Wastewater Treatment Laboratory

Week 1

Course #2212

Fleming Training Center
Your Partner in Clean Water

TN Department of Environment & Conservation

September 15-18, 2020

Wastewater Treatment Laboratory -- Week 1
Course #2212
September 14-18, 2020

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Monday, September 14:

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, September 15:

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

Wednesday, September 16:

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

Thursday, September 17:

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

Friday, September 18:

8:30 BOD Analysis — Takeoff and Calculations
10:30 Need to Know Info
11:00 Exam Review
12:00 Lunch
1:00 Final Exam — Lab Practical

Fleming Training Center
Your Partner in Clean Water

TN Department of Environment & Conservation

Table of Contents

Section 1:

Laboratory Basics 3

Lab Safety 4

GHS of Classification, Labeling, and SDS 24

Lab Equipment 36

Laboratory Vocabulary 49

Cleaning Glassware 54

How to Read a Meniscus 55

Sampling 56

Section 2:

Dissolved Oxygen 63

Section 3:

Biochemical Oxygen Demand 99

Section 4:

Chemical Oxygen Demand 155

Section 5:

Solids 169

Section 6:

Turbidity 201

Section 7:

pH 215

Section 8:

Bacteriological Analyses 237

Section 9:

QA/QC 281

Section 10:

Certification Exam Need to Know 301

Section 11:

Answers to Review Questions 321

Section 1
Lab Basics

Wastewater Laboratory Class

Laboratory Safety

Safety

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

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

Laboratory Hazards

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

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

Infectious Materials

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

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

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

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 ($H_4C_2O_2$)

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)

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

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

Explosive or Flammable Materials

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

Personal Protective Equipment

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

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

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

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

Personal Safety and Hygiene

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

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

Personal Safety and Hygiene

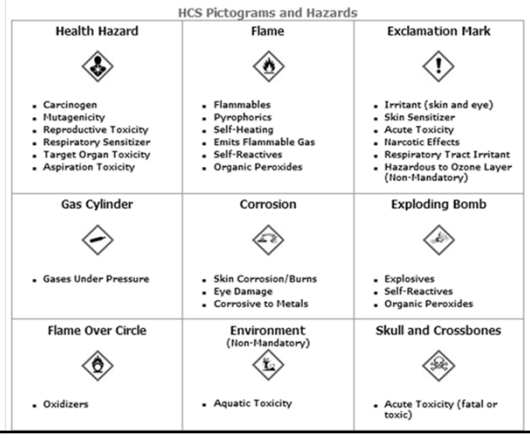
11. Never look into the open end of a container during a reaction or when heating a container
12. Always check labels on bottles to make sure you selected the proper chemical
    - Unlabeled containers only allowed if the are dispensed immediately after use
13. Never handle chemicals with bare hands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

Personal Safety and Hygiene

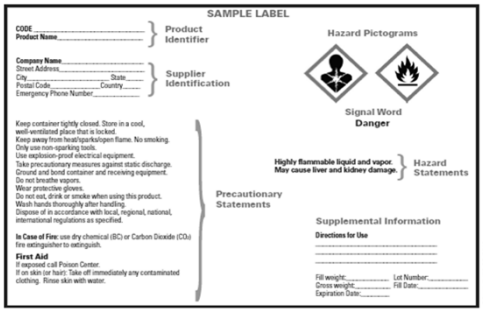
14. Unsafe glassware is the largest single cause of accidents in the lab
    - Fire polish chips – slowly heat chipped area until it reaches a temp at which glass will begin to melt, remove from heat, allow to cool
    - Never hold glassware (or equipment) with bare hands while heating
      - Use gloves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

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



Manufacturer Container Label



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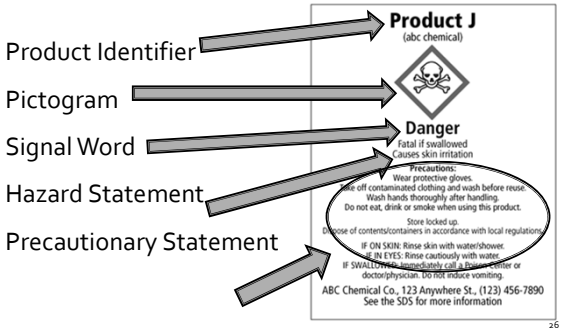
Manufacturer Container Label



26

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

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

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

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

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

Chemical Storage

Moving chemicals

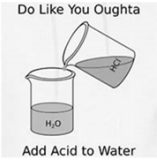
- Use cradles or tilters for carboys or other large chemical vessels
- Use a trussed hand truck for transporting cylinders of compressed gas
- Never roll a cylinder by its valve
- Clamp securely into place to prevent shifting or toppling
- Carry flammable liquids in safety cans
  - Gloves, safety shoes,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

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

Proper lab technique

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



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Any Questions?

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 4 types of Personal Protective Equipment.

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 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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This InfoSheet is not a standard or regulation, and it creates no new legal obligations. It contains recommendations as well as descriptions of mandatory safety and health standards. The recommendations are advisory in nature, informational in content, and are intended to assist employers in providing a safe and healthful workplace. The *Occupational Safety and Health Act* requires employers to comply with safety and health standards and regulations promulgated by OSHA or by a state with an OSHA-approved state plan. In addition, the Act's General Duty Clause, Section 5(a)(1), requires employers to provide their employees with a workplace free from recognized hazards likely to cause death or serious physical harm.

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® Occupational Safety and Health Administration
www.osha.gov 1-800-321-6742

OSHA FS-3461 8/2011
DSG

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

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_2O		
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

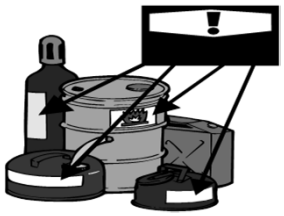
- A chemical in a gaseous state that will ignite spontaneously in air at a temperature of 130ºF
  - For example:
    - Arsine
    - Silane
    - Metal carbonyls (dicobalt octacarbonyl, nickel carbonyl)
    - Diborane

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

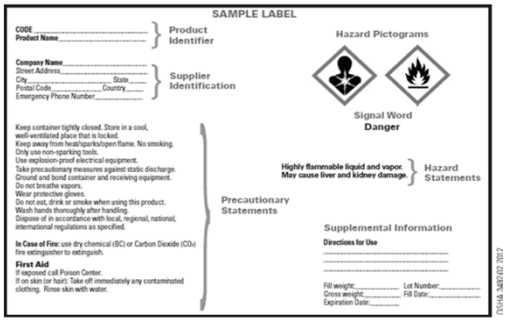
Labeling is the Law

13

Incoming Containers

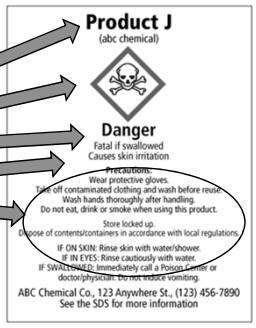
- The requirement to have labels is unchanged
- But the label content has changed
- The chemical manufacturer, distributor, or importer must label a container with
  - Product identifier
  - Signal word
  - Hazard statement(s)
  - Pictogram
  - Precautionary statement(s)
  - Name, address, telephone 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

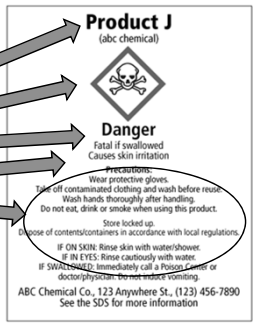


_____A
_____B
_____C
_____D
_____E

Precautionary Statement
Pictogram
Product Identifier
Signal Word
Hazard Statement

16

Manufacturer Container Label Requirement



Product Identifier _____A
Pictogram _____B
Signal Word _____C
Hazard Statement _____D
Precautionary Statement _E

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

18

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

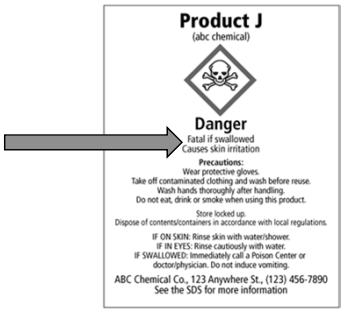
19

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

20

Hazard Statement



21

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

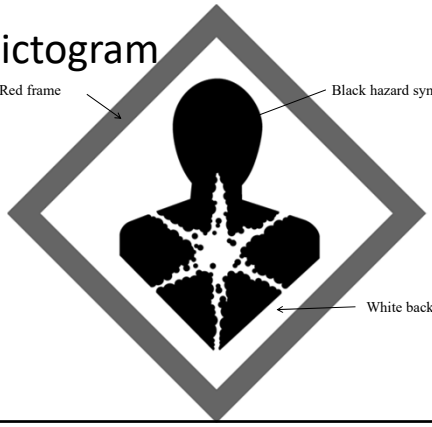
23

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"

24

Pictogram



25

Health Hazard

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

26

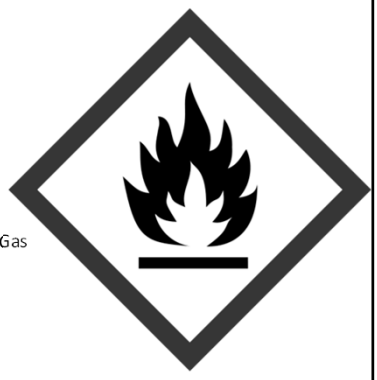
Skull and Crossbones

- Acute Toxicity

27

Flame

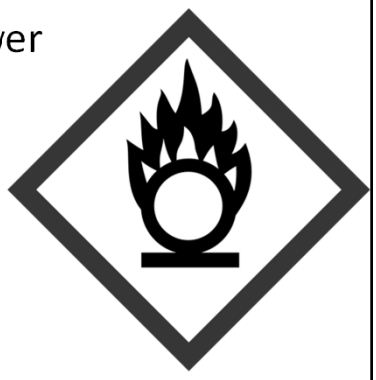
- Flammables
- Pyrophorics
- Self-Heating
- Emits Flammable Gas
- Self Reactives
- Organic Peroxides



28

Flame Over Circle

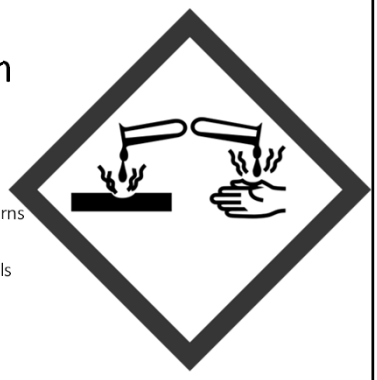
- Oxidizers



29

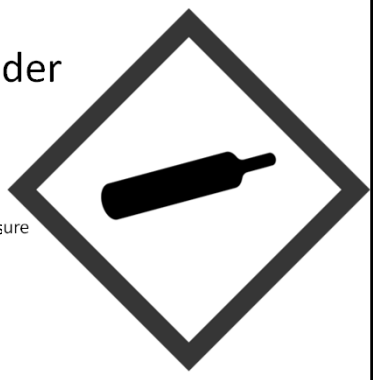
Corrosion

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



30

Gas Cylinder



- Gases Under Pressure

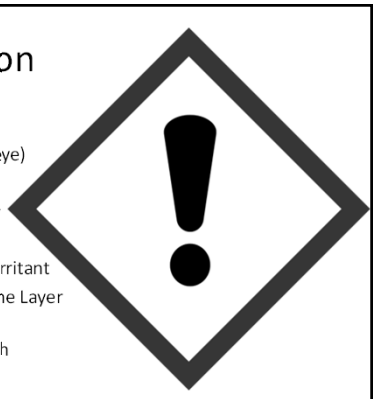
31

Exploding Bomb

- Explosives
- Self-Reactives
- Organic Peroxides

32

Exclamation Mark

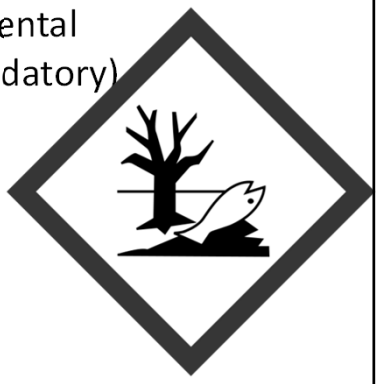


- Irritant (skin and eye)
- Skin Sensitizer
- Acute Toxicity-low
- Narcotic Effects
- Respiratory Tract Irritant
- Hazardous to Ozone Layer (-non-mandatory)
- (Low degree health hazard)

33

Environmental (non-mandatory)

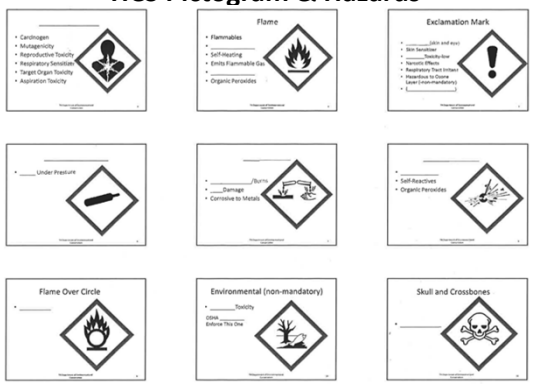
OSHA Does Not Enforce This One



- Aquatic Toxicity

34

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)

36

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

37

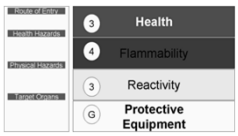
Transfer Container Labeling Exemption Continues

- 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 <u>and</u>
    - Contents used up in one shift

Employers must comply by June 1, 2016

38

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

39

Workplace Labeling

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

GHS Category	GHS Hazard	HMIS/NFPA Category	HMIS/NFPA 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

40

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.

41

SAFETY DATA SHEETS (SDS)

42

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**

43

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

Information in these sections will not be enforced by OSHA

44

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

45

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

46

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

47

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

48

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

49

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

50

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

51

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

52

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

53

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

54

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.

55

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

56

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

57

Section 14

Information in this section will not be enforced by OSHA

Section 14 – Transport Information

DOT - Not Regulated

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

58

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

59

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

60

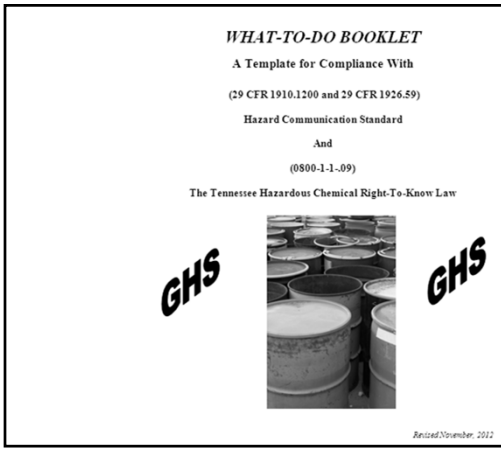
EFFECTS ON OTHER STANDARDS

61

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	

62



63

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**

64

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:

65

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

67

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.

68

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**

69

Laboratory Equipment

Identification, Handling, and Cleaning

1

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

2

Beakers

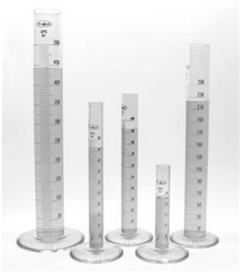
Used for:

- Mixing
- Measuring approximate volumes
- ~10% accuracy

3

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

5

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

6

Types of Pipets

1. Volumetric
2. Measuring
   - Mohr
   - Serological

7

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

8

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

9

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

10

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

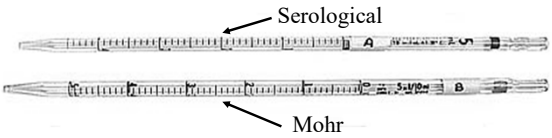
11

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

12

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



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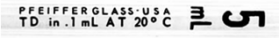
Specifications on a Measuring Pipet

- 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

14

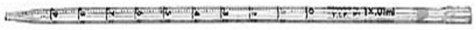
5 in 1/10 ml
TD 20°C

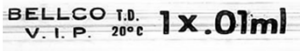


- Specifications on a pipet as shown above indicate that the pipet is calibrated in 1/10ml divisions and will deliver up to 5.0 ml within published tolerance levels at 20°C

15

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

16

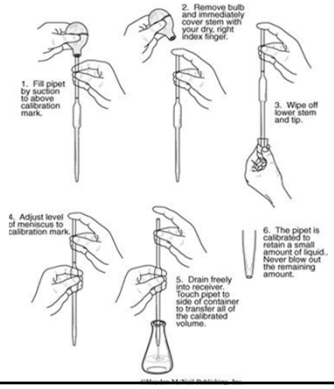
Using Pipets

- 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

17

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

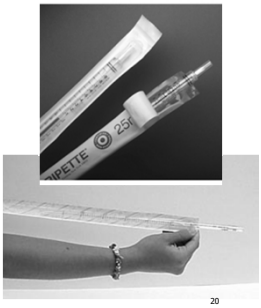


Handling and Disposing of Pipets

- 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

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



20

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

21

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

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

23

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

24

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

25

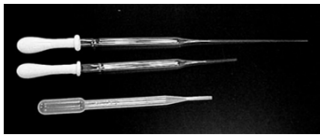
Other Pipet Bulbs

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

26

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



27

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

28

Flasks

- Distilling Flask

- Florence (Flat Bottom) Flask

29

Flasks

- Erlenmeyer Flasks
- Filter Flask

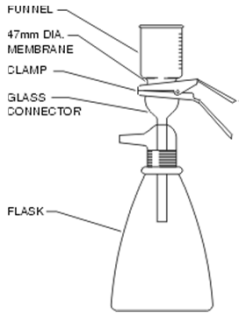
30

How Scientific Glassware is made

- Glass blowing video
- Machine made video

31

Filter Apparatus



- Vacuum Pump

32

Bottles

- Dilution Bottles
- Sample Bottles

33

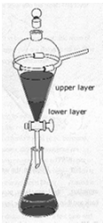
Bottles

- Reagent Bottles
- Weighing Bottles

34

Funnels

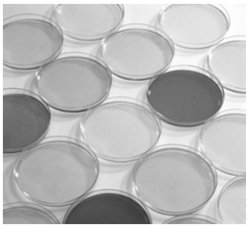
- Separatory
- Buchner



- General

Petri Dish/Dessicator

- Petri Dish
  - Culturing container
- Desiccators
  - Dust and moisture free



36

Evaporating Dish/Crucible

- Evaporating Dish
- Crucible

37

Centrifuge

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

38

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

39

Refrigerators

Walk-in cooler

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

40

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

41

Incubators

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

42

UV Sterilizer

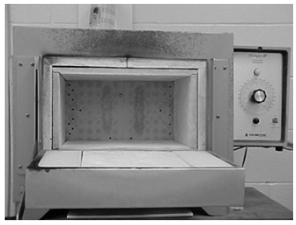
- Use in Bacterial Lab to sterilize test equipment
- 3 minutes time

Drying Oven

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

44

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

45

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

46

Water Still

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

47

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

48

Balances

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

49

pH Meter

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

50

Spectrophotometer

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

51

Colorimeters

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

52

Amperometric Titrator

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

53

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

54

Chemical Storage

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

55

Flammable Cabinet

Flammable chemicals should be kept in a flammable cabinet

56

Safety Equipment

PPE (Personal Protective Equipment):

- Goggles
- Gloves
- Aprons
- Wear safety clothing

57

Eye Wash and Shower

- Should be checked weekly

58

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

59

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

60

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

61

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

62

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.

63

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

64

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

65

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

66

Laboratory Equipment – Review Questions

1. Which type of glassware is used for mixing and measuring approximate volumes?

2. Which type of glassware is calibrated to provide the most accurate volume measurement?

3. Give an example of when you would need to use the type of glassware from question #2 in the lab.

4. Mark whether or not the following types of pipets should be blown out (the last drop is forced out with the bulb).
    a. Volumetric –
    b. Mohr –
    c. Serological –

5. Explain the differences between "TC" and "TD" glassware.

6. Pipet bulbs can easily become contaminated and should be handled as such. True or False?

7. What is the proper way to read a meniscus?

8. What do you do with used glass pipets?

9. How long should glassware/equipment remain in the UV sterilization box in order to be properly sterilized?

10. What is the standard temperature setting for sterilization in an autoclave?

11. BOD incubators should be kept at what temperature? And how often should temperature be checked (for all appliances)?

12. What is the difference between a top loading balance and an analytical balance?

13. List the PPE that could be required in a wastewater laboratory.

14. Acids and Bases can be stored together in the same cabinet. True or False?

15. How often should eye wash stations and emergency showers be checked?

16. What type of detergent should be used to clean laboratory glassware?

17. List the standard procedure for washing glassware.

18. What is the purpose of acid washing glassware?

19. List the steps that are required for acid washing glassware.

20. How often should laboratory grade water be tested and the (in house) system inspected?

Laboratory Vocabulary

>	Greater than; DO > 5 mg/L would be read: DO greater than 5 mg/L
<	Less than; DO < 5 mg/L would be read: Do less than 5 mg/L
Aliquot	Representative portion of a sample. Often, an equally divided portion of a sample
Ambient Temperature	Temperature of the surroundings
Amperometric	A method of measurement that records electric current flowing or generated, rather than recording voltage. Amperometric titration is a means of measuring concentrations of certain substances in water.
Anaerobic Environment	A condition where "free" or dissolved oxygen is NOT present in aquatic environment.
Aseptic	Free from the living germs of disease, fermentation or putrefaction. Sterile.
Blank	A bottle containing only dilution water or distilled water, but the sample being tested is not added. Tests are frequently run on a sample and a blank and the differences are compared.
Buffer	A solution or liquid whose chemical makeup neutralizes acids or bases without a great change in pH.
Buffer Capacity	A measure of the capacity of a solution or liquid to neutralize acids or bases. This is a measure of the capacity of water or wastewater for offering a resistance to changes in pH.
Colorimetric Measurement	A means of measuring unknown concentration of water quality indicators in a sample by measuring the sample's color intensity. The color of the sample after the addition of specific chemicals (reagents) is compared with colors of known concentrations.

Composite (Proportional) Samples A collection of individual samples obtained at regular intervals, usually every one or two hours during a 24-hour time span. Each individual sample is combined with the others in proportion to the flow when the sample was collected. The resulting mixture (composite sample) forms a representative sample and is analyzed to determine the average conditions during that sampling period.

Compound A pure substance composed of two or more elements whose composition is constant. For example, table salt (sodium chloride, NaCl) is a compound.

Desiccator A closed container that heated weighing or drying dishes are placed to cool in a dry environment. The dishes may be empty or they may contain a sample. Desiccators contain a substance, such as anhydrous calcium sulfate, which absorbs moisture and keeps the relative humidity near zero so that the dish or sample will not gain weight from absorbed moisture.

Distillate In the distillation sample, a portion is evaporated; the part that is condensed afterwards is the distillate.

Element A substance that cannot be separated into substances of other kinds by ordinary chemicals means. For example, sodium (Na) is an element.

End Point Samples are titrated to the end point. This means that a chemical is added, drop-by-drop, to a sample until a certain color change (blue to clear, for example) occurs that is called the end point of the titration. In addition to a color change, an end point may be reached by the formation of a precipitate or the reaching of a specified pH. An end point may be detected by the use of an electronic device such as a pH meter.

Flame Polished Melted by a flame to smooth out irregularities. Sharp or broken edges of glass (such as the end of a glass tube) are rotated in a flame until the edge melts slightly and becomes smooth.

Grab Sample A single sample of water or wastewater taken at neither a set time nor flow.

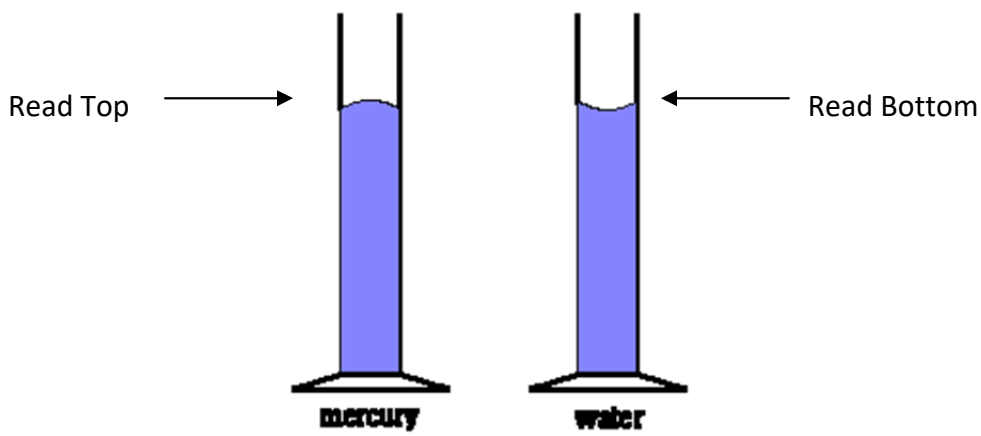
Gravimetric A means of measuring unknown concentrations of water quality indicators in a sample by weighing a precipitate or residue of the sample.

Indicator (Chemical) A substance that gives a visible change, usually of color, at a desired point in a chemical reaction, generally at a specified end point.

***M* or Molar** A molar solution consists of one gram molecular weight of a compound dissolved in enough water to make one liter of solution. A gram molecular weight is the molecular weight of a compound in grams. For example, the molecular weight of sulfuric acid (H_2SO_4) is 98. A 1 *M* solution of sulfuric acid would consist of 98 grams of H_2SO_4 dissolved in enough distilled water to make one liter of solution.

MPN Most Probable Number of coliform group organisms per unit volume. Expressed at a density of population of organisms per 100 mL.

Meniscus The curved top of a column of liquid (water, oil, mercury) in a small tube. When the liquid wets the sides of the container (as with water), the curve forms a valley. When the confining sides are not wetted (as with mercury), the curve forms a hill or upward bulge.



Molecular Weight The molecular weight of a compound in grams is the sum of the atomic weights of the elements in the compound. The molecular weight of sulfuric acid (H_2SO_4) in grams is 98.

ELEMENT	ATOMIC WEIGHT	NUMBER OF ATOMS	MOLECULAR WEIGHT
H	1	2	2
S	32	1	32
O	16	4	64
		Total Weight	98

Molecule The smallest portion of an element or compound that still retains or exhibits all the properties of the substance.

***N* or Normal** A normal solution contains one gram equivalent weight of reactant (compound) per liter of solution. The equivalent weight of an acid is that weight that contains one-gram atom of ionizable hydrogen or its chemical equivalent. For example, the equivalent weight of sulfuric acid (H_2SO_4) is 49 (98 divided by 2 because there are two replaceable hydrogen ions). A 1 *N* solution of sulfuric acid would consist of 49 grams of H_2SO_4 dissolved in enough water to make one liter of solution.

Oxidation The addition of oxygen, removal of hydrogen, or the removal of electrons from an element or compound. The opposite of reduction.

Percent Strength The amount of a substance that is dissolved in a solution compared with the amount that could be dissolved in the solution, expressed as a percent.

pH An expression of the intensity of the alkaline or acid condition of a water. Mathematically, pH is the logarithm (base 10) of the reciprocal of the hydrogen ion concentration. The pH may range from 0-14, where 0 is most acidic, 14 most alkaline and 7 is neutral. Natural waters usually have a pH between 6.5 and 8.5.

Precipitate To separate (a substance) out in a solid form from a solution, as by the use of a reagent.

Reagent A substance that takes part in a chemical reaction and is used to measure, detect or examine other substances.

Reduction The addition of hydrogen, removal of oxygen, or the addition of electrons to an element or compound. The opposite of oxidation.

Representative Sample A portion of material or water identical in content to that in the larger body of material or water being sampled.

Solution A liquid mixture of dissolved substances. In a solution, it is impossible to see all the separate parts.

Standard Solution A solution that the exact concentration of a chemical or compound is known.

Titrate A chemical solution of known strength is added on a drop by drop basis until a color change, precipitate or pH change in the sample is observed (end point). This is the process of adding the chemical solution to completion of the reaction as signaled by the end point.

Turbidity Units Expressed in Nephelometric Turbidity Units (NTU) when measure by a nephelometric (reflected light) instrument.

Volumetric A means of measuring unknown concentrations of water quality indicators in a sample by determining the volume of titrant or liquid reagent needed to complete particular reactions.

Cleaning Glassware

We often overlook the importance of clean glassware in the lab. We think if it looks clean, it must be clean. But there may be residues on the glassware that can affect our results. Since we use those results for both running the plant and reporting water quality to the state, it is important that those results be as accurate as possible.

For many purposes in the water treatment lab, washing in a detergent such as Alconox is sufficient. However, some analyses and some glassware require special cleaning procedures to ensure removal of all residues. Residues of minerals and other substances can build up on glassware, causing erroneous test results. Always follow the recommendations for cleaning glassware and sample containers, and always use the suggested type of sample container.

The following is a partial list of special cleaning procedures for laboratory glassware used for chemical analyses:

Sample containers:

When collecting samples for metals analyses, special cleaning of the containers is necessary to prevent residues from affecting results. Clean the sample bottles by thoroughly washing with laboratory soap (preferably phosphate-free), followed by an acid wash and multiple rinses with distilled or deionized water. Do not use glass sample bottles for metals analysis.

Sample cells and cuvets:

Wash thoroughly using laboratory soap (preferably phosphate-free), followed by an acid wash and multiple rinses with distilled or deionized water. Allow to air dry or wipe with a Kim-wipe, don't use paper towels.

Flasks, beakers, etc used for metals analysis:

Wash thoroughly using laboratory soap (preferably phosphate-free), followed by an acid wash and multiple rinses with distilled or deionized water.

Pipets:

Soak overnight in a solution of Alconox. Rinse thoroughly using a pipet washer.

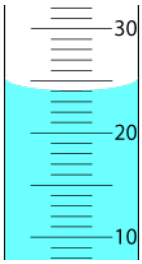
Procedure for Acid Washing Glassware

If acid washing is required, follow these steps:

- Clean the glassware using laboratory detergent (phosphate-free)
- Rinse with tap water
- Rinse with 1:1 hydrochloric acid solution or 1:1 nitric acid solution
- Rinse well with distilled water
- Air dry

❖ Note: always use gloves and safety goggles when handling acids

How to Read a Meniscus



When using Graduated Cylinders the students must first learn how to read the meniscus. The meniscus is formed when the sides of the cylinder pull the water up the sides. This is due to adhesion. You should always read the bottom of the meniscus

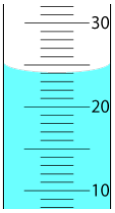
Steps to reading Graduated Cylinders:

1. Place the graduated cylinder on a level surface with the measurement lines facing you.
2. The water in a cylinder will form a curve called the meniscus
3. Your eye should be level with the top of the liquid
4. Find the bottom of the curved meniscus in the water. This should be in the center of the graduated cylinder.
5. Follow the lowest point at the surface of the water to the wall of the graduated cylinder. Read the volumetric scale at this point.

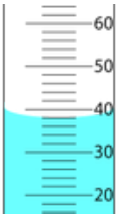
If you have trouble seeing the meniscus, try holding a black card against the opposite side of the graduated cylinder at the same height as the water.

Plastic cylinders may differ and the liquid level will have a flat surface. In that case you still read the center, not the edges.

Samples:



This reading would be 24



This reading would be 39

SAMPLING

Wastewater Lab Class

Fleming Training Center | TN Department of Environment & Conservation

1

Why Sample?

1. Meet compliance requirements
2. Process control
3. Ensure public safety and protect the environment

Photo: www.tpomag.com

2

Proper Sampling = Good Data

1. Sample is representative
2. Proper sampling techniques
3. Proper sample preservation
4. Chain of custody procedure

3

Sampling Plan

Questions to consider before making a sampling plan:

1. Why is the sample being collected?
2. What tests need to be run on the sample?
3. Where is the sample going to be collected from?
4. How is the sample going to be collected?
5. When does the sample need to be collected/analyzed?
6. Who is going to analyze the sample?

