OIA-1677-09.[44]
	Manual micro-diffusion and colorimetry			D4282-15.	
25. Fluoride - Total, mg/L	Manual distillation,[6] followed by any of the following:		4500-F^- B-2011	D1179-16 (A).	
	Electrode, manual		4500-F^- C-2011	D1179-16 (B).	
	Electrode, automated				I-4327-85.[2]
	Colorimetric, (SPADNS)		4500-F^- D-2011.		
	Automated complexone		4500-F^- E-2011.		
	Ion Chromatography	300.0, Rev 2.1 (1993) and 300.1, Rev 1.0 (1997)	4110 B-2011 or C-2011	D4327-17	993.30.[3]
	CIE/UV		4140 B-2011	D6508-15	D6508, Rev. 2.[54]
26. Gold - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011.		
	AA furnace	231.2 (Issued 1978)[1]	3113 B-2010.		
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	DCP				See footnote.[34]
27. Hardness - Total, as $CaCO_3$, mg/L	Automated colorimetric	130.1 (Issued 1971)[1].			
	Titrimetric (EDTA)		2340 C-2011	D1126-17	973.52B[3], I-1338-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Ca plus Mg as their carbonates, by any approved method for Ca and Mg (See Parameters 13 and 33), provided that the sum of the lowest point of quantitation for Ca and Mg is below the NPDES permit requirement for Hardness		2340 B-2011.		
28. Hydrogen ion (pH), pH units	Electrometric measurement		4500-H^+ B-2011	D1293-99 (A or B)	973.41,[3] I-1586-85.[2]
	Automated electrode	150.2 (Dec. 1982)[1]			See footnote,[21] I-2587-85.[2]
29. Iridium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011.		
	AA furnace	235.2 (Issued 1978)[1].			
	ICP/MS		3125 B-2011.		
30. Iron - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011 or 3111 C-2011	D1068-15 (A)	974.27,[3] I-3381-85.[2]
	AA furnace		3113 B-2010	D1068-15 (B).	
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	DCP[36]			D4190-15	See footnote.[34]
	Colorimetric (Phenanthroline)		3500-Fe B-2011	D1068-15 (C)	See footnote.[22]
31. Kjeldahl Nitrogen[5] - Total, (as N), mg/L	Manual digestion[20] and distillation or gas diffusion, followed by any of the following:		4500-N_{org} B-2011 or C-2011 and 4500-NH_3 B-2011	D3590-17 (A)	I-4515-91.[45]
	Titration		4500-NH_3 C-2011		973.48.[3]
	Nesslerization			D1426-15 (A).	
	Electrode		4500-NH_3 D-2011 or E-2011	D1426-15 (B).	
	Semi-automated phenate	350.1, Rev. 2.0 (1993)	4500-NH_3 G-2011 4500-NH_3 H-2011		
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction based methods		4500-NH_3 F-2011		See footnote.[60]
	Automated gas diffusion, followed by conductivity cell analysis				Timberline Ammonia-001.[74]
	Automated gas diffusion followed by fluorescence detector analysis				FIAlab 100.[82]
	Automated Methods for TKN that do not require manual distillation				
	Automated phenate, salicylate, or other substituted phenols in Berthelot reaction based methods colorimetric (auto digestion and distillation)	351.1 (Rev. 1978)[1]			I-4551-78.[8]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Semi-automated block digestor colorimetric (distillation not required)	351.2, Rev. 2.0 (1993)	4500-N_{org} D-2011	D3590-17 (B)	I-4515-91[45]
	Block digester, followed by Auto distillation and Titration				See footnote.[39]
	Block digester, followed by Auto distillation and Nesslerization				See footnote.[40]
	Block Digester, followed by Flow injection gas diffusion (distillation not required)				See footnote.[41]
	Digestion with peroxdisulfate, followed by Spectrophotometric (2,6-dimethyl phenol)				Hach 10242.[76]
	Digestion with persulfate, followed by Colorimetric				NCASI TNTP W10900.[77]
32. Lead - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011 or 3111 C-2011	D3559-15 (A or B)	974.27,[3] I-3399-85.[2]
	AA furnace		3113 B-2010	D3559-15 (D)	I-4403-89.[51]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	DCP[36]			D4190-15	See footnote.[34]
	Voltammetry[11]			D3559-15 (C).	
	Colorimetric (Dithizone)		3500-Pb B-2011.		
33. Magnesium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011	D511-14 (B)	974.27,[3] I-3447-85.[2]
	ICP/AES	200.5, Rev. 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	DCP				See footnote.[34]
	Ion Chromatography			D6919-17.	
34. Manganese - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011	D858-17 (A or B)	974.27,[3] I-3454-85.[2]
	AA furnace		3113 B-2010	D858-17 (C).	
	STGFAA	200.9, Rev. 2.2 (1994)			
	ICP/AES[36]	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	DCP[36]			D4190-15	See footnote.[34]
	Colorimetric (Persulfate)		3500-Mn B-2011		920.203.[3]
	Colorimetric (Periodate)				See footnote.[23]
35. Mercury - Total, mg/L	Cold vapor, Manual	245.1, Rev. 3.0 (1994)	3112 B-2011	D3223-17	977.22,[3] I-3462-85.[2]
	Cold vapor, Automated	245.2 (Issued 1974)[1].			
	Cold vapor atomic fluorescence spectrometry (CVAFS)	245.7 Rev. 2.0 (2005)[17]			I-4464-01.[71]
	Purge and Trap CVAFS	1631E[43].			
36. Molybdenum - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 D-2011		I-3490-85.[2]
	AA furnace		3113 -2010		I-3492-96.[47]
	ICP/AES	200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]
	DCP				See footnote.[34]
37. Nickel - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011 or 3111 C-2011	D1886-14 (A or B)	I-3499-85[2]
	AA furnace		3113 B-2010	D1886-14 (C)	I-4503-89.[51]
	STGFAA	200.9, Rev. 2.2 (1994)			

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	ICP/AES[36]	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05[70] I-4472-97.[81]
	DCP[36]			D4190-15	See footnote.[34]
38. Nitrate (as N), mg/L	Ion Chromatography	300.0, Rev. 2.1 (1993) and 300.1, Rev. 1.0 (1997)	4110 B-2011 or C-2011	D4327-17	993.30.[3]
	CIE/UV	4140 B-2011	D6508-15	D6508, Rev. 2.[54]	
	Ion Selective Electrode		4500-NO_3^- D-2016.		
	Colorimetric (Brucine sulfate)	352.1 (Issued 1971)[1]			973.50,[3] 419D[17], p. 28.[9]
	Spectrophotometric (2,6-dimethylphenol)				Hach 10206[75]
	Nitrate-nitrite N minus Nitrite N (See parameters 39 and 40)				
39. Nitrate-nitrite (as N), mg/L	Cadmium reduction, Manual		4500-NO_3^- E-2016	D3867-16 (B).	
	Cadmium reduction, Automated	353.2, Rev. 2.0 (1993)	4500-NO_3^- F-2016 4500-NO_3^- I-2016	D3867-16 (A)	I-2545-90.[51]
	Automated hydrazine		4500-NO_3^- H-2016.		
	Reduction/Colorimetric				See footnote.[62]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	Ion Chromatography	300.0, Rev. 2.1 (1993) and 300.1, Rev. 1.0 (1997)	4110 B-2011 or C-2011	D4327-17	993.30.[3]
	CIE/UV		4140 B-2011	D6508-15	D6508, Rev. 2.[54]
	Enzymatic reduction, followed by automated colorimetric determination			D7781-14	I-2547-11.[72] I-2548-11.[72] N07-0003.[73]
	Enzymatic reduction, followed by manual colorimetric determination		4500-NO_3^- J-2018.		
	Spectrophotometric (2,6-dimethylphenol)				Hach 10206.[75]
40. Nitrite (as N), mg/L	Spectrophotometric: Manual		4500-NO_2^- B-2011		See footnote.[25]
	Automated (Diazotization)				I-4540-85,[2] See footnote.[62] I-2540-90.[80]
	Automated (*bypass cadmium reduction)	353.2, Rev. 2.0 (1993)	4500-NO_3^- F-2016 4500-NO_3^- I-2016	D3867-16 (A)	I-4545-85.[2]
	Manual (*bypass cadmium or enzymatic reduction)		4500-NO_3^- E-2016, 4500-NO_3^- J-2018	D3867-16 (B).	
	Ion Chromatography	300.0, Rev. 2.1 (1993) and 300.1, Rev. 1.0 (1997)	4110 B-2011 or C-2011	D4327-17	993.30.[3]
	CIE/UV		4140 B-2011	D6508-15	D6508, Rev. 2.[54]
	Automated (*bypass Enzymatic reduction)			D7781-14	I-2547-11[72] I-2548-11[72] N07-0003.[73]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
41. Oil and grease - Total recoverable, mg/L	Hexane extractable material (HEM): *n*-Hexane extraction and gravimetry	1664 Rev. A; 1664 Rev. B[42]	5520 B-2011[38].		
	Silica gel treated HEM (SGT-HEM): Silica gel treatment and gravimetry	1664 Rev. A; 1664 Rev. B[42]	5520 B-2011[38] and 5520 F-2011[38].		
42. Organic carbon - Total (TOC), mg/L	Combustion		5310 B-2014	D7573-09(17)	973.47,[3] p. 14.[24]
	Heated persulfate or UV persulfate oxidation		5310 C-2014 5310 D-2011	D4839-03(17)	973.47,[3,] p. 14.[24]
43. Organic nitrogen (as N), mg/L	Total Kjeldahl N (Parameter 31) minus ammonia N (Parameter 4)				
44. Ortho-phosphate (as P), mg/L	Ascorbic acid method:				
	Automated	365.1, Rev. 2.0 (1993)	4500-P F-2011 or G-2011		973.56,[3] I-4601-85,[2] I-2601-90.[80]
	Manual, single-reagent		4500-P E-2011	D515-88 (A)	973.55.[3]
	Manual, two-reagent	365.3 (Issued 1978)[1].			
	Ion Chromatography	300.0, Rev. 2.1 (1993) and 300.1, Rev. 1.0 (1997)	4110 B-2011 or C-2011	D4327-17	993.30.[3]
	CIE/UV		4140 B-2011	D6508-15	D6508, Rev. 2.[54]
45. Osmium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 D-2011.		