4

Considerations

- Collection
- Volume
- Storage and preservation
- Sample points
- Sampling frequency

- Include Sampling Plan in SOP

5

Representative Sample

- A sample portion of material, water, or wastestream that is as nearly identical in content and consistency as possible to that in the larger body being sampled.
- Samples should be taken from the wastestream where it is well mixed.
- Frequency:
  - To represent typical weekdays
  - Varied from day to day within the week

6

Grab Sample

- Single sample
  - *NPDES definition = a single influent or effluent sample collected at a particular time*
- Represents portion of water or wastewater at any one time
  - NOT the average
- Residual chlorine, dissolved oxygen, coliforms, *E. coli,* pH and temperature

7

Composite Sample

- Collected at regular intervals
  - *NPDES definition = a combination of not less than 8 influent or effluent portions, of at least 100 mL, collected over a 24-hour period.*
  - Under certain circumstances a lesser time period may be allowed, but in no case, less than 8 hours

8

Composite Sample

- Taking samples throughout the day, every 1 – 2 hours
- Once collected, refrigerated at 6°C
  - Bacterial decomposition
- In proportion to existing flow
  - As flow increases, more sample is collected
- Combined to form sample representative of entire flow for period

9

Composite Sample

- Refrigerated and thoroughly mixed
  - Solids greater than one-quarter inch excluded
  - If not remixed, errors of 25 – 50% may occur

- Measure flow and sample volume
- BOD, total N, settleable solids
- NEVER use composite sample for bacterial analysis

10

Sludge Sample

- Sludge solids vary considerably with pumping time
- Composite samples
- As a sludge sample stands, the solids and liquid will separate
- Do not store in sealed container
  - Digestion can produce gas
- Refrigeration is critical to prevent bacterial action

11

Sampling Guidelines

- Representative
  - Well mixed
- Proper container
  - 40 CFR 136 Table II
- Do not contaminate the lid
- Preservative and/or dechlorinating agent

12

Sampling Guidelines

- Hold by base
- Turn into current
- Avoid air bubbles

- Label containers with:

1. Sample Location
2. Date and Time of collection
3. Name of collector
4. Any other pertinent information

13

Sample Labeling

Location: 196 E. Main Street, Springfield, TN

Date / Time: 9/22/2018 @ 8:15 AM

Sampled by: BS (Bob Smith)

Comments: grab sample
pH < 2 with H_2SO_4 and stored at 4°C

14

Sample Labeling

- Special care must be taken not to contaminate samples.
  - This includes storing samples in a secure location to preclude conditions which could alter the properties of the sample. Samples shall be custody sealed during long-term storage or shipment.
- Documentation of field sampling is done in a bound logbook.

15

Chain of Custody

- Written record to trace possession and handling of samples from collection to reporting
  - In case of legal litigation
- Used when sending out samples to contract lab
- Should identify who handled sample from collection to transport to storage to analysis to destruction
  - Including dates, times, initials, addresses, etc.

16

Chain of Custody

- Collected samples are in the custody of the sampler or sample custodian until the samples are relinquished to another party.
  - If samples are transported by the sampler, they will remain under his/her custody or be secured until they are relinquished.

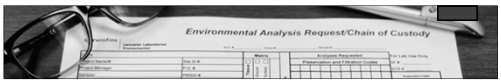
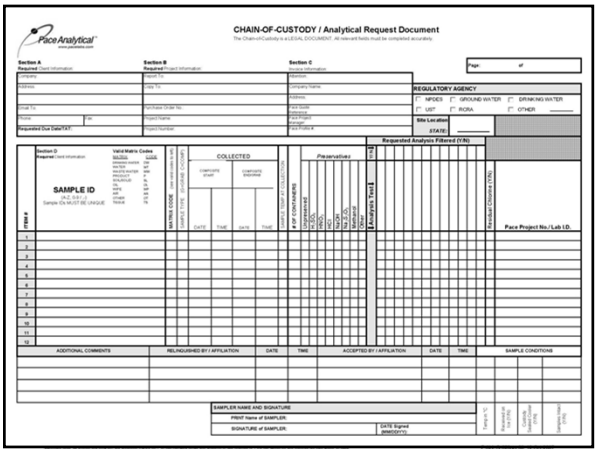


Photo: www.eurofinsus.com

17



Sample Volume

- Depends on test procedure
- Headspace for mixing
- Preservative
- QA/QC comparisons

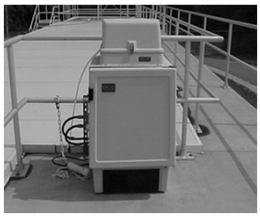
19

Sampling Point Selection

- Flow well mixed
- Exclude large particles (>1/4 inch)
- Exclude floating matter
- Readily accessible & in safe area

20

Sampling Devices



Automatic:

- Timesaver
- Composite: set to collect specific volumes over a period of time
- Clean intake line regularly to prevent growth of bacteria or algae

21

Sampling Devices

Manual:

- Dippers
- Weighted bottle sampler
- Whirl-pak® bags
- Jugs

22

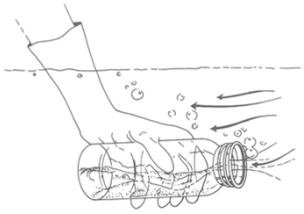
Sampling Devices

- Okay to improvise as long as the device can be properly cleaned
- Does this look like an approved device?

Sampling Devices: Approved or Not?

24

Subsurface Sampling

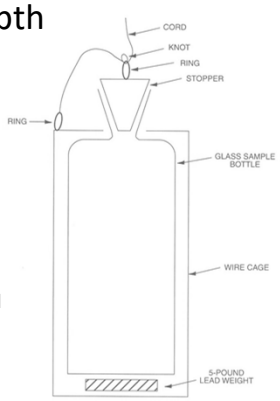


- Grasp container at base
- Plunge bottle mouth down into water
  - To avoid introducing any floating material
- Position mouth of bottle into current and away from hand
- Tip bottle slightly upward to allow air to exit so bottle can fill

25

Homemade Depth Sampler

- Collection from basins, tanks, lakes, reservoirs
- Pre-marked steel cable
- Pre-measured/marked rope
  - non-smearing ink/paint
- A jerk on the cord will remove the stopper and allow the bottle to fill



26

Most Common Sources of Error

- Improper sampling
- Poor or improper sample preservation
- Lack of sufficient mixing during compositing and testing

27

Preservation Techniques

- Refrigeration at 6°C
- pH<2:
  - Using HCl
  - Using H_2SO_4
  - Using HNO_3
- pH>9 using NaOH
- pH>12 using NaOH

28

Preservation

- Less time between collection and analysis = more reliable data
- Sample deterioration starts immediately after collection for most wastewaters
- Residual chlorine (TRC) and temperature require immediate analysis

29

Any Questions?

?

30

Sampling – Review Questions

1. There are many things to take into consideration prior to collecting a sample, including those listed below. Explain where you would look to answer questions about these considerations.
    a. Collection:
    b. Volume:
    c. Storage and Preservation:
    d. Sample points:
    e. Sampling frequency:

2. In order to get good data, you must follow proper sampling procedure. The sample you collect must be _____.

3. What is the difference between a Grab sample and a Composite sample?

4. List some tests that require Grab samples.

5. List some tests that require Composite samples.

6. Automatic composite samplers must be periodically cleaned to prevent what?

7. Why must composite samples be refrigerated? At what temperature should they be stored?

8. What information should be included on the label that is attached to your sample container?

9. Documentation of field sampling is done in a bound logbook.
    a. True
    b. False

10. What is a Chain of Custody and when would you most likely encounter one as an operator?

11. Improvised sampling devices are allowed as long as they can be properly cleaned.
    a. True
    b. False

12. When choosing a sampling point location, which of the following statements is **not** true?
    a. The flow should be well mixed
    b. The site should be readily accessible and in a safe location
    c. The site should be in a corner where the water is still and easy to collect
    d. Any floating matter or large particles should be excluded.

13. When you are performing subsurface sampling, you should position the mouth of the bottle away from the current.
    a. True
    b. False

14. List the most common sources of error when sampling.

15. Sample deterioration starts about 8 hours after collection for most wastewater.
    a. True
    b. False

Section 2
Dissolved
Oxygen

Dissolved Oxygen Measurement

Wastewater Laboratory Class

Fleming Training Center 1

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

Fleming Training Center 2

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

Fleming Training Center 3

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

Fleming Training Center 4

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

Fleming Training Center 6

Dissolved Oxygen

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

Fleming Training Center 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.***

Fleming Training Center 8

Saturation Concentration

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

Table 9.13 Saturation concentrations for wastewater DO under different conditions

Temperature	Wastewater mg/L[a] 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

Fleming Training Center 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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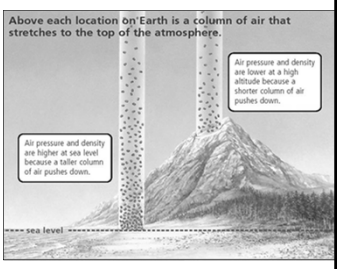
Barometric Pressure (BP)

- 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



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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

Fleming Training Center 12

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 $\div 25.4 =$ inches of Hg

Fleming Training Center 13

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

Fleming Training Center 14

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

Fleming Training Center 15

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)

Fleming Training Center 16

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.

Fleming Training Center 18

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/

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)

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
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:

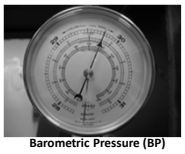
$$°F = [(°C)(1.8) + 32]$$

- To convert from Fahrenheit to Celsius:

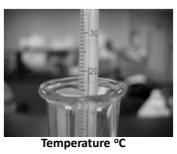
$$°C = \frac{(°F - 32)}{1.8}$$

Barometer on site

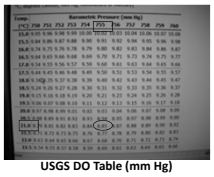
Easiest way to do this - Direct Read from Barometer & Thermometer in the lab to determine DO from USGS DO table



Barometric Pressure (BP) UNCORRECTED



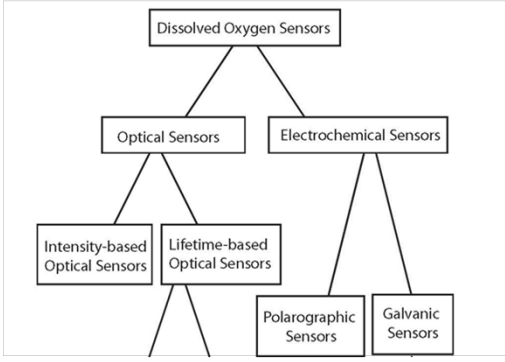
Temperature °C



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)



Fleming Training Center 25

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)

Fleming Training Center 26

Types of Measurement

- DO probes and meters are the most commonly used method

Fleming Training Center 27

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

Fleming Training Center 28

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

Fleming Training Center 29

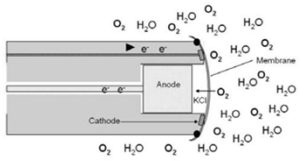


Figure 1. In electrochemical sensors, O_2 molecules diffuse through the membrane to the sensor at a rate proportional to the pressure difference across it.

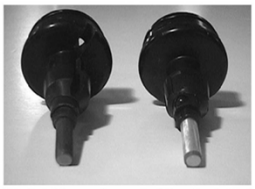


Figure 3. On the right is a properly prepared cathode and anode. Note the light silver appearance of the anode and matte finish of the gold cathode.

Fleming Training Center 30

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

Fleming Training Center 31

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

Fleming Training Center 33

Remember - Hold Time

- 15 minutes – Note time of collection <u>and</u> time of analysis!

Fleming Training Center 34

Optical Method

Luminescent Dissolved Oxygen Probe

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

Fleming Training Center 35

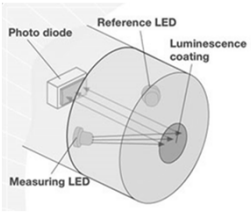
Optical Method

- 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

Fleming Training Center 36

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



Fleming Training Center 37

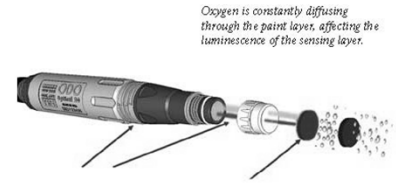


Figure 9. Illustration of how a YSI optical sensor measures oxygen.

Fleming Training Center 38

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

Fleming Training Center 39

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

Fleming Training Center 40

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.

Fleming Training Center 41

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

Fleming Training Center 42

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

Fleming Training Center 43

Taking D.O. Readings

Fleming Training Center 44

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

Fleming Training Center 45

Common Deficiencies

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

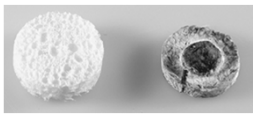


Figure 6. The calibration bottle on the right is clean. The calibration bottle on the left actually has algae growing in it which could affect the calibration values.

Fleming Training Center 46

DO Hach Method 10360, LDO Measurement Oct. 2011

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

Fleming Training Center 47

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.

Fleming Training Center 48

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.

Fleming Training Center 49

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.

Fleming Training Center 50

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.**

Fleming Training Center 51

DO Hach Method 10360, LDO Measurement Oct. 2011

- 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 [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.

Fleming Training Center 52

DO Hach Method 10360, LDO Measurement Oct. 2011

- 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

Fleming Training Center 53

DO Hach Method 10360, LDO Measurement Oct. 2011

- Continuing Calibration Verification (CCV) continued
  - Real people language – prepare dilution water that is air-saturated and analyze bottles and compare to the theoretical dissolved oxygen concentration (± 0.2 mg/L) after daily samples or with each batch of 20 samples or less in an 8-hour period.
- Batch Size
  - 9.3.1 – … with each analytical batch of 20 samples or less in an 8 hour period.

Fleming Training Center 54

DO SM4500-O G – 2001 Membrane Electrode Method

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

Fleming Training Center 55

DO SM4500-O G – 2001 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.

Fleming Training Center 56

DO SM4500-O G – 2001 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.

Fleming Training Center 57

DO SM4500-O G – 2001 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%
  - **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.**

Fleming Training Center 58

DO SM4500-O G – 2001 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.

Fleming Training Center 59

DO SM4500-O G – 2001 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.

Fleming Training Center 60

Temperature

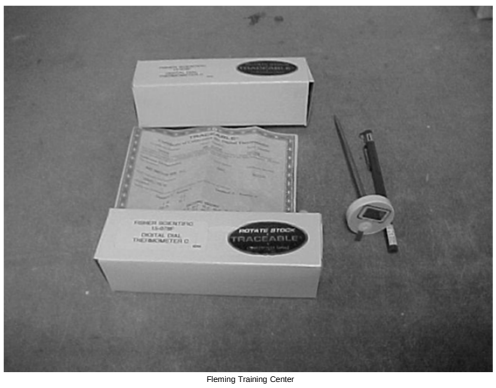
- NIST (National Institute of Standards & Technology) traceable thermometer
- Scale marked in 0.1˚C
- Calibrate annually by checking against a NIST certified thermometer
- Corrections can be made up to ± 4˚C

Fleming Training Center 61

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

Fleming Training Center 62



Fleming Training Center 63

Temperature SM2550 B – 2000 Thermometric Measurement

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

Fleming Training Center 64

Any Questions?

Fleming Training Center 65

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.

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

U.S. Department of the Interior | U.S. Geological Survey
URL: http://water.usgs.gov/cgi-bin/dotables
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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 = $\frac{(°F-32)}{1.8}$
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.)

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

   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) Solution 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°F
    d) Solution temperature = 20°C

USE THIS TABLE TO ANSWER REVIEW QUESTION # 12

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)	380	390	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580
2.0	6.87	7.05	7.24	7.42	7.60	7.79	7.97	8.15	8.34	8.52	8.70	8.89	9.07	9.25	9.43	9.62	9.80	9.98	10.17	10.35	10.53
2.5	6.78	6.96	7.14	7.32	7.50	7.68	7.86	8.04	8.22	8.40	8.58	8.76	8.94	9.13	9.31	9.49	9.67	9.85	10.03	10.21	10.39
3.0	6.68	6.86	7.04	7.22	7.40	7.58	7.75	7.93	8.11	8.29	8.47	8.65	8.82	9.00	9.18	9.36	9.54	9.72	9.89	10.07	10.25
3.5	6.59	6.77	6.94	7.12	7.30	7.47	7.65	7.83	8.00	8.18	8.35	8.53	8.71	8.88	9.06	9.23	9.41	9.59	9.76	9.94	10.11
4.0	6.50	6.68	6.85	7.03	7.20	7.37	7.55	7.72	7.90	8.07	8.24	8.42	8.59	8.76	8.94	9.11	9.29	9.46	9.63	9.81	9.98
4.5	6.42	6.59	6.76	6.93	7.10	7.28	7.45	7.62	7.79	7.96	8.13	8.31	8.48	8.65	8.82	8.99	9.16	9.34	9.51	9.68	9.85
5.0	6.33	6.50	6.67	6.84	7.01	7.18	7.35	7.52	7.69	7.86	8.03	8.20	8.37	8.54	8.71	8.87	9.04	9.21	9.38	9.55	9.72
5.5	6.25	6.42	6.58	6.75	6.92	7.09	7.25	7.42	7.59	7.76	7.92	8.09	8.26	8.43	8.59	8.76	8.93	9.10	9.26	9.43	9.60
6.0	6.17	6.33	6.50	6.66	6.83	7.00	7.16	7.33	7.49	7.66	7.82	7.99	8.15	8.32	8.48	8.65	8.81	8.98	9.14	9.31	9.47
6.5	6.09	6.25	6.42	6.58	6.74	6.91	7.07	7.23	7.40	7.56	7.72	7.89	8.05	8.21	8.38	8.54	8.70	8.86	9.03	9.19	9.35
7.0	6.01	6.17	6.33	6.50	6.66	6.82	6.98	7.14	7.30	7.46	7.62	7.79	7.95	8.11	8.27	8.43	8.59	8.75	8.91	9.08	9.24
7.5	5.94	6.09	6.25	6.41	6.57	6.73	6.89	7.05	7.21	7.37	7.53	7.69	7.85	8.01	8.17	8.33	8.49	8.64	8.80	8.96	9.12
8.0	5.86	6.02	6.18	6.33	6.49	6.65	6.81	6.96	7.12	7.28	7.44	7.59	7.75	7.91	8.07	8.22	8.38	8.54	8.70	8.85	9.01
8.5	5.79	5.94	6.10	6.25	6.41	6.57	6.72	6.88	7.03	7.19	7.34	7.50	7.66	7.81	7.97	8.12	8.28	8.43	8.59	8.74	8.90
9.0	5.72	5.87	6.02	6.18	6.33	6.49	6.64	6.79	6.95	7.10	7.25	7.41	7.56	7.72	7.87	8.02	8.18	8.33	8.48	8.64	8.79
9.5	5.65	5.80	5.95	6.10	6.25	6.41	6.56	6.71	6.86	7.01	7.17	7.32	7.47	7.62	7.77	7.93	8.08	8.23	8.38	8.53	8.69
10.0	5.58	5.73	5.88	6.03	6.18	6.33	6.48	6.63	6.78	6.93	7.08	7.23	7.38	7.53	7.68	7.83	7.98	8.13	8.28	8.43	8.58
10.5	5.51	5.66	5.81	5.96	6.10	6.25	6.40	6.55	6.70	6.85	7.00	7.14	7.29	7.44	7.59	7.74	7.89	8.04	8.18	8.33	8.48
11.0	5.44	5.59	5.74	5.88	6.03	6.18	6.33	6.47	6.62	6.77	6.91	7.06	7.21	7.35	7.50	7.65	7.80	7.94	8.09	8.24	8.38
11.5	5.38	5.52	5.67	5.81	5.96	6.11	6.25	6.40	6.54	6.69	6.83	6.98	7.12	7.27	7.41	7.56	7.70	7.85	7.99	8.14	8.29
12.0	5.32	5.46	5.60	5.75	5.89	6.03	6.18	6.32	6.47	6.61	6.75	6.90	7.04	7.18	7.33	7.47	7.62	7.76	7.90	8.05	8.19
12.5	5.25	5.39	5.54	5.68	5.82	5.96	6.11	6.25	6.39	6.53	6.67	6.82	6.96	7.10	7.24	7.39	7.53	7.67	7.81	7.95	8.10
13.0	5.19	5.33	5.47	5.61	5.75	5.89	6.04	6.18	6.32	6.46	6.60	6.74	6.88	7.02	7.16	7.30	7.44	7.58	7.72	7.86	8.01
13.5	5.13	5.27	5.41	5.55	5.69	5.83	5.97	6.11	6.25	6.38	6.52	6.66	6.80	6.94	7.08	7.22	7.36	7.50	7.64	7.78	7.92
14.0	5.07	5.21	5.35	5.49	5.62	5.76	5.90	6.04	6.17	6.31	6.45	6.59	6.73	6.86	7.00	7.14	7.28	7.41	7.55	7.69	7.83
14.5	5.01	5.15	5.29	5.42	5.56	5.70	5.83	5.97	6.11	6.24	6.38	6.51	6.65	6.79	6.92	7.06	7.20	7.33	7.47	7.60	7.74
15.0	4.96	5.09	5.23	5.36	5.50	5.63	5.77	5.90	6.04	6.17	6.31	6.44	6.58	6.71	6.85	6.98	7.12	7.25	7.39	7.52	7.66
15.5	4.90	5.04	5.17	5.30	5.44	5.57	5.70	5.84	5.97	6.10	6.24	6.37	6.50	6.64	6.77	6.91	7.04	7.17	7.31	7.44	7.57
16.0	4.85	4.98	5.11	5.24	5.38	5.51	5.64	5.77	5.90	6.04	6.17	6.30	6.43	6.57	6.70	6.83	6.96	7.09	7.23	7.36	7.49
16.5	4.79	4.92	5.05	5.19	5.32	5.45	5.58	5.71	5.84	5.97	6.10	6.23	6.36	6.49	6.63	6.76	6.89	7.02	7.15	7.28	7.41
17.0	4.74	4.87	5.00	5.13	5.26	5.39	5.52	5.65	5.78	5.91	6.04	6.17	6.30	6.43	6.55	6.68	6.81	6.94	7.07	7.20	7.33
17.5	4.69	4.82	4.94	5.07	5.20	5.33	5.46	5.59	5.72	5.84	5.97	6.10	6.23	6.36	6.49	6.61	6.74	6.87	7.00	7.13	7.26

18.0	4.64	4.76	4.89	5.02	5.15	5.27	5.40	5.53	5.65	5.78	5.91	6.04	6.16	6.29	6.42	6.54	6.67	6.80	6.93	7.05	7.18
18.5	4.59	4.71	4.84	4.96	5.09	5.22	5.34	5.47	5.59	5.72	5.85	5.97	6.10	6.22	6.35	6.48	6.60	6.73	6.85	6.98	7.10
19.0	4.54	4.66	4.79	4.91	5.04	5.16	5.29	5.41	5.54	5.66	5.78	5.91	6.03	6.16	6.28	6.41	6.53	6.66	6.78	6.91	7.03
19.5	4.49	4.61	4.74	4.86	4.98	5.11	5.23	5.35	5.48	5.60	5.72	5.85	5.97	6.09	6.22	6.34	6.47	6.59	6.71	6.84	6.96
20.0	4.44	4.56	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
20.5	4.39	4.51	4.64	4.76	4.88	5.00	5.12	5.24	5.36	5.49	5.61	5.73	5.85	5.97	6.09	6.21	6.33	6.46	6.58	6.70	6.82
21.0	4.35	4.47	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
21.5	4.30	4.42	4.54	4.66	4.78	4.90	5.02	5.14	5.25	5.37	5.49	5.61	5.73	5.85	5.97	6.09	6.21	6.33	6.45	6.57	6.68
22.0	4.26	4.37	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

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

$$\frac{1 \text{ ampoule } (2.0 \text{ mL}) \times 0.450 \text{ mg/mL GGA}}{300 \text{ mL final volume}} \times 1000 \text{ mL/L} = 3.0 \text{ mg/L GGA per bottle}$$

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

Initial LDO Probe Calibration

Reference: *Hach Method 10360 Luminescence Measurement of Dissolved Oxygen in Water and Wastewater and for Use in the Determination of BOD5 and cBOD5, Revision 1.2, September 2011*

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.

7.1.4 The stopper may now be removed from the BOD bottle and the 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 oxygen detection process (EPA Validation study, 2004). Therefore, do not adjust the luminescence measurement to that of Winker or membrane 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 be 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 (± 20°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.

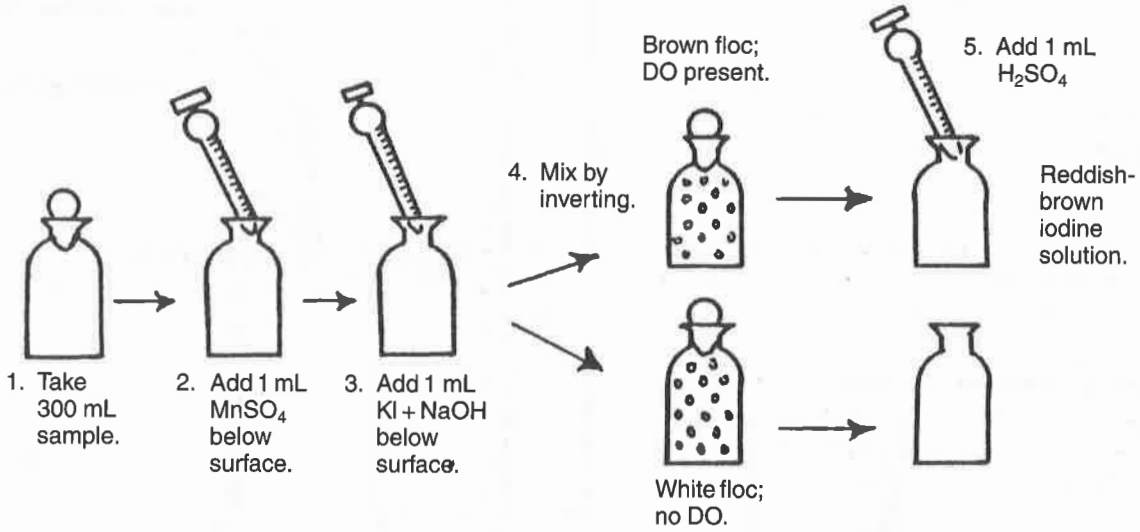
7.2.4 At the completion of aeration, let water re-equilibrate to room temperature (± 20°C) for 30 minutes and note the barometric pressure of the laboratory during preparation.

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

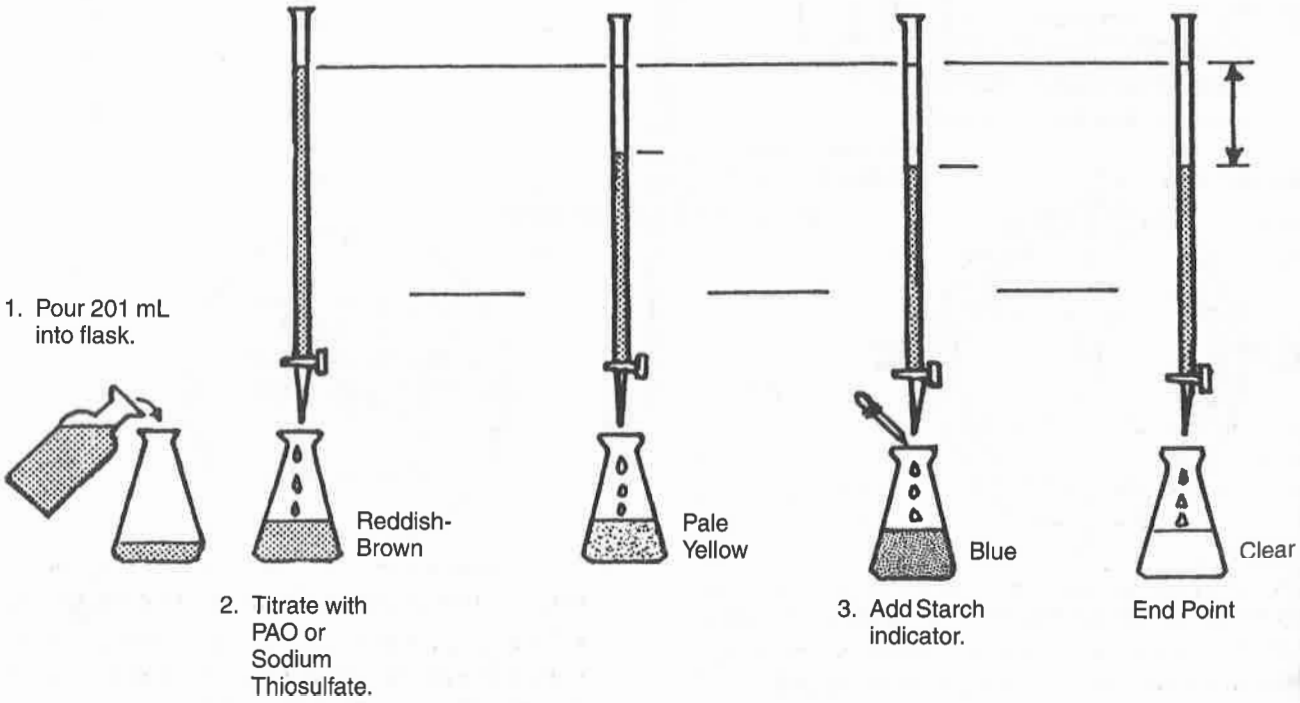
7.2.6 Calculate the theoretical dissolved oxygen concentration using a dissolved oxygen table such as Hitchman (1978).

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 be equally effective.

OUTLINE OF PROCEDURE FOR DO



Titration of Iodine Solution:



Section 3
Biochemical Oxygen Demand

BIOCHEMICAL OXYGEN DEMAND
BOD

1

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

WHAT IS BOD?

- Biochemical Oxygen Demand is the amount of oxygen, expressed in mg/L or parts per million (ppm), that bacteria take from the water when they oxidize organic matter.

  (Hach, Clifford; R. Klein; C. Gibbs. Introduction to Biochemical Oxygen Demand. Hach Company, 1997.)

- The BOD test measures the amount of oxygen used by microorganisms as they use the food in wastewater when placed in a controlled temperature for 5 days.

3

WHAT IS BOD?

- BOD is a measure of organic strength of the wastewater:
  - Changes in dissolved oxygen concentration are used as an indirect measure of organic content.
  - The amount of oxygen used by the bacteria as they stabilize the organic matter
- In the presence of free oxygen, aerobic bacteria use the organic matter in wastewater as "food."
- The BOD test is an estimate of the "food" available in the sample.
  - The more "food" present, the more DO will be required

4

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

5

HISTORY OF BOD

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

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."

7

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.

8

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.

9

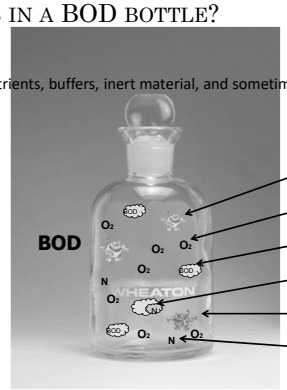
BOD Timeline

- 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

10

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)

11

What is BOD?

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

, Food, and O_2

- How much would a 300 mL BOD sample have all these?

12

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 $C_4H_7O_4$**N** – Glutamic Acid

13

WHAT IS BOD?

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

Food – Reduced forms of nitrogen, exert nitrogenous oxygen demand

14

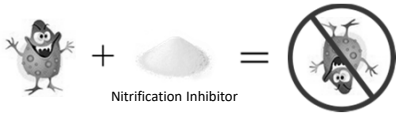
WHAT IS BOD?

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

15

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



16

WHAT IS BOD?

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

17

WHAT IS BOD?

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

18

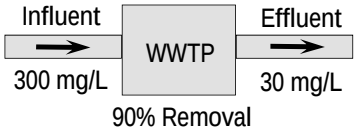
Why is BOD Important?

- 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.

19

Why is BOD Important?

- BOD Removal Efficiency
  - BOD of effluent versus influent



20

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.

21

BOD Test Requirements

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

22

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 %

23

How is BOD Measured?

Dilution Method

BODTrak

24

(Not EPA Approved for Permit Compliance)

BOD Calculations

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

25

BOD Dilution Method

26

BOD – Dilution Method

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

27

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

28

Dilution Water

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

29

Dilution Water Source Water

- 22nd 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
  - Free of heavy metals
    - Ex: copper

30

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

31

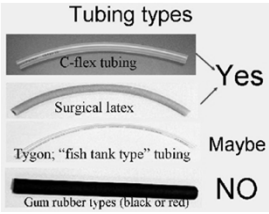
Dilution Water Method Criteria

- 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
- Run one nitrification inhibitor blank
- Total of three blanks
  - One dilution water blank at beginning
  - ~~One nitrification blank at beginning~~
  - One dilution water blank at end

32

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)



33

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

34

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.

35

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

36

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.

37

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

38

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)

39

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

40

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

41

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

42

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

43

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.

44

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)
  - 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

45

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

46

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

47

Determining Range and Sample Volume

48

Determining Range and Sample Volume

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

49

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

50

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

51

Determining Range and Sample Volume

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

52

Determining Range and Sample Volume

Table 2 Determining Minimum Sample Volume

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	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

Table 3 Determining Maximum Sample Volume

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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

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

54

Determining Range and Sample Volume

Calculation of Range of Acceptable BOD results

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

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

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

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

55

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 = $\frac{\text{Initial Dissolved Oxygen – 1.0}}{\text{Sample Volume (SV)}}$ X 300mL

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

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

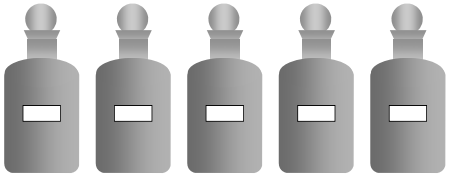
56

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

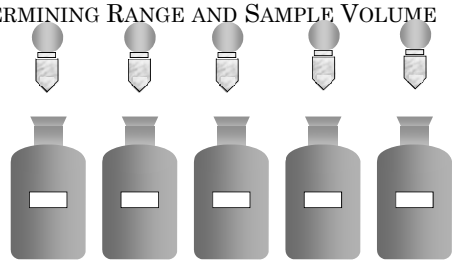
57

Determining Range and Sample Volume



58

Determining Range and Sample Volume



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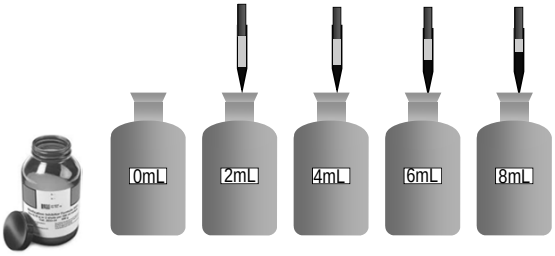
Determining Range and Sample Volume



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

60

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.

61

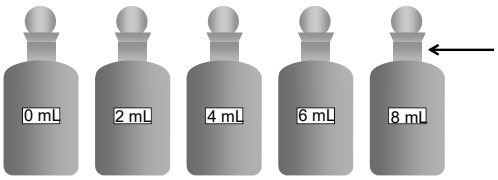
Determining Range and Sample Volume

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

62

Determining Range and Sample Volume

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



There should be

63

Obtaining Data Points

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

64

Obtaining Data Points

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

65

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

66

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)

67

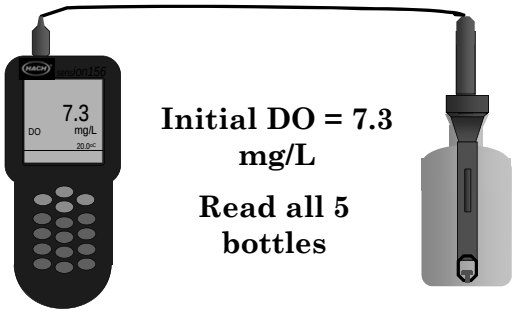
Obtaining Data Points

68

Obtaining Data Points

69

Obtaining Data Points



Initial DO = 7.3 mg/L

Read all 5 bottles

70

Obtaining Data Points

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

71

Obtaining Data Points

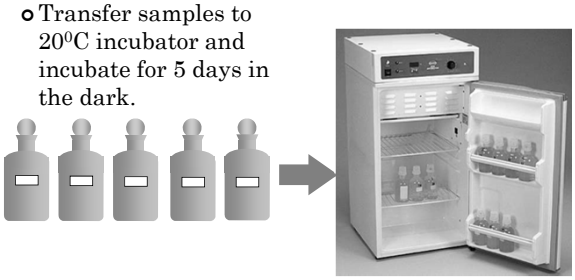
- Fill area in around stopper with dilution water.
- Cover with plastic BOD bottle cap.

You need a water seal!

72

Obtaining Data Points

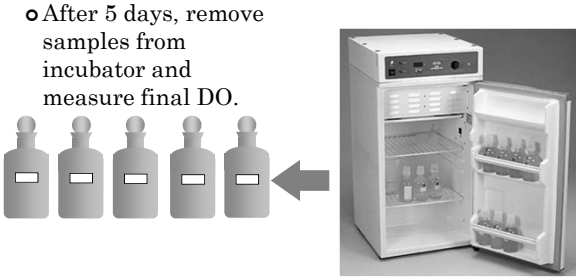
- Transfer samples to 20^0C incubator and incubate for 5 days in the dark.



73

Obtaining Data Points

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

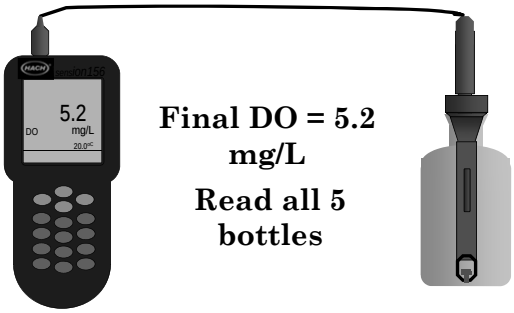


74

Obtaining Data Points

75

Obtaining Data Points



Final DO = 5.2 mg/L

Read all 5 bottles

76

Obtaining Data

- Plug data into equation:

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

77

Obtaining Data

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

$$P = 2/300 = 0.00667$$

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

$$BOD_5, \text{ mg/L} = 315 \text{ mg/L}$$

78

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

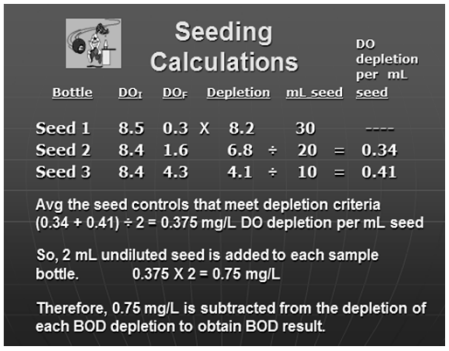
B = DO of seed

P = Sample volume/300

F = Ratio of seed in sample to seed in control

79

Obtaining Data



80

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

81

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

82

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

83

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

84

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

85

Running Standards

- Be careful purchasing GGA from Hach, ***some*** are not the same concentration as specified in Standard Methods
  - Standard Methods - 150 mg/L each GGA
  - Hach BOD Standard – 300mg/L each GGA (divide by 2)
  - Hach ezGGA – gives 198 mg/L BOD results

86

Running Standards

- The concentration of standard does NOT equal BOD.
  - 150mg/L each = 198 ± 30.5 mg/L BOD
  - 300mg/L each = 396 ± 61 mg/L BOD

87

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.

88

QA/QC Steps

- DOC
- Method Blank
- Lab Fortified Blank
- Duplicates
- ICAL/CCV (Dissolved Oxygen probe)
- Control Charts
- Corrective Action
- QC Acceptance
- Batch size: 20
- QC Frequency depends on Permit

89

BOD5/cBOD5 SM5210 B – 2011 & 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)

90

BOD5/cBOD5 SM5210 B – 2011 & 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….

91

BOD5/cBOD5 SM5210 B – 2011 & 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.

92

BOD5/cBOD5 SM5210 B – 2011 & Hach Method 10360

- Method Blanks
  - Real people language: analyze dilution water
    - Preferably one at the beginning and one at end
    - 2014 Update – Removed – If reporting CBOD5 results, then also add one Nitrification Inhibitor (NI Blank)
  - Run daily (day of)
  - Target value is less than 0.20 mg/L (preferably less than 0.10 mg/L)

93

BOD5/cBOD5 SM5210 B – 2011 & 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
  - **2014 Update** - If permit requires cBOD, add nitrification inhibitor (NI) to one GGA bottle once/quarter (or more often if the Lot # of NI changes), which should be equal to 164 ± 30.7 mg/L

94

BOD5/cBOD5 SM5210 B – 2011 & 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.**

95

BOD5/cBOD5 SM5210 B – 2011 & Hach Method 10360

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

96

BOD5/cBOD5 SM5210 B – 2011 & 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

97

BOD5/cBOD5 SM5210 B – 2011 & 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)

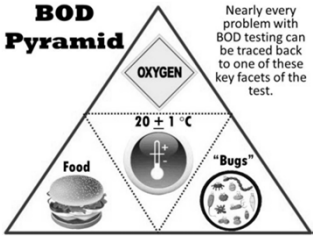
98

BOD5/cBOD5 SM5210 B – 2011 & Hach Method 10360

- QC Acceptance Criteria
  - Blanks < 0.20 mg/L
  - GGA = 198 ± 30.5 mg/L (if running cBOD, add NI to one bottle once/quarter or more often if NI Lot# changes, and it should = 164 ± 30.7 mg/L)
  - 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

99

Any Questions?



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

TDEC - Fleming Training Center

100

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:

The following is an excerpt from Standard Methods for the Examination of Water and Wastewater, 23rd edition and is to be used for instructional purposes only.

5210 BIOCHEMICAL OXYGEN DEMAND (BOD)*

5210 A. Introduction

1. General Discussion

The biochemical oxygen demand (BOD) determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.

Measurements of oxygen consumed in a 5-d test period (5-d BOD or BOD_5, 5210B), oxygen consumed after 60 to 90 d of incubation (ultimate BOD or UBOD, 5210C), and continuous oxygen uptake (respirometric method, 5210D) are described here. Many other variations of oxygen demand measurements exist, including using shorter and longer incubation periods and tests to determine rates of oxygen uptake. Alternative seeding, dilution, and incubation conditions can be chosen to mimic receiving-water conditions, thereby providing an estimate of the environmental effects of wastewaters and effluents.

The UBOD measures the oxygen required for the total degradation of organic material (ultimate carbonaceous demand) and/or the oxygen to oxidize reduced nitrogen compounds (ultimate nitrogenous demand). UBOD values and appropriate kinetic descriptions are needed in water quality modeling studies such as 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.

2. Carbonaceous Versus Nitrogenous BOD

A number of factors, for example, 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 is no way to include adjustments or corrections to account for the effect of these factors.

Oxidation of reduced forms of nitrogen, such as ammonia and organic nitrogen, can be mediated by microorganisms and exert nitrogenous demand. Nitrogenous demand historically has been considered an interference in the determination of BOD, and the inclusion of ammonia in the dilution water contributes an external source of nitrogenous demand. The interference from nitrogenous demand can now be prevented by an inhibitory chemical.[1] If an inhibiting chemical is not used, the oxygen demand measured 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 (Section 4500-NH_3); 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 is subject to considerable error. Chemical inhibition of nitrogenous demand provides a more direct and more reliable measure of carbonaceous demand.

The extent of oxidation of nitrogenous compounds during the 5-d incubation period depends on the concentration and type of microorganisms capable of carrying out this oxidation. Such organisms usually are not present in raw or settled primary sewage in sufficient numbers to oxidize sufficient quantities of reduced nitrogen forms in the 5-d BOD test. Many biological treatment plant effluents contain sufficient numbers of nitrifying organisms to cause nitrification in BOD tests. Because oxidation of nitrogenous compounds can occur in such samples, inhibition of nitrification as directed in 5210B.5*e*) is recommended for samples of secondary effluent, for samples seeded with secondary effluent, and for samples of polluted waters.

3. Reference

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

* Approved by Standard Methods Committee, 2001. Editorial revisions, 2011. Joint Task Group: 21st Edition—James C. Young (chair), George T. Bowman, Sabry M. Kamhawy, Terry G. Mills, Marlene Patillo, Ray C. Whittemore.

5210 B. 5-Day BOD Test

1. General Discussion

The method consists of filling with diluted and seeded sample, to overflowing, an airtight bottle of specified size and incubating it at the specified temperature for 5 d. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. Because the initial DO is determined shortly after the dilution is made, 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 glass bottles having 60 mL or greater capacity (300-mL bottles having a ground-glass stopper and a flared mouth are preferred). Clean bottles with a detergent, rinse thoroughly, and drain before use.

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

3. Reagents

Prepare reagents in advance but discard 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 water, preferably sterilized, for making all solutions.

a. Phosphate buffer solution: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4Cl in about 500 mL distilled 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 distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L.

b. Magnesium sulfate solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.

c. Calcium chloride solution: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.

d. Ferric chloride solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.

e. Acid and alkali solutions, 1*N*, for neutralization of caustic or acidic waste samples.

1) Acid—Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.

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

f. Sodium sulfite solution: Dissolve 1.575 g Na_2SO_3 in 1000 mL distilled water. This solution is not stable; prepare daily.

g. Nitrification inhibitor:

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

2) Allylthiourea (ATU) solution—Dissolve 2.0 g allylthiourea ($C_4H_8N_2S$) in about 500 mL water and dilute to 1 L. Store at 4°C. The solution is stable for not more than 2 weeks.

h. Glucose-glutamic acid 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 distilled water and dilute to 1 L. Prepare fresh immediately before use unless solution is maintained in a sterile condition. Store all glucose-glutamic acid mixtures at 4°C or lower. Commercial preparations may be used but concentrations may vary.

i. Ammonium chloride solution: Dissolve 1.15 g NH_4Cl in about 500 mL distilled 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, tap, or natural water for making 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 is begun within 2 h of collection, cold storage is unnecessary. If analysis is not started within 2 h of sample collection, keep sample at or below 4°C from the time of collection. Begin analysis within 6 h of collection; when this is not possible because the sampling site is distant from the laboratory, store at or below 4°C and report length and temperature of storage with the results. In no case start analysis more than 24 h after grab sample collection. When samples are to be used for regulatory purposes make every effort to deliver samples for analysis within 6 h of collection.

2) Composite samples—Keep samples at or below 4°C during compositing. Limit compositing period to 24 h. Use the same criteria as for storage of grab samples, starting the measurement of holding time from end of compositing period. State storage time and conditions as part of the results.

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 7.0 to 7.2 using a solution of sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) of such strength that the quantity of reagent does not dilute the sample by more than 0.5%. Exceptions may be justified with natural waters when the BOD is to be measured at in-situ pH values. The pH of dilution water 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. In some samples chlorine will dissipate within 1 to 2 h of standing in the light. This dissipation often occurs during sample transport and handling. For samples in which chlorine residual does not dissipate in a reasonably short time, destroy chlorine residual 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, 10 mL potassium iodide (KI) solution (10 g /100 mL) per 1000 mL sample and titrating with Na_2SO_3 solution to the starch-iodine end point for residual. Add to neutralized sample the proportional volume of Na_2SO_3 solution determined by the above test, mix, and after 10 to 20 min check sample for residual chlorine. (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, for example, plating wastes, contain toxic metals. Such samples often require special study and treatment.

4) Samples supersaturated with DO—Samples containing DO concentration above saturation at 20°C may be encountered in cold waters or in water where photosynthesis occurs. To prevent loss of oxygen during incubation of such samples, reduce DO to

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

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

5) Samples containing hydrogen peroxide—Hydrogen peroxide remaining in samples from some industrial bleaching processes such as 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 for sufficient time to allow the hydrogen peroxide to dissipate before setting up BOD tests. Check adequacy of peroxide removal by observing dissolved oxygen 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 the 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—distilled, tap, or receiving water. Make sure the water is free of heavy metals, specifically copper, and toxic substances, such as chlorine, that can interfere with BOD measurements. Protect source water quality by using clean glassware, tubing, and bottles. Deionized water often contains sufficient amounts of organics and microorganisms to cause failure of the dilution water quality control check (5210B.6*c*). Source water may be stored before use as long as the prepared dilution water (5210B.5*a*) meets quality control 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 cause deterioration in others. Storage of prepared dilution water (5210B.5*h*) for more than 24 h after adding nutrients, minerals, and buffer is not recommended unless dilution water blanks consistently meet quality control limits. Discard stored source water if the dilution water blank shows more than 0.20 mg/L DO depletion in 5 d (5210B.6*c*).

d. Preparation of seed suspension: It is necessary to have present in each BOD bottle a population of microorganisms capable of oxidizing the biodegradable organic matter in the sample. Domestic wastewater, unchlorinated or otherwise undisinfected effluents from biological wastewater treatment plants, and surface waters receiving wastewater discharges usually contain satisfactory microbial populations. Some samples (for example, some untreated industrial wastes, disinfected wastes, high-temperature wastes, wastes having pH values less than 6 or greater than 8, or wastes stored more than 6 h after collection) do not contain a sufficient microbial population. Seed such samples by adding a population of suitable microorganisms. The preferred seed is obtained from a biological treatment system processing the waste. 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 point of discharge. When effluent or mixed liquor from a biological treatment process is used as a seed source, inhibition of nitrification is recommended. Do not use seed from effluents that have been disinfected by chlorine or other means. Commercial seed sources may be used but are more likely to be unadapted to the wastewater constituents. Do not filter seed sources; filtering removes the seed microorganisms.

When acclimated seed sources are not available, 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 performance of the seed in BOD tests on the sample. BOD values that increase with time of 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 dissolved oxygen concentration is at least 7.5 mg/L before using water for BOD tests. If not, add DO by shaking bottle or by aerating 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 the dilution water blanks show a DO depletion greater than 0.20 mg/L, obtain a satisfactory water by improving purification or use water from another source. Do not add oxidizing agents or expose dilution water to ultraviolet light in attempts to bring the dilution blank into range.

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

c. Preparation of dilutions: Using the dilution water prepared as in ¶ *a* above, make at least three dilutions of prepared sample estimated to produce a residual DO of at least 1.0 mg/L and a DO uptake of at least 2.0 mg/L after a 5-d incubation. Five dilutions are recommended if experience with a particular sample does not produce at least three bottles having acceptable minimum DO depletions and residual limits (5210B.6*a*). 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 the DO technique and the number of replicates desired. Prepare dilutions in volumetric containers (Class A glass or equivalent) and then transfer to BOD bottles or prepare directly in BOD bottles. Either dilution method can be combined with any DO measurement technique.

1) Dilutions prepared in volumetric containers—Using a wide-tipped pipet, add desired amount of prepared sample to individual volumetric cylinders or flasks. Mix the sample well immediately before pipetting to avoid loss of solids by settling. For dilutions greater than 1:100 make a primary dilution before making final dilution in the bottle. Fill cylinders or flasks at least two-thirds full of dilution water 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 the cylinder or flask during transfer.

2) Dilutions prepared directly in BOD bottles—Using a wide-tip volumetric pipet, add the desired sample volume to individual BOD bottles. Fill each BOD bottle approximately two-thirds full with dilution water. Add appropriate amounts of seed suspension (¶ *d* below) and nitrification inhibitor (¶ *e* below) to the individual BOD bottles. When a bottle contains more than 67% of the 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–e*) 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 the dilution vessels or to individual BOD bottles before final dilution as described in ¶ *c* above. Do not add seed directly to wastewater samples if they contain materials that are toxic before dilution. 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 a suitable amount of microorganisms. Do not filter seed suspension before use. Agitate the 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 the seed added to each bottle generally should be between 0.6 and 1.0 mg/L, but 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 suspension is required to achieve 198 ± 30.5 mg/L BOD in the glucose-glutamic acid 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 nitrogen inhibition and the chemical used when reporting results. (NOTE: TCMP is the preferred nitrification inhibitor but requires handling and transfer in a solid form. Allylthiourea is not always effective in inhibiting nitrification within the 5-d incubation period and concentrations above 2 mg/L may cause increases in carbonaceous BOD measurements. ATU concentrations above 2 mg/L also can adversely affect the azide modification of the iodometric method). Seed all samples to which nitrification inhibitor has been added. The amount of seed should be consistent with that required to achieve GGA test results in the range of 198 ± 30.5 mg/L (5210B.6*b*).

1) Nitrification inhibition using 2-chloro-6-(trichloromethyl) pyridine (TCMP)—Add 10 mg TCMP/L to diluted sample or 3 mg TCMP to each 300-mL bottle or sample dilution vessel, or proportional amounts to other sized bottles, after initial sample dilution but before final filling of the 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 the sample if not mixed well). Some commercial TCMP formulations are not 100% TCMP; adjust dosage appropriately.

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

f. Sealing of bottles: Complete filling of each bottle by adding enough dilution water that insertion of the stopper leaves no bubbles in the bottle. Mix the sample by turning the bottle manually several times unless a DO probe having a stirrer is used immediately 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 the water seal during incubation.

g. Determination of initial DO: Use the azide modification of the iodometric method (Section 4500-O.C) or the membrane electrode method (Section 4500-O.G) to determine initial DO on all sample dilutions, dilution water blanks, and, where appropriate, seed controls. Replace any displaced contents with sufficient 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 the membrane electrode method is used, take care to eliminate drift in calibration between initial and final DO readings. If the azide modification of the titrimetric iodometric method is used, prepare an extra bottle for initial DO determination for each sample dilution.

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

i. Determination of final DO: After 5 d ± 6 h of incubation, determine DO in all sample dilutions, and in all blanks and checks as in ¶ 6*b–d*, using the azide modification of the titrimetric method or the membrane electrode method.

6. Quality Control Checks

The quality control 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, giving a minimum DO depletion of 2.0 mg/L and a residual DO of at least 1.0 mg/L after 5 d of incubation are considered to produce valid data, because at least 2.0 mg oxygen/uptake L is required to give a meaningful measure of oxygen uptake and at least 1.0 mg/L must remain throughout the test to ensure that insufficient DO does not affect the rate of oxidation of waste constituents. Exceptions occur for reporting purposes only when the depletions for tests using undiluted samples in all bottles fall below 2.0 mg/L and when the residual DO in all dilutions is less than 1.0 mg/L (5210B.7). When using membrane electrodes for measuring DO, make frequent calibration checks to ensure accurate DO readings (Section 4500-0.G.3*a*).

b. Glucose-glutamic acid check: The glucose-glutamic acid check is the primary basis for establishing accuracy and precision of the BOD test and is the principal measure of seed quality and set-up procedure. Together with each batch of 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-glutamic acid solution (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 glucose-glutamic acid concentrations; adjust doses accordingly. Add nitrification inhibitor if seed is obtained from a source that is nitrifying. Evaluate data as described in 5210B.8, Precision and Bias. The resulting average BOD for the three bottles, after correction for dilution and seeding, must fall into the range of 198 ± 30.5 mg/L. If the average value falls outside this range, evaluate the cause and make appropriate corrections. Consistently high values can indicate the use of too much seed suspension, contaminated dilution water, or the occurrence of nitrification; consistently low values can indicate poor seed quality or quantity or the presence of a toxic material. If low values persist, prepare a new mixture of glucose and glutamic acid and check the sources of dilution water and source of seed.

c. Dilution water quality check: With each batch of samples incubate one or more bottles of dilution water that contains nutrient, mineral, and buffer solutions but no seed or nitrification inhibitor. This dilution water blank serves as a check on quality of unseeded dilution water and cleanliness of incubation bottles. Determine initial and final DO as in 5210B.5*g* and *i*. The DO uptake in 5 d must not be more than 0.20 mg/L and preferably not more than 0.10 mg/L, before making seed corrections. If the dilution water blank exceeds 0.20 mg/L, discard all data for tests using this dilution water or clearly identify such samples in data records.

d. Seed control: Determine BOD of the seed suspension as for any other sample. This is the *seed control.* Ideally, make three dilutions of seed such that the smallest quantity gives at least 2.0 mg/L DO depletion and the largest quantity results in at least 1.0 mg/L DO residual after 5 d of incubation. Determine the DO uptake per milliliter of seed added to each bottle using either the slope method or the ratio method. For the slope method, plot DO depletion in milligrams per liter versus milliliters of seed for all seed control bottles having a 2.0 mg/L depletion and 1.0 minimum residual DO. The plot should present a straight line for which the slope indicates DO depletion per milliliter of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.20 mg/L (see ¶ *c* above). For the ratio method, divide the DO depletion by the volume of seed in milliliters 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. Seed dilutions showing widely varying depletions per milliliter of seed (±30%) suggest the presence of toxic substances or large particulates in the seed suspension. In this case, check or change the seed source.

7. Data Analysis and Reporting

a. Calculations:

1) For each test bottle having 2.0 mg/L minimum DO depletion and at least 1.0 mg/L residual DO, 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 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.

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.

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

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

In the above calculations, do not make corrections for DO uptake by the dilution water blank during incubation. This correction is unnecessary if dilution water meets the blank criteria stipulated in 5210B.6*c*. If the dilution water does not meet these criteria, proper corrections are difficult; do not record results or, as a minimum, mark them as not meeting quality control criteria.

b. Reporting: Average the test results for all qualified bottles within each dilution series. Report the result as BOD_5 if nitrification is not inhibited. Report results as CBOD_5 if nitrification is inhibited. 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 the following quality control parameters is not met:

- Dilution water blank exceeds 0.20 mg/L (5210B.6*c*),
- Glucose-glutamic acid check falls outside acceptable limits (5210B.6*b*),
- 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.6*d*), or
- Minimum DO is less than 1.0 mg/L [5210B.7*a*3)].

8. Precision and Bias

There is no measurement for establishing bias of the BOD procedure. The glucose-glutamic acid check prescribed in 5210B.6*b* is intended to be a reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. Single-laboratory tests using a 300-mg/L mixed glucose-glutamic acid 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 glucose and glutamic acid in the total concentration range of 3.3 to 231 mg/L. The regression equations for mean value, X, and standard deviation, S, from these studies were:

$$X = 0.658 \text{ (added concentration, mg/L)} + 0.280 \text{ mg/L}$$

$$S = 0.100 \text{ (added concentration, mg/L)} + 0.547 \text{ mg/L}$$

For the 300-mg/L mixed primary standard, the average 5-d BOD would be 198 mg/L with a standard deviation of 30.5 mg/L. When nitrification inhibitors are used, GGA test results falling outside the 198 ± 30.5 control limit quite often indicate use of incorrect amounts of seed. Adjust amount of seed added to the GGA test to achieve results falling within this range.

a. Control limits: Because of many factors affecting BOD tests in multilaboratory studies and the resulting extreme variability in 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 control limits by performing a minimum of 25 glucose-glutamic acid checks (5210B.6*b*) over a period of several weeks or months and calculating the mean and standard deviation. Use the mean ±3 standard deviations as the control limit for future glucose-glutamic acid checks. Compare calculated control limits to the single-laboratory tests presented above and to interlaboratory results. If the glucose-glutamic acid test results are outside the range of 198 ± 30.5, re-evaluate the control limits and investigate source of the problem. If measured BOD for a glucose-glutamic acid check is outside the accepted control limit range, reject tests made with that seed and dilution water or identify such tests clearly in all data records and reports.

b. Working range and detection 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.

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

- The lower detection limit for unseeded samples that require dilution ($S = 0$; $P < 1.0$) is 2 mg/L multiplied by the dilution factor as established by the requirement for a minimum DO depletion of 2 mg/L.
- The lower limit for seeded samples that require dilution ($S > 0$; $P < 1.0$) is approximately 1 mg/L as established by the minimum depletion of 2.0 mg/L minus the maximum seed correction, which should be less than about 1 mg/L.
- 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).
- The lower detection limit for seeded samples that require no dilution ($S > 0$; $P = 1.0$) is 0 mg/L, as established by the difference between the sample DO depletion and the seed correction.

9. References

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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. The user should be familar 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] Dissolved oxygen (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 improved 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 with oxygen demands exceeding the DO available in air-saturated water. Therefore, it is necessary either to dilute the sample or to monitor DO frequently to ensure that low DO or anaerobic conditions do not occur. When DO concentrations approach 2 mg/L, the sample should be reaerated.

Because bacterial growth requires nutrients such as nitrogen, phosphorus, and trace metals, the necessary amounts may be added to the dilution water together with buffer to ensure that pH remains in a range suitable for bacterial growth and seed to

BOD_5 of Soda Lab Set-up

Equipment Needed:

1. Calibrated LDO probe/meter
2. Plastic tote to hold BOD bottles
3. 10 BOD bottles with stoppers and caps
4. 25 mL graduated cylinder
5. 100 (or 1,000) mL graduated cylinder
6. 1000 mL beaker
7. Stir plate with stir bar
8. Seed material (1 Polyseed capsule)
9. Dilution water
    - DI water was aerated overnight, then Hach BOD Nutrient Buffer Pillows (1 buffer pillow per 4 liters of water) were added and water was mixed well just prior to set-up.
10. 2 GGA (Glucose Glutamic Acid) standard ampules (EZGGA)
11. Litmus paper
12. Glass rod
13. 1N Sodium Hydroxide (NaOH)

To prepare the Seed, add one Polyseed capsule to 500 mL dilution water (do NOT use DI water). Add stir bar and stir for 1 hour.

To check the pH of our sample prior to set-up, use glass rod to wet the litmus paper. Add 1N NaOH as needed to bring the pH up to 6-8.

Summary of experiment:

We will be testing the BOD of soda. We expect the results to be high, therefore we are diluting the sample prior to analysis (a 1:100 dilution is created by adding 1 mL soda into a 100 mL volumetric flask and then filling up to the line with DI water). A total of 10 BOD bottles will be set up: 3 Sample dilutions, 2 Blanks, 2 GGA Standards, and 3 Seed Controls. The initial DO of all bottles will be read and recorded on a bench sheet, the samples will then be incubated in the dark at 20°C+/-1 for 5 days, the samples will be removed from the incubator, the final DO of all bottles will be read and recorded on the bench sheet, and finally the BOD results will be calculated.

Things to keep in mind:

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

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				GGA			BLANKS	
				DUP					
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

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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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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.

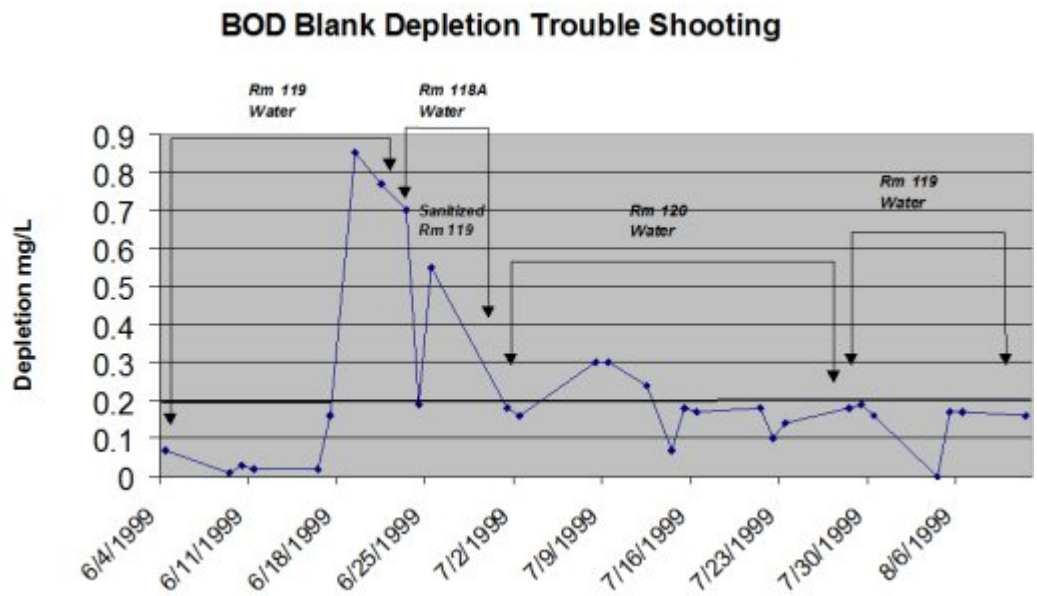
Activated charcoal in deionizer systems are also prone to contamination problems. They can quickly become contaminated with bacteria and mold, and can slough-off oxygen demanding material.

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		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				DUP	UP STREAM			DUP	DOWN STREAM			DUP	BLANKS	
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°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°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.

Source: http://www.polyseed.com/misc/bodtechreq.pdf

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.

Source: http://www.polyseed.com/misc/addhintstech.pdf

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.

Source: http://www.polyseed.com/misc/bodtech.pdf

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:

$$\frac{1500}{COD} = X \text{ (Sample Size in mL)}$$

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)

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2

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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.

3

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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)

4

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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

5

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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

6

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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

7

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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

8

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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

9

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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

10

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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

11

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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

12

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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

13

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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

14

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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

15

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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,

    COD, mg/L = $\frac{(\text{mg } O_2 \text{ in final vol.})(1000)}{\text{mL sample}}$

16

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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

17

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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)

18

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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

19

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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.

20

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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.

21

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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.

22

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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

23

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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

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**

3 to 150 mg/L COD (LR) **TNTplus™ 821/822**

20 to 1500 mg/L COD (HR)

Scope and application: For water, wastewater; digestion is required.

[1] COD ranges 3–150 mg/L and 20– 1500 mg/L COD are USEPA approved (5220 D) for wastewater analyses, Federal Register, April 21, 1980, 45(78), 26811-26812
[2] Jirka, A.M.; Carter, M.J., Analytical Chemistry, 1975, 47(8), 1397

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 applicable instruments that can use TNTplus vials.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for TNTplus vials

Instrument	Adapters	Light shield
DR 6000, DR 5000	—	—
DR 3900	—	LZV849
DR 3800, DR 2800	—	LZV646
DR 1900	9609900 or 9609800 (A)	—

Before starting

DR 3900, DR 3800, DR 2800: Install the light shield in Cell Compartment #2 before this test is started.
Review the safety information and the expiration date on the package.
The recommended temperature for samples and reagents is 15–25 °C (59–77 °F).
The recommended temperature for reagent storage is 15–25 °C (59–77 °F).
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.
Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Be prepared to wash spills with running water.
The reagents that are used in this test contain mercury. Collect the reacted samples for proper disposal.
Analyze reagent blanks as a quality check for accurate results at low concentrations or when the reagents were in storage for long periods of time. For the best results, analyze one blank (at minimum) per lot of reagents. Refer to Blanks for colorimetric determination on page 3. Run all tests (the samples and the blank) with the same lot of vials. The lot number is on the container label.
Keep unused (light sensitive) vials in a closed box.
Use the DRB reactor with 13-mm wells for the digestion. If the reactor has 16-mm wells, insert adapter sleeves into the wells.
DR 1900: Go to All Programs>LCK or TNTplus Methods>Options to select the TNTplus number for the test. Other instruments automatically select the method from the barcode on the vial.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
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.