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	AA furnace	252.2 (Issued 1978)[1].			
46. Oxygen, dissolved, mg/L	Winkler (Azide modification)		4500-O (B-F)-2016	D888-12 (A)	973.45B,[3] I-1575-78.[8]
	Electrode		4500-O G-2016	D888-12 (B)	I-1576-78.[8]
	Luminescence-Based Sensor		4500-O H-2016	D888-12 (C)	See footnote.[63] See footnote.[64]
47. Palladium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011.		
	AA furnace	253.2 (Issued 1978)[1].			
	ICP/MS		3125 B-2011.		
	DCP				See footnote.[34]
48. Phenols, mg/L	Manual distillation,[26] followed by any of the following:	420.1 (Rev. 1978)[1]	5530 B-2010	D1783-01(12).	
	Colorimetric (4AAP) manual	420.1 (Rev. 1978)[1]	5530 D-2010[27]	D1783-01(12) (A or B).	
	Automated colorimetric (4AAP)	420.4 Rev. 1.0 (1993)			
49. Phosphorus (elemental), mg/L	Gas-liquid chromatography				See footnote.[28]
50. Phosphorus - Total, mg/L	Digestion,[20] followed by any of the following:		4500-P B (5)-2011		973.55.[3]
	Manual	365.3 (Issued 1978)[1]	4500-P E-2011	D515-88 (A).	
	Automated ascorbic acid reduction	365.1 Rev. 2.0 (1993)	4500-P (F-H)-2011		973.56,[3] I-4600-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	ICP/AES [4 36]	200.7, Rev. 4.4 (1994)	3120 B-2011		I-4471-97.[50]
	Semi-automated block digestor (TKP digestion)	365.4 (Issued 1974)[1]		D515-88 (B)	I-4610-91.[48]
	Digestion with persulfate, followed by Colorimetric				NCASI TNTP W10900.[77]
51. Platinum - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011.		
	AA furnace	255.2 (Issued 1978)[1].			
	ICP/MS		3125 B-2011.		
	DCP				See footnote.[34]
52. Potassium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011		973.53,[3] I-3630-85.[2]
	ICP/AES	200.7, Rev. 4.4 (1994)	3120 B-2011.		
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	Flame photometric		3500-K B-2011.		
	Electrode		3500-K C-2011.		
	Ion Chromatography			D6919-17.	
53. Residue - Total, mg/L	Gravimetric, 103-105°		2540 B-2015		I-3750-85.[2]
54. Residue - filterable, mg/L	Gravimetric, 180°		2540 C-2015	D5907-13	I-1750-85.[2]
55. Residue - non-filterable (TSS), mg/L	Gravimetric, 103-105° post-washing of residue		2540 D-2015	D5907-13	I-3765-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
56. Residue - settleable, ml/L	Volumetric (Imhoff cone), or gravimetric		2540 F-2015.		
57. Residue - Volatile, mg/L	Gravimetric, 550°	160.4 (Issued 1971)[1]	2540 E-2015		I-3753-85.[2]
58. Rhodium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration, or		3111 B-2011.		
	AA furnace	265.2 (Issued 1978)[1].			
	ICP/MS		3125 B-2011.		
59. Ruthenium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration, or		3111 B-2011.		
	AA furnace	267.2[1].			
	ICP/MS		3125 B-2011.		
60. Selenium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA furnace		3113 B-2010	D3859-15 (B)	I-4668-98.[49]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12.	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05[70] I-4472-97.[81]
	AA gaseous hydride		3114 B-2011, or 3114 C-2011	D3859-15 (A)	I-3667-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
61. Silica - Dissolved,[37] mg/L	0.45-micron filtration followed by any of the following:				
	Colorimetric, Manual		4500-SiO_2 C-2011	D859-16	I-1700-85.[2]
	Automated (Molybdosilicate)		4500-SiO_2 E-2011 or F-2011		I-2700-85.[2]
	ICP/AES	200.5, Rev. 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994)	3120 B-2011		I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
62. Silver - Total,[4 31] mg/L	Digestion,[4 29] followed by any of the following:				
	AA direct aspiration		3111 B-2011 or 3111 C-2011		974.27,[3] p. 37,[9] I-3720-85.[2]
	AA furnace		3113 B-2010		I-4724-89.[51]
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4472-97.[81]
	DCP				See footnote.[34]
63. Sodium - Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011		973.54,[3] I-3735-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	ICP/AES	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011		I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	DCP				See footnote.[34]
	Flame photometric		3500-Na B-2011.		
	Ion Chromatography			D6919-17.	
64. Specific conductance, micromhos/cm at 25 °C	Wheatstone bridge	120.1 (Rev. 1982)[1]	2510 B-2011	D1125-95(99) (A)	973.40,[3] I-2781-85.[2]
65. Sulfate (as SO_4), mg/L	Automated colorimetric	375.2, Rev. 2.0 (1993)	4500-SO_4^{2-} F-2011 or G-2011		
	Gravimetric		4500-SO_4^{2-} C-2011 or D-2011		925.54.[3]
	Turbidimetric		4500-SO_4^{2-} E-2011	D516-16.	
	Ion Chromatography	300.0, Rev. 2.1 (1993) and 300.1, Rev. 1.0 (1997)	4110 B-2011 or C-2011	D4327-17	993.30$_3$, I-4020-05[70]
	CIE/UV		4140 B-2011	D6508-15	D6508, Rev. 2.[54]
66. Sulfide (as S), mg/L	Sample Pretreatment		4500-S^{2-} B, C-2011.		
	Titrimetric (iodine)		4500-S^{2-} F-2011		I-3840-85.[2]
	Colorimetric (methylene blue)		4500-S^{2-} D-2011.		
	Ion Selective Electrode		4500-S^{2-} G-2011	D4658-15.	

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
67. Sulfite (as SO_3), mg/L	Titrimetric (iodine-iodate)		4500-SO_3^{2-} B-2011.		
68. Surfactants, mg/L	Colorimetric (methylene blue)		5540 C-2011	D2330-02.	
69. Temperature, °C	Thermometric		2550 B-2010		See footnote.[32]
70. Thallium-Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011.		
	AA furnace	279.2 (Issued 1978)[1]	3113 B-2010.		
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES	200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12.	
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4471-97[50] I-4472-97.[81]
71. Tin-Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 B-2011		I-3850-78.[8]
	AA furnace		3113 B-2010.		
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES	200.5, Rev. 4.2 (2003)[68]; 200.7, Rev. 4.4 (1994).			
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
72. Titanium-Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 D-2011.		
	AA furnace	283.2 (Issued 1978)[1].			
	ICP/AES	200.7, Rev. 4.4 (1994).			
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14.[3]
	DCP				See footnote.[34]
73. Turbidity, NTU[53]	Nephelometric	180.1, Rev. 2.0 (1993)	2130 B-2011	D1889-00	I-3860-85[2] See footnote.[65] See footnote.[66] See footnote.[67]
74. Vanadium-Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration		3111 D-2011.		
	AA furnace		3113 B-2010	D3373-17.	
	ICP/AES	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05.[70]
	DCP			D4190-15	See footnote.[34]
	Colorimetric (Gallic Acid)		3500-V B-2011.		
75. Zinc-Total,[4] mg/L	Digestion,[4] followed by any of the following:				
	AA direct aspiration[36]		3111 B-2011 or 3111 C-2011	D1691-17 (A or B)	974.27,[3] p. 37,[9] I-3900-85.[2]

Parameter	Methodology[58]	EPA[52]	Standard methods[84]	ASTM	USGS/AOAC/Other
	AA furnace	289.2 (Issued 1978)[1].			
	ICP/AES[36]	200.5, Rev. 4.2 (2003);[68] 200.7, Rev. 4.4 (1994)	3120 B-2011	D1976-12	I-4471-97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994)	3125 B-2011	D5673-16	993.14,[3] I-4020-05[70] I-4472-97.[81]
	DCP[36]			D4190-15	See footnote.[34]
	Colorimetric (Zincon)		3500 Zn B-2011		See footnote.[33]
76. Acid Mine Drainage		1627[69].			

Table IB Notes:

[1] Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020. Revised March 1983 and 1979, where applicable. U.S. EPA.

[2] Methods for Analysis of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resource Investigations of the U.S. Geological Survey, Book 5, Chapter A1., unless otherwise stated. 1989. USGS.

[3] Official Methods of Analysis of the Association of Official Analytical Chemists, Methods Manual, Sixteenth Edition, 4th Revision, 1998. AOAC International.