Items to collect

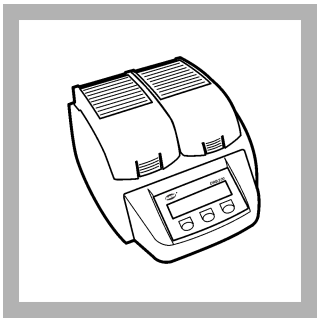
Description	Quantity
COD TNTplus™ Reagent Set, LR or HR	1
DRB200 reactor with 13-mm wells	1
Blender, 2-speed	1
Pipet, adjustable volume, 1.0–5.0 mL	1
Pipet tips, for 1.0–5.0 mL pipet	1
Test tube rack	1

Refer to Consumables and replacement items on page 5 for order information.

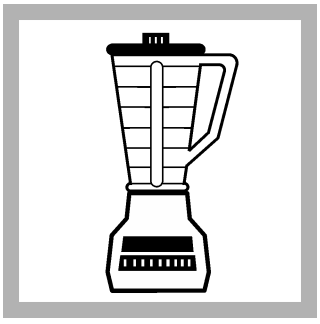
Sample collection and storage

- 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 (about 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at or below 6 °C (43 °F) for up to 28 days.
- Correct the test result for the dilution caused by the volume additions.

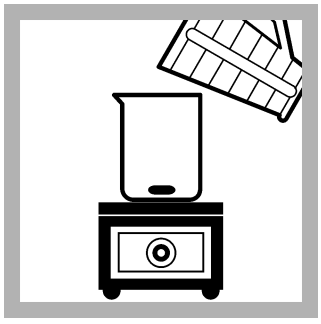
Test procedure



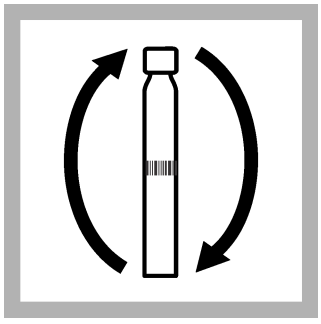
1. Set the DRB200 reactor power to on. Set the temperature to 150 °C.



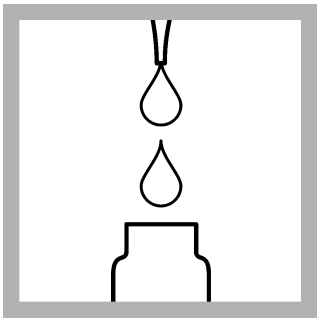
2. Measure 100 mL of sample in a blender. Blend for 30 seconds or until homogenized.
If the sample does not have suspended solids, ignore this step.



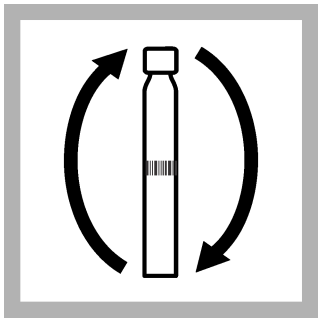
3. Pour the homogenized sample into a 250-mL beaker and stir slowly with a magnetic stir plate.
If the sample does not have suspended solids, ignore this step.



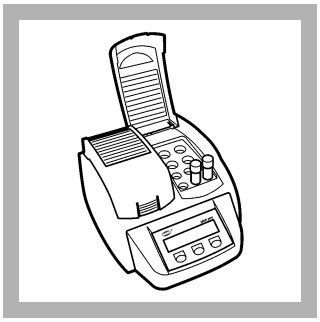
4. Invert a test vial several times to mix.



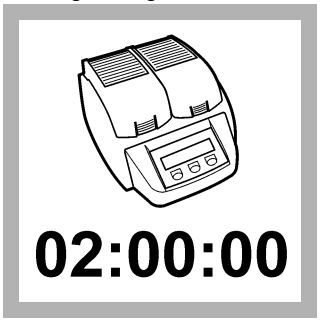
5. Use a pipet to add 2.0 mL of sample to the test vial.



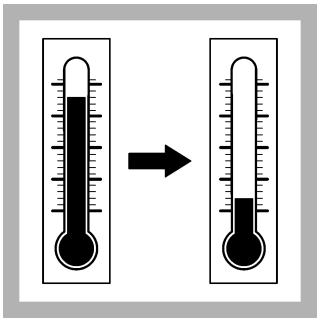
6. Hold the vial by the cap, over a sink. Invert gently several times to mix. **The vial gets very hot during mixing.**



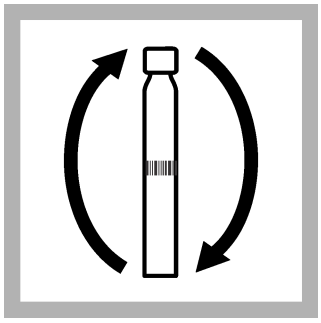
7. Insert the vial in the preheated DRB200 reactor. Close the lid.



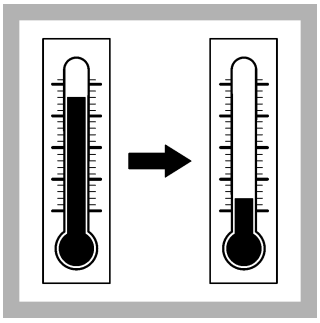
8. Keep the vial in the reactor for 2 hours.



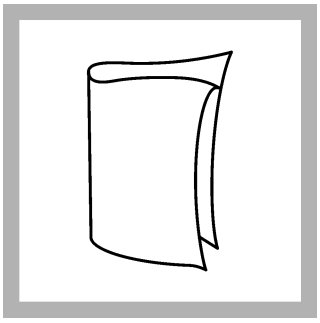
9. When the timer expires, set the reactor power to off. Let the temperature decrease for about 20 minutes to 120 °C or less.



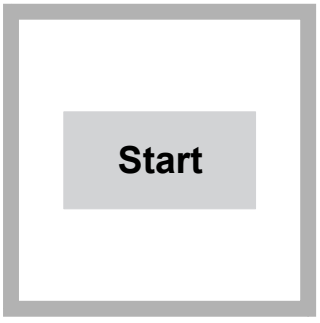
10. Hold the vial by the cap and invert gently several times while the vial is still hot.



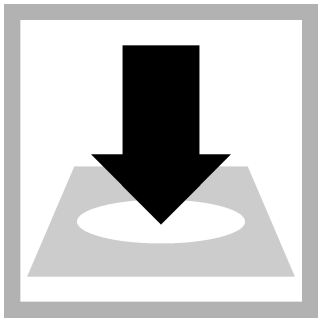
11. Put the vial in a test tube rack. Let the temperature of the vial decrease to room temperature.



12. Clean the vial.



13. DR 1900 only: Select program 821 (LR) or 822 (HR). Refer to Before starting on page 1.



14. Insert the vial into the cell holder. DR 1900 only: Push **READ**.
Results show in mg/L COD.

Blanks for colorimetric determination

Analyze reagent blanks as a quality check for accurate results at low concentrations or when the reagents were in storage for long periods of time. For the best results, analyze one blank (at minimum) per lot of reagents. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results automatically with the reagent blank adjust option. Use the blank again for other measurements with the same lot of vials. For storage, keep the blanks in a dark location. Monitor the decomposition of the blanks by periodically measuring its concentration. Measure the reagent blank value when a new lot of reagent is used.

To subtract the value of the blanks from a series of measurements:

1. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Clean the vial, then put it in the cell holder. Close the lid.
2. Set the reagent blank function to on. The measured value of the reagent blank is shown.

   Note: *As an alternative, record or enter the reagent blank value at a different time. Push the highlighted reagent blank box and use the keypad to enter the value.*

Interferences

Chloride is the primary interference in this test method and results in a positive interference. Each COD vial contains mercuric sulfate that will eliminate chloride interference to a maximum of 2000 mg/L Cl^-.

Accuracy check

Standard solution method—LR

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- COD Standard Solution, 1000-mg/L COD
- 100-mL volumetric flask, Class A
- 10.0-mL volumetric pipet, Class A and pipet filler safety bulb
- Deionized water

1. Prepare a 100-mg/L COD standard solution as follows:
   a. Use a pipet to add 10.0 mL of a 1000-mg/L COD standard solution into the volumetric flask.
   b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the prepared standard solution.
3. Compare the expected result to the actual result.

   Note: *The factory calibration can be adjusted slightly with the standard 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 slight variations in the reagents or instruments.*

Standard solution method—HR

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- COD Standard Solution, 300-mg/L, 800-mg/L or 1000-mg/L COD or Oxygen Demand Standard (contains 617-mg/L COD) or Wastewater Influent Standard Solution, Mixed Parameter (contains 500-mg/L COD)

1. Use the test procedure 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 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 slight variations in the reagents or instruments.*

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
barcode (TNTplus 821, LR)	75 mg/L COD	72–78 mg/L COD	—
barcode (TNTplus 822, HR)	750 mg/L COD	736–764 mg/L COD	—

Summary of Method

The mg/L COD results are defined as the mg of O_2 consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for 2 hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}). With this method, the amount of yellow Cr^{6+} that remains is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences. The measurement wavelength is 420 nm for the LR or 620 nm for the HR.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
COD TNTplus™ Reagent Set, LR, 3 to 150 mg/L COD	1–2 vials	25/pkg	TNT821
COD TNTplus™ Reagent Set, HR, 20 to 1500 mg/L COD	1–2 vials	25/pkg	TNT822

Required apparatus

Description	Quantity/test	Unit	Item no.
Blender, 2-speed, 120 VAC option	1	each	2616100
Blender, 2-speed, 240 VAC option	1	each	2616102
DRB 200 Reactor, 115 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB20001
DRB 200 Reactor, 230 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB20005
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Light shield, DR 3800, DR 2800, DR 2700	1	each	LZV646
Light shield, DR 3900	1	each	LZV849

Recommended standards

Description	Unit	Item no.
COD Standard Solution, 300-mg/L	200 mL	1218629
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
Wastewater Effluent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4^{3-}, COD, SO_4^{2-}, TOC	500 mL	2833249
Wastewater Influent Standard Solution, Mixed Parameter, for NH_3-N, NO_3-N, PO_4, COD, SO_4, TOC	500 mL	2833149

Optional reagents and apparatus

Description	Unit	Item no.
Flask, volumetric, Class A, 100-mL glass	each	1457442
Reactor adapter sleeves, 16 mm to 13 mm diameter, for TNTplus vials	5/pkg	2895805
Sampling bottle with cap, low density polyethylene, 500-mL	12/pkg	2087079
Sulfuric Acid, concentrated, ACS	500 mL	97949
Test tube rack, polyethylene, for 13-mm OD vials, 90 holes	each	2497900
Water, deionized	4 L	27256

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Section 5
Solids

Analysis of Solids in Wastewater

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)

Wastewater Solids Removal

- Most suspended solids are organic
- Oxygen Demand
- Serve as refuge for harmful bacteria
- Unsightly appearance

Solids Testing

- Why should I care about solids tests?
  - Assessment of compliance
  - Control of Biological and Physical treatment process
    - Poor data = Poor decisions

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

Standard Methods 2540B-G

- Sources of error
  - Sampling
  - Sub sampling
  - Measuring
  - Filter/Bowl preparation
  - Filter/Bowl handling
- Remedies
  - MIX WELL
  - Measure quickly

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

- 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, small filter
  - Avoid prolonged filtration times >5-7 min.
- 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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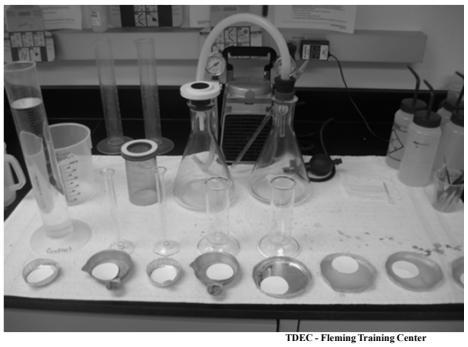
TSS SM2540 D – 2011
Dried at 103-105°C



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TSS SM2540 D – 2011
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
  - Record the initial weight of the filter & pan after drying
  - Repeat this cycle of drying, desiccating and weighing until a constant weight has been reached

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TSS SM2540 D – 2011
Dried at 103-105°C

- Sample Analysis:
  - Choose a sample volume to yield between 2.5 - 200 mg dried residue
  - 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
  - Filter sample

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TSS SM2540 D – 2011
Dried at 103-105°C

- Sample Analysis – continued:
  - 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
  - Weigh filter until a constant weight is obtained

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TSS SM2540 D – 2011
Dried at 103-105°C

- Fats, Oil, and Grease
  - May interfere due to difficulty in drying to a constant weight in time range.
- Duplicates should weigh within 5% of their average weight
- 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 – 2011
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 – 2011
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)

TDEC - Fleming Training Center 21

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**.

TDEC - Fleming Training Center 24

TSS – Portable and Inline

TDEC - Fleming Training Center 25

TSS SM2540 D – 2011 Dried at 103-105°C

- DOC
- LRB
- LFB
- Dup
- ICAL
- MDL *2017 Update*
- Corrective Action
- QC Acceptance
- Batch Size
- QC Frequency

TDEC - Fleming Training Center 26

TSS SM2540 D – 2011 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 an 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 – 2011 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
    - **2014 Update – 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 – 2011 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: $\frac{(A - B)\ (1000)}{\text{Vol. used}} = \frac{(10\ \text{mg})\ (1000)}{100\ \text{mL}} = 100\ \text{mg/L}$

*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 – 2011 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 technicians initials
    - Recertify reference weights as specified in the certificate of calibration or at least every 5 years.

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TSS SM2540 D – 2011
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 10% basis, one for every 10 samples
  - Calculate %RPD
    - Target value should be close to the first value and have a small RPD (less than 15%)
  - **2014 Update – For reporting purposes, average sample and duplicate.**

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TSS SM2540 D – 2011
Dried at 103-105°C

- **2014 Update** - Create and maintain control charts if you have 20-30 data points within 90 days.
  - If you do not meet the above criteria, follow QC Acceptance Criteria below.
    - Blanks < 2.5 mg/L
    - LFB ± 15%
    - RPD < 15%
- **2017 Update** – Method Detection Limit
  - MDL_b – Blanks only
    - MDL Calculator on FTC website

32

SS SM2540 F – 2011
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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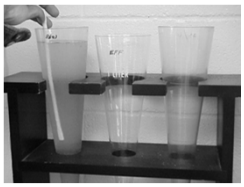
SS SM2540 F – 2011
Settleable Solids

Sample	Common Range, mL/L
Influent	8 – 20
Primary Effluent	0.1 – 3
Secondary Effluent	0.1 – 0.5

TDEC - Fleming Training Center 34

SS SM2540 F – 2011
Settleable Solids

- Dup
- Corrective Action
- Batch Size
- QC Frequency



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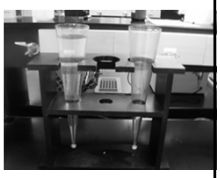
SS SM2540 F – 2011
Settleable Solids

- 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).
  - **2014 Update – For reporting purposes, average sample and duplicate.**



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SS SM2540 F – 2011 Settleable Solids

- **2014 Update** - 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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Any Questions?

TDEC - Fleming Training Center 41

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?

<u>Classroom portion:</u>

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?

The following is an excerpt from Standard Methods for the Examination of Water and Wastewater, 23rd edition and is to be used for instructional purposes only.

2540 SOLIDS*

2540 A. Introduction

Solids refer to matter suspended or dissolved in water or wastewater. Solids may affect water or effluent quality adversely 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. For these reasons, a limit of 500 mg dissolved solids/L is desirable for drinking waters. Highly mineralized waters also are unsuitable for many industrial applications. Waters high in suspended solids may be esthetically unsatisfactory for such purposes as bathing. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

1. Definitions

"Total solids" is the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids includes "total suspended solids," the portion of total solids retained by a filter, and "total dissolved solids," the portion that passes through the filter.

The type of filter holder, the pore size, porosity, area, and thickness of the filter and the physical nature, particle size, and amount of material deposited on the filter are the principal factors affecting separation of suspended from dissolved solids. "Dissolved solids" is the portion of solids that passes through a filter of 2.0 μm (or smaller) nominal pore size under specified conditions. "Suspended solids" is the portion retained on the filter.

"Fixed solids" is the term applied to the residue of total, suspended, or dissolved solids after heating to dryness for a specified time at a specified temperature. The weight loss on ignition is called "volatile solids." Determinations of fixed and volatile solids do not distinguish precisely between inorganic and organic matter because the loss on ignition is not confined to organic matter. It includes losses due to decomposition or volatilization of some mineral salts. Better characterization of organic matter can be made by such tests as total organic carbon (Section 5310), BOD (Section 5210), and COD (Section 5220).

"Settleable solids" is the term applied to the material settling out of suspension within a defined period. It may include floating material, depending on the technique (2540F.3*b*).

2. Sources of Error and Variability

Sampling, subsampling, and pipeting two-phase or three-phase samples may introduce serious errors. Make and keep such samples homogeneous during transfer. Use special handling to insure sample integrity when subsampling. Mix small samples with a magnetic stirrer. If suspended solids are present, pipet with wide-bore pipets. If part of a sample adheres to the sample container, consider this in evaluating and reporting results. Some samples dry with the formation of a crust that prevents water evaporation; special handling is required to deal with this. Avoid using a magnetic stirrer with samples containing magnetic particles.

The temperature at which the residue is dried has an important bearing on results, because weight losses due to volatilization of organic matter, mechanically occluded water, water of crystallization, and gases from heat-induced chemical decomposition, as well as weight gains due to oxidation, depend on temperature and time of heating. Each sample requires close attention to desiccation after drying. Minimize opening desiccator because moist air enters. Some samples may be stronger desiccants than those used in the desiccator and may take on water.

Residues dried at 103 to 105°C may retain not only water of crystallization but also some mechanically occluded water. Loss of CO_2 will result in conversion of bicarbonate to carbonate. Loss of organic matter by volatilization usually will be very slight. Because removal of occluded water is marginal at this temperature, attainment of constant weight may be very slow.

Residues dried at 180 ± 2°C will lose almost all mechanically occluded water. Some water of crystallization may remain, especially if sulfates are present. Organic matter may be lost by volatilization, but not completely destroyed. Loss of CO_2 results from conversion of bicarbonates 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°C yields values for dissolved solids closer to those obtained through summation of individually determined mineral species than the dissolved solids values secured through drying at the lower temperature.

To rinse filters and filtered solids and to clean labware use Type III water. Special samples may require a higher quality water; see Section 1080.

Results for residues high in oil or grease may be questionable because of the difficulty of drying to constant weight in a reasonable time.

To aid in quality assurance, analyze samples in duplicate. Dry samples to constant weight if possible. This entails multiple drying-cooling-weighing cycles for each determination.

Analyses performed for some special purposes may demand deviation from the stated procedures to include an unusual constituent with the measured solids. Whenever such variations of technique are introduced, record and present them with the results.

3. Sample Handling and Preservation

Use resistant-glass or plastic bottles, provided that the material in suspension does not adhere to container walls. Begin analysis as soon as possible because of the impracticality of preserving the sample. Refrigerate sample at 4°C up to the time of analysis to minimize microbiological decomposition of solids. Preferably do not hold samples more than 24 h. In no case hold sample more than 7 d. Bring samples to room temperature before analysis.

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* Approved by Standard Methods Committee, 1997. Editorial revisions, 2011. Joint Task Group: 20th Edition—Brannon H. Wilder (chair), Harold S. Costa, Christine M. Kosmowski, William E. Purcell.

4. Selection of Method

Methods B through F are suitable for the determination of solids in potable, surface, and saline waters, as well as domestic and industrial wastewaters in the range up to 20 000 mg/L.

Method G is suitable for the determination of solids in sediments, as well as solid and semisolid materials produced during water and wastewater treatment.

5. 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. Publ. 600/4-79-020, rev. Mar. 1983. Environmental Monitoring and Support Lab., U.S. Environmental Protection Agency, Cincinnati, Ohio.

2540 B. Total Solids Dried at 103–105°C

1. General Discussion

a. Principle: A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105°C. The increase in weight over that of the empty dish represents the total solids. The results may not represent the weight of actual dissolved and suspended solids in wastewater samples (see above).

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 the sample if it is determined that their inclusion is not desired in the final result. Disperse visible floating oil and grease with a blender before withdrawing a sample portion for analysis. Because excessive residue in the dish may form a water-trapping crust, limit sample to no more than 200 mg residue (see 2540A.2).

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

2. Apparatus

a. Evaporating dishes: Dishes of 100-mL capacity made of one of the following materials:

1) Porcelain, 90-mm diam.
2) Platinum—Generally satisfactory for all purposes.
3) High-silica glass.*

b. Muffle furnace for operation at 550°C.

c. Steam bath.

d. Desiccator, provided with a desiccant containing a color indicator of moisture concentration or an instrumental indicator.

e. Drying oven, for operation at 103 to 105°C.

f. Analytical balance, capable of weighing to 0.1 mg.

g. Magnetic stirrer with TFE stirring bar.

h. Wide-bore pipets.†

i. Graduated cylinder.

j. Low-form beaker.‡

3. Procedure

a. Preparation of evaporating dish: If volatile solids are to be measured ignite clean evaporating dish at 550°C for 1 h in a muffle furnace. If only total solids are to be measured, heat clean dish to 103 to 105°C for 1 h. Store and cool dish in desiccator until needed. Weigh immediately before use.

b. Sample analysis: Choose a sample volume that will yield a residue between 2.5 and 200 mg. Pipet a measured volume of well-mixed sample, during mixing, to a preweighed dish. For homogeneous samples, pipet from the approximate midpoint of the container but not in the vortex. Choose a point both middepth and midway between wall and vortex. Evaporate to dryness on a steam bath or in a drying oven. Stir sample with a magnetic stirrer during transfer. If necessary, add successive sample portions to the same dish after evaporation. When evaporating in a drying oven, lower temperature to approximately 2°C below boiling to prevent splattering. Dry evaporated sample for at least 1 h in an oven at 103 to 105°C, cool dish in desiccator to balance temperature, and weigh. Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. When weighing dried sample, be alert to change in weight due to air exposure and/or sample degradation. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

4. Calculation

$$\text{mg total solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = 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.
† Kimble Nos. 37005 or 37034B, or equivalent.
‡ Class B or better.

2540 C. Total Dissolved Solids Dried at 180°C

1. General Discussion

a. Principle: A well-mixed sample is filtered through a standard glass fiber filter, and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180°C. The increase in dish weight represents the total dissolved solids. This procedure may be used for drying at other temperatures.

The results may not agree with the theoretical value for solids calculated from chemical analysis of sample (see above). Approximate methods for correlating chemical analysis with dissolved solids are available.[1] The filtrate from the total suspended solids determination (2540D) may be used for determination of total dissolved solids.

b. Interferences: See 2540A.2 and 2540B.1*b*. Highly mineralized waters with a considerable calcium, magnesium, chloride, and/or sulfate content may be hygroscopic and require prolonged drying, proper desiccation, and rapid weighing. Samples high in bicarbonate require careful and possibly prolonged drying at 180°C to insure complete conversion of bicarbonate to carbonate. Because excessive residue in the dish may form a water-trapping crust, limit sample to no more than 200 mg residue.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

2. Apparatus

Apparatus listed in 2540B.2*a–h* is required, and in addition:

*a. Glass-fiber filter disks** without organic binder.

b. Filtration apparatus: One of the following, suitable for the filter disk selected:

1) *Membrane filter funnel.*

2) *Gooch crucible,* 25-mL 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, of sufficient capacity for sample size selected.

d. Drying oven, for operation at 180 ± 2°C.

3. Procedure

a. Preparation of glass-fiber filter disk: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up into filtration apparatus. Apply vacuum and wash disk with three successive 20-mL volumes of reagent-grade water. Continue suction to remove all traces of water. Discard washings.

b. Preparation of evaporating dish: If volatile solids are to be measured, ignite cleaned evaporating dish at 550°C for 1 h in a muffle furnace. If only total dissolved solids are to be measured, heat clean dish to 180 ± 2°C for 1 h in an oven. Store in desiccator until needed. Weigh immediately before use.

c. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If more than 10 min are required to complete filtration, increase filter size or decrease sample volume.

d. Sample analysis: Stir sample with a magnetic stirrer and pipet a measured volume onto a glass-fiber filter with applied vacuum. Wash with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Transfer total filtrate (with washings) to a weighed evaporating dish and evaporate to dryness on a steam bath or in a drying oven. If necessary, add successive portions to the same dish after evaporation. Dry evaporated sample for at least 1 h in an oven at 180 ± 2°C, cool in a desiccator to balance temperature, and weigh. Repeat drying cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. If volatile solids are to be determined, follow procedure in 2540E.

4. Calculation

$$\text{mg total dissolved solids/L} = \frac{(A - B) \times 1000}{\text{sample volume, mL}}$$

where:

A = 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. U.S. Geological Surv., Washington, D.C.

* Whatman grade 934AH; Gelman type A/E; Millipore type AP40; E-D Scientific Specialties grade 161; Environmental Express Pro Weigh; or other products that give demonstrably equivalent results. Practical filter diameters are 2.2 to 12.5 cm.
† Gelman No. 4201 or equivalent.

2540 D. Total Suspended Solids Dried at 103–105°C

1. General Discussion

a. Principle: A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume. To obtain an estimate of total suspended solids, calculate the difference between total dissolved solids and total solids.

b. Interferences: See 2540A.2 and 2540B.1*b*. Exclude large floating particles or submerged agglomerates of nonhomogeneous materials from the sample if it is determined that their inclusion is not representative. Because excessive residue on the filter may form a water-entrapping crust, limit the sample size to that yielding no more than 200 mg residue. For samples high in dissolved solids thoroughly wash the filter to ensure removal of dissolved material. Prolonged filtration times resulting from filter clogging may produce high results owing to increased colloidal materials captured on the clogged filter.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

2. Apparatus

Apparatus listed in 2540B.2 and C.2 is required, except for evaporating dishes, steam bath, and 180°C drying oven. In addition:

Aluminum weighing dishes.

3. Procedure

a. Preparation of glass-fiber filter disk: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water, turn vacuum off, and discard washings. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish. If a Gooch crucible is used, remove crucible and filter combination. Dry in an oven at 103 to 105°C for 1 h. If volatile solids are to be measured, ignite at 550°C for 15 min in a muffle furnace. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less. Store in desiccator until needed.

b. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 min, increase filter diameter or decrease sample volume.

c. Sample analysis: Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of reagent-grade water to seat it. Stir sample with a magnetic stirrer at a speed to shear larger particles, if practical, to obtain a more uniform (preferably homogeneous) particle size. Centrifugal force may separate particles by size and density, resulting in poor precision when point of sample withdrawal is varied. While stirring, pipet a measured volume onto the seated glass-fiber filter. For homogeneous samples, pipet from the approximate midpoint of container but not in vortex. Choose a point both middepth and midway between wall and vortex. Wash filter with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Samples with high dissolved solids may require additional washings. Carefully remove filter from filtration apparatus and transfer to an aluminum weighing dish as a support. Alternatively, remove the crucible and filter combination from the crucible adapter if a Gooch crucible is used. Dry for at least 1 h at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing 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. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. If volatile solids are to be determined, 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 = 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.

Chanin, G., E.H. Chow, R.B. Alexander & J. Powers. 1958. Use of glass fiber filter medium in the suspended solids determination. *Sewage Ind. Wastes* 30:1062.

Nusbaum, I. 1958. New method for determination of suspended solids. *Sewage Ind. Wastes* 30:1066.

Smith, A.L. & A.E. Greenberg. 1963. Evaluation of methods for determining suspended solids in wastewater. *J. Water Pollut. Control Fed.* 35:940.

WYCKOFF, B.M. 1964. Rapid solids determination using glass fiber filters. *Water Sewage Works* 111:277.

NATIONAL COUNCIL OF THE PAPER INDUSTRY FOR AIR AND STREAM IMPROVEMENT. 1975. A Preliminary Review of Analytical Methods for the Determination of Suspended Solids in Paper Industry Effluents for Compliance with EPA-NPDES Permit Terms. Spec. Rep. No. 75-01. National Council of the Paper Industry for Air & Stream Improvement, New York, N.Y.

NATIONAL COUNCIL OF THE PAPER INDUSTRY FOR AIR AND STREAM IMPROVEMENT. 1977. A Study of the Effect of Alternate Procedures on Effluent Suspended Solids Measurement. Stream Improvement Tech. Bull. No. 291, National Council of the Paper Industry for Air & Stream Improvement, New York, N.Y.

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 Method B, C, or D is ignited to constant weight at 550°C. The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

b. Interferences: Negative errors in the volatile solids may be produced by loss of volatile matter during drying. Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subject to considerable error. In such cases, measure for suspect volatile components by another test, for example, total organic carbon (Section 5310). Highly alkaline residues may react with silica in sample or silica-containing crucibles.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Tables 2020:I and II.

2. Apparatus

See Sections 2540B.2, C.2, and D.2.

3. Procedure

Ignite residue produced by Method 2540B, C, or D to constant weight in a muffle furnace at a temperature of 550°C. Ignite a blank glass fiber filter along with samples. Have furnace up to temperature before inserting sample. Usually, 15 to 20 min ignition are required for 200 mg residue. However, more than one sample and/or heavier residues may overtax the furnace and necessitate longer ignition times. Let dish or filter disk cool partially in air until most of the heat has been dissipated. Transfer to a desiccator for final cooling in a dry atmosphere. Do not overload desiccator. Weigh dish or disk as soon as it has cooled to balance temperature. Repeat cycle of igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight. Weight loss of the blank filter is an indication of unsuitability of a particular brand or type of filter for this analysis.

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 = weight of residue + dish before ignition, mg,
B = 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

Settleable solids in surface and saline waters as well as domestic and industrial wastes may be determined and reported on either a volume (mL/L) or a weight (mg/L) basis.

The QC practices considered to be an integral part of each method are summarized in Table 2020:II.

2. Apparatus

The volumetric test requires only an Imhoff cone. The gravimetric test requires all the apparatus listed in 2540D.2 and a glass vessel with a minimum diameter of 9 cm.

3. Procedure

a. Volumetric: Fill an Imhoff cone to the 1-L mark with a well-mixed sample. Settle for 45 min, gently agitate sample near the sides of the cone with a rod or by spinning, settle 15 min longer, and record volume of settleable solids in the cone as milliliters per liter. If the settled matter contains pockets of liquid between large settled particles, estimate volume of these and subtract from volume of settled solids. The practical lower limit of measurement depends on sample composition and generally is in the range of 0.1 to 1.0 mL/L. Where a separation of settleable and floating materials occurs, do not estimate the floating material as settleable matter. Replicates usually are not required.

Where biological or chemical floc is present, the gravimetric method (3*b*) is preferred.

b. Gravimetric:

1) Determine total suspended solids as in 2540D.

2) Pour a well-mixed sample into a glass vessel of not less than 9 cm diam using not less than 1 L and sufficient sample to give a depth of 20 cm. Alternatively use a glass vessel of greater diameter and a larger volume of sample. Let stand quiescent for 1 h and, without disturbing the settled or floating material, siphon 250 mL from center of container at a point halfway between the surface of the settled material and the liquid surface. Determine total suspended solids (milligrams per liter) of this supernatant liquor (2540D). These are the nonsettleable solids.

4. Calculation

$$\text{mg settleable solids/L} = \text{mg total suspended solids/L} - \text{mg nonsettleable solids/L}$$

5. Precision and Bias

Precision and bias data are not now available.

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 is applicable to the determination of total solids and its fixed and volatile fractions in such solid and semisolid 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 true also for wastewater, the effect tends to be more pronounced with sediments, and especially with 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. Make all weighings quickly because wet samples tend to lose weight by evaporation. After drying or ignition, residues often are very hygroscopic and rapidly absorb moisture from the air. Highly alkaline residues may react with silica in the samples or silica-containing crucibles.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 2020:II.

2. Apparatus

All the apparatus listed in 2540B.2 is required except that a magnetic stirrer and pipets are not used and a balance capable of weighing to 10 mg may be used.

3. Procedure

a. Total solids:

1) Preparation of evaporating dish—If volatile solids are to be measured, ignite a clean evaporating dish at 550°C for 1 h in a muffle furnace. If only total solids are to be measured, heat dish at 103 to 105°C for 1 h in an oven. Cool in desiccator, weigh, and store in desiccator until ready for use.