[4] For the determination of total metals (which are equivalent to total recoverable metals) the sample is not filtered before processing. A digestion procedure is required to solubilize analytes in suspended material and to break down organic-metal complexes (to convert the analyte to a detectable form for colorimetric analysis). For non-platform graphite furnace atomic absorption determinations, a digestion using nitric acid (as specified in Section 4.1.3 of Methods for Chemical Analysis of Water and Wastes) is required prior to analysis. The procedure used should subject the sample to gentle acid refluxing, and at no time should the sample be taken to dryness. For direct aspiration flame atomic absorption (FLAA) determinations, a combination acid (nitric and hydrochloric acids) digestion is preferred, prior to analysis. The approved total recoverable digestion is described as Method 200.2 in Supplement I of "Methods for the Determination of Metals in Environmental Samples" EPA/600R-94/111, May, 1994, and is reproduced in EPA Methods 200.7, 200.8, and 200.9 from the same Supplement. However, when using the gaseous hydride technique or for the determination of certain elements such as antimony, arsenic, selenium, silver, and tin by non-EPA graphite furnace atomic absorption methods, mercury by cold vapor atomic absorption, the noble metals and titanium by FLAA, a specific or modified sample digestion procedure may be required, and, in all cases the referenced method write-up should be consulted for specific instruction and/or cautions. For analyses using inductively coupled plasma-atomic emission spectrometry (ICP-AES), the direct current plasma (DCP) technique or EPA spectrochemical techniques (platform furnace AA, ICP-AES, and ICP-MS), use EPA Method 200.2 or an approved alternate procedure (e.g., CEM microwave digestion, which may be used with certain analytes as indicated in Table IB); the total recoverable digestion procedures in EPA Methods 200.7, 200.8, and 200.9 may be used for those respective methods. Regardless of the digestion procedure, the results of the analysis after digestion procedure are reported as "total" metals.

[5] Copper sulfate or other catalysts that have been found suitable may be used in place of mercuric sulfate.

[6] Manual distillation is not required if comparability data on representative effluent samples are on file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies. In general, the analytical method should be consulted regarding the need for distillation. If the method is not clear, the laboratory may compare a minimum of 9 different sample matrices to evaluate the need for distillation. For each matrix, a matrix spike and matrix spike duplicate are analyzed both with and without the distillation step (for a total of 36 samples, assuming 9 matrices). If results are comparable, the laboratory may dispense with the distillation step for future analysis. Comparable is defined as < 20% RPD for all tested matrices). Alternatively, the two populations of spike recovery percentages may be compared using a recognized statistical test.

[7] Industrial Method Number 379-75 WE Ammonia, Automated Electrode Method, Technicon Auto Analyzer II. February 19, 1976. Bran & Luebbe Analyzing Technologies Inc.

[8] The approved method is that cited in Methods for Determination of Inorganic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 5, Chapter A1. 1979. USGS.

[9] American National Standard on Photographic Processing Effluents. April 2, 1975. American National Standards Institute.

[10] In-Situ Method 1003-8-2009, Biochemical Oxygen Demand (BOD) Measurement by Optical Probe. 2009. In-Situ Incorporated.

[11] The use of normal and differential pulse voltage ramps to increase sensitivity and resolution is acceptable.

[12] Carbonaceous biochemical oxygen demand ($CBOD_5$) must not be confused with the traditional BOD_5 test method which measures “total 5-day BOD.” The addition of the nitrification inhibitor is not a procedural option but must be included to report the $CBOD_5$ parameter. A discharger whose permit requires reporting the traditional BOD_5 may not use a nitrification inhibitor in the procedure for reporting the results. Only when a discharger's permit specifically states $CBOD_5$ is required can the permittee report data using a nitrification inhibitor.

[13] OIC Chemical Oxygen Demand Method. 1978. Oceanography International Corporation.

[14] Method 8000, Chemical Oxygen Demand, Hach Handbook of Water Analysis, 1979. Hach Company.

[15] The back-titration method will be used to resolve controversy.

[16] Orion Research Instruction Manual, Residual Chlorine Electrode Model 97-70. 1977. Orion Research Incorporated. The calibration graph for the Orion residual chlorine method must be derived using a reagent blank and three standard solutions, containing 0.2, 1.0, and 5.0 mL 0.00281 N potassium iodate/100 mL solution, respectively.

[17] Method 245.7, Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, EPA-821-R-05-001. Revision 2.0, February 2005. US EPA.

[18] National Council of the Paper Industry for Air and Stream Improvement (NCASI) Technical Bulletin 253 (1971) and Technical Bulletin 803, May 2000.

[19] Method 8506, Bicinchoninate Method for Copper, Hach Handbook of Water Analysis. 1979. Hach Company.

[20] When using a method with block digestion, this treatment is not required.

[21] Industrial Method Number 378-75WA, Hydrogen ion (pH) Automated Electrode Method, Bran & Luebbe (Technicon) Autoanalyzer II. October 1976. Bran & Luebbe Analyzing Technologies.

[22] Method 8008, 1,10-Phenanthroline Method using FerroVer Iron Reagent for Water. 1980. Hach Company.

[23] Method 8034, Periodate Oxidation Method for Manganese, Hach Handbook of Wastewater Analysis. 1979. Hach Company.

[24] Methods for Analysis of Organic Substances in Water and Fluvial Sediments, Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 5, Chapter A3, (1972 Revised 1987). 1987. USGS.

[25] Method 8507, Nitrogen, Nitrite-Low Range, Diazotization Method for Water and Wastewater. 1979. Hach Company.

[26] Just prior to distillation, adjust the sulfuric-acid-preserved sample to pH 4 with 1 + 9 NaOH.

[27] The colorimetric reaction must be conducted at a pH of 10.0 ± 0.2.

[28] Addison, R.F., and R.G. Ackman. 1970. Direct Determination of Elemental Phosphorus by Gas-Liquid Chromatography, *Journal of Chromatography*, 47(3):421-426.

[29] Approved methods for the analysis of silver in industrial wastewaters at concentrations of 1 mg/L and above are inadequate where silver exists as an inorganic halide. Silver halides such as the bromide and chloride are relatively insoluble in reagents such as nitric acid but are readily soluble in an aqueous buffer of sodium thiosulfate and sodium hydroxide to pH of 12. Therefore, for levels of silver above 1 mg/L, 20 mL of sample should be diluted to 100 mL by adding 40 mL each of 2 M $Na_2S_2O_3$ and NaOH. Standards should be prepared in the same manner. For levels of silver below 1 mg/L the approved method is satisfactory.

[30] The use of EDTA decreases method sensitivity. Analysts may omit EDTA or replace with another suitable complexing reagent provided that all method-specified quality control acceptance criteria are met.

[31] For samples known or suspected to contain high levels of silver (e.g., in excess of 4 mg/L), cyanogen iodide should be used to keep the silver in solution for analysis. Prepare a cyanogen iodide solution by adding 4.0 mL of concentrated NH_4OH, 6.5 g of KCN, and 5.0 mL of a 1.0 N solution of I_2 to 50 mL of reagent water in a volumetric flask and dilute to 100.0 mL. After digestion of the sample, adjust the pH of the digestate to >7 to prevent the formation of HCN under acidic conditions. Add 1 mL of the cyanogen iodide solution to the sample digestate and adjust the volume to 100 mL with reagent water (NOT acid). If cyanogen iodide is added to sample digestates, then silver standards must be prepared that contain cyanogen iodide as well. Prepare working standards by diluting a small volume of a silver stock solution with water and adjusting the pH>7 with NH_4OH. Add 1 mL of the cyanogen iodide solution and let stand 1 hour. Transfer to a 100-mL volumetric flask and dilute to volume with water.

[32] "Water Temperature-Influential Factors, Field Measurement and Data Presentation," Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 1, Chapter D1. 1975. USGS.

[33] Method 8009, Zincon Method for Zinc, Hach Handbook of Water Analysis, 1979. Hach Company.

[34] Method AES0029, Direct Current Plasma (DCP) Optical Emission Spectrometric Method for Trace Elemental Analysis of Water and Wastes. 1986-Revised 1991. Thermo Jarrell Ash Corporation.

[35] In-Situ Method 1004-8-2009, Carbonaceous Biochemical Oxygen Demand (CBOD) Measurement by Optical Probe. 2009. In-Situ Incorporated.

[36] Microwave-assisted digestion may be employed for this metal, when analyzed by this methodology. Closed Vessel Microwave Digestion of Wastewater Samples for Determination of Metals. April 16, 1992. CEM Corporation

[37] When determining boron and silica, only plastic, PTFE, or quartz laboratory ware may be used from start until completion of analysis.

[38] Only use *n*-hexane (*n*-Hexane - 85% minimum purity, 99.0% min. saturated C6 isomers, residue less than 1 mg/L) extraction solvent when determining Oil and Grease parameters - Hexane Extractable Material (HEM), or Silica Gel Treated HEM (analogous to EPA Methods 1664 Rev. A and 1664 Rev. B). Use of other extraction solvents is prohibited.

[39] Method PAI-DK01, Nitrogen, Total Kjeldahl, Block Digestion, Steam Distillation, Titrimetric Detection. Revised December 22, 1994. OI Analytical.

[40] Method PAI-DK02, Nitrogen, Total Kjeldahl, Block Digestion, Steam Distillation, Colorimetric Detection. Revised December 22, 1994. OI Analytical.

[41] Method PAI-DK03, Nitrogen, Total Kjeldahl, Block Digestion, Automated FIA Gas Diffusion. Revised December 22, 1994. OI Analytical.

[42] Method 1664 Rev. B is the revised version of EPA Method 1664 Rev. A. U.S. EPA. February 1999, Revision A. Method 1664, *n*-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated *n*-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry. EPA-821-R-98-002. U.S. EPA. February 2010, Revision B. Method 1664, *n*-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated *n*-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry. EPA-821-R-10-001.

[43] Method 1631, Revision E, Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, EPA-821-R-02-019. Revision E. August 2002, U.S. EPA. The application of clean techniques described in EPA's Method 1669: *Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels*, EPA-821-R-96-011, are recommended to preclude contamination at low-level, trace metal determinations.

[44] Method OIA-1677-09, Available Cyanide by Ligand Exchange and Flow Injection Analysis (FIA). 2010. OI Analytical.

[45] Open File Report 00-170, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Ammonium Plus Organic Nitrogen by a Kjeldahl Digestion Method and an Automated Photometric Finish that Includes Digest Cleanup by Gas Diffusion. 2000. USGS.

[46] Open File Report 93-449, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Chromium in Water by Graphite Furnace Atomic Absorption Spectrophotometry. 1993. USGS.