2) Sample analysis

a) Fluid samples—If the sample contains enough moisture to flow more or less readily, stir to homogenize, place 25 to 50 g in a prepared evaporating dish, and weigh. Evaporate to dryness on a water bath, dry at 103 to 105°C for 1 h, cool to balance temperature in an individual desiccator containing fresh desiccant, and weigh. Repeat heating, cooling, desiccating, and weighing procedure until the weight change is less than 4% or 50 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

b) Solid samples—If the sample consists of discrete pieces of solid material (dewatered sludge, for example), take cores from each piece with a No. 7 cork borer or pulverize the entire sample coarsely on a clean surface by hand, using rubber gloves. Place 25 to 50 g in a prepared evaporating dish and weigh. Place in an oven at 103 to 105°C overnight. Cool to balance temperature in a desiccator and weigh. Repeat drying (1 h), cooling, weighing, and desiccating steps until weight change is less than 4% or 50 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

b. Fixed and volatile solids: Transfer the dried residue from ¶(2)a) above to a cool muffle furnace, heat furnace to 550°C, and ignite for 1 h. (If the residue contains large amounts of organic

matter, first ignite it over a gas burner and under an exhaust hood in the presence of adequate air to lessen losses due to reducing conditions and to avoid odors in the laboratory.) Cool in desiccator to balance temperature and weigh. Repeat igniting (30 min), cooling, desiccating and weighing steps until the weight change is less than 4% or 50 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

4. Calculation

$$\% \text{ total solids} = \frac{(A - B) \times 100}{C - B}$$

$$\% \text{ volatile solids} = \frac{(A - D) \times 100}{A - B}$$

$$\% \text{ fixed solids} = \frac{(D - B) \times 100}{A - B}$$

where:

A = weight of dried residue + dish, mg,
B = weight of dish,
C = weight of wet sample + dish, mg, and
D = weight of residue + dish after ignition, mg.

5. Precision and Bias

Precision and bias data are not now available.

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.

TSS Dried at 103-105°C Standard Methods Exercise

Total _____ Solids
SM ____ D-2011

1. A sample that is _____ is filtered through a weighed standard glass-fiber filter.
2. The residue retained on the filter is ____ to a constant weight at ___ to ___°C.
3. Exclude large floating _____ or submerged agglomerates of nonhomogeneous _____ from the sample if their inclusion is not _____.
4. Because _____ on the filter may form a water-_____ crust, limit the _____ to that yielding no more than _____ residue.
5. For samples ____ in _____ thoroughly ____ the filter to ensure removal of dissolved material.
6. *Preparation of _____ filter disk:*
7. If _____ glass fiber filter disks are used, eliminate this step.
8. Insert disk with _____ up in filtration apparatus.
9. Apply vacuum and wash disk with _____portions of _____ water.
10. Continue suction to remove _____ of water, turn vacuum off, and _____ washings.
11. Remove filter from _____ apparatus and transfer to an _____ weighing dish.
12. Dry in an oven at 103 to 105°C for ____.
13. If _____ solids are to be measure, ignite at _____ for 15 min in a _____.
14. Cool in _____ to balance temperature and _____.
15. _____ cycle of _____ or igniting, _____, _____, and _____ until a _____ weight is obtained or until weight change is _____ of the previous weighing or _____, whichever is ____.
16. Store in _____ until needed.
17. *Selection of filter and _____:*
18. Choose sample _____ to yield between 2.5 and 200 __ dried residue.
19. If volume filtered fails to meet _____ yield, _____ sample volume up to ___.
20. If _____ takes more than _____. increase filter diameter or _____ sample volume.
21. *Sample _____:*
22. Assemble filtering apparatus and filter and being _____.
23. ___ filter with a small _____ of _____ water to ____ it.

24. Stir sample with magnetic stirrer at a speed to shear larger particles, _____, to obtain a more uniform (_____) particle size.
25. _____ force may separate particles by _____, resulting in _____ when point of sample withdrawal is varied.
26. While _____, pipet a measured volume onto the seated glass-fiber filter.
27. For homogeneous samples, pipet from approximate _____ of container but ___ in _____.
28. Choose a point both _____ and _____ between wall and vortex.
29. Wash filter with _____ successive _____ volumes of reagent-grade water, allow _____ drainage between washings, and _____ suction for about ____ after filtration is _____.
30. Samples with _____ dissolved solids may require additional _____.
31. Carefully remove filter from filtration apparatus and _____ to an aluminum weighing dish as a _____.
32. Dry for _____ at 103 to 105°C in an oven, cool in a desiccator to balance temperature, and weight.
33. _____ the <u>cycle</u> of drying, cooling, desiccating, and weight until a constant weight is obtained or until the weight change is less than ____ of the previous weight or _____ whichever is less.
34. Analyze _____ of all samples in _____.
35. _____ determinations should agree within ___ of their _____ weight.

2540 D. Total Suspended Solids Dried at 103-105°

Reference: Standard Method 2540 D., 22nd Edition, 1997, Editorial revisions, 2011.

Definitions:

"Suspended solids" is the portion retained on the filter after filtration.

Principles:

A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103-105°C. The increase in weight of the filter represents the total suspended solids.

Supplies Needed:

- Glass fiber filters, 47 mm
- Aluminum weighing dishes
- Marker
- Vacuum flask
- Vacuum pump
- Membrane filter funnel/filtration apparatus
- DI water
- Tweezers
- 100 mL graduated cylinder
- 10 mL graduated cylinder
- Drying oven, set to 103-105°C
- Analytical balance capable of weighing to 0.1 mg
- Desiccator, with indicating desiccant
- Lab Fortified Blank (LFB), aka Standard
- Timer
- Sample

Step 1 - Preparation of glass-fiber filter disk:

1. Pre-label aluminum dishes (Ex: Method Blank, Standard, Sample, Sample Dup)
2. Place a glass fiber filter, wrinkled side up, in filtration apparatus.
3. Turn on vacuum pump.
4. Wash the filter with 3 successive 20 mL portions of DI water. Continue suction to remove all traces of water.
5. Turn off vacuum pump.
6. Remove filter from filtration apparatus and transfer to aluminum weighing dish. *Note: only use tweezers to touch filters from this point on.
7. Place in 103-105°C oven for 1 hour.

8. Remove from oven, place in desiccator. Cool in desiccator to balance temperature (allow to come to room temperature).
9. Remove from desiccator, remove filter from aluminum weighing dish, and weigh the filter using an analytical balance.
10. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained; or until the weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.
11. Store filters (in aluminum weighing dish) in desiccator until needed.

Step 2 – Sample Analysis:

Note: Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 minutes, increase filter diameter or decrease sample volume.

1. Assemble filtering apparatus and vacuum pump set-up.
2. Place a prepared and pre-weighed filter on the filtration apparatus.
3. Turn on vacuum.
4. Wet filter with a small amount of DI water to seat the filter.
5. Thoroughly mix sample to obtain a representative portion. Use graduated cylinder to measure sample portion. Add the measured volume of sample to filtering apparatus and allow to filter through.
   To obtain homogenous particle size: Stir sample with magnetic stirrer at a speed to shear larger particles. While stirring, pipet a measured volume onto the seated glass fiber filter. For homogenous samples, pipet from the approximate midpoint of beaker, but not in vortex.
6. Rinse the graduated cylinder with 3 successive 10 mL volumes of DI water, adding each to the filtering apparatus, and allowing complete drainage between washings. Continue suction for about 3 minutes after filtration is complete.
7. Carefully remove filter (using tweezers) and transfer to aluminum weighing dish as a support.
8. Dry in an oven at 103-105°C for 1 hour.
9. Remove from oven, place in desiccator. Cool in desiccator to balance temperature (allow to come to room temperature).
10. Remove from desiccator, remove filter from aluminum weighing dish, and weigh the filter using an analytical balance.
11. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained; or until the weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.

QA/QC:

*Note: the following includes the “Real people language” from the Total Suspended Solids Guidance Document listed on the Fleming Training Center website. Please see actual document for detailed list of QA/QC requirements.

1. Initial Demonstration of Capability (DOC)

- Each analyst/operator needs to analyze 4 samples of a TSS standard.
- Keep a folder for each analyst with signed documentation that the analyst has read and understands all appropriate SOPs and methods.
- Recommended that back-up analyst do this once a year.

2. Initial Calibration Verification (ICV)
   - Check balance daily (day of analysis) with at least 2 working weights that bracket the normal usage range and record results on bench sheet or separate log book.

3. Method Blank
   - On a 5% basis, filter 100mL distilled water through prepared filter. (Should be less than 2.5 mg/L)

4. Laboratory Fortified Blank (LFB), a.k.a. Standard
   - Analyze TSS Standard sample that can be prepared from recipe (see Total Suspended Solids Guidance Document on Fleming Training Center website for recipe) or bought premade.
   - Run on a 5% basis.

5. Duplicate
   - Analyze 2 samples for TSS (Ex: filter 100mL of effluent through first filter, then filter another 100mL of effluent through second filter. Dry, cool, weigh. Figure RPD for both samples.)
   - Target value should be close to the first value and have a small RPD (less than %)
   - Analyze at least 10% of all samples in duplicate.

6. QC Acceptance Criteria
   - Blanks < 2.5mg/L
   - LFB +/- 15%
   - RPD +/- 15%

7. Method Detection Limit
   - Annual verification using data collected within the past 24 months. Include all data from Ongoing Data Collections and the initial MDL where appropriate (<24 months old). A minimum of 7 data points are required for both MLD_S and method blanks. However, **for TSS only the method blanks** are required to determine the MDL.
   - Ongoing Method Blank Data Collection – population should include all routine method blanks analyzed with each batch during the course of sample analysis
   - Refer to MDL guidance for complete requirements.

Batch Size: Influent and Effluent are 2 different samples. See Total Suspended Solids Guidance Document on Fleming Training Center Website for more information.

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

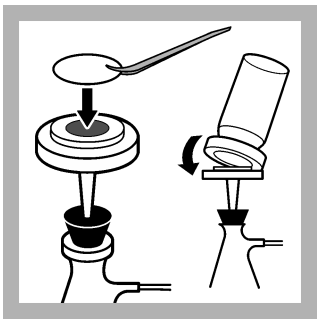
Description	Quantity
Analytical balance	1
Cylinder, graduated, 100 mL	1
Desiccator with desiccant	1
Drying oven	1
Filter flask	1
Filter holder	1
Filter, 47-mm	1
Furnace, muffle	1
Rubber policeman for 3/16 in. rod (user-supplied)	1
Tongs	1
Tweezers	1
Watch glass	1
Watch glass	1
Water, deionized	varies

Refer to Consumables and replacement items on page 5 for order information.

Sample collection and storage

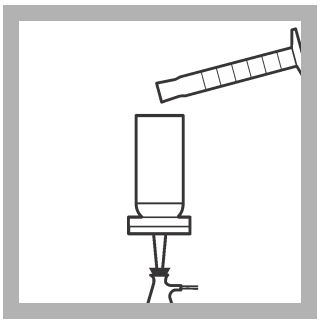
- Collect samples in clean glass or plastic bottles.
- To preserve samples for later analysis, keep the samples at or below 6 °C (43 °F) for up to 7 days.
- Let the sample temperature increase to room temperature before analysis.

Test procedure—Total Non-filterable Solids, Method 8158

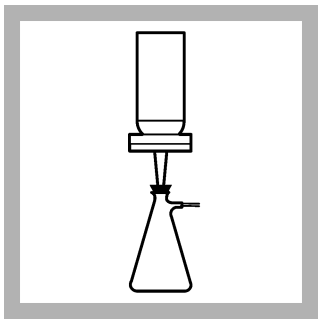


1. Use tweezers to put a fiber filter disc in the filter holder.

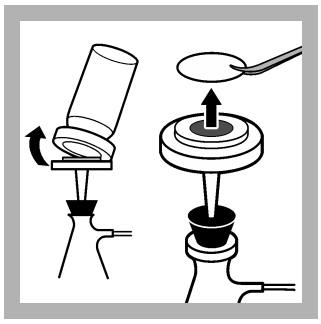
Put the filter holder assembly in the filtering flask.



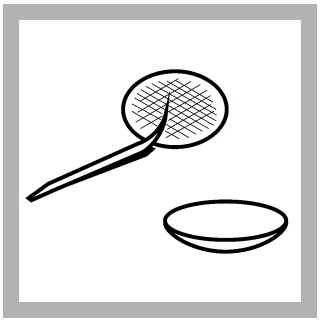
2. Use a graduated cylinder to add 100 mL of deionized water to the filtering flask.



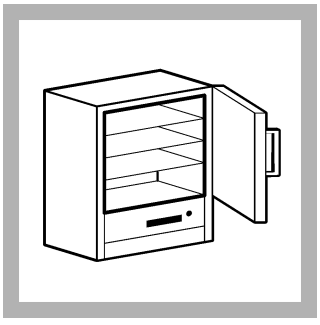
3. Apply vacuum to the flask until all of the water is pulled through the filter.



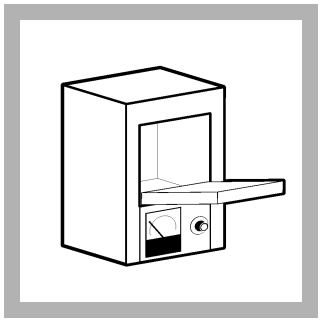
4. Slowly release the vacuum from the filtering system. Remove the fiber filter disc from the filter holder.



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.

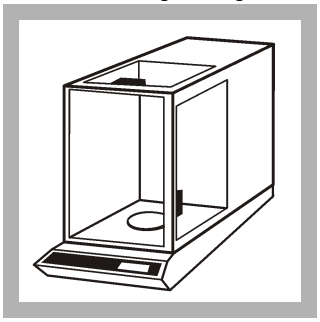


7. If Volatile Non-filterable Solids are also measured, use tongs to put the watch glass with the fiber filter disc into a preheated muffle furnace at 550 °C (1022 °F) for 15 minutes. Discard this step if Volatile Non-filterable Solids are not measured.

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.*

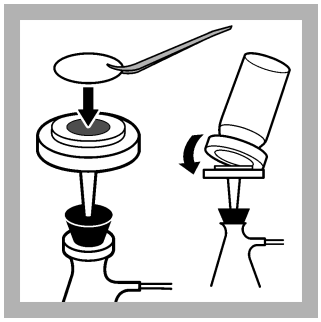
8. Use metal tongs to remove the watch glass with the fiber filter disc from the drying oven or muffle furnace and put in a desiccator. Immediately cover the desiccator. Do not seal the desiccator until the watch glass temperature has decreased a little, because pressure from the hot air inside can push the cover off.

Let the fiber filter disc and watch glass temperature decrease to room temperature.

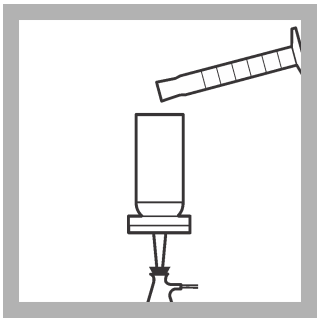


9. Remove the watch glass with the fiber filter disc from the desiccator and put it adjacent to the analytical balance.

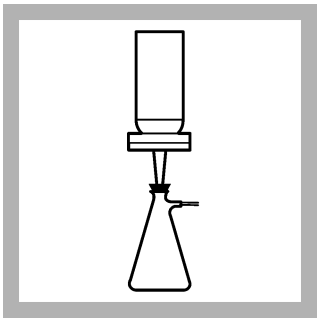
Use tweezers to remove the fiber filter disc from the watch glass. Weigh the fiber filter disc to the nearest 0.1 mg (0.0001 g). Record this mg value as B.



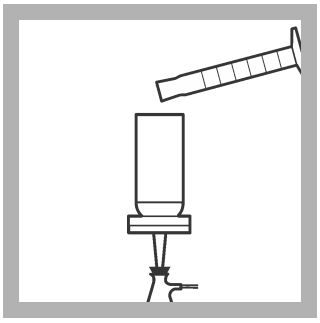
10. Put the fiber filter disc in the filter holder/filtering system again. Use deionized water to bond the fiber filter disc to the filter holder.



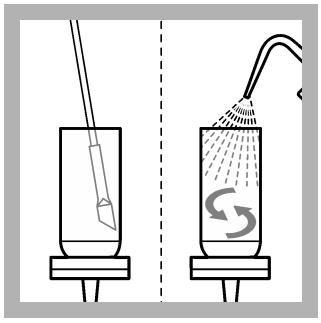
11. Use a graduated cylinder to add 100 mL (or more, if the solids content is low) of well-mixed, representative water sample.



12. Apply vacuum to the flask until all of the water is pulled through the filter.



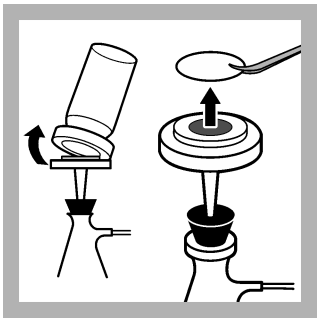
13. Add three different 10-mL aliquots of deionized water. Wait until each aliquot is pulled through the filter before the next one is added.



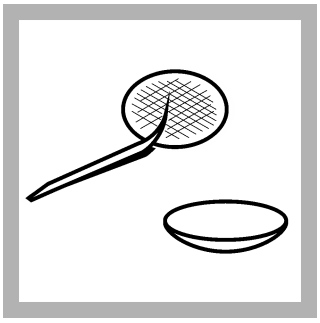
14. Move all of the remaining material that stays on the sides or bottom lip of the filter holder on the filter.

Use a rubber policeman on the end of a stirring rod as a scraper to remove the solids.

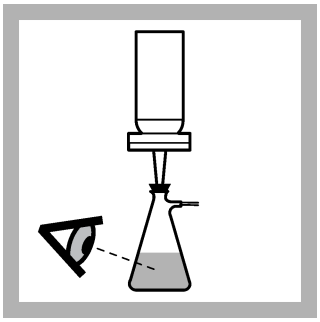
Use small amounts of deionized water to pull the solids down on the fiber filter disc.



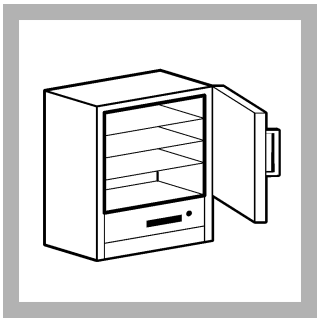
15. Slowly release the vacuum from the filtering system. Remove the fiber filter disc from the filter holder.



16. Put the fiber filter disc in a watch glass.



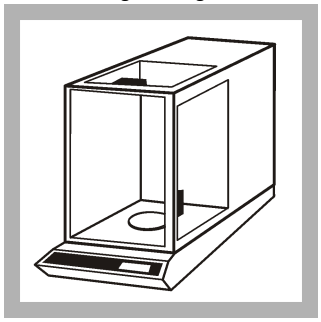
17. Examine the filtrate (filtered water in flask) to make sure that the solids are caught on the fiber filter disc.



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.

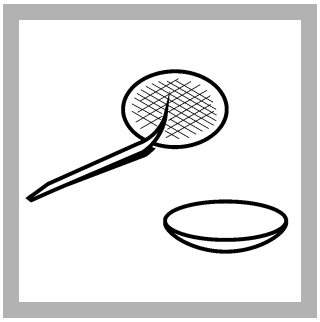
19. Use metal tongs to remove the watch glass with the fiber filter disc from the drying oven or muffle furnace and put in a desiccator. Immediately cover the desiccator. Do not seal the desiccator until the watch glass temperature has decreased a little, because pressure from the hot air inside can push the cover off.

Let the fiber filter disc and watch glass temperature decrease to room temperature.

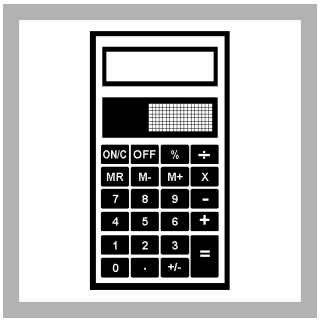


20. Remove the watch glass with the fiber filter disc from the desiccator and put it adjacent to the analytical balance.

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

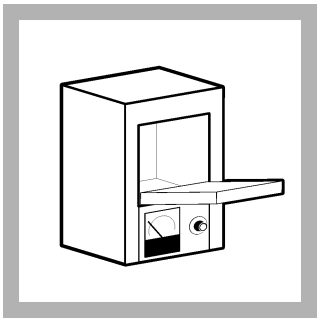
B = 81.5 mg

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

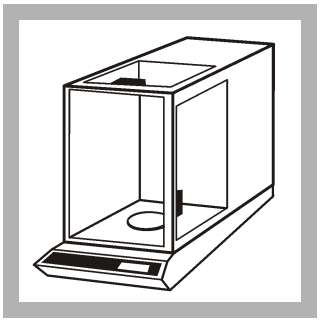


1. Put the watch glass and filter disc from the Total Non-filterable Solids procedure (step 21) in a preheated muffle furnace at 550 °C (1022 °F) for 15 minutes.

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.*

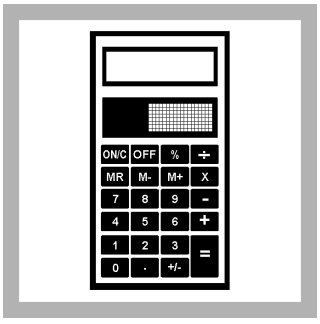
2. Use metal tongs to remove the watch glass with the fiber filter disc from the drying oven or muffle furnace and put in a desiccator. Immediately cover the desiccator. Do not seal the desiccator until the watch glass temperature has decreased a little, because pressure from the hot air inside can push 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

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Total Suspended Solids (TSS) Sample Worksheet

Filter Preparation				
Filter/Pan #	Initial Filter Weight, g (B)	Subsequent Filter Weight, g (B)	Difference between 1st and 2nd filter weights (should be <4% or <0.0005g)	Analyst/Date/Time

Sample Analysis					
Filter/Pan #	Sample Volume, mL (C)	Initial Filter + Dried Sample Weight, g (A)	Subsequent Filter + Dried Sample Weight, g (A)	Difference between 1st and 2nd filter weights (should be <4% or <0.0005g)	Analyst/Date/ Time

Sample Calculations				
Filter/Pan #	Filter Weight, g (B)	Sample Volume, mL	Filter + Dried Sample Weight, g (A)	TSS

$$TSS = \frac{(A-B)(1{,}000{,}000)}{C}$$

Where:
A = weight of filter + dried residue, g
B = weight of filter, g

Please refer to oven temperature log book for corresponding oven temperatures.

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

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

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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

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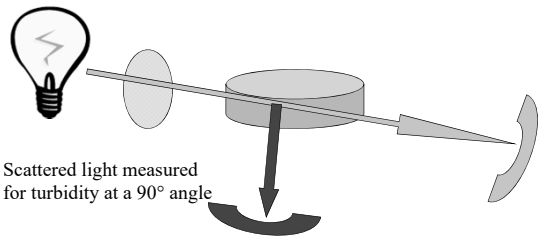
Turbidimeters



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

Turbidimeters

- Newer technology: Hach TU5 Series
  - Collects an array of results at 90° all the way around the sample cell
  - Full circle (360° x 90°)

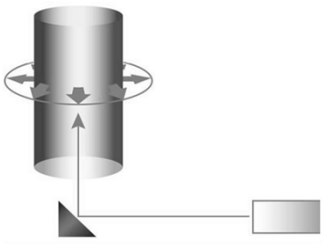


Figure 1: Schematics of the 360° by 90° turbidity measurement system

Source: https://www.hach.com/TU5

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

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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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Instruments

Hach 2100N Laboratory Benchtop Turbidimeter

Hach 2100Q Portable Turbidimeter

8

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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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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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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Any Questions?

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?

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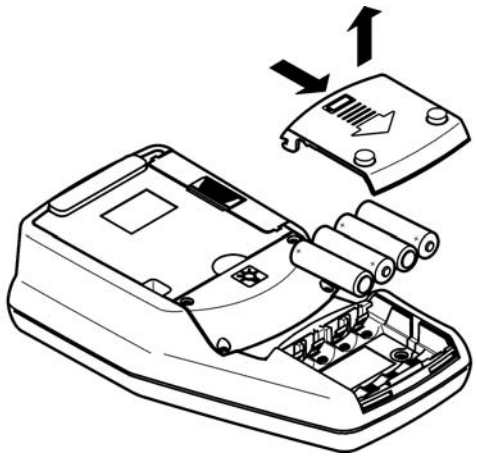
2100Q and 2100Q*is*

12/2017, Edition 4

User Manual

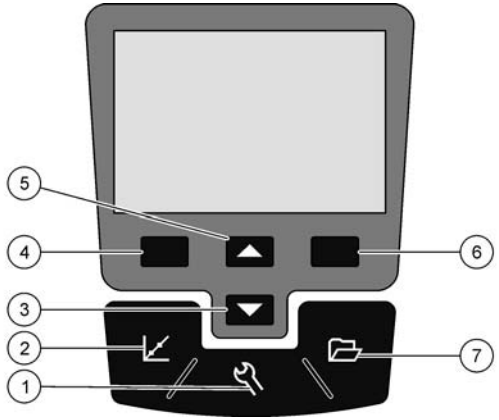
Figure 3 Battery installation



User interface and navigation

User interface

Figure 4 Keypad description

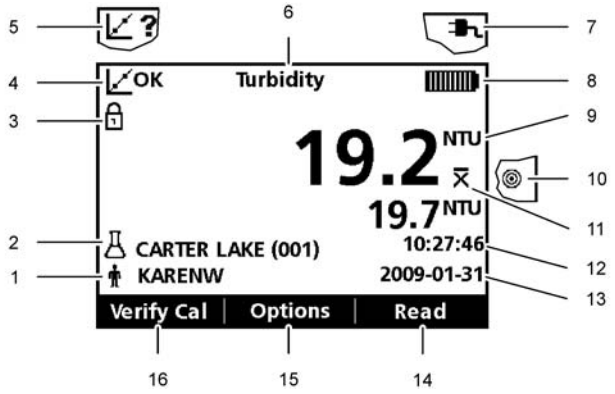


1 **SETTINGS** key: select menu options for setting up the meter	5 **UP** key: scroll through menus, enter numbers and letters
2 **CALIBRATION** key: shows calibration screen, start calibration, select cal options	6 **RIGHT** key (contextual): read turbidity sample, selects or confirms options, opens/jumps to sub-menus
3 **DOWN** key: scroll through menus, enter numbers and letters	7 **DATA MANAGEMENT** key: view, delete or transfer stored data
4 **LEFT** key (contextual): access for calibration verification, cancels or exits the current menu screen to the previous menu screen	

Display description

The measurement screen shows the turbidity, unit, calibration status, date and time, operator ID (if setup) and sample ID (if setup). Refer to Figure 5.

Figure 5 Single screen display



1	Operator identification	**9**	NTU (Nephelometric Turbidity Unit) or FNU (Formazin Turbidity Unit)
2	Sample identification	**10**	Reading mode: Rapidly Settling Turbidity (Target icon)
3	Stability or display lock indicator	**11**	Reading mode: Signal Average (X-bar icon)
4	Calibration status indicator (Calibration OK=pass)	**12**	Time
5	Calibration status indicator (Calibration ?=fail)	**13**	Date
6	Parameter title	**14**	Read (contextual: OK, Select)
7	AC power icon	**15**	Options (contextual)
8	Battery icon	**16**	Verification calibration

Navigation

The meter contains a Settings menu, Reading Options menu, Calibration Options menu and Calibration Verification Options menu to change various options. Use the **UP** and **DOWN** keys to highlight different options. Push the **RIGHT** key to select an option. There are two ways to change options:

1. Select an option from a list: Use the **UP** and **DOWN** keys to select an option. If check boxes are shown, more than one option can be selected. Push the **LEFT** key under Select.

   Note: *To deselect check boxes, push the **LEFT** key under Deselect.*
2. Enter an option value using the arrow keys:
   Push the **UP** and **DOWN** keys to enter or change a value.
3. Push the **RIGHT** key to advance to the next space.
4. Push the **RIGHT** key under **OK** to accept the value.

Startup

Turn the meter on and off

Push the **ON/OFF** key to turn on or turn off the meter. If the meter does not turn on, make sure that the batteries, or the module, are properly installed or that the AC power supply is properly connected to an electrical outlet.

Note: *The Auto-Shutoff option can also be used to turn off the meter. Refer to* Power management *on page 12.*

Change the language

There are three options to set the language:

- The display language is selected when the meter is powered on for the first time.
- The display language is selected when the power key is pushed and held.
- The language can be changed from the Settings menu.

1. Select a language from the list. Confirm with **OK**.
2. Push **Done** when the update is complete.

Change the date and time

The date and time can be changed from the Date & Time menu.

1. Push the **SETTINGS** key and select Date & Time.
2. Update the time and date information:

Option	Description
Format	Select one of the formats for the date and time: yyyy-mm-dd 24h yyyy-mm-dd 12h dd-mm-yyyy 24h dd-mm-yyyy 12h mm/dd/yyyy 24h mm/dd/yyyy 12h
Date	Enter the current date
Time	Enter the current time

The current date and time will be shown on the display.

After the date and time setup, the meter is ready to take a reading.

Standard operation

Use a sample ID

The sample ID tag is used to associate readings with a particular sample location. If assigned, stored data will include this ID.

1. Select **Sample ID** in the Settings menu.
2. Select, create or delete a sample ID:

Option	Description
Current ID	Select an ID from a list. The current ID will be associated with sample data until a different ID is selected.
Create a New Sample ID	Enter a name for a new sample ID.
Delete Sample ID	Delete an existing sample ID.

Use an operator ID

The operator ID tag associates readings with an individual operator. All stored data will include this ID.

1. Select **Operator ID** in the Settings menu.
2. Select, create or delete an operator ID:

Option	Description
Current ID	Select an ID from a list. The current ID will be associated with sample data until a different ID is selected.
Create a New Operator ID	Enter a name for a new operator ID (maximum 10 names can be entered).
Delete Operator ID	Delete an existing operator ID.

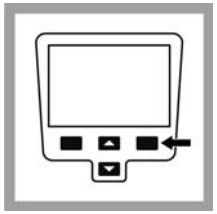
Calibrate the turbidimeter with StablCal® Standards

Note: *For best accuracy use the same sample cell or four matched sample cells for all readings during calibration. Insert the sample cell in the instrument cell*

compartment so the diamond or orientation mark aligns with the raised orientation mark in front of the cell compartment.

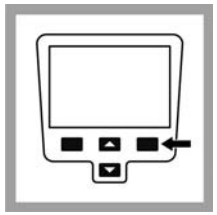
1. Push the **CALIBRATION** key to enter the Calibration mode. Follow the instructions on the display.
Note: *Gently invert each standard before inserting the standard.*

2. Insert the 20 NTU StablCal Standard and close the lid.
Note: *The standard to be inserted is bordered.*

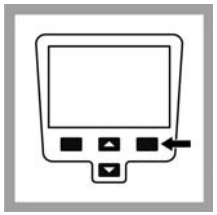


3. Push **Read**. The display shows Stabilizing and then shows the result.

4. Repeat Step 2 and 3 with the 100 NTU and 800 NTU StablCal Standard.
Note: *Push **Done** to complete a 2 point calibration.*



5. Push **Done** to review the calibration details.



6. Push **Store** to save the results.
After a calibration is complete, the meter automatically goes into the Verify Cal mode. Refer to Calibration verification (Verify Cal) on page 16.

Turbidity measurement

⚠ WARNING

Potential explosion and fire hazard. This turbidimeter is designed for water based samples. Do not measure solvent or combustible based samples.

Readings can be taken with the Normal reading mode, Signal Average mode or in the Rapidly Settling Turbidity mode. Refer to Reading modes on page 17 for more information. For accurate turbidity readings use clean sample cells and remove air bubbles (degassing).

Measurement notes

Proper measurement techniques are important in minimizing the effects of instrument variation, stray light and air bubbles. Use the following measurement notes for proper measurements.

Instrument

- Make sure that the meter is placed on a level, stationary surface during the measurement.
  Note: *Do not hold the meter in the hand during measurement.*
- Always close the sample compartment lid during measurement, calibration and storage.
- Remove sample cell and batteries from the instrument if the instrument is stored for an extended time period (more than a month).
- Keep the sample compartment lid closed to prevent the entry of dust and dirt.

Sample cells

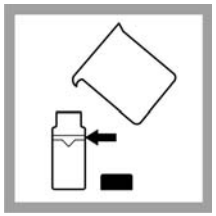
- Always cap the sample cell to prevent spillage of the sample into the instrument.
- Always use clean sample cells in good condition. Dirty, scratched or damaged cells can cause inaccurate readings.
- Make sure that cold samples do not “fog” the sample cell.
- Store sample cells filled with distilled or deionized water and cap tightly.

Measurement

- Measure samples immediately to prevent temperature changes and settling. Before a measurement is taken, always make sure that the sample is homogeneous throughout.
- Avoid sample dilution when possible.
- Avoid operation in direct sunlight.

Turbidity measurement procedure

Note: *Before a measurement is taken, always make sure that the sample is homogeneous throughout.*



1. Collect a representative sample in a clean container. Fill a sample cell to the line (about 15 mL). Take care to handle the sample cell by the top. Cap the cell.

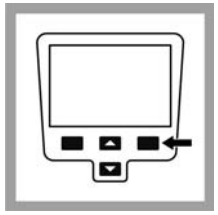
2. Wipe the cell with a soft, lint-free cloth to remove water spots and fingerprints.

3. Apply a thin film of silicone oil. Wipe with a soft cloth to obtain an even film over the entire surface (Apply silicone oil to a sample cell on page 17).

4. Push the **Power** key to turn the meter on. Place the instrument on a flat, sturdy surface.

Note: *Do not hold the instrument while making measurements.*

5. Gently invert and then insert the sample cell in the instrument cell compartment so the diamond or orientation mark aligns with the raised orientation mark in front of the cell compartment. Close the lid.



6. Push **Read**. The display shows Stabilizing then the turbidity in NTU (FNU). The result is shown and stored automatically. Additional information is available on the manufacturer's website.

Data management

About stored data

The following types of data are stored in the data log:

- Reading Log: stores automatically each time a sample reading is taken (500 records).
- Calibration Log: stores only when **Store** is selected at the end of a calibration (25 records).
- Verify Cal Log: stores only after **Done** is selected at the end of a verification calibration (250 records).

When the data log becomes full, the oldest data point is deleted when more data is added to the log.

View data log

The data log contains Reading Log, Calibration Log and Verify Cal log. All logs can be sorted by date.

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.

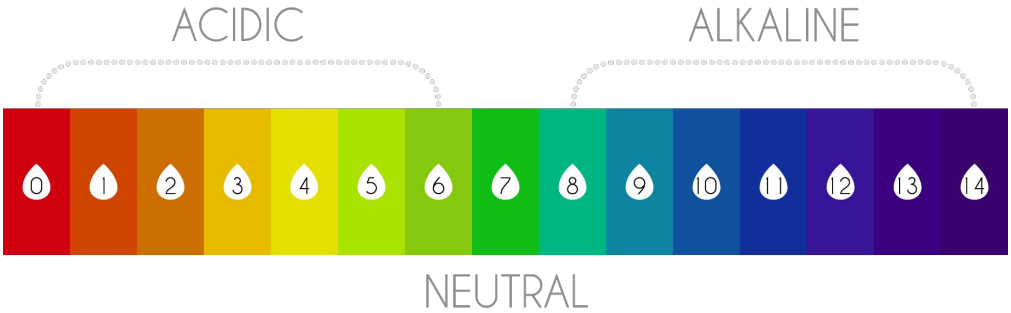
Diagnostic Codes

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

Section 7
pH



pH

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

pH Measurement

- pH is typically measured with a meter and probe
- This is an electrochemical method of analysis

pH Theory

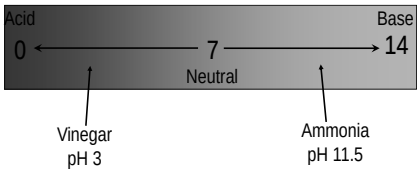
- Acid
  - increases the hydrogen ion (H+) concentration in a solution
- Base
  - increases the hydroxide ion (OH-) concentration in a solution

pH Theory

- pH is defined as the negative log of the molar hydrogen ion concentration in aqueous solution

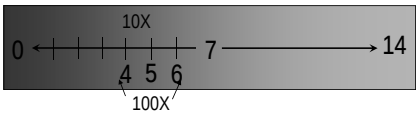
$$pH = -\log [H^+]$$

pH Scale



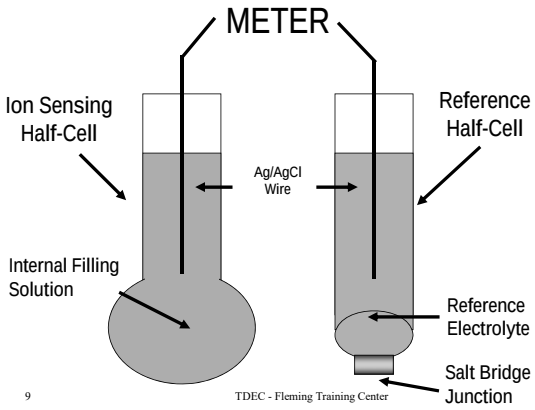
pH Scale

- 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



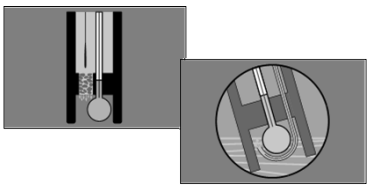
How Does a pH Probe Work?

- Probe measures hydrogen ion concentration
  - Two electrodes in probe:
    a) sensing half-cell,
    b) reference half-cell



Reference Half-Cell

- Dispenses reference solution which completes circuit for meter

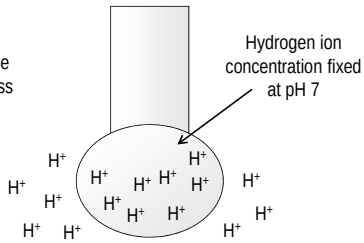


Sensing Half-Cell

pH 7 Solution

H^+ conc. the same both inside and outside glass bulb

*No potential develops



Sensing Half-Cell

pH 7 Solution

H^+ conc. the same both inside and outside glass bulb

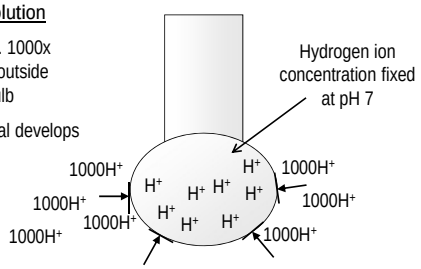
*No potential develops

Sensing Half-Cell

pH 4 Solution

H^+ conc. 1000x greater outside glass bulb

*Potential develops



13 TDEC - Fleming Training Center

Sensing Half-Cell

pH 4 Solution

H^+ conc. 1000x greater outside glass bulb

*Potential develops

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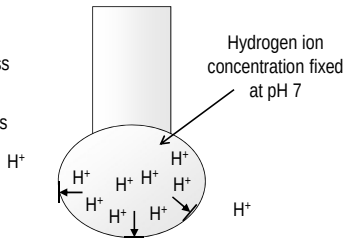
Sensing Half-Cell

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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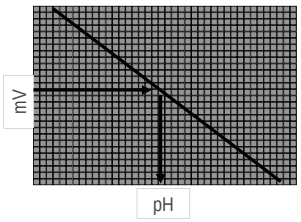
Calibration

- 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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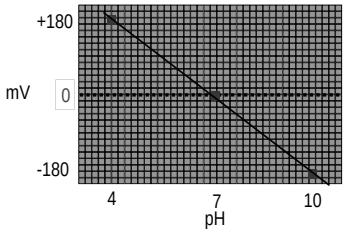
Calibration



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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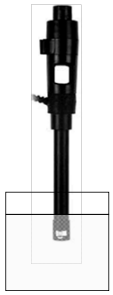
New Probe

- 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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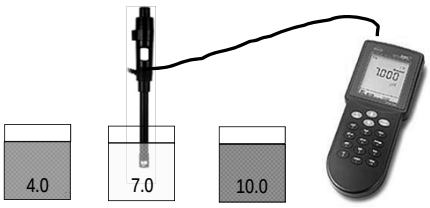
Calibrate

- 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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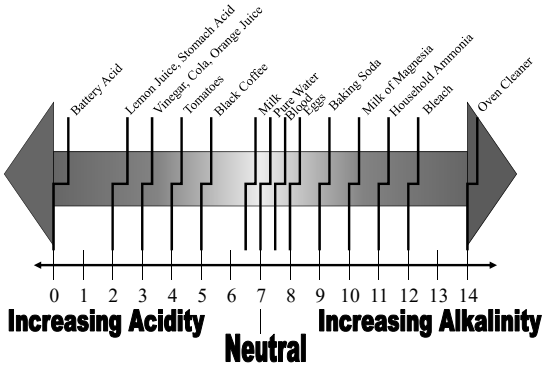
Troubleshooting

- 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

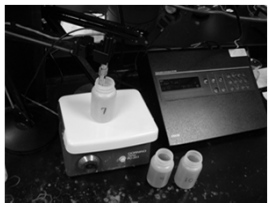
Cleaning

- 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



pH SM4500-H⁺ B – 2011 Electrometric Method

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



pH SM4500-H⁺ B – 2011 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.

pH SM4500-H⁺ B – 2011 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

pH SM4500-H^+ B – 2011 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 TDEC - Fleming Training Center

The following is an excerpt from Standard Methods for the Examination of Water and Wastewater, 23rd edition and is to be used for instructional purposes only.

4500-H^+ pH VALUE*

4500-H^+ A. Introduction

1. 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, e.g. (acid–base neutralization, water softening, precipitation, coagulation, disinfection, and corrosion control) is pH-dependent. pH is used in alkalinity and carbon dioxide measurements and many other acid–base equilibria. At a given temperature the *intensity* of the acidic or basic character of a solution is indicated by pH or hydrogen ion activity. Alkalinity and acidity are the acid- and base-neutralizing capacities of a water and usually are expressed as milligrams $CaCO_3$ per liter. 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-L sample by 1 unit. pH as defined by Sorenson[1] is $-\log [H^+]$; it is the "intensity" factor of acidity. Pure water is very slightly ionized and at equilibrium the ion product is

$$[H^+][OH^-] = K_w = 1.01 \times 10^{-14} \text{ at } 25°C \quad (1)$$

and

$$[H^+] = [OH^-] = 1.005 \times 10^{-7}$$

where:

$[H^+]$ = activity of hydrogen ions, moles/L,
$[OH^-]$ = activity of hydroxyl ions, moles/L, and
K_w = ion product of water.

Because of ionic interactions in all but very dilute solutions, it is necessary to use the "activity" of an ion and not its molar concentration. Use of the term pH assumes that the activity of the hydrogen ion, a_{H^+}, is being considered. The *approximate* equivalence to molarity, $[H^+]$ can be presumed only in very dilute solutions (ionic strength <0.1).

A logarithmic scale is convenient for expressing a wide range of ionic activities. Equation 1 in logarithmic form and corrected to reflect activity is:

$$(-\log_{10} a_{H^+}) + (-\log_{10} a_{OH^-}) = 14 \quad (2)$$

or

$$pH + pOH = pK_w$$

where:

pH† = $\log_{10} a_{H+}$ and
pOH = $\log_{10} a_{OH^-}$.

Equation 2 states that as pH increases pOH decreases correspondingly and vice versa because pK_w is constant for a given temperature. At 25°C, pH 7.0 is neutral, the activities of the hydrogen and hydroxyl ions are equal, and each corresponds to an approximate activity of 10^{-7} moles/L. The neutral point is temperature-dependent and is pH 7.5 at 0°C and pH 6.5 at 60°C.

The pH value of a highly dilute solution is approximately the same as the negative common logarithm of the hydrogen ion concentration. 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.

2. Reference

1. SORENSON, S. 1909. Über die Messung und die Bedeutung der Wasserstoff ionen Konzentration bei Enzymatischen Prozessen. *Biochem. Z.* 21:131.

* Approved by Standard Methods Committee, 2000. Editorial revisions, 2011.

† p designates $-\log_{10}$ of a number.

4500-H^+ B. Electrometric Method

1. General Discussion

a. Principle: The basic principle of electrometric pH measurement is determination of the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode. The hydrogen electrode consists of a platinum electrode across which hydrogen gas is bubbled at a pressure of 101 kPa. Because of difficulty in its use and the potential for poisoning the hydrogen electrode, the glass electrode commonly is used. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the pH of different buffers. Sample pH is determined by extrapolation.

Because single ion activities, such as a_{H^+}, cannot be measured, pH is defined operationally on a potentiometric scale. The pH

measuring instrument is calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using National Institute of Standards and Technology (NIST) buffers having assigned values so:

$$pH_B = -\log_{10} a_{H^+}$$

where:

pH_B = assigned pH of NIST buffer.

The operational pH scale is used to measure sample pH and is defined as:

$$pH_x = pH_B \pm \frac{F(E_x - E_s)}{2.303\ RT}$$

where:

pH_x = potentiometrically measured sample pH,
F = Faraday: 9.649×10^4 coulomb/mole,
E_x = sample emf, V,
E_s = buffer emf, V,
R = gas constant; 8.314 joule/(mole °K), and
T = absolute temperature, °K.

NOTE: Although the equation for pH_x appears in the literature with a plus sign, the sign of emf readings in millivolts for most pH meters manufactured in the United States is negative. The choice of negative sign is consistent with the IUPAC Stockholm convention concerning the sign of electrode potential.[1,2]

The activity scale gives values that are higher than those on Sorenson's scale by 0.04 units:

$$pH\ (activity) = pH\ (Sorenson) + 0.04$$

The equation for pH_x assumes that the emf of the cells containing the sample and buffer is due solely to hydrogen ion activity unaffected by sample composition. In practice, samples will have varying ionic species and ionic strengths, both affecting H^+ activity. This imposes an experimental limitation on pH measurement; thus, to obtain meaningful results, the differences between E_x and E_s should be minimal. Samples must be dilute aqueous solutions of simple solutes (<0.2*M*). (Choose buffers to bracket the sample.) Determination of pH cannot be made accurately in nonaqueous media, suspensions, colloids, or high-ionic-strength solutions.

b. Interferences: The glass electrode is relatively free from interference from color, turbidity, colloidal matter, oxidants, reductants, or high salinity, except for a sodium error at pH > 10. Reduce this error by using special "low sodium error" electrodes.

pH measurements are affected by temperature in two ways: mechanical effects that are caused by changes in the properties of the electrodes and chemical effects caused by equilibrium changes. In the first instance, the Nernstian slope increases with increasing temperature and electrodes take time to achieve thermal equilibrium. This can cause long-term drift in pH. Because chemical equilibrium affects pH, standard pH buffers have a specified pH at indicated temperatures.

Always report temperature at which pH is measured.

c. Quality control (QC): The QC practices considered to be an integral part of each method are summarized in Table 4020:I.

2. Apparatus

a. pH meter consisting of potentiometer, a glass electrode, a reference electrode, and a temperature-compensating device. A circuit is completed through the potentiometer when the electrodes are immersed in the test solution. Many pH meters are capable of reading pH or millivolts and some have scale expansion that permits reading to 0.001 pH unit, but most instruments are not that precise.

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.

Although manufacturers provide operating instructions, the use of different descriptive terms may be confusing. For most instruments, there are two controls: intercept (set buffer, asymmetry, standardize) and slope (temperature, offset); their functions are shown diagramatically in Figures 4500-H+:1 and 2. The intercept control shifts the response curve laterally to pass through the isopotential point with no change in slope. This permits bringing the instrument on scale (0 mV) with a pH 7 buffer that has no change in potential with temperature.

The slope control rotates the emf/pH slope about the isopotential point (0 mV/pH 7). To adjust slope for temperature without disturbing the intercept, select a buffer that brackets the sample with pH 7 buffer and adjust slope control to pH of this buffer. The instrument will indicate correct millivolt change per unit pH at the test temperature.

b. Reference electrode consisting of a half cell that provides a constant electrode potential. Commonly used are calomel and silver: silver-chloride electrodes. Either is available with several types of liquid junctions.

The liquid junction of the reference electrode is critical because at this point the electrode forms a salt bridge with the sample or buffer and a liquid junction potential is generated that in turn affects the potential produced by the reference electrode. Reference electrode junctions may be annular ceramic, quartz, or asbestos fiber, or the sleeve type. The quartz type is most widely used. The asbestos fiber type is not recommended for strongly basic solutions. Follow the manufacturer's recommendation on use and care of the reference electrode.

Refill nonsealed electrodes with the correct electrolyte to proper level and make sure junction is properly wetted.

c. Glass electrode: The sensor electrode is a bulb of special glass containing a fixed concentration of HCl or a buffered chloride solution in contact with an internal reference electrode. Upon immersion of a new electrode in a solution, the outer bulb surface becomes hydrated and exchanges sodium ions for hydrogen ions to build up a surface layer of hydrogen ions. This, together with the repulsion of anions by fixed, negatively charged silicate sites, produces at the glass–solution interface a potential that is a function of hydrogen ion activity in solution.

Several types of glass electrodes are available. Combination electrodes incorporate the glass and reference electrodes into a single probe. Use a "low sodium error" electrode that can operate at high temperatures for measuring pH > 10 because standard glass electrodes yield erroneously low values. For measuring pH

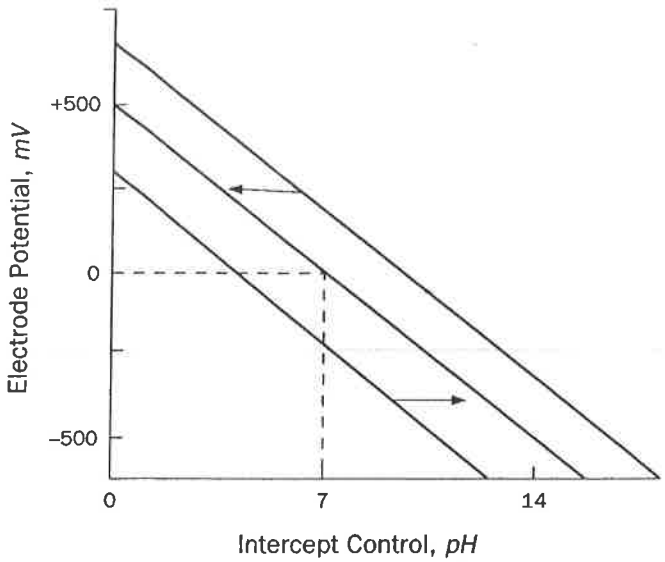


Figure 4500-H+:1. Electrode potential vs. pH. Intercept control shifts response curve laterally.

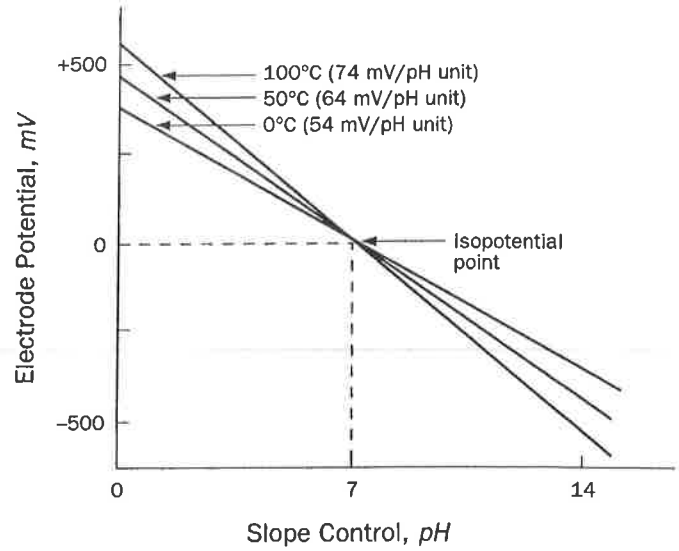


Figure 4500-H+:2. Typical pH electrode response as a function of temperature.

< 1 standard glass electrodes yield erroneously high values; use liquid membrane electrodes instead.

d. Beakers: Preferably use polyethylene or TFE* beakers.

e. Stirrer: Use either a magnetic, TFE-coated stirring bar or a mechanical stirrer with inert plastic-coated impeller.

f. Flow chamber: Use for continuous flow measurements or for poorly buffered solutions.

3. Reagents

a. General preparation: Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work by weighing the amounts of chemicals specified in Table 4500-H+:I, dissolving in distilled water at 25°C, and diluting to 1000 mL. This is particularly important for borate and carbonate buffers.

Boil and cool distilled water having a conductivity of less than 2 μmhos/cm. To 50 mL, add 1 drop of saturated KCl solution suitable for reference electrode use. If the pH of this test solution is between 6.0 and 7.0, use it to prepare all standard solutions.

Dry KH_2PO_4 at 110 to 130°C for 2 h before weighing but do not heat unstable hydrated potassium tetroxalate above 60°C nor dry the other specified buffer salts.

Although ACS-grade chemicals generally are satisfactory for preparing buffer solutions, use certified materials available from the National Institute of Standards and Technology when the greatest accuracy is required. For routine analysis, use commercially available buffer tablets, powders, or solutions of tested quality. In preparing buffer solutions from solid salts, ensure complete solution.

As a rule, select and prepare buffer solutions classed as primary standards in Table 4500-H+:I; reserve secondary standards for extreme situations encountered in wastewater measurements.

Consult Table 4500- H+:II for accepted pH of standard buffer solutions at temperatures other than 25°C. In routine use, store buffer solutions and samples in polyethylene bottles. Replace buffer solutions every 4 weeks.

b. Saturated potassium hydrogen tartrate solution: Shake vigorously an excess (5 to 10 g) of finely crystalline $KHC_4H_4O_6$ with 100 to 300 mL distilled water at 25°C in a glass-stoppered bottle. Separate clear solution from undissolved material by decantation or filtration. Preserve for 2 months or more by adding one thymol crystal (8 mm diam) per 200 mL solution.

c. Saturated calcium hydroxide solution: Calcine a well-washed, low-alkali grade $CaCO_3$ in a platinum dish by igniting for 1 h at 1000°C. Cool, hydrate by slowly adding distilled water with stirring, and heat to boiling. Cool, filter, and collect solid $Ca(OH)_2$ on a fritted glass filter of medium porosity. Dry at 110°C, cool, and pulverize to uniformly fine granules. Vigorously shake an excess of fine granules with distilled water in a stoppered polyethylene bottle. Let temperature come to 25°C after mixing. Filter supernatant under suction through a sintered glass filter of medium porosity and use filtrate as the buffer solution. Discard buffer solution when atmospheric CO_2 causes turbidity to appear.

d. Auxiliary solutions: 0.1*N* NaOH, 0.1*N* HCl, 5*N* HCl (dilute five volumes 6*N* HCl with one volume distilled water), and acid potassium fluoride solution (dissolve 2 g KF in 2 mL conc H_2SO_4 and dilute to 100 mL with distilled water).

4. Procedure

a. Instrument calibration: In each case, follow manufacturer's instructions for pH meter and for storage and preparation of electrodes for use. Recommended solutions for short-term storage of electrodes vary with type of electrode and manufacturer, but generally have a conductivity greater than 4000 μmhos/cm. Tap water is a better substitute than distilled water, but pH 4 buffer is best for the single glass electrode and saturated KCl is preferred for a calomel and Ag/AgCl reference electrode. Satu-

* Teflon or equivalent

TABLE 4500-H+:I. PREPARATION OF pH STANDARD SOLUTIONS[3]

Standard Solution (molality)	pH at 25°C	Weight of Chemicals Needed/1000 mL Pure Water at 25°C
Primary standards:		
Potassium hydrogen tartrate (saturated at 25°C)	3.557	> 7 g $KHC_4H_4O_6$*
0.05 potassium dihydrogen citrate	3.776	11.41 g $KH_2C_6H_5O_7$
0.05 potassium hydrogen phthalate	4.004	10.12 g $KHC_8H_4O_4$
0.025 potassium dihydrogen phosphate + 0.025 disodium hydrogen phosphate	6.863	3.387 g KH_2PO_4 + 3.533 g Na_2HPO_4†
0.008 695 potassium dihydrogen phosphate + 0.030 43 disodium hydrogen phosphate	7.415	1.179 g KH_2PO_4 + 4.303 g Na_2HPO_4†
0.01 sodium borate decahydrate (borax)	9.183	3.80 g $Na_2B_4O_7 \cdot 10H_2O$†
0.025 sodium bicarbonate + 0.025 sodium carbonate	10.014	2.092 g $NaHCO_3$ + 2.640 g Na_2CO_3
Secondary standards:		
0.05 potassium tetroxalate dihydrate	1.679	12.61 g $KH_3C_4O_8 \cdot 2H_2O$
Calcium hydroxide (saturated at 25°C)	12.454	> 2 g $Ca(OH)_2$*

* Approximate solubility.
† Prepare with freshly boiled and cooled distilled water (carbon-dioxide-free).

rated KCl is the preferred solution for a combination electrode. Keep electrodes wet by returning them to storage solution whenever pH meter is not in use.

Before use, remove electrodes from storage solution, rinse, blot dry with a soft tissue, place in initial buffer solution, and set the isopotential point (4500-H+.B.2*a*). Select a second buffer within 2 pH units of sample pH and bring sample and buffer to same temperature, which may be the room temperature; a fixed temperature, such as 25°C; or the temperature of a fresh sample. Remove electrodes from first buffer, rinse thoroughly with distilled water, blot dry, and immerse in second buffer. Record temperature of measurement and adjust temperature dial on meter so meter indicates pH value of buffer at test temperature (this is a slope adjustment).

Use the pH value listed in the tables for the buffer used at the test temperature. Remove electrodes from second buffer, rinse thoroughly with distilled water and dry electrodes as indicated above. Immerse in a third buffer below pH 10, approximately

TABLE 4500-H+:II. STANDARD pH VALUES[3]

	Primary Standards							Secondary Standards	
Temperature °C	Tartrate (Saturated)	Citrate (0.05*M*)	Phthalate (0.05*M*)	Phosphate (1:1)	Phosphate (1:3.5)	Borax (0.01*M*)	Bicarbonate-Carbonate (0.025*M*)	Tetroxalate (0.05*M*)	Calcium Hydroxide (Saturated)
0			4.003	6.982	7.534	9.460	10.321	1.666	
5			3.998	6.949	7.501	9.392	10.248	1.668	
10			3.996	6.921	7.472	9.331	10.181	1.670	
15			3.996	6.898	7.449	9.276	10.120	1.672	
20			3.999	6.878	7.430	9.227	10.064	1.675	
25	3.557	3.776	4.004	6.863	7.415	9.183	10.014	1.679	12.454
30	3.552		4.011	6.851	7.403	9.143	9.968	1.683	
35	3.549		4.020	6.842	7.394	9.107	9.928	1.688	
37			4.024	6.839	7.392	9.093			
40	3.547		4.030	6.836	7.388	9.074	9.891	1.694	
45	3.547		4.042	6.832	7.385	9.044	9.859	1.700	
50	3.549		4.055	6.831	7.384	9.017	9.831	1.707	
55	3.554		4.070					1.715	
60	3.560		4.085					1.723	
70	3.580		4.12					1.743	
80	3.609		4.16					1.766	
90	3.650		4.19					1.792	
95	3.674		4.21					1.806	

3 pH units different from the second; the reading should be within 0.1 unit for the pH of the third buffer. If the meter response shows a difference greater than 0.1 pH unit from expected value, look for trouble with the electrodes or potentiometer (4500-H^+.B.5*a* and *b*).

The purpose of standardization is to adjust the response of the glass electrode to the instrument. When only occasional pH measurements are made, standardize instrument before each measurement. When frequent measurements are made and the instrument is stable, standardize less frequently. If sample pH values vary widely, standardize for each sample with a buffer having a pH within 1 to 2 pH units of the sample.

b. Sample analysis: Establish equilibrium between electrodes and sample by stirring sample to ensure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes after cleaning by dipping them into sample for 1 min. Blot dry, immerse in a fresh portion of the same sample, and read pH.

With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

5. Troubleshooting

a. Potentiometer: To locate trouble source, disconnect electrodes and, using a short-circuit strap, connect reference electrode terminal to glass electrode terminal. Observe change in pH when instrument calibration knob is adjusted. If potentiometer is operating properly, it will respond rapidly and evenly to changes in calibration over a wide scale range. A faulty potentiometer will fail to respond, will react erratically, or will show a drift upon adjustment. Switch to the millivolt scale on which the meter should read zero. If inexperienced, do not attempt potentiometer repair other than maintenance as described in instrument manual.

b. Electrodes: If potentiometer is functioning properly, look for the instrument fault in the electrode pair. Substitute one electrode at a time and cross-check with two buffers that are about 4 pH units apart. A deviation greater than 0.1 pH unit indicates a faulty electrode. Glass electrodes fail because of scratches, deterioration, or accumulation of debris on the glass surface. Rejuvenate electrode by alternately immersing it three times each in 0.1*N* HCl and 0.1*N* NaOH. If this fails, immerse tip in KF solution for 30 s. After rejuvenation, soak in pH 7.0 buffer overnight. Rinse and store in pH 7.0 buffer. Rinse again with distilled water before use. Protein coatings can be removed by soaking glass electrodes in a 10% pepsin solution adjusted to pH 1 to 2.

To check reference electrode, oppose the emf of a questionable reference electrode against another one of the same type that is known to be good. Using an adapter, plug good reference electrode into glass electrode jack of potentiometer; then plug questioned electrode into reference electrode jack. Set meter to read millivolts and take readings with both electrodes immersed in the same electrolyte (KCl) solution and then in the same buffer solution. The millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used (i.e., silver:silver-chloride against calomel or vice versa) the reading will be 44 ± 5 mV for a good reference electrode.

Reference electrode troubles generally are traceable to a clogged junction. Interruption of the continuous trickle of electrolyte through the junction causes increase in response time and drift in reading. Clear a clogged junction by applying suction to the tip or by boiling tip in distilled water until the electrolyte flows freely when suction is applied to tip or pressure is applied to the fill hole. Replaceable junctions are available commercially.

6. Precision and Bias

By careful use of a laboratory pH meter with good electrodes, a precision of ±0.02 pH unit and an accuracy of ±0.05 pH unit can be achieved. However, ±0.1 pH unit represents the limit of accuracy under normal conditions, especially for measurement of water and poorly buffered solutions. For this reason, report pH values to the nearest 0.1 pH unit. A synthetic sample of a Clark and Lubs buffer solution of pH 7.3 was analyzed electrometrically by 30 laboratories with a standard deviation of ±0.13 pH unit.

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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.

Fleming Training Center

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				

Month_____ **pH Meter** _____

Fleming Training Center

Method: SM 4500-H^+B-2011
pH Sample Record

Date	Time	Sample Location	Temp. of Solution	Date of Last Calibration	Measured pH	Analyst Initials

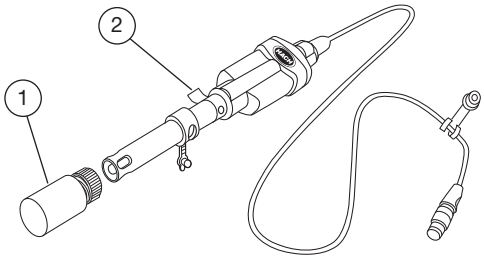
Month_____ **pH Meter** _____

pH Value	H^+ Concentration Relative to Pure Water	Example
0	10 000 000	battery acid
1	1 000 000	concentrated sulfuric acid
2	100 000	lemon juice, vinegar
3	10 000	orange juice, soda
4	1 000	tomato juice, acid rain
5	100	black coffee, bananas
6	10	urine, milk
7	1	pure water
8	0.1	sea water, eggs
9	0.01	baking soda
10	0.001	Great Salt Lake, milk of magnesia
11	0.000 1	ammonia solution
12	0.000 01	soapy water
13	0.000 001	bleach, oven cleaner
14	0.000 000 1	liquid drain cleaner

pH ELECTRODE CLEANING & MAINTENANCE GUIDE

Throughout the life of your pH electrode, you want to make sure you are getting the best performance and most accurate results possible. However minor items like air bubbles, crystallization, low electrolyte solution, KCl leakage and minor contamination can cause issues. Issues which can result in a lower confidence in the results being provided and additional purchases of replacement electrodes. This guide will provide tips to help you maintain and keep your pH electrodes clean, extending your electrode's life, while allowing you to maintain a high level of confidence in your readings.

1. First Use & Conditioning



pH electrodes are typically shipped with a storage cap to protect and hydrate the glass bulb (1). Refillable pH electrodes are additionally shipped with the refill hole sealed to prevent electrolyte leakage during shipment. This is often a piece of tape which must be removed before first use (2). During shipment, the electrolyte can move or shift, sometimes allowing air bubbles into the glass bulb, or drying of the reference element.

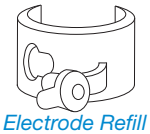
Air in the pH Glass Bulb

Because of this, it is recommended that the pH electrode be conditioned before first use. After unpacking, check for sufficient electrolyte inside the electrode and refill if necessary (for refillable electrodes). Then check that the glass bulb is completely filled with electrolyte and that there are no visible air bubbles. If there are, shake the electrode in a downward motion to force air bubbles out of the glass bulb.

The pH electrode will then need to be conditioned per the electrode's user manual (typically this requires soaking the electrode for several minutes in the sample or pH buffers). The response time for a new, conditioned pH electrode in pH buffers at 25°C is typically less than 30 seconds.

2. Closing & Opening the KCl Refill Hole

Refillable pH electrodes have a refill hole where electrolyte can be added. The level or height of the inner liquid is important for a refillable pH electrode junction.

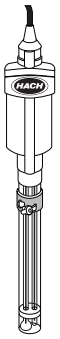


Electrode Refill Hole Stopper

If sufficient electrolyte solution is inside the electrode, the hydrostatic pressure of the liquid column will allow electrolyte to flow out of the electrode at a faster flow rate, and additionally will prevent sample solution from going inside the electrode.