[47] Open File Report 97-198, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Molybdenum by Graphite Furnace Atomic Absorption Spectrophotometry. 1997. USGS.

[48] Open File Report 92-146, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Total Phosphorus by Kjeldahl Digestion Method and an Automated Colorimetric Finish That Includes Dialysis. 1992. USGS.

[49] Open File Report 98-639, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Arsenic and Selenium in Water and Sediment by Graphite Furnace-Atomic Absorption Spectrometry. 1999. USGS.

[50] Open File Report 98-165, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Elements in Whole-water Digests Using Inductively Coupled Plasma-Optical Emission Spectrometry and Inductively Coupled Plasma-Mass Spectrometry. 1998. USGS.

[51] Open File Report 93-125, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Inorganic and Organic Constituents in Water and Fluvial Sediments. 1993. USGS.

[52] Unless otherwise indicated, all EPA methods, excluding EPA Method 300.1, are published in U.S. EPA. May 1994. Methods for the Determination of Metals in Environmental Samples, Supplement I, EPA/600/R-94/111; or U.S. EPA. August 1993. Methods for the Determination of Inorganic Substances in Environmental Samples, EPA/600/R-93/100. EPA Method 300.1 is U.S. EPA. Revision 1.0, 1997, including errata cover sheet April 27, 1999. Determination of Inorganic Ions in Drinking Water by Ion Chromatography.

[53] Styrene divinyl benzene beads (e.g., AMCO-AEPA-1 or equivalent) and stabilized formazin (e.g., Hach StablCal™ or equivalent) are acceptable substitutes for formazin.

[54] Method D6508-15, Test Method for Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte. 2015. ASTM

[55] Kelada-01, Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate, EPA 821-B-01-009, Revision 1.2, August 2001. U.S. EPA. Note: A 450-W UV lamp may be used in this method instead of the 550-W lamp specified if it provides performance within the quality control (QC) acceptance criteria of the method in a given instrument. Similarly, modified flow cell configurations and flow conditions may be used in the method, provided that the QC acceptance criteria are met.

[56] QuikChem Method 10-204-00-1-X, Digestion and Distillation of Total Cyanide in Drinking and Wastewaters using MICRO DIST and Determination of Cyanide by Flow Injection Analysis. Revision 2.2, March 2005. Lachat Instruments.

[57] When using sulfide removal test procedures described in EPA Method 335.4-1, reconstitute particulate that is filtered with the sample prior to distillation.

[58] Unless otherwise stated, if the language of this table specifies a sample digestion and/or distillation “followed by” analysis with a method, approved digestion and/or distillation are required prior to analysis.

[59] Samples analyzed for available cyanide using OI Analytical method OIA-1677-09 or ASTM method D6888-16 that contain particulate matter may be filtered only after the ligand exchange reagents have been added to the samples, because the ligand exchange process converts complexes containing available cyanide to free cyanide, which is not removed by filtration. Analysts are further cautioned to limit the time between the addition of the ligand exchange reagents and sample filtration to no more than 30 minutes to preclude settling of materials in samples.

[60] Analysts should be aware that pH optima and chromophore absorption maxima might differ when phenol is replaced by a substituted phenol as the color reagent in Berthelot Reaction (“phenol-hypochlorite reaction”) colorimetric ammonium determination methods. For example, when phenol is used as the color reagent, pH

optimum and wavelength of maximum absorbance are about 11.5 and 635 nm, respectively - see, Patton, C.J. and S.R. Crouch. March 1977. *Anal. Chem.* 49:464-469. These reaction parameters increase to pH > 12.6 and 665 nm when salicylate is used as the color reagent - see, Krom, M.D. April 1980. *The Analyst* 105:305-316.

[61] If atomic absorption or ICP instrumentation is not available, the aluminon colorimetric method detailed in the 19th Edition of *Standard Methods for the Examination of Water and Wastewater* may be used. This method has poorer precision and bias than the methods of choice.

[62] Easy (1-Reagent) Nitrate Method, Revision November 12, 2011. Craig Chinchilla.

[63] Hach Method 10360, Luminescence Measurement of Dissolved Oxygen in Water and Wastewater and for Use in the Determination of BOD_5 and $CBOD_5$. Revision 1.2, October 2011. Hach Company. This method may be used to measure dissolved oxygen when performing the methods approved in Table IB for measurement of biochemical oxygen demand (BOD) and carbonaceous biochemical oxygen demand (CBOD).

[64] In-Situ Method 1002-8-2009, Dissolved Oxygen (DO) Measurement by Optical Probe. 2009. In-Situ Incorporated.

[65] Mitchell Method M5331, Determination of Turbidity by Nephelometry. Revision 1.0, July 31, 2008. Leck Mitchell.

[66] Mitchell Method M5271, Determination of Turbidity by Nephelometry. Revision 1.0, July 31, 2008. Leck Mitchell.

[67] Orion Method AQ4500, Determination of Turbidity by Nephelometry. Revision 5, March 12, 2009. Thermo Scientific.

[68] EPA Method 200.5, Determination of Trace Elements in Drinking Water by Axially Viewed Inductively Coupled Plasma-Atomic Emission Spectrometry, EPA/600/R-06/115. Revision 4.2, October 2003. U.S. EPA.

[69] Method 1627, Kinetic Test Method for the Prediction of Mine Drainage Quality, EPA-821-R-09-002. December 2011. U.S. EPA.

[70] Techniques and Methods Book 5-B1, Determination of Elements in Natural-Water, Biota, Sediment and Soil Samples Using Collision/Reaction Cell Inductively Coupled Plasma-Mass Spectrometry, Chapter 1, Section B, Methods of the National Water Quality Laboratory, Book 5, Laboratory Analysis, 2006. USGS.

[71] Water-Resources Investigations Report 01-4132, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Organic Plus Inorganic Mercury in Filtered and Unfiltered Natural Water with Cold Vapor-Atomic Fluorescence Spectrometry, 2001. USGS.

[72] USGS Techniques and Methods 5-B8, Chapter 8, Section B, Methods of the National Water Quality Laboratory Book 5, Laboratory Analysis, 2011 USGS.

[73] NECi Method N07-0003, “Nitrate Reductase Nitrate-Nitrogen Analysis,” Revision 9.0, March 2014, The Nitrate Elimination Co., Inc.

[74] Timberline Instruments, LLC Method Ammonia-001, “Determination of Inorganic Ammonia by Continuous Flow Gas Diffusion and Conductivity Cell Analysis,” June 2011, Timberline Instruments, LLC.

[75] Hach Company Method 10206, “Spectrophotometric Measurement of Nitrate in Water and Wastewater,” Revision 2.1, January 2013, Hach Company.

[76] Hach Company Method 10242, “Simplified Spectrophotometric Measurement of Total Kjeldahl Nitrogen in Water and Wastewater,” Revision 1.1, January 2013, Hach Company.

[77] National Council for Air and Stream Improvement (NCASI) Method TNTP-W10900, “Total (Kjeldahl) Nitrogen and Total Phosphorus in Pulp and Paper Biologically Treated Effluent by Alkaline Persulfate Digestion,” June 2011, National Council for Air and Stream Improvement, Inc.

[78] The pH adjusted sample is to be adjusted to 7.6 for NPDES reporting purposes.

[79] I-2057-85 U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chap. A11989, Methods for Determination of Inorganic Substances in Water and Fluvial Sediments, 1989.

[80] Methods I-2522-90, I-2540-90, and I-2601-90 U.S. Geological Survey Open-File Report 93-125, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Determination of Inorganic and Organic Constituents in Water and Fluvial Sediments, 1993.

[81] Method I-1472-97, U.S. Geological Survey Open-File Report 98-165, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory-Determination of Inorganic and Organic Constituents in Water and Fluvial Sediments, 1998.

[82] FIAlab Instruments, Inc. Method FIAlab 100, "Determination of Inorganic Ammonia by Continuous Flow Gas Diffusion and Fluorescence Detector Analysis", April 4, 2018, FIAlab Instruments, Inc.

[83] MACHEREY-NAGEL GmbH and Co. Method 036/038 NANOCOLOR® COD LR/HR, "Spectrophotometric Measurement of Chemical Oxygen Demand in Water and Wastewater", Revision 1.5, May 2018, MACHEREY-NAGEL GmbH and Co. KG.

[84] Please refer to the following applicable Quality Control Sections: Part 2000 Methods, Physical and Aggregate Properties 2020 (2017); Part 3000 Methods, Metals, 3020 (2017); Part 4000 Methods, Inorganic Nonmetallic Constituents, 4020 (2014); Part 5000 Methods, and Aggregate Organic Constituents, 5020 (2017). These Quality Control Standards are available for download at *www.standardmethods.org* at no charge.

[85] Each laboratory may establish its own control limits by performing at least 25 glucose-glutamic acid (GGA) checks over several weeks or months and calculating the mean and standard deviation. The laboratory may then use the mean ± 3 standard deviations as the control limit for future GGA checks. However, GGA acceptance criteria can be no wider than 198 ± 30.5 mg/L for BOD_5. GGA acceptance criteria for CBOD must be either 198 ± 30.5 mg/L, or the lab may develop control charts under the following conditions:

• Dissolved oxygen uptake from the seed contribution is between 0.6-1.0 mg/L.

• Control charts are performed on at least 25 GGA checks with three standard deviations from the derived mean.

• The RSD must not exceed 7.5%.

• Any single GGA value cannot be less than 150 mg/L or higher than 250 mg/L.