Typically, the faster the electrolyte flow rate, the faster the pH stabilization time. However this is dependent on the junction type used. Infiltration of the sample solution can contaminate the reference element and cause unstable or unreliable measurements.

Open the refill hole plug during use and close it when not in use.



Refillable pH Electrode with Stopper

3. Regular Maintenance

There are some key signs that will help the electrode user understand when to clean.

- Slow stabilization times
- Erroneous or erratic readings
- Difficulty in calibrating

Proper maintenance will ensure faster measurements, improve pH accuracy and extend the lifetime of the electrode.

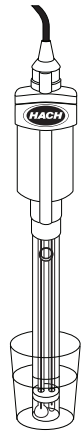
Regular pH electrode maintenance requires that a pH electrode be stored in the recommended pH storage solution between measurements, and that the electrolyte filling solution be refilled as necessary. For best electrode performance, do not let the reference junction dry out.

The pH electrode will need to be cleaned regularly, depending on the nature of the sample. The most suitable cleaning solution is one which interacts in a more selective way with the contamination. Typically fats, oils and grease (FOG) contaminations should be cleaned with a non-ionic surfactant solution or methanol, proteins such as food stuff should be cleaned with an acidic pepsin cleaning solution and mineral deposits should be cleaned with an acidic cleaning solution. Refer to your user manual for your electrode's specific guidelines; however, typical cleaning/maintenance guidelines are as follows:

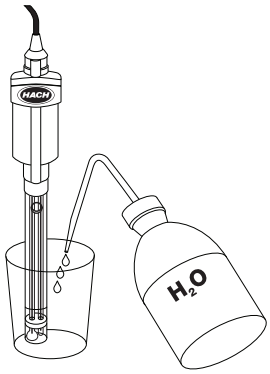
Proteins: Cleaned with an acidic pepsin cleaning solution.

Fats, oils: Cleaned with a non-ionic surfactant solution or methanol.

Afterward, rinse the electrode with deionized water, then store in the recommended pH storage solution.



Electrode in Beaker



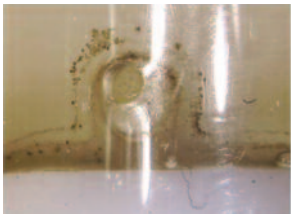
Rinse Electrode

Hach Electrode Cleaning Solution

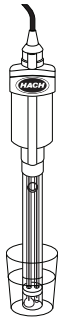
4. Regular Cleaning of the Glass Bulb and Reference Junction

For optimal response time, it may be necessary to clean the pH electrode's glass bulb and reference junction of contaminants and precipitations. The pH electrode will need to be cleaned regularly following the steps outlined in the pH electrode manual. Typically, it is recommended to soak the electrode for a few minutes in warm deionized water, or in a cleaning solution (Hach Electrode Cleaning Solution) to maintain the reference junction.

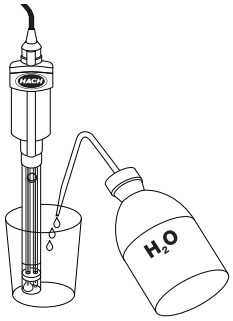
Correctly Functioning Ceramic Junction Outflow of Electrolyte (Red Dye)



Contaminated Reference Junction



Electrode in Beaker

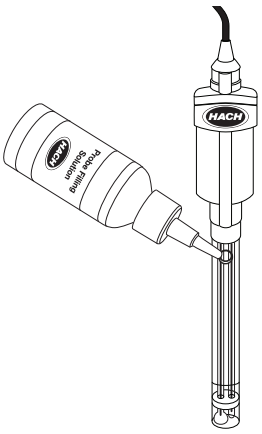


Rinse Electrode

Hach Electrode Cleaning Solution

5. Refilling pH Electrode Electrolyte

Refer to the specific pH electrode manual to determine the correct electrode filling solution to use. Remove the filling hole plug, and fill the pH electrode with electrolyte to just below the refill hole. Leave a small amount of space below the refill hole to help minimize electrolyte leakage and build up of KCl crystallization at the filling hole. Replace the filling hole plug (or leave open if next step is to take a measurement).



Refill with KCl

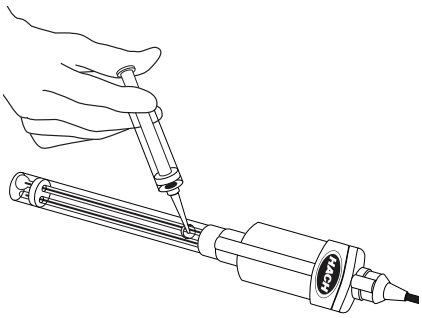
KCl

KCl w/Ag

Sat. KCl

6. Exchange of Electrolyte Filling Solution

If the electrolyte filling solution is contaminated, or if a different electrolyte is needed, use a syringe with needle to empty the entire fluid contents of the electrode. Withdraw the filling solution slowly and carefully to ensure that the pH electrode is not damaged during this process. Refer to the pH electrode manual to determine the correct replacement filling solution to use to refill the electrode.

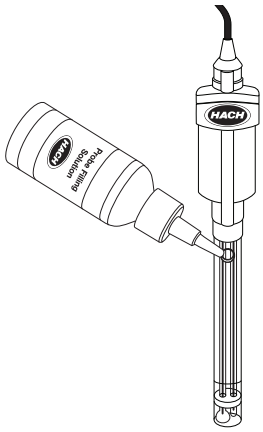


Remove Fluid Contents of Electrode

KCl

KCl w/Ag

Sat. KCl

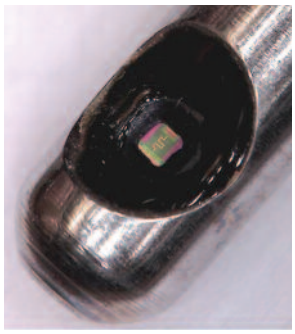


Refill with KCl

7. Cleaning ISFET pH Sensors

Using Toothbrush to Clean an ISFET pH Sensor

ISFET pH sensors are robust and can be cleaned with a toothbrush, even on the ISFET surface. Do NOT use sharp tools, abrasive materials, or cleaning powder. A mild soap or detergent can be used to help remove oil or fat. Pepsin cleaning solution should routinely be used for samples that contain proteins. After cleaning, rinse completely with deionized water.



Clean ISFET pH Sensor

Don't forget to clean the reference junction as well. Since ISFET electrodes are stored dry and are commonly exposed to food stuff, they commonly get KCl crystallization (see Tip 13) and dirty reference junction (see Tip 4).

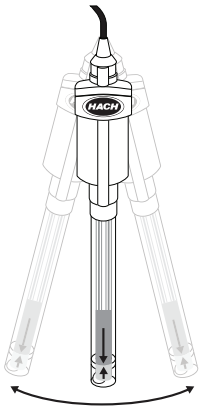
Make sure that the electrode cap is always placed on the electrode tip when not in use. This will create a humid environment to prevent the reference junction from drying out and clogging.

8. Air Bubble in Glass Bulb

The electrolyte inside a pH electrode can shift during shipment, during horizontal storage, and from general use. This movement can allow air bubbles into the glass bulb causing erratic readings or calibration errors. Before each use, it is recommended to inspect that the glass bulb is completely filled with electrolyte and that there are no visible air bubbles.

If the pH electrode has visible air bubbles, shake the electrode in a downward motion like a thermometer. This will force air bubbles out of the glass bulb.

Air in the pH Glass Bulb



Shaking the Electrode

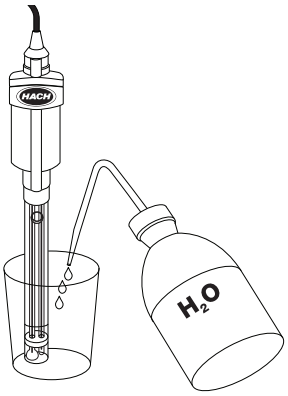
9. KCl Leakage & Salt Build-Up

Salt crystals can accumulate when water in a salt solution evaporates during contact with air (1) (2). As a result, liquid junctions of the pH electrode can occasionally become clogged (3) if not stored properly. The salt crystallization is not harmful to the electrode, nor does it inhibit the electrode's performance if treated properly. These salt crystals can be removed by rinsing the area with warm water (4). Salt crystals can also be minimized by properly covering the reference junction(s) with storage solution when the electrode is not in use, along with maintaining the electrolyte level in refillable electrode (5).

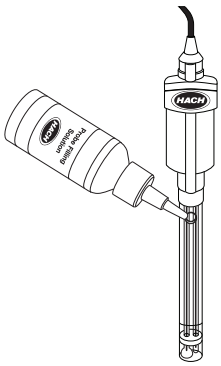
(1) KCl salt crystallization

(2) KCl salt crystallization

(3) KCl salt crystallization



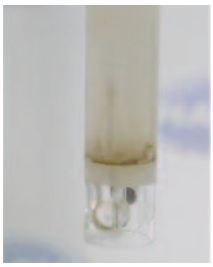
(4) Rinse Electrode



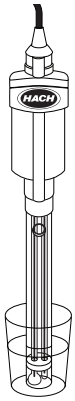
(5) Refill Electrode with KCl

10. Contamination Inside the Electrode

Some samples can cause unintended biological growth inside the pH electrode. This contamination affects the performance of the pH electrode. Thiourea solution (example: Hach KS410 Solution) can remove this type of organic growth. Allow the electrode to soak for a few hours in thiourea, followed by a thorough rinsing with deionized water.



Dirty Gel Electrolyte in Hach IntelliCAL PHC101 pH Electrode



Electrode in Beaker

Hach KS410 Solution

Clean Gel Electrolyte in Hach IntelliCAL PHC101 pH Electrode

11. Dirty Glass Bulb

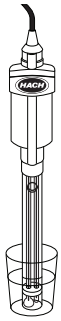
Dirty samples or samples that remain on the glass surface can result in incorrect pH readings. Refer to your user manual for specific guidelines for the most suitable cleaning solution for the specific contamination you have; however, a typical cleaning procedure for a dirty glass bulb is as follows:

General contaminants: Soak the glass bulb for up to 16 hours in Hach Electrode Cleaning Solution. Rinse in deionized water. Soak the electrode in pH 4 buffer for up to 20 minutes, then rinse with deionized water.

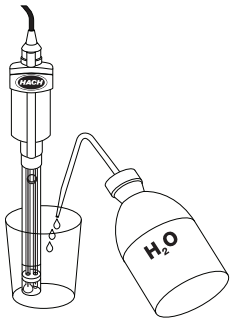
Dirty Glass pH Bulb

Dirty Glass pH Bulb

Dirty Glass pH Bulb



Electrode in Beaker



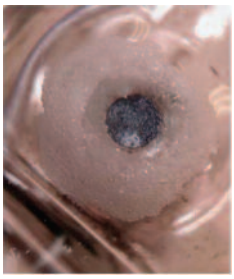
Rinse Electrode

Hach Electrode Cleaning Solution

12. Sulfide Precipitation

Sulfide and silver ions can react to form a dark precipitation inside of refillable pH electrodes. This precipitate can block the reference junction. Soak the pH electrode in thiourea solution (example: Hach KS410 Solution) for a few minutes to remove the blockage.

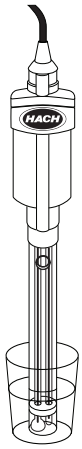
Ceramic Pin Junction Blocked by Sulfide Precipitation



Ceramic Pin Junction Blocked by Sulfide Precipitation

Ceramic Pin Junction Cleared of Sulfide Precipitation by Hach KS410 Solution

Ceramic Pin Junction Cleared of Sulfide Precipitation by Hach KS410 Solution



Electrode in Beaker

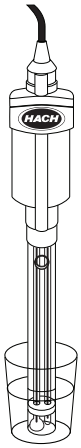
Hach KS410 Solution

13. KCl Crystallization Inside the Electrode

The most common electrolyte in Hach pH electrodes is 3 molar KCl. In some instances, such as low temperature, the KCl may crystallize inside the electrode. If the KCl crystals block the ion exchange with the reference element the electrode may read incorrect pH values.

Soak the electrode in a warm (45°C) KCl filling solution or buffer. Allow the electrode to cool down to room temperature. This should cause the KCl crystals to dissolve.

KCl Crystals Blocking Reference Junction



Electrode in Beaker

Hach Solutions for Your pH Electrodes

2965249 Electrode Cleaning Solution (500 mL)

2964449 Electrode Rinse Solution, Non-Ionic Surfactant (500 mL)

2975149 Acid Electrode Cleaning Solution (500 mL)

2756549 Fill or Storage Solution, 3M KCl (500 mL)

2841700 Fill Solution, 3M KCl Saturated w/ AgCl (30 mL)

2965026 Fill Solution, 2.44M Viscous KCl (59 mL)

For more information on Hach's pH electrodes, please visit us at: hach.com/HQdGuide-pH

To order any needed pH electrode solutions, please call Hach at: 800-227-4224

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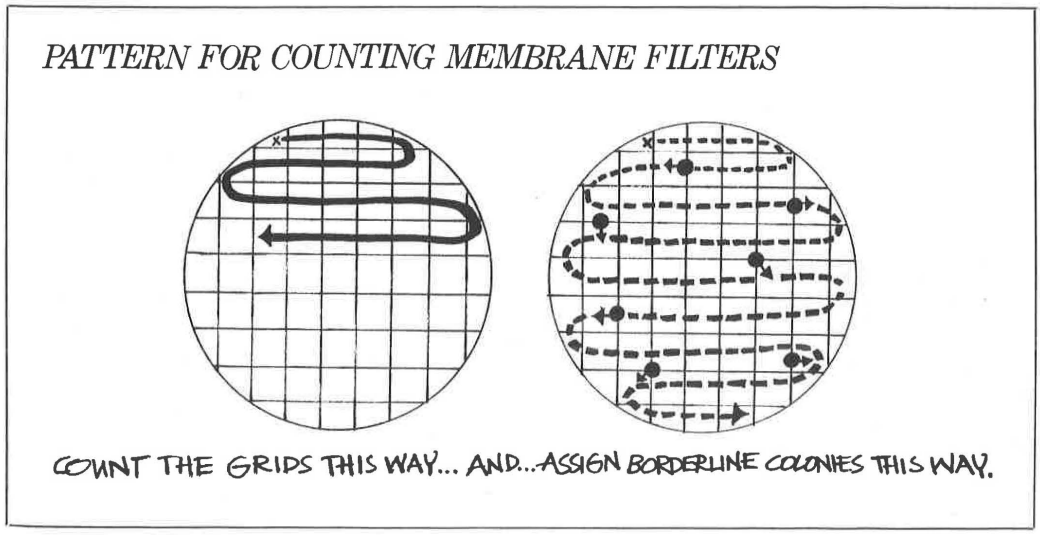
Be Right™

Section 8
Bacteriological Analyses



Bacteriological Analysis

1 **Wastewater Laboratory Class**

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

2

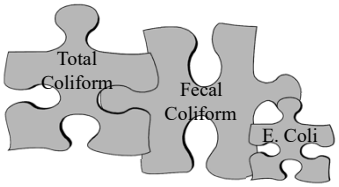
Coliform Bacteria

- 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

3

Family Portrait

- Indicators of water contamination



4

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

Safety

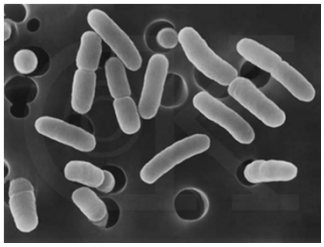
- Follow standard safety practices appropriate to microbiological laboratories.
- Materials suspected of containing viable bacteria should be decontaminated using an autoclave or by using an appropriate disinfectant before discarding.

6

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

7



Fecal Coliform

8

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

Interferences

- No interferences
- Excess particulates may cause colonies to grow together on a crowded filter or slow the sample filtration process.

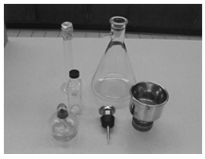
10

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

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

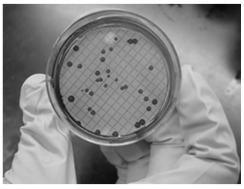
Sampling

- 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.

13

Fecal Data Analysis

- Visually determine colony counts on membrane filters.
- Verify using 10-15 X binocular wide-field microscope.
- Fecal coliforms appear blue.



14

Fecal Data Interpretation

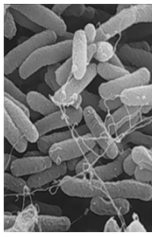
- Incubation time is 24 ± 2 hrs.
- Fecal coliform density reported as number of colonies per 100 mL of sample.
- NPDES permit limit: monthly average of 200/100 mL; daily maximum of 1000/100 mL.

15

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).

16



Escherichia coli (E.coli)

17 **Colilert**

Colilert® & Colilert-18®

MPN Method

- Add substrate to a 100 mL sample
- If making dilutions, use sterile DI water, not sterile buffered water.

18

COLILERT® & COLILERT-18®

- Shake sample vigorously. Wait for bubbles to dissipate.
- Pour into QuantiTray.

19

COLILERT® & COLILERT-18®

- Seal sample in Quanti-Tray
- Incubate at **35±0.5°**C for 18 hrs (Colilert-18) OR 24 hrs (Colilert)

20

COLILERT® & COLILERT-18®

- Examine tray for appropriate color change
- Yellow is an indicator of total coliforms

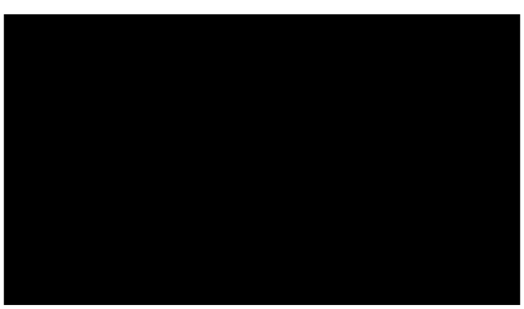
Left: The 97 well QuantiTray 2000 will count up to 2419 cfu without dilution.
Right: The 51 well QuantiTray will count up to 200 cfu without dilution.

21

COLILERT® & COLILERT-18®

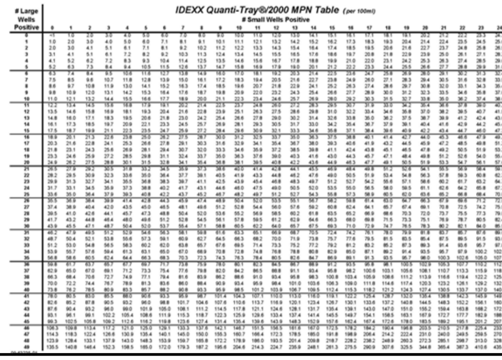
- Examine positive total coliform for fluorescence using a UV light in a dark environment
- Fluorescence is a positive indicator for E. coli
- Calculate MPN value according to the table provided with the QuantiTray

22



23

COLILERT® & COLILERT-18®



IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

Small Wells Positive

# Large 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	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	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	11.5	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	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
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	33.8	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	22.5	23.7	24.8	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	13.5	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	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	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	37.5	38.7	39.9	41.2	42.4	43.6
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	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	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	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	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	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
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	42.8	44.3	45.9	47.4	48.9	50.4	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	56.9	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	43.9	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	63.8	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	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	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	115.9	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	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
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	116.9	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	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
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	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
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	307.6	325.5	344.8	365.4	387.3	410.6	435.2

24

COLILERT® & COLILERT-18®

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

Small Wells Positive

# Large 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	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	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	39.9	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	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
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	56.1	57.4	58.6	59.9	61.2	62.4	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	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	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	74.6	76.3	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	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	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	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
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	118.9	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	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	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	115.2	117.8	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	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
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	152.6	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	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	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	325.7
44	193.6	199.3	205.1	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	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	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	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	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	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

25

ESCHERICHIA COLI (E.COLI)

Modified mTEC Agar with Membrane Filtration

26

EPA METHOD 1603

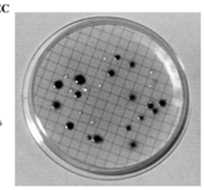
- Membrane Filter – modified mTEC agar
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)
- Place sample in a petri dish with modified mTEC agar
- Invert dish and incubate for **35± 0.5°C for 2 hours**
  - Resuscitates injured or stressed bacteria
- 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

27

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

Modified mTEC



Count magenta colonies as *E. coli*. These are easily discerned from non-target colonies which are clear or beige.

28

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

29

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

30

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***.

31

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***.

32

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***.

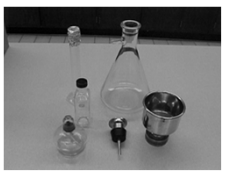
33

ESCHERICHIA COLI (E.COLI)

34 **m-ColiBlue24® with Membrane Filtration**

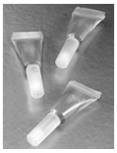
M-COLIBLUE24®

- Membrane Filter
- Filter sample dilutions through a 47mm diameter sterile, white, grid marked filter (0.45µm pore size)



M-COLIBLUE24®

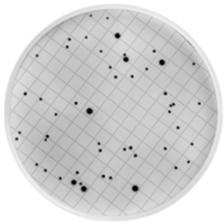
- Place sample in a petri dish with absorbent pad containing 2 mL mColiBlue 24 broth
- Invert dish and incubate at **35± 0.5°C for 24 ±2 hours**



36

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



37

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.

38

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.

39

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

40

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*

41

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*	

42

M-ColiBlue24® Trouble-Shooting Guide, Hach Company, www.Hach.com

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

43

E. COLI

- Two Approved Methods
  - SM 9223 B – 2004 IDEXX Colilert Quanti-Tray
  - Hach Method 10029 – m -ColiBlue24® -

44

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

45

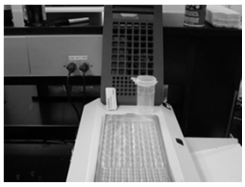
SM 9020 B. QC GUIDELINES

- 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

46

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)



47

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

48

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)

49

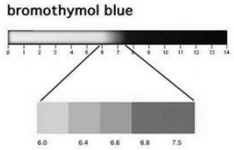
SM 9020 B. QC GUIDELINES

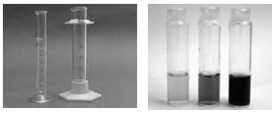
- Lab Equipment – continued
  - Membrane filters and pads (if MF procedure is used) – 9020 B.5.i.3
    - Check filters for brittleness if lot is held for one or more years

50

SM 9020 B. QC GUIDELINES

- Lab Supplies
  - Glassware – 9020 8.5.a
    - 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





51

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

52

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

53

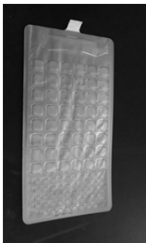
SM 9020 B. QC GUIDELINES

- 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.

54

SM 9020 B. QC GUIDELINES

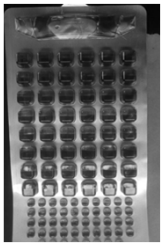
- Lab Supplies – continued
  - Multi-well trays and sealers – 9020 B.5.e
    - **2014 Update– analyze a method blank once per lot (of sterile water, media, bottles and trays) or once per quarter, whichever is more frequent, to demonstrate sterility.**



55

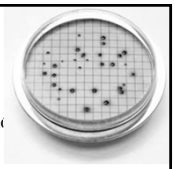
SM 9020 B. QC GUIDELINES

- 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.
    - **2014 Update – 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.



56

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.

57

SM 9020 B. QC GUIDELINES

- 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.
  - Duplicate Analyses – Perform duplicate analyses on a 5% basis (1 in 20 samples) or once a month, whichever is more frequent.

58

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.

59

MEMBRANE FILTER COUNTS

60

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

61

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

$$\text{Count per 100 mL} = \frac{40}{5} \times 100 = 800/100 \text{ mL}$$

62

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

63

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

$$\frac{2600 + 3600}{2} = 3100/100 \text{ mL}$$

64

More Than One Acceptable Count

- If more than one dilution, independently carry counts to final reporting units, then average for final reported value

65

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

66

More Than One Acceptable Count

- Independently carry each MF count to a count per 100 mL

$\frac{55}{0.05} \times 100 = 110{,}000/100\text{ mL}$ $\frac{30}{0.03} \times 100 = 100{,}000/100\text{ mL}$

- Then calculate the arithmetic mean of these counts to obtain the final reported value

$$\frac{110{,}000 + 100{,}000}{2} = 105{,}000/100\text{ mL}$$

67

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

$\frac{14}{1.0} \times 100 = 1400/100\text{ mL}$

68

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**

69

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.

70

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**

71

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.

72

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**

73

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

74

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**

75

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

76

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

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

78

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

79

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*

m-ColiBlue24 Broth PourRite Ampules[1]

Method 10029

Membrane Filtration

Scope and application: For potable water, nonpotable water, recreation water and wastewater.

[1] USEPA approved.

Test preparation

Before starting

Let the media in PourRite ampules increase to room temperature before the ampule is opened.
Set the temperature of the incubator to 35 ± 0.5 °C (95 ± 0.9 °F). Let the incubator temperature become stable, then add the samples.
Wash hands thoroughly with soap and water.
Use a germicidal cloth, bactericidal spray, weak bleach solution or weak iodine solution to clean the work area.
Make sure that all of the materials that come in contact with samples are sterile.
During filtration, remove the vacuum as soon as the funnel is empty so that the membrane filter does not become dry.
As an alternative to the filter assembly with flask, use a sterile, disposable filter unit.

Items to collect

Description	Quantity
Broth ampule, m-ColiBlue24	1
Sterile buffered dilution water	1
Membrane filter, 0.45 micron	1
Petri dish with absorbent pad, 47-mm	1
Filtration apparatus with aspirator or pump	1
Forceps, sterilized	1
Incubator	1
Microscope, low-power	1
Pipet(s) for dilution or for sample volumes less than 100 mL, if necessary	1

Refer to Consumables and replacement items on page 6 for order information.

Sample collection

- Use a sterile glass or plastic container such as a Whirl-Pak bag that contains sterilized sodium thiosulfate. The sodium thiosulfate is not necessary if the sample does not contain a residual disinfectant.
- Open the sample containers immediately before collection and close immediately after collection. Do not put the lid or cap down. Do not touch the lip or inner surfaces of the container. Do not rinse the containers before use.
- To collect a potable water sample from a faucet, spigot, hydrant or pump, let the water flow at a moderate rate for 2–3 minutes. Remove the screens or aerators. Do not use faucets or spigots that have a bad seal or that show a leak between components.
- To collect a non-potable sample from a river, lake or reservoir, hold the container below the water surface, then remove the cap. As an alternative, remove the cap and push the container, mouth down, below the water surface to prevent the collection of surface scum. Put the mouth of the container into the current. Fully fill the container below the water surface.
- Collect a minimum of 100 mL of sample. Keep a minimum of 2.5 cm (1 inch) of air space in the container.
- Write the sample information on the container and start the analysis as soon as possible.
- If immediate analysis is not possible, keep the sample at or below 10 °C (50 °F) for a maximum of 8 hours. Do not let the sample freeze.

Sample volumes

Use a sample volume that is applicable to the sample type. For samples with a low level of bacteria such as finished, potable water, use 100 mL of sample. Use less sample for non-potable water or water that contains more bacteria.

When the approximate bacteria level is unknown, analyze three different sample volumes. Use the results from the sample volume that shows approximately 20 to 200 colonies for each membrane filter.

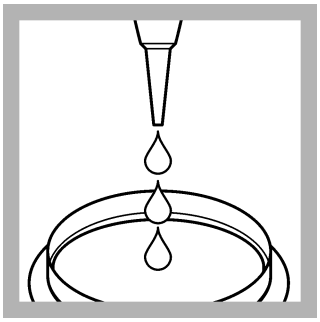
When the sample volume is less than 20 mL (diluted or undiluted), add 10 mL of sterile buffered dilution water to the filter funnel before the vacuum is applied. The additional dilution water helps to apply the bacteria equally across the membrane filter.

Sample dilution

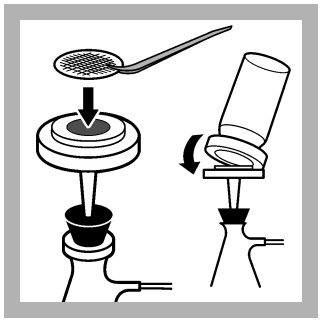
Dilute samples that contain a high level of bacteria so that approximately 20 to 200 bacteria colonies grow on the membrane filter. Use the steps that follow to make serial dilutions of the sample.

1. Wash hands thoroughly with soap and water.
2. Invert the sample container for 30 seconds (approximately 25 times).
3. Open a bottle of sterile buffered dilution water.
4. Use a sterile pipet to add 11 mL of sample into the dilution water bottle.
5. Put the cap on the dilution water bottle and invert for 30 seconds (25 times). This is a 10x dilution (sample is diluted by a factor of 10).
6. Add 11 mL of the 10-fold dilution to another dilution bottle (100x dilution). Mix well.
7. Add 11 mL of the 100-fold dilution to the third bottle (1000x dilution). Mix well.
8. If necessary, continue to dilute the sample.

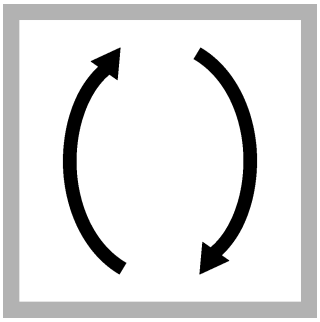
Membrane filtration test procedure



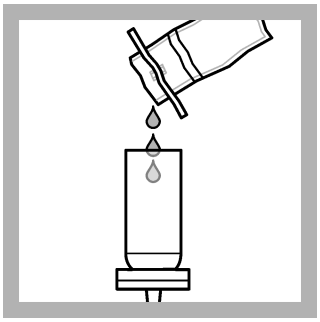
1. Invert one m-ColiBlue24 broth ampule 2 to 3 times. Open the ampule. Lift the lid of a petri dish and carefully pour the contents equally on the absorbent pad.



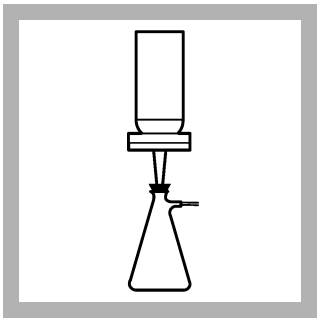
2. Set up the membrane filtration apparatus. Use a sterile forceps to put a membrane filter in the assembly. Make sure that the grid side is up.



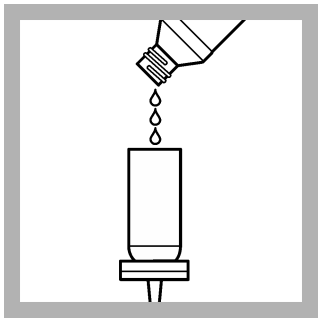
3. Invert the sample or the diluted sample for 30 seconds (25 times) to make sure that the sample is mixed well.



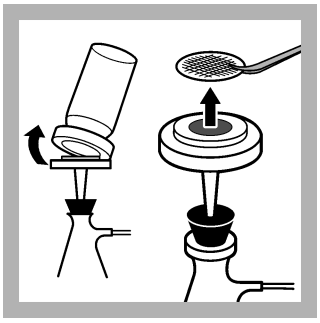
4. Pour or use a pipet to add the sample into the funnel. If the volume is less than 20 mL, add 10 mL of sterile buffered dilution water to the funnel.



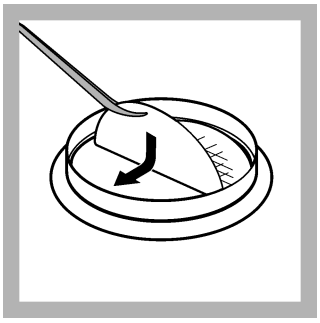
5. Apply the vacuum until the funnel is empty. Stop the vacuum.



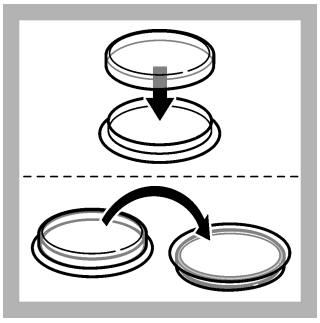
6. Rinse the funnel with 20 to 30-mL of sterile buffered dilution water. Apply the vacuum. Rinse the funnel two more times.



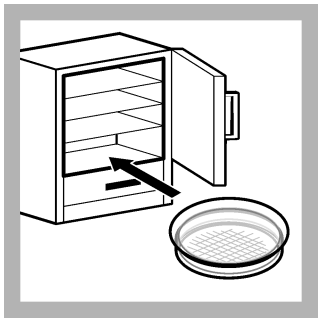
7. Stop the vacuum when the funnel is empty. Remove the funnel from the filter assembly. Use sterile forceps to lift the membrane filter.



8. Put the membrane filter on the absorbent pad. Let the membrane filter bend and fall equally across the absorbent pad to make sure that air bubbles are not caught below the filter.