Table IC - List of Approved Test Procedures for Non-Pesticide Organic Compounds

Parameter[1]	Method	EPA[27]	Standard methods	ASTM	Other
1. Acenaphthene	GC	610			
	GC/MS	625.1, 1625B	6410 B-2000		See footnote[9], p. 27.
	HPLC	610	6440 B-2005	D4657-92 (98)	
2. Acenaphthylene	GC	610			
	GC/MS	625.1, 1625B	6410 B-2000		See footnote[9], p. 27.
	HPLC	610	6440 B-2005	D4657-92 (98)	
3. Acrolein	GC	603			
	GC/MS	624.1,[4] 1624B			
4. Acrylonitrile	GC	603			

(xx) Water-Resources Investigation Report 01-4098, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Moderate-Use Pesticides and Selected Degradates in Water by C-18 Solid-Phase Extraction and Gas Chromatography/Mass Spectrometry. 2001. Table ID, Note 13.

(xxi) Water-Resources Investigations Report 01-4132, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Organic Plus Inorganic Mercury in Filtered and Unfiltered Natural Water With Cold Vapor-Atomic Fluorescence Spectrometry. 2001. Table IB, Note 71.

(xxii) Water-Resources Investigation Report 01-4134, Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory - Determination of Pesticides in Water by Graphitized Carbon-Based Solid-Phase Extraction and High-Performance Liquid Chromatography/Mass Spectrometry. 2001. Table ID, Note 12.

(xxiii) Water Temperature - Influential Factors, Field Measurement and Data Presentation, Techniques of Water-Resources Investigations of the U.S. Geological Survey, Book 1, Chapter D1. 1975. Table IB, Note 32.

(39) Waters Corporation, 34 Maple Street, Milford MA 01757, Telephone: 508-482-2131, Fax: 508-482-3625.

(i) Method D6508, Test Method for Determination of Dissolved Inorganic Anions in Aqueous Matrices Using Capillary Ion Electrophoresis and Chromate Electrolyte. Revision 2, December 2000. Table IB, Note 54.

(ii) [Reserved]

(c) Under certain circumstances, the Director may establish limitations on the discharge of a parameter for which there is no test procedure in this part or in 40 CFR parts 405 through 499. In these instances the test procedure shall be specified by the Director.

(d) Under certain circumstances, the Administrator may approve additional alternate test procedures for nationwide use, upon recommendation by the Alternate Test Procedure Program Coordinator, Washington, DC.

(e) Sample preservation procedures, container materials, and maximum allowable holding times for parameters are cited in Tables IA, IB, IC, ID, IE, IF, IG, and IH are prescribed in Table II. Information in the table takes precedence over information in specific methods or elsewhere. Any person may apply for a change from the prescribed preservation techniques, container materials, and maximum holding times applicable to samples taken from a specific discharge. Applications for such limited use changes may be made by letters to the Regional Alternative Test Procedure (ATP) Program Coordinator or the permitting authority in the Region in which the discharge will occur. Sufficient data should be provided to assure such changes in sample preservation, containers or holding times do not adversely affect the integrity of the sample. The Regional ATP Coordinator or permitting authority will review the application and then notify the applicant and the appropriate State agency of approval or rejection of the use of the alternate test procedure. A decision to approve or deny any request on deviations from the prescribed Table II requirements will be made within 90 days of receipt of the application by the Regional Administrator. An analyst may not modify any sample preservation and/or holding time requirements of an approved method unless the requirements of this section are met.

Table II - Required Containers, Preservation Techniques, and Holding Times

Parameter number/name	Container [1]	Preservation [2 3]	Maximum holding time [4]
Table IA - Bacterial Tests			
1-4. Coliform, total, fecal, and *E. coli*	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22 23]
5. Fecal streptococci	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22]
6. Enterococci	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22]
7. *Salmonella*	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22]
Table IA - Aquatic Toxicity Tests			
8-11. Toxicity, acute and chronic	P, FP, G	Cool, ≤6 °C [16]	36 hours.
Table IB - Inorganic Tests			
1. Acidity	P, FP, G	Cool, ≤6 °C [18]	14 days.
2. Alkalinity	P, FP, G	Cool, ≤6 °C [18]	14 days.

Parameter number/name	Container [1]	Preservation [2 3]	Maximum holding time [4]
4. Ammonia	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH <2	28 days.
9. Biochemical oxygen demand	P, FP, G	Cool, ≤6 °C [18]	48 hours.
10. Boron	P, FP, or Quartz	HNO_3 to pH <2	6 months.
11. Bromide	P, FP, G	None required	28 days.
14. Biochemical oxygen demand, carbonaceous	P, FP G	Cool, ≤6 °C [18]	48 hours.
15. Chemical oxygen demand	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH <2	28 days.
16. Chloride	P, FP, G	None required	28 days.
17. Chlorine, total residual	P, G	None required	Analyze within 15 minutes.
21. Color	P, FP, G	Cool, ≤6 °C [18]	48 hours.
23-24. Cyanide, total or available (or CATC) and free	P, FP, G	Cool, ≤6 °C [18], NaOH to pH >10 [5 6], reducing agent if oxidizer present	14 days.
25. Fluoride	P	None required	28 days.
27. Hardness	P, FP, G	HNO_3 or H_2SO_4 to pH <2	6 months.
28. Hydrogen ion (pH)	P, FP, G	None required	Analyze within 15 minutes.
31, 43. Kjeldahl and organic N	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH <2	28 days.
Table IB - Metals [7]			
18. Chromium VI	P, FP, G	Cool, ≤6 °C [18], pH = 9.3-9.7 [20]	28 days.
35. Mercury (CVAA)	P, FP, G	HNO_3 to pH <2	28 days.
35. Mercury (CVAFS)	FP, G; and FP-lined cap [17]	5 mL/L 12N HCl or 5 mL/L BrCl [17]	90 days.[17]
3, 5-8, 12, 13, 19, 20, 22, 26, 29, 30, 32-34, 36, 37, 45, 47, 51, 52, 58-60, 62, 63, 70-72, 74, 75. Metals, except boron, chromium VI, and mercury	P, FP, G	HNO_3 to pH <2, or at least 24 hours prior to analysis [19]	6 months.
38. Nitrate	P, FP, G	Cool, ≤6 °C [18]	48 hours.
39. Nitrate-nitrite	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH <2	28 days.
40. Nitrite	P, FP, G	Cool, ≤6 °C [18]	48 hours.

Parameter number/name	Container [1]	Preservation [2 3]	Maximum holding time [4]
41. Oil and grease	G	Cool to ≤6 °C [18], HCl or H_2SO_4 to pH <2	28 days.
42. Organic Carbon	P, FP, G	Cool to ≤6 °C [18], HCl, H_2SO_4, or H_3PO_4 to pH <2	28 days.
44. Orthophosphate	P, FP, G	Cool, to ≤6 °C [18 24]	Filter within 15 minutes; Analyze within 48 hours.
46. Oxygen, Dissolved Probe	G, Bottle and top	None required	Analyze within 15 minutes.
47. Winkler	G, Bottle and top	Fix on site and store in dark	8 hours.
48. Phenols	G	Cool, ≤6 °C [18], H_2SO_4 to pH <2	28 days.
49. Phosphorus (elemental)	G	Cool, ≤6 °C [18]	48 hours.
50. Phosphorus, total	P, FP, G	Cool, ≤6 °C [18], H_2SO_4 to pH <2	28 days.
53. Residue, total	P, FP, G	Cool, ≤6 °C [18]	7 days.
54. Residue, Filterable (TDS)	P, FP, G	Cool, ≤6 °C [18]	7 days.
55. Residue, Nonfilterable (TSS)	P, FP, G	Cool, ≤6 °C [18]	7 days.
56. Residue, Settleable	P, FP, G	Cool, ≤6 °C [18]	48 hours.
57. Residue, Volatile	P, FP, G	Cool, ≤6 °C [18]	7 days.
61. Silica	P or Quartz	Cool, ≤6 °C [18]	28 days.
64. Specific conductance	P, FP, G	Cool, ≤6 °C [18]	28 days.
65. Sulfate	P, FP, G	Cool, ≤6 °C [18]	28 days.
66. Sulfide	P, FP, G	Cool, ≤6 °C [18], add zinc acetate plus sodium hydroxide to pH >9	7 days.
67. Sulfite	P, FP, G	None required	Analyze within 15 minutes.
68. Surfactants	P, FP, G	Cool, ≤6 °C [18]	48 hours.
69. Temperature	P, FP, G	None required	Analyze within 15 minutes.
73. Turbidity	P, FP, G	Cool, ≤6 °C [18]	48 hours.

Table IC - Organic Tests [8]

Parameter number/name	Container [1]	Preservation [2 3]	Maximum holding time [4]
13, 18-20, 22, 24, 25, 27, 28, 34-37, 39-43, 45-47, 56, 76, 104, 105, 108-111, 113. Purgeable Halocarbons	G, FP-lined septum	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5], HCl to pH 2 [9]	14 days.[9]
26. 2-Chloroethylvinyl ether	G, FP-lined septum	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5]	14 days.
6, 57, 106. Purgeable aromatic hydrocarbons	G, FP-lined septum	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5], HCl to pH 2 [9]	14 days.[9]
3, 4. Acrolein and acrylonitrile	G, FP-lined septum	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$, pH to 4-5 [10]	14 days.[10]
23, 30, 44, 49, 53, 77, 80, 81, 98, 100, 112. Phenols [11]	G, FP-lined cap	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$	7 days until extraction, 40 days after extraction.
7, 38. Benzidines [11 12]	G, FP-lined cap	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5]	7 days until extraction.[13]
14, 17, 48, 50-52. Phthalate esters [11]	G, FP-lined cap	Cool, ≤6 °C [18]	7 days until extraction, 40 days after extraction.
82-84. Nitrosamines [11 14]	G, FP-lined cap	Cool, ≤6 °C [18], store in dark, 0.008% $Na_2S_2O_3$ [5]	7 days until extraction, 40 days after extraction.
88-94. PCBs [11]	G, FP-lined cap	Cool, ≤6 °C [18]	1 year until extraction, 1 year after extraction.
54, 55, 75, 79. Nitroaromatics and isophorone [11]	G, FP-lined cap	Cool, ≤6 °C [18], store in dark, 0.008% $Na_2S_2O_3$ [5]	7 days until extraction, 40 days after extraction.
1, 2, 5, 8-12, 32, 33, 58, 59, 74, 78, 99, 101. Polynuclear aromatic hydrocarbons [11]	G, FP-lined cap	Cool, ≤6 °C [18], store in dark, 0.008% $Na_2S_2O_3$ [5]	7 days until extraction, 40 days after extraction.
15, 16, 21, 31, 87. Haloethers [11]	G, FP-lined cap	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5]	7 days until extraction, 40 days after extraction.

Parameter number/name	Container [1]	Preservation [2 3]	Maximum holding time [4]
29, 35-37, 63-65, 73, 107. Chlorinated hydrocarbons [11]	G, FP-lined cap	Cool, ≤6 °C [18]	7 days until extraction, 40 days after extraction.
60-62, 66-72, 85, 86, 95-97, 102, 103. CDDs/CDFs [11]	G	See footnote 11	See footnote 11.
Aqueous Samples: Field and Lab Preservation	G	Cool, ≤6 °C [18], 0.008% $Na_2S_2O_3$ [5], pH <9	1 year.
Solids and Mixed-Phase Samples: Field Preservation	G	Cool, ≤6 °C [18]	7 days.
Tissue Samples: Field Preservation	G	Cool, ≤6 °C [18]	24 hours.
Solids, Mixed-Phase, and Tissue Samples: Lab Preservation	G	Freeze, ≤−10 °C	1 year.
114-118. Alkylated phenols	G	Cool, <6 °C, H_2SO_4 to pH <2	28 days until extraction, 40 days after extraction.
119. Adsorbable Organic Halides (AOX)	G	Cool, <6 °C, 0.008% $Na_2S_2O_3$, HNO_3 to pH <2	Hold *at least* 3 days, but not more than 6 months.
120. Chlorinated Phenolics	G, FP-lined cap	Cool, <6 °C, 0.008% $Na_2S_2O_3$, H_2SO_4 to pH <2	30 days until acetylation, 30 days after acetylation.
Table ID - Pesticides Tests			
1-70. Pesticides [11]	G, FP-lined cap	Cool, ≤6 °C [18], pH 5-9 [15]	7 days until extraction, 40 days after extraction.
Table IE - Radiological Tests			
1-5. Alpha, beta, and radium	P, FP, G	HNO_3 to pH <2	6 months.
Table IH - Bacterial Tests			
1, 2. Coliform, total, fecal	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22]
3.*E. coli*	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22]
4. Fecal streptococci	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22]
5. Enterococci	PA, G	Cool, <10 °C, 0.008% $Na_2S_2O_3$ [5]	8 hours.[22]

Parameter number/name	Container [1]	Preservation [2 3]	Maximum holding time [4]
Table IH - Protozoan Tests			
6. *Cryptosporidium*	LDPE; field filtration	1-10 °C	96 hours.[21]
7. *Giardia*	LDPE; field filtration	1-10 °C	96 hours.[21]

[1] "P" is for polyethylene; "FP" is fluoropolymer (polytetrafluoroethylene [PTFE]; Teflon®), or other fluoropolymer, unless stated otherwise in this Table II; "G" is glass; "PA" is any plastic that is made of a sterilizable material (polypropylene or other autoclavable plastic); "LDPE" is low density polyethylene.

[2] Except where noted in this Table II and the method for the parameter, preserve each grab sample within 15 minutes of collection. For a composite sample collected with an automated sample (e.g., using a 24-hour composite sample; see 40 CFR 122.21(g)(7)(i) or 40 CFR part 403, appendix E), refrigerate the sample at ≤6 °C during collection unless specified otherwise in this Table II or in the method(s). For a composite sample to be split into separate aliquots for preservation and/or analysis, maintain the sample at ≤6 °C, unless specified otherwise in this Table II or in the method(s), until collection, splitting, and preservation is completed. Add the preservative to the sample container prior to sample collection when the preservative will not compromise the integrity of a grab sample, a composite sample, or aliquot split from a composite sample within 15 minutes of collection. If a composite measurement is required but a composite sample would compromise sample integrity, individual grab samples must be collected at prescribed time intervals (e.g., 4 samples over the course of a day, at 6-hour intervals). Grab samples must be analyzed separately and the concentrations averaged. Alternatively, grab samples may be collected in the field and composited in the laboratory if the compositing procedure produces results equivalent to results produced by arithmetic averaging of results of analysis of individual grab samples. For examples of laboratory compositing procedures, see EPA Method 1664 Rev. A (oil and grease) and the procedures at 40 CFR 141.24(f)(14)(iv) and (v) (volatile organics).

[3] When any sample is to be shipped by common carrier or sent via the U.S. Postal Service, it must comply with the Department of Transportation Hazardous Materials Regulations (49 CFR part 172). The person offering such material for transportation is responsible for ensuring such compliance. For the preservation requirement of Table II, the Office of Hazardous Materials, Materials Transportation Bureau, Department of Transportation has determined that the Hazardous Materials Regulations do not apply to the following materials: Hydrochloric acid (HCl) in water solutions at concentrations of 0.04% by weight or less (pH about 1.96 or greater; Nitric acid (HNO_3) in water solutions at concentrations of 0.15% by weight or less (pH about 1.62 or greater); Sulfuric acid (H_2SO_4) in water solutions at concentrations of 0.35% by weight or less (pH about 1.15 or greater); and Sodium hydroxide (NaOH) in water solutions at concentrations of 0.080% by weight or less (pH about 12.30 or less).

[4] Samples should be analyzed as soon as possible after collection. The times listed are the maximum times that samples may be held before the start of analysis and still be considered valid. Samples may be held for longer periods only if the permittee or monitoring laboratory have data on file to show that, for the specific types of samples under study, the analytes are stable for the longer time, and has received a variance from the Regional ATP Coordinator under § 136.3(e). For a grab sample, the holding time begins at the time of collection. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR part 403, appendix E), the holding time begins at the time of the end of collection of the composite sample. For a set of grab samples composited in the field or laboratory, the holding time begins at the time of collection of the last grab sample in the set. Some samples may not be stable for the maximum time period given in the table. A permittee or monitoring laboratory is obligated to hold the sample for a shorter time if it knows that a shorter time is necessary to maintain sample stability. See § 136.3(e) for details. The date and time of collection of an individual grab sample is the date and time at which the sample is collected. For a set of grab samples to be composited, and that are all collected on the same calendar date, the date of collection is the date on which the samples are collected. For a set of grab samples to be composited, and that are collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14-15. For a composite sample collected automatically on a given date, the date of collection is the date on which the sample is collected. For a composite sample collected automatically, and that is collected across two calendar dates, the date of collection is the dates of the two days; e.g., November 14-15. For static-renewal toxicity tests, each grab or composite sample may also be used to prepare test solutions for renewal at 24 h, 48 h, and/or 72 h after first use, if stored at 0-6 °C, with minimum head space.

[5] ASTM D7365-09a specifies treatment options for samples containing oxidants (e.g., chlorine) for cyanide analyses. Also, Section 9060A of Standard Methods for the Examination of Water and Wastewater (23rd edition) addresses dechlorination procedures for microbiological analyses.

[6] Sampling, preservation and mitigating interferences in water samples for analysis of cyanide are described in ASTM D7365-09a (15). There may be interferences that are not mitigated by the analytical test methods or D7365-09a (15). Any technique for removal or suppression of interference may be employed, provided the laboratory demonstrates that it more accurately measures cyanide through quality control measures described in the analytical test method. Any removal or suppression technique not described in D7365-09a (15) or the analytical test method must be documented along with supporting data.

[7] For dissolved metals, filter grab samples within 15 minutes of collection and before adding preservatives. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR part 403, appendix E), filter the sample within 15 minutes after completion of collection and before adding preservatives. If it is known or suspected that dissolved sample integrity will be compromised during collection of a composite sample collected automatically over time (e.g., by interchange of a metal between dissolved and suspended forms), collect and filter grab samples to be composited (footnote 2) in place of a composite sample collected automatically.

[8] Guidance applies to samples to be analyzed by GC, LC, or GC/MS for specific compounds.

[9] If the sample is not adjusted to pH 2, then the sample must be analyzed within seven days of sampling.

[10] The pH adjustment is not required if acrolein will not be measured. Samples for acrolein receiving no pH adjustment must be analyzed within 3 days of sampling.

[11] When the extractable analytes of concern fall within a single chemical category, the specified preservative and maximum holding times should be observed for optimum safeguard of sample integrity (*i.e.,* use all necessary preservatives and hold for the shortest time listed). When the analytes of concern fall within two or more chemical categories, the sample may be preserved by cooling to ≤6 °C, reducing residual chlorine with 0.008% sodium thiosulfate, storing in the dark, and adjusting the pH to 6-9; samples preserved in this manner may be held for seven days before extraction and for forty days after extraction. Exceptions to this optional preservation and holding time procedure are noted in footnote 5 (regarding the requirement for thiosulfate reduction), and footnotes 12, 13 (regarding the analysis of benzidine).

[12] If 1,2-diphenylhydrazine is likely to be present, adjust the pH of the sample to 4.0 ± 0.2 to prevent rearrangement to benzidine.

[13] Extracts may be stored up to 30 days at <0 °C.

[14] For the analysis of diphenylnitrosamine, add 0.008% $Na_2S_2O_3$ and adjust pH to 7-10 with NaOH within 24 hours of sampling.

[15] The pH adjustment may be performed upon receipt at the laboratory and may be omitted if the samples are extracted within 72 hours of collection. For the analysis of aldrin, add 0.008% $Na_2S_2O_3$.

[16] Place sufficient ice with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when the samples arrive, immediately measure the temperature of the samples and confirm that the preservation temperature maximum has not been exceeded. In the isolated cases where it can be documented that this holding temperature cannot be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature. Aqueous samples must not be frozen. Hand-delivered samples used on the day of collection do not need to be cooled to 0 to 6 °C prior to test initiation.

[17] Samples collected for the determination of trace level mercury (<100 ng/L) using EPA Method 1631 must be collected in tightly-capped fluoropolymer or glass bottles and preserved with BrCl or HCl solution within 48 hours of sample collection. The time to preservation may be extended to 28 days if a sample is oxidized in the sample bottle. A sample collected for dissolved trace level mercury should be filtered in the laboratory within 24 hours of the time of collection. However, if circumstances preclude overnight shipment, the sample should be filtered in a designated clean area in the field in accordance with procedures given in Method 1669. If sample integrity will not be maintained by shipment to and filtration in the laboratory, the sample must be filtered in a designated clean area in the field within the time period necessary to maintain sample integrity. A sample that has been collected for determination of total or dissolved trace level mercury must be analyzed within 90 days of sample collection.

[18] Aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. Also, for purposes of NPDES monitoring, the specification of "≤ °C" is used in place of the "4 °C" and "<4 °C" sample temperature requirements listed in some methods. It is not necessary to measure the sample temperature to three significant figures (1/100th of 1 degree); rather, three significant figures are specified so that rounding down to 6 °C may not be used to meet the ≤6 °C requirement. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

[19] An aqueous sample may be collected and shipped without acid preservation. However, acid must be added at least 24 hours before analysis to dissolve any metals that adsorb to the container walls. If the sample must be analyzed within 24 hours of collection, add the acid immediately (see footnote 2). Soil and sediment samples do not need to be preserved with acid. The allowances in this footnote supersede the preservation and holding time requirements in the approved metals methods.

[20] To achieve the 28-day holding time, use the ammonium sulfate buffer solution specified in EPA Method 218.6. The allowance in this footnote supersedes preservation and holding time requirements in the approved hexavalent chromium methods, unless this supersession would compromise the measurement, in which case requirements in the method must be followed.

[21] Holding time is calculated from time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

[22] Sample analysis should begin as soon as possible after receipt; sample incubation must be started no later than 8 hours from time of collection.

[23] For fecal coliform samples for sewage sludge (biosolids) only, the holding time is extended to 24 hours for the following sample types using either EPA Method 1680 (LTB-EC) or 1681 (A-1): Class A composted, Class B aerobically digested, and Class B anaerobically digested.

[24] The immediate filtration requirement in orthophosphate measurement is to assess the dissolved or bio-available form of orthophosphorus (*i.e.,* that which passes through a 0.45-micron filter), hence the requirement to filter the sample immediately upon collection (*i.e.,* within 15 minutes of collection).

[38 FR 28758, Oct. 16, 1973]

EDITORIAL NOTE

Editorial Note: For Federal Register citations affecting § 136.3, see the List of CFR Sections Affected, which appears in the Finding Aids section of the printed volume and at *www.govinfo.gov*.

Section 11
Answers to Review Questions

Answers to Review Questions

Laboratory Safety – p.

1. Infectious Materials, Poisons, Explosions, Cuts and Bruises, Electric Shock, Toxic fumes, Fire, Burns
2. Someone should always be there to help you in case you should have an accident that blinds you, leaves you unconscious, or starts a fire you cannot handle. If necessary, have someone check on you regularly.
3. True
4. You can dispose of small amounts of corrosive acids by pouring the neutralized acid down a corrosion-resistant sink (to the sewer) and using large quantities of water to dilute and flush the acid.
5. Safety Data Sheet
6. 30 years
7. A signal word is used to indicate the relative level of severity of hazard and alert the reader to a potential hazard. Danger = more severe hazard, Warning = less severe hazard
8. Within one minute of contact, flush with copious amounts of water for at least 20 minutes. Consult our doctor.
9. Immediately wash the area with water and neutralize the acid with sodium bicarbonate (baking soda) or bicarbonate
10. Gloves, safety glasses, apron, lab coat, face shield, closed toe shoes
11. Fume hood
12. Vinegar neutralizes bases, baking soda neutralizes acids
13. Weekly
14. False
15. If incompatible chemicals are inadvertently mixed, a fire, explosion, or toxic release can easily occur.
16. Glass plate, wet towel, wet blanket
17. A –ordinary combustibles, B – flammable and combustible liquids, C- energized electrical equipment, D – combustible metals
18. Pull, Aim, Squeeze, Sweep
19. The potable water source could become contaminated with bacteria or dangerous chemicals. The air gap is the best method because it completely eliminates the cross connection entirely.
20. A reproductive toxin that may cause damage to the fetus.

Laboratory Equipment – p.

1. Beakers
2. Volumetric glassware
3. When you are creating standards
4. Volumetric – do not blow out; Mohr – do not blow out; Serological – yes, blow out
5. TC (To Contain) will accurately measure how much of a liquid is held in the container. TD (To Deliver) will measure the amount that will be poured from the container.
6. True
7. Pipet – hold it up to eye level; Larger glassware –set it on a flat surface and bend down to read at eye level

8. Place them into soapy water, tip up in a pipet cleaner. Or lay them into a dish filled with soapy water. Phosphate free, lab grade soap should be used.
9. 3 minutes
10. 121 degrees C at 15 psi for 15 min
11. 20 +/- 1 degree C; record temperatures twice daily with at least 4 hours apart
12. Analytical is more precise, can weigh down to 0.0001g vs. 0.01g for a top loading balance.
13. Goggles, gloves, aprons, safety clothing (including closed toe shoes)
14. False
15. Weekly
16. Phosphate free (ex: Alconox)
17. Detergent, tap water, rinse 3 times with DI water, air dry
18. To remove any built-up residue that could be causing water to bead up. To ensure glassware is as clean as possible.
19. Clean glassware using lab detergent (phosphate free); rinse with tap water; rinse with 1:1 hydrochloric acid or nitric acid (1:1 means equal part distilled water and acid); rinse well with distilled water; let air dry
20. Monthly

Sampling – p.

1. a) 40 CFR 136 Table II b) 40 CFR 136 Table II c) 40 CFR 136 Table II d) NPDES permit e) NPDES permit
2. Representative of the wastestream
3. A grab sample is a single influent or effluent sample collected at that exact moment in time. It is not an average. A composite sample is a combination of not less than 8 influent or effluent portions, of at least 100 ml, collected over a 24 hour period. It is combined to form a sample that is representative of the entire flow for a set period of time.
4. TRC, DO, coliforms, E.coli, pH, temperature
5. BOD, total N, settleable solids
6. To prevent growth of bacteria or algae
7. To prevent bacterial decomposition; at 6 degrees C
8. Location of where the sample was taken, date/time, sampled by (with operators name or initials), and any important comments (such as preservatives that were added or pH adjustments that were made)
9. True
10. COC is a written record to trace possession and handling of samples from collection to reporting. It should identify who handled the sample from collection, through transport, to storage, to analysis, to final destruction. An operator would need to fill out a COC if they are sending their samples out to a contract lab for analysis.
11. True
12. C
13. False
14. Improper sampling, poor or improper sampling preservation, and lack of sufficient mixing during compositing and testing
15. True

Dissolved Oxygen – p.

1. 15 minutes
2. Useful for maintaining a stream fit for swimming, fishing, and/or as a source of potable water; DO must be present for fish and aquatic life; DO level must be kept high; DO level must be kept above permit minimum limit; the flavor of water is improved by DO; Low DO levels can have harmful effects on receiving waters, cause suffocation of fish and promote growth of harmful bacteria; Presence of DO in drinking water can contribute to corrosion of piping systems; Low or zero DO levels at the bottom of lakes or reservoirs often cause flavor and odor problems in drinking water
3. BP, Temperature, Other substances dissolved in the water
4. The pressure of the column of air above us.
5. 29.92, 28.82, 29.3
6. 599, 649, 758
7. 62.6, 68, 84.2
8. 12.8, 18.3, 21.4
9. 28.87 in Hg
10. 20 degrees C, oxygen solubility ~8.71 mg/L
11. Oxygen solubility ~8.42 (round to 28.298 on the table)
12. Oxygen solubility ~3.40 mg/L; the initial DO needs to be between 7.5 – 9.0 mg/L, therefore the sample would not meet quality criteria. Also, GGA typically consumes about 4.0 mg/L of DO, so you couldn't possibly meet the QC criteria of at least 1.0 mg/L DO remaining at the end of the 5 day test.

Biochemical Oxygen Demand – p.

1. To check the quality of the dilution water
2. Glucose Glutamic Acid, 198mg/L +/- 30
3. 5 days incubation at 20°C +/- 1 in darkness
4. Less than or equal to 9.0 mg/L
5. Immediately before you are going to set up your samples. Add the buffer solution and then shake vigorously to ensure saturation.
6. Oxidized effluents; Samples that were collected after chlorination/disinfection point (the microorganisms have been killed); Toxic effluents; Samples with insufficient microorganisms; CBOD samples (since you added NI)
7. (varies)
8. If you make pH adjustments, are you required to seed the sample? An alkaline or acidic environment can prevent bacteria from growing during the course of the test. pH 6.0-8.0 is the preferred range for the microorganisms. Yes, you must seed the sample if it has been adjusted.
9. The chlorine would kill the bacteria.
10. It helps form a seal and prevent evaporation.
11. Carbonaceous and Nitrogenous (Carbonaceous often referred to as "first stage BOD" and Nitrogenous as "second stage BOD")
12. The Carbonaceous BOD. You must add NI to prevent the nitrogen demand from occurring. It prevents Nitrosomonas from oxidizing ammonia to nitrite.

13. Tubing constructed of gum rubber type (black) or some fish tank (Tygon) tubing; Slime growth in the delivery tubing; Poor water quality/improperly maintained DI system; Poorly cleaned bottles or dilution water carboys; Improperly calibrated probe; Poor quality air for aeration
14. The amount of DO uptake contributed by the seed (the amount of dissolved oxygen the seed bacteria are using up.)
15. 0.6 -1.0 mg/L
16. No, the blank depletion is greater than 0.2 and the sample did not deplete at least 2.0 mg/L
17. Blank depletion must be: less than or equal to 0.2mg/L
    Initial DO must be: less than or equal to 9.0mg/L
    Samples must deplete at least: 2.0mg/L
    Samples must have at least: 1.0mg/L DO remaining at end of test

Chemical Oxygen Demand (COD) – p.

1. The strength of a wastewater in terms of its chemical oxygen demand. It is a good estimate of the first stage oxygen demand. It also measures the organic matter content of a sample that is susceptible to oxidation by strong chemical oxidants.
2. Plastic or glass, although glass is preferred. Even a trace amount of organic matter can cause a gross error. Use plastic bottles only if they are known to be free of organic contamination.
3. Composite
4. Mercury (specifically, Mercuric sulfate), which removes the Chloride interference.
5. Can measure the strength of organic waste that is too toxic for BOD; Results are quicker – 2 hours; Fewer interferences (Chloride); Stable methodology; Easy to run
6. The tests use different methods of oxidation. BOD uses microorganisms to oxidize and they are very sensitive to variation in pH, temp, etc that could impede the oxidation process. COD uses chemicals to oxidize and they are unaffected by variations in the water, and will oxidize regardless.
7. a. It does not measure the rate of biodegradability of organic matter
   b. It cannot be used to predict the effects of an effluent on the DO in receiving waters
   c. It is difficult to predict the treatability of a particular wastewater by biological processes
   d. It contains toxic and corrosive reagents that require special handling and disposal
8. You must collect empirical data (both COD and BOD) for the same water samples collected over the same period of time; and you must graph that data to determine whether or not a correlation exists.
9. Potassium dichromate in 50% sulfuric acid.
10. Reported as mg/L COD, defined as the milligrams of oxygen consumed per liter of sample
11. COD test includes both biodegradable and non-biodegradable substances. (BOD only biodegradable)

Solids – p.

1. Samples must be kept homogenous during transfer.
2. Yes, use 2 hour maximum window (of sample sitting out at room temp) from sample collection to start of sample analysis.

3. True
4. 47 mm glass fiber filter, 1.5µm
5. Wrinkled side up
6. 3 successive 20 mL portions of reagent grade water
7. They are placed in 103-105°C oven for I hour, cooled in the desiccator to balance temperature and then weighed.
8. A constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.
9. In the desiccator.
10. Moisture from fingers can add moisture to the filter and cause a weighing error.
11. To seat it.
12. Wash filter with 3 successive 10 mL volumes of DI water, allowing complete drainage between washings.
13. About 3 minutes after filtration is complete.
14. True
15. Total suspended solids = The portion of total solids retained by a filter
    Total dissolved solids = The portion of solids that passes through the filter.
    Fixed solids = The residue of total, suspended, or dissolved solids after heating to dryness for a specified time at a specified temperature.
    Volatile solids = the weight loss (from the fixed solids measurement) on ignition in a muffle furnace
    Settleable solids = the material settling out of suspension within a defined period. It may include floating material, depending on the technique.
16. 2.5 and 200 mg dried residue (0.2 grams max)
17. Increase filter diameter, or decrease sample volume.
18. Most suspended solids are organic- will lead to higher oxygen demand (BOD)
    Suspended solids serve as a refuge for harmful bacteria – negatively impact the UV disinfection and Chlorine disinfection process
    Unsightly appearance – causes turbidity and scum build-up both inside the plant and in the receiving waters.
    If discharged into the receiving waters, it can have a negative impact on the aquatic life – choking gills, smothering eggs

<u>Turbidity – p.</u>

1. The measure of the clarity of water.
2. Suspended solids and colloidal matter – particulates such as silt, clay, organic matter, algae and other microorganisms
3. Very small particles that settle very slowly, if at all.
4. Erosion, stormwater run-off, construction, mining, agriculture
5. False
6. Turbidity can support growth of microorganisms,
   It reduces the effectiveness of chlorination,
   It can interfere with chemical and microbiological analysis,
   It is unacceptable for aesthetic reasons,
   It can negatively impact coagulation and filtration,
   It's unacceptable for most industrial water because it can clog sensitive filters,

It can reduce light reaching to lower depths of receiving waters and inhibit the growth of aquatic plants,
It can hinder the ability of fish to absorb dissolved oxygen

7. NTU (Nephelometric Turbidity Unit)
8. It will smooth out scratches that would scatter light and give false readings.
9. To prevent changes in sample characteristics – microorganisms can add/destroy turbidity and change the readings
10. Formazin solution – a stable synthetic material with uniform particle size

pH – p.

1. We used 3, but only 2 are required. If using 2 however, you must be able to bracket your expected sample range in order for calibration to be valid.
2. Temperature, Automatic Temperature Compensation
3. Saturated KCL (potassium chloride)
4. Carbon dioxide entrainment
5. Ammonia, COD, TKN, Nitrate-Nitrite, Oil and Grease, Total phosphorus
6. <2 , H_2SO_4
7. Calcium Carbonate
8. Calcium carbonate is a base because it falls on the alkaline side of the pH scale. Calcium carbonate provides alkalinity, which serves to neutralize acids and provide buffering capacity.
9. 15 minutes
10. Electrometric measurement, 4500 – H^+ B – 2011
11. Daily
12. Buffer solutions may deteriorate as a result of mold growth or contamination.
13. The acidic or alkaline character of a solution.
14. Hydrogen
15. Hydroxide
16. 10x
17. 10,000 times
18. The sensing half-cell is where the electrical potential develops and this is what the meter actually reads. The reference half-cell dispenses reference solution which completes the (electrical) circuit.
19. No potential develops because the Hydrogen concentration is the same both inside and outside of the glass bulb.
20. mV (Millivolt)
21. A decade is a 10 fold concentration change. It also equals 1 pH unit.

Bacteriological Analysis – p.

1. mColiBlue-24 (Hach Method 10029) with membrane filtration and Colilert Test Kit (MPN) Procedure (SM 9223B-2004). Hach Method 10029 uses mColi Blue 24 broth, the Colilert Test uses Colilert media. Both tests are for *E.coli* (and Total Coliform with Colilert)
2. The mColi Blue test requires sterile buffered water (made using Hach Dilution Water Concentrate, APHA), whereas the Colilert method just requires sterile DI water.

3. Recent rain events and a visual assessment of the water to include turbidity and debris present (also, if there is a lot of I/I present, you should do more dilutions)
4. 3 minute sterilization to kill any bacteria that are left on the funnel so as to not contribute/confound the results of the next dilution. The blank that is run at the end is used to test the effectiveness of the UV box.
5. Blue
6. Yellow = total coliforms, fluorescence (plus yellow) = *E. coli*
7. To clean and wrap the membrane filtration apparatus in brown paper and autoclave to sterilize.
8. Autoclave
9. Most Probable Number
10. True
11. 44.5 +/- 0.2 °C
12. ? 8 hours hold time, cool < 10°C with 0.008% $Na_2S_2O_3$[5]
13. It removes any residual chlorine from the sample, $Na_2S_2O_3$[5]
14. The geometric mean of the density of fecal coliform in the samples shall be less than either 2 million MPN per gram of total solids or 2 million cfu per gram of total solids.
15. The geometric mean of the density of fecal coliform in the samples shall be less than either 2,000,000 Most Probable Number per gram of total solids (dry weight basis) or 2,000,000 Colony Forming Units per gram of total solids (dry weight basis).
16. 35 +/- 0.5°C for 24 +/- 2 hours
17. You could use a boiling water bath, remember to let metal cool completely in before adding sample (or it could kill your bacteria), keep the water at a rolling boil, and always use long-handled tongs to transfer hot equipment
18. 126 colonies/100mL monthly average, or daily max of 487 or 947/100mL depending on permit.
19. Range of 20-80 colonies
20. True

<u>QA/QC – p.</u>

1. Representative
2. Precision = repeatability, being able to get the same results time after time.
   This is associated with duplicates.
   Accuracy = the closeness of test results to the correct (known) value.
   This is associated with Lab Fortified Blanks or Standards.
3. False
4. You must go by the editorial revision date on the method (which is listed in 40 CFR 136). That date is located at the bottom of the Introduction (Section A) in the Standard Method.
5. True
6. 40 CFR 136
7. DOC Demonstration of Capability
   MDL Method Detection Limit
   LRB Laboratory Reagent Blank
   LFB Laboratory Fortified Blank
   LFM/LFMD Laboratory Fortified Matrix/Duplicate

"Dup" Duplicate

ICV Initial Calibration Verification

CCV Continuing Calibration Verification

8. Demonstration of Capability (DOC)
9. Method Detection Limit (MDL)
10. MDLs: Minimum of 2 spiked samples on each instrument per quarter, therefore a minimum of 8 per year; MDLb: no additional samples are required, but you should be entering your routine method blanks into the MDL calculator
11. Laboratory Fortified Blank or Standard
12. To determine if there are interferences in the effluent matrix
13. If you have 20-30 data points within 90 days
14. When QC data falls outside the acceptance limits or exhibits a trend towards falling outside the acceptance limits, both of which indicate an error in the analytical process.
15. False - They must take corrective action promptly to determine and eliminate the source of error. They should not report the data until the cause of the problem is identified and either corrected or qualified.
16. Make any necessary changes/adjustments (such as changes to the facility or staff), Document any new revisions, and Have all analysts read/review the SOP and include documentation that they have undergone the yearly refresher.
17. True