9. Put the lid on the petri dish and invert the petri dish.



10. Incubate the inverted petri dish at 35 ± 0.5 °C (95 ± 0.9 °F) for 24 hours.

11. Remove the petri dish from the incubator. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter. Refer to Interpret and report the coliform results on page 5.

Optional testing of red colonies

The m-ColiBlue24 Broth is made so that coliforms other than E. coli form red colonies. The percentage of red colonies that are false positives (non-coliforms) is equivalent to the

percentage of sheen colonies grown on m-Endo Broth that are false positives (non-coliforms). So, a confirmation procedure is not necessary.

Some varieties of the non-coliform bacteria *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. can form on m-ColiBlue24 Broth and form red colonies. Use the oxidase method to quickly identify between these bacteria and total coliforms. *Pseudomonas*, *Vibrio*, and *Aeromonas* spp. are oxidase-positive. Total coliforms and *Escherichia coli* are oxidase-negative. If the sample has high levels of interfering bacteria, do an oxidase method to identify which of the red colonies are total coliforms.

Two oxidase methods follow. Count the red and blue colonies on the m-ColiBlue24 Broth membrane filter before the oxidase method is started.

Oxidase method 1

Use this method to easily and quickly analyze membrane filters that have numerous colonies. Use this method after 24 hours of incubation on m-ColiBlue24 Broth.

Research[1] shows that the oxidase method cannot be done on media where acidification of the media occurs during bacterial growth. The m-ColiBlue24 Broth is made so that acidification of the medium does not occur. As a result, the method can analyze many colonies at the same time for their oxidase reaction.

1. Remove the lid from the petri dish.
2. Invert the lid.
3. Put the lid on the bench top.
4. Use a dropper to put 0.5 mL of Difco SpotTest Oxidase Reagent in the center of the inverted lid.
5. Use sterile forceps to move the membrane filter from the pad in the petri dish to the inverted lid.

   After 10 to 15 seconds, the filter absorbs the Difco SpotTest Oxidase Reagent. The oxidase-positive colonies change from red to purple. This purple color shows in the colony or adjacent to the colony. Oxidase-negative colonies stay red.
6. After the initial 10 to 15 second reaction time, count the red colonies that are purple in 30 seconds or less. Use a 10 to 15x microscope to count the number of bacteria colonies on the membrane filter.

   For easy colony counting, put a spare lid on the petri dish lid. Use a felt-tip pen to put a mark on each purple colony. After 30 seconds, count the marks.

   Note: *The red oxidase-negative colonies start to change to a purple color after 30 seconds after the initial 10 to 15 second reaction time.*

Bacteria are not killed with this method, so colonies can be selected for streaking and for more testing.

Colonies that are blue after the initial 24-hour incubation are almost always *E. coli*. So, confirmation with the oxidase method is not necessary.

Oxidase method 2

This method is the official oxidase test 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 on Tryptic Soy Agar.
2. Incubate the Tryptic Soy Agar plates at 35 °C (95 °F) for 18 to 24 hours or until the isolated colonies form.

[1] 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.

3. Soak a piece of filter paper with Difco SpotTest Oxidase Reagent. This reagent has a stabilized solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride.

   Note: *As an alternative, use a dropper to add oxidase reagent directly on colonies on Tryptic Soy Agar. Oxidase-positive colonies change from pink to purple.*

4. Use a sterile nichrome inoculating needle to move 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 cause false-positive results. Wooden applicator sticks work well.*

   Oxidase-negative colonies will not react with the reagent, but oxidase-positive colonies cause the reagent to change to dark purple within 10 seconds.

5. Count the oxidase-negative colonies as total coliform bacteria.

Interpret and report the coliform results

Report the coliform density as the number of colonies in 100 mL of sample. For total coliforms, use a sample volume that gives 20–80 coliform colonies on the membrane filter. For fecal coliforms, use a sample volume that gives 20–60 fecal coliform colonies on the membrane filter.

If there are more than 200 colonies, dilute the sample and use the diluted sample in the test procedure. Use the sample volume before dilution in the coliform density determination.

1. Use the microscope to look at the colonies on the membrane filter. Count the number of isolated coliform colonies.
2. Determine the coliform density as follows:

Membrane filter(s)	Coliform density determination
One membrane filter	Coliform colonies in 100 mL = Coliform colonies counted ÷ mL sample × 100 *Example: 50 coliform colonies were counted. The sample volume was 20 mL. The coliform density is 50 ÷ 20 mL × 100 = 250 coliforms in 100 mL of sample.*
Multiple filters, dilutions or duplicates for each sample	Average coliform colonies in 100 mL = Sum of coliform colonies in all samples ÷ sum of mL sample × 100 *Example: Two 50-mL samples gave 5 colonies on one filter and 9 colonies on another filter. The coliform density is (5 + 9) ÷ (50 + 50) × 100 = 14 coliforms in 100 mL of sample.*

3. If colonies are not isolated or if there are more than 200 colonies of all types:
   a. Report the results as "Confluent growth with or without coliforms" when the bacteria grows together across some or all of the membrane filter.
   b. Do the test procedure again with half the sample volume. If the total number of colonies (coliforms plus non-coliforms) is more than 200 for each membrane or the colonies are not isolated, report the results as "Too numerous to count" (TNTC).
   c. Do the test procedure again with a dilution that gives approximately 50 coliform colonies and not more than 200 colonies of all types.

Controls for coliform bacteria tests

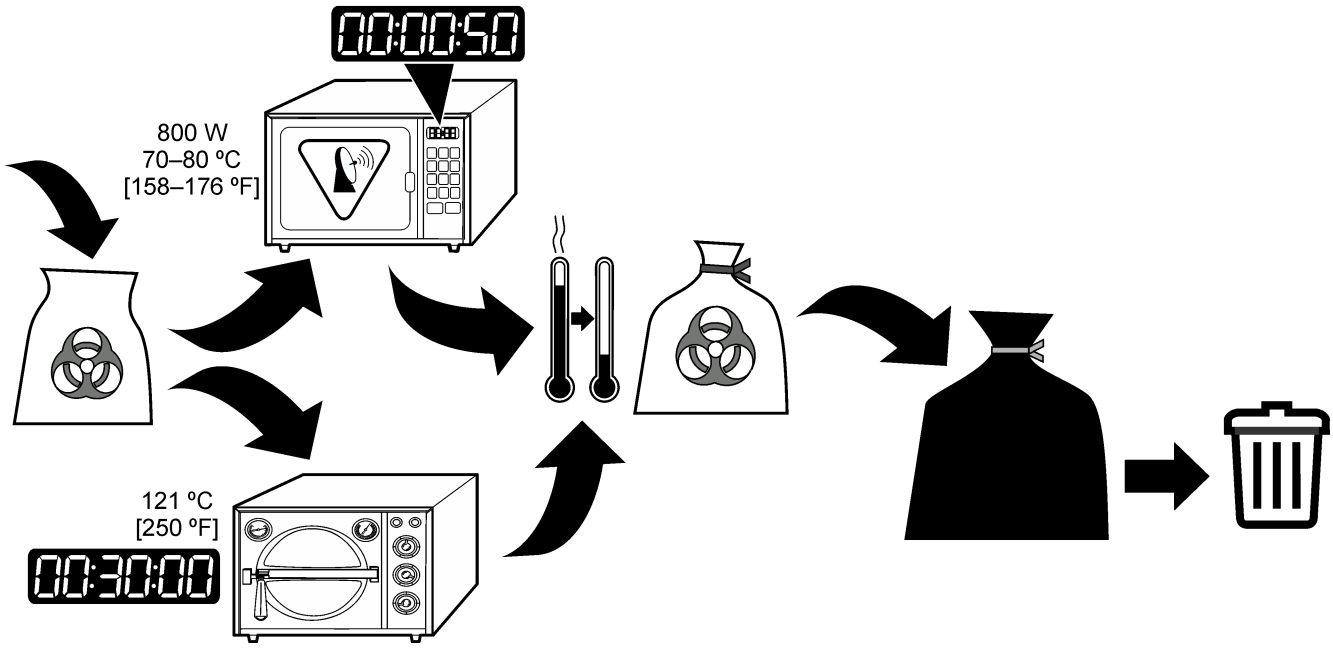
Positive and negative controls validate that the test gives a positive result when coliform bacteria are in the sample and a negative result when coliform bacteria are not in the sample. *Pseudomonas aeruginosa* is recommended as a negative control and *Escherichia coli* is recommended as a positive control.

Bacteria disposal

Make sure to kill the cultured bacteria before disposal. Refer to Figure 1 and the information that follows.

- Microwave—Add 1–2 mL of hypochlorite (bleach) solution to each test container. If a container has a lid, do not close it too tightly. Put the container in the microwave at 70–80 °C (158–176 °F) for 50 seconds. Wait 10 to 15 minutes. Pour the liquid down the drain.
- Autoclave—Put the used test containers in a contaminated items bag or biohazard bag to prevent leaks. Do not seal the bag. Put the bag in the autoclave at 121 °C (250 °F) for 30 minutes at 1.0 bar (15 psi) of pressure. When the bag is cool, seal it and put it into a garbage bag. Make sure to tie the garbage bag tightly.

Figure 1 Bacteria disposal



Summary of method

The m-ColiBlue24 Broth can be used to analyze drinking water, bottled water, beverages, surface water, well water, groundwater, waste water, recreational waters and process water for ultrapure, chemical processing and pharmaceutical applications.

Count all of the red and blue colonies as total coliforms. Count all of the blue colonies as *E. coli*. Blue colonies can be blue to purple.

Note: *Sometimes only the center of a colony shows color. Count a colony with any red color as red. Count a colony with any blue as a blue colony. Red colonies can be different in color intensity.*

The membrane filtration procedure is used for samples that are low in turbidity and have low bacteria counts. The sample is poured through a membrane filter. The bacteria in the sample stays on the membrane filter. The membrane filter is moved to a petri dish that contains a nutritional broth or agar. During incubation, the bacteria grow and form colonies on the membrane filter. After incubation, the filter is examined with a microscope for bacteria colonies.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
m-ColiBlue24® broth ampules, glass	1	20/pkg	2608420
m-ColiBlue24® broth ampules, plastic	1	50/pkg	2608450

Consumables and replacement items (continued)

Description	Quantity/test	Unit	Item no.
m-ColiBlue24®, prepared agar plates	1	15/pkg	2805215
Dilution water, buffered, 99 mL, sterile[2]	1	25/pkg	1430598

Required apparatus

Description	Unit	Item no.
Ampule breaker, PourRite™	each	2484600
Membrane filter holder, magnetic, 300-mL funnel	each	1352900
Filter pump, aspirator	each	213100
Flask, filtering, glass, 1000 mL	each	54653
Forceps, stainless steel	each	2141100
Membrane filter, 0.45 micron, 47 mm diameter, sterile	200/pkg	1353001
Membrane filter, 0.45 micron, 47 mm diameter, sterile EO (ethylene oxide)	150/pkg	2936100
Microscope, compound	each	2947050
Petri dish with absorbent pad, for 47-mm membrane filters, sterile	100/pkg	1471799
Petri dish with absorbent pad, for 47-mm membrane filters, sterile EO (ethylene oxide)	150/pkg	25248000
Stopper, rubber, size 8, for filtration assembly	6/pkg	211908
Pipet, TenSette®, 1.0–10.0 mL	each	1970010
Pipet tips, TenSette, 1.0–10.0 mL, sterile, individually wrapped	50/pkg	2558996
Tubing, rubber, 7.9 mm (5/16-in.) inside diameter	3.66 m (12 ft)	56019

Incubators

Description	Unit	Item no.
Laboratory incubator, culture, 110 VAC	each	2619200
Laboratory incubator, culture, 230 VAC	each	2619202
Portable incubator with 12 VDC power socket	each	2569900
AC power supply for portable incubator, 110–240 VAC	each	2968100
Battery pack, rechargeable, for portable incubator 12 VDC	each	2580300
Portable incubator rack, general purpose/petri dish	each	2580502

Sample collection

Description	Unit	Item no.
Sampling bags, Whirl-Pak® with dechlorinating reagent, 177 mL	100/pkg	2075333
Sampling bags, Whirl-Pak without dechlorinating reagent, 207 mL	100/pkg	2233199
Sampling bottles, sterilized, with dechlorinating agent, 100-mL sample	100/pkg	8888006
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	12/pkg	2495012
Sampling bottles, sterilized, without dechlorinating reagent, 100-mL sample	50/pkg	2495050
Sample transport kit, includes 100 sample bags with dechlorinating agent, refrigerant pack, rack and 9-L cooler	each	2568700

[2] Buffered dilution water is prepared with magnesium chloride and potassium dihydrogen phosphate.

Optional reagents and apparatus

Description	Unit	Item no.
m-ColiBlue24® Broth, glass bottle	100 mL	2608442
Disposable filter funnels with membrane filters, sterile	150/pkg	2586300
Pipet, serological, 10–11 mL, sterile, disposable	25/pkg	209798
Pipet, serological, 2 mL, sterile, glass	35/pkg	2093136
Pipet filler, safety bulb	each	1465100
Support base for disposable filter funnels	each	2586201
Vacuum pump, hand-operated	each	1428300

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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° 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° 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° C or by heating in an oven for at least 1 hour at 170° 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° 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° 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-

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)

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:

North/South America: 1 207 556 4496/1 800 321 0207

Europe: 00800 4339 9111

UK: +44 (0) 1638 676800

China: +86 21 61279528

Japan: 03 5301 6800

Australia: 1300 443 399

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*.

*Colilert, Defined Substrate Technology, DST and Quanti-Tray are trademarks or registered trademarks of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries. Quanti-Cult is a trademark or registered trademark of Remel Inc.
Patent information: idexx.com/patents.
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Wastewater Lab Class Bacteriological Tests

Test	Sample Source	Sample Volume	Media	# of Colonies		Dilution factor	per 100 mL
Blank	Dilution Water	100 mL	mColiBlue				
E. coli	River water		mColiBlue				
E. coli	River water		mColiBlue				
E. coli	River water		mColiBlue				
Blank	Dilution Water	100 mL	mColiBlue				

				Small wells	Large wells	Dilution factor	MPN
Colilert	River water	100 mL	Colilert QuantiTray				
		10:100 (1:10)				10	
		1:100				100	

				Small wells	Large wells	Dilution factor	MPN
Colilert	River water	100 mL	Colilert QuantiTray				
		10:100 (1:10)				10	
		1:100				100	

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:

$$\frac{110{,}000 + 100{,}000}{2} = 105{,}000\text{ mL}$$

<u>If All MF Counts are Below the Lower Limit</u>

<u>Select the Most Nearly Acceptable Count</u>

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

<u>If Counts from All Membranes are Zero</u>

<u>Calculate Using Count from Largest Filtration Volume</u>

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:

$$\frac{110}{0.01} \times 100 = 1{,}100{,}000/100 \text{ mL}$$ 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.

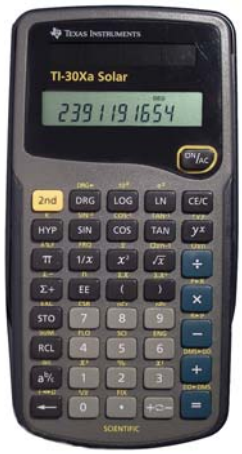
C. If there are any individual samples reported as >, drop the > signs when calculating the geometric mean. However, report the geometric mean as a > values.

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.

- 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.

<u>Method Performance Characteristics</u> (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.

m-ColiBlue24 is a registered trademark and patented product of Hach Company, US Patents 5,650,290 and 5,849,515

Section 9
QA/QC

2017 Updates for 40 CFR 136 and QA/QC Program

Wastewater Laboratory Class

1

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

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

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)

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

Quality Assurance

- QA refers to a total program for ensuring the reliability of data
  - A plan for lab operations that specifies the measures used to produce good data
  - SM: A lab operations program that specifies the measures required to produce defensible data with known precision and accuracy

Quality Assurance Programs

- 2 Primary Functions:
  1. Continually monitor the reliability (accuracy and precision) of the results reported
     - "How good (accurate and precise) are the results obtained?"
     - The *determination* of quality
  2. Control of quality – to meet the program requirements for reliability

Quality Assurance Programs

- Defined in a QA Manual, written procedures, work instructions, and records
- Should include:
  1. A policy that defines the statistical level of confidence used to express data precision and bias
  2. Method Detection Levels (MDLs)
  3. Minimum Reporting Limits (MRLs)
  4. All QA policies and QC processes to ensure and demonstrate the lab's competence and ensure and document the quality of its data

Quality Control

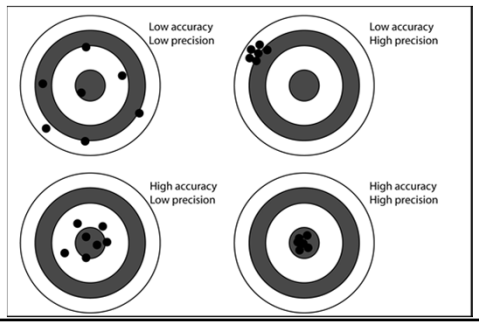
- QC is a function of QA
  - The routine application of procedures for controlling the accuracy and precision of the measurement process
  - Includes:
    - Proper calibration of instruments
    - Use of the appropriate analytical procedures
    - The use of analytical grade reagents

Why are we doing this?

- A QA/QC program consists of procedures that ensure the precision and accuracy of tests performed on a daily basis.
- *Precision* – repeatability; being able to get the same results time after time
  - Shooting at a target and hitting the same spot repeatedly
- *Accuracy* – closeness of test results to the correct (known) value
  - Shooting at a target and hitting bull's eye

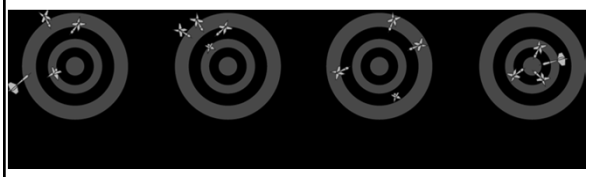
10

Accuracy vs. Precision



11

Which archer has accuracy? Which has precision?



12

NPDES Permit

- Section 1.2.3 Test Procedures
  - b. Unless otherwise noted in the permit, all pollutant parameters shall be determined according to methods prescribed in Title 40, Code of Federal Regulations, Part 136, as amended, promulgated pursuant to Section 304 (h) of the Act.
- Section 2.1.4 Proper O&M
  - a. ….proper O&M also includes …. appropriate quality assurance procedures.

13

You Have Heard it All Before

- More Rules
- More Testing
- More Paperwork…
- But everything we do is regulated

12 Steps of QA:

- Applies to Permit Required Testing
- Not Process Control Testing
- Avoid it by using a Commercial Lab for these tests
  - Facility should still have SOPs for Sampling and Shipping
- It is not as bad as we once thought

14

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

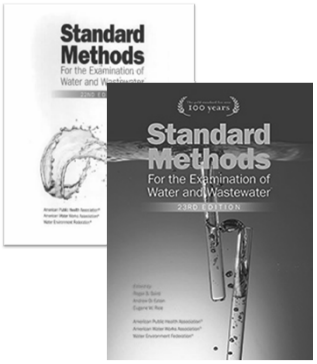
15

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

16

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

17

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

18

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

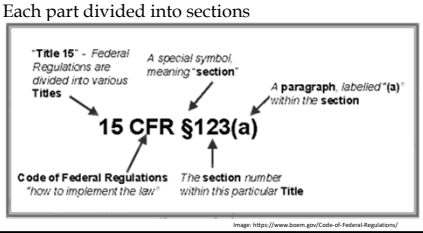


Image: https://www.boem.gov/Code-of-Federal-Regulations/

19

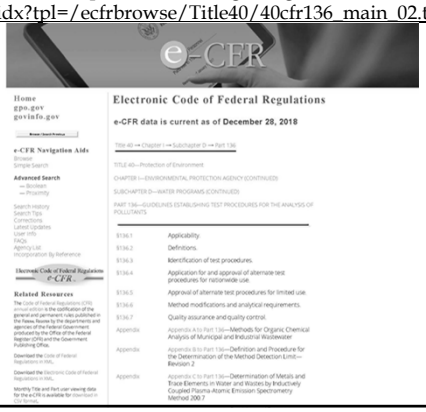
Code of Federal Regulations (CFR)

- 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!

20

- Online: https://www.ecfr.gov/cgi-bin/text-idx?tpl=/ecfrbrowse/Title40/40cfr136_main_02.tpl



21

40 CFR 136 Updates

2012

- Standard Methods approved by date not Edition
- Section 136.7 Quality Assurance and Quality Control

2017

- Definition and Procedure for the Determination of the Method Detection Limit—Revision 2

Federal Register May 18, 2012

22

Method Updates in Part 136

- **Ammonia SM4500-NH_3**-1997 updated to 2011
- **BOD5 SM5210 B**-2001 updated to 2011
- **Fecal coliform SM9222 D** -1997 updated to 2006.
- **Nitrate-N SM4500-NO_3 D** -2000 updated to 2011 (electrode)
- **Nitrite-N SM4500-NO_2 B** -2000 updated to 2011
- **Oxygen SM4500-O G** -2001 updated to 2011
- **pH SM4500-H^+ B** -2000 updated to 2011
- **Temperature SM2550B** -2000 updated to 2010
- **Total Phosphorus SM4500-P** -1999 updated to 2011
- **Total Residual Chlorine SM4500-Cl G** -2000 updated to 2011
- **TSS SM2540D** -1997 updated to 2011

23

40 CFR 136 (03-12-2007)

TABLE IB.—LIST OF APPROVED INORGANIC TEST PROCEDURES

Parameter	Methodology[58]	Reference (method number or page)				
		EPA [36, 52]	Standard methods (18th, 19th)	Standard methods (20th)	Standard methods online	ASTM
1. Acidity, as $CaCO_3$, mg/L	Electrometric endpoint or phenolphthalein endpoint.		2310 B(4a)	2310 B(4a)	2310 B(4a)–97	D1067–92, 02
2. Alkalinity, as $CaCO_3$, mg/L	Electrometric or Colorimetric titration to pH 4.5, manual, or automatic	310.2 (Rev. 1974)[1].	2320 B	2320 B	2320 B–97	D1067–92, 02
3. Aluminum—Total,[4] mg/L	Digestion[4] followed by:					
	AA direct aspiration[34]		3111 D		3111 D–99	
	AA furnace		3113 B		3113 B–99.	
	STGFAA	200.9, Rev. 2.2 (1994).				
	ICP/AES [35]	200.7, Rev. 4.4 (1994).	3120 B	3120 B	3120 B–99	
	ICP/MS	200.8, Rev. 5.4 (1994).				D5673–03
	Direct Current Plasma (DCP)[36]					D4190–94, 99
	Colorimetric (Eriochromine cyanine R)		3500–Al D	3500–Al B	3500–Al B–01.	
	Manual, distillation (at pH 9.5)[4] followed by:	350.1, Rev. 2.0 (1993).	4500–NH $_3$	4500–NH_3 B	4500–NH_3 B–97	
	Nesslerization		4500–NH_3 C (18th only).			D1426–98, 03 (A)
	Titration		4500–NH_3 C (19th) and 4500–NH_3 E (18th).	4500–NH_3 C	4500–NH_3 C–97.	
	Electrode		4500–NH_3 D or E (19th) and 4500–NH_3 F or G (18th).	4500–NH_3 D or E	4500–NH_3 D or E–97.	D1426–98, 03 (B).
	Automated phenate, or	350.1[40], Rev. 2.0 (1993).	4500–NH_3 G (19th) and 4500–NH_3 H (18th).	4500–NH_3 G	4500–NH_3 G–97	
	Automated electrode					
	Ion Chromatography					D6919–03.

40 CFR 136 (05-21-2012)

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES

Parameter	Methodology[58]	EPA[52]	Standard methods	ASTM	USGS/AOAC/Other
1. Acidity, as $CaCO_3$, mg/L.	Electrometric endpoint or phenolphthalein end-point.		2310 B–1997	D1067–06	I–1020–85.[2]
2. Alkalinity, as $CaCO_3$, mg/L.	Electrometric or Colorimetric titration to pH 4.5, Manual.		2320 B–1997	D1067–06	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[35]		3111 D–1999 or 3111 E–1999, 3113 B–2004.		I–3051–85.[2]
	AA furnace				
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[69] 200.7, Rev. 4.4 (1994).	3120 B–1999	D1976–07	I–4471–97.[50]
	ICP/MS	200.8, Rev. 5.4 (1994).	3125 B–2009	D5673–05	993.14,[3] I–4471–97.[50]
	Direct Current Plasma (DCP)[36].			D4190–08	See footnote.[34]
	Colorimetric (Eriochrome cyanine R).		3500–Al B–2001.		
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–1997		973.49[3].
	Nesslerization			D1426–08 (A)	973.49[3], I–3520–85.[2]
	Titration		4500–NH_3 C–1997.		
	Electrode		4500–NH_3 D–1997 or E–1997.	D1426–08 (B).	
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction based methods.		4500–NH_3 F–1997		See footnote.[60]
	Automated phenate,	350.1,[30] Rev. 2.0	4500–NH_3 G–1997		I–4523–85.[2]

25

40 CFR 136 (Eff 09-27-2017)

Federal Register / Vol. 82, No. 165 / Monday, August 28, 2017 / Rules and Regulations 40849

[36] On a monthly basis, at least ten blue colonies from the medium 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.

TABLE IB—LIST OF APPROVED INORGANIC TEST PROCEDURES

Parameter	Methodology[58]	EPA[52]	Standard methods	ASTM	USGS/AOAC/other
1. Acidity, as $CaCO_3$, mg/L.	Electrometric endpoint or phenolphthalein end-point.		2310 B–2011	D1067–11	I–1020–85.[2]
2. Alkalinity, as $CaCO_3$, mg/L.	Electrometric or Colorimetric titration to pH 4.5, Manual.		2320 B–2011	D1067–11	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[35]		3111 D–2011 or 3111 E–2011. 3113 B–2010.		I–3051–85.[2]
	AA furnace				
	STGFAA	200.9, Rev. 2.2 (1994).			
	ICP/AES[36]	200.5, Rev 4.2 (2003);[69] 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	D5673–10	993.14,[3] I–4471–97.[50]
	Direct Current Plasma (DCP)[36].			D4190–08	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			D1426–08 (A)	973.49.[3] I–3520–85.[2]
	Titration		4500–NH_3 C–2011.		
	Electrode		4500–NH_3 D–2011 or E–2011.	D1426–08 (B).	
	Manual phenate, salicylate, or other substituted phenols in Berthelot reaction based methods.		4500–NH_3 F–2011		See footnote.[60]
	Automated phenate, sa-	350.1,[30] Rev. 2.0 (1993)	4500–NH_3 G–2011,		I–4523–85.[2]

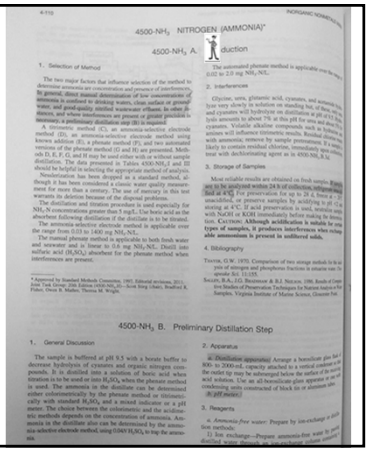
26

Standard Methods Changes

- More recent revision of a Standard Method
- Noted in on-line version
- Located at the end of section "A" of each Standard Method in hard copy
- Many new revisions of Standard Methods added

27

- Where's Waldo?

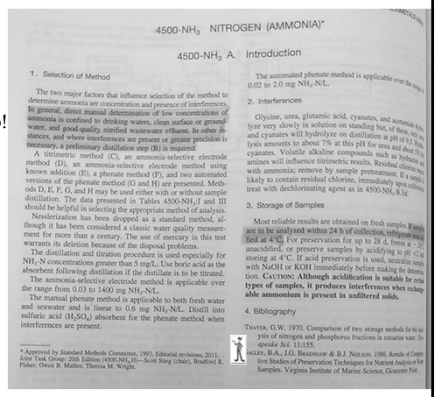


4500-NH_3 NITROGEN (AMMONIA)*

4500-NH_3 A. Introduction

4500-NH_3 B. Preliminary Distillation Step

- There's Waldo!



4500-NH_3 NITROGEN (AMMONIA)*

4500-NH_3 A. Introduction

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…

30

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**

31

12 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. Unwritten 13th Step – SOP – Standard Operating Procedures need to be written and followed for all lab sampling and analyses

32

Not all of these items apply to all tests, there are many exceptions!

Can you defend what you do?

- How do you interpret your Permit language or the Rule?
- Can you defend that interpretation, will a judge or jury support you?
- What do Regulators say and what is written?
  - Is it clear?
  - Don't be afraid to ask Why?
  - Don't be afraid to ask for directives in writing.

33

What You Are Already Doing

- Most Labs are already doing lots of QA/QC stuff
- Write down what you do….SOP
- Summarize QC Data
  - Table Form
  - Average, Max, Min.
  - Control Charts

34

Demonstration of Capability

- DOC
- Standard Methods 1020.B.1
  - As a minimum, include a reagent blank and at least 4 LFBs at a concentration between 10 times the MDL and the midpoint of a calibration curve.
- What tests does this apply to?
  - Ammonia, BOD/CBOD, Chlorine, pH, DO, Total Phosphorus, TSS

35

Demonstration of Capability

- Analyst should prepare the standard*
  - Example: Analyst can make 1 L of 1.0 mg/L by diluting down from 100 mg/L or 1000 mg/L and then pour up 4-100 mL aliquots
- Optional: use "blind" samples (samples with a concentration unknown to them) prepared by someone else
  - Preferred by some regulators

36

Demonstration of Capability

- How often?
  - At least once for each analyst.
    - Recommended yearly for backup analyst who does not perform tests frequently
  - Each analyst should have a file kept on their training within and for the lab.
  - Something to keep along with these records is a signed form (documentation) that analyst has read and understands all appropriate SOPs and Methods
    - Analyst could sign off on individual SOPs as part of the DOC process

37

Demonstration of Capability

- **2014 Update**
  - **DMRQA's were removed as acceptable DOC**
    - **Analysts have had plenty of time, there should be at least 4 standards that have been analyzed and within limits to demonstrate capability.**
    - **Discharge Monitoring Report – Quality Assurance Study Program**

38

Method Detection Limit (MDL)

- New definition of the MDL is: "The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results."

39

What is an MDL study?

- It is a calculation that statistically gives the lowest concentration that a lab/facility can "see", that is detect an analyte *with 99% confidence that it is distinguishable from a method blank*
- Not practical for some analyses (Ex: BOD_5)
- As detector sensitivity improves, the background contamination of the lab, consumable supplies, and equipment can be more important in determining the detection limit than the sensitivity of the instrument
- The result is the MDL (method detection level)
- **2014 Update – this is your reporting limit**

40

Method Detection Limit

- Estimation of the detection limit for variety of physical and chemical methods
- What tests does this apply to (for most municipal WWTPs)?
  - Ammonia, Chlorine and Total Phosphorus
    - There may be others
- How often?
  - Annually – but at least every 13 months
- Ongoing data collection and MDL validation is now required quarterly

41

Method Detection Limit

- *The implied requirement that the calculated MDL must be no less than one-tenth the spike value in Revision 1.11 of the MDL procedure* ***has been eliminated in Revision 2, so laboratories will not have to redo the MDL procedure if the calculated MDL is lower than expected.***
- Latest update effective September 27, 2017
  - Required to calculate the MDL using spiked samples and blank samples for multiple instruments (MDL samples are a reference matrix, such as reagent water, spiked with a known and consistent quantity of the analyte.)
  - Required to check their MDL values once a quarter instead of just once per year
  - Recalculation of the MDL is required annually (at least once every thirteen months)

42

Method Detection Limit

- Standard Methods 1020.B.4
  - As a starting point for selecting the concentration to use when determining the MDL, use an estimate of five times the estimated true detection level
  - Prepare and analyze at least seven portions of this solution over a 3-day period to ensure the MDL determination is more representative of routine measurements as performed in the laboratory
  - Ideally use pooled data from several analysts rather than data from one analyst

43

Method Detection Limit

- Determine Initial MDL:
  - Process a minimum of 7 spiked samples and 7 method blank samples
  - Must be prepared in at least 3 batches on 3 separate dates and analyzed on 3 separate dates (Preparation and analysis may be on the same day)
  - If multiple instruments will be assigned the same MDL, sample analyses must be distributed across all of the instruments
    - Minimum two spiked and two blank samples per instrument, analyzed on different dates
  - Compute MDL_s – value based on spiked samples
  - Compute MDL_b – value based on method blank samples
  - Initial MDL is the greater of MDL_s or MDL_b

44

How MDL Studies are Performed

- Ongoing Data Collection:
  - Procedure uses method blanks, as well as spiked samples to calculate MDL
  - MDL_s: value calculated from the spiked samples
    - Min 2 spiked samples on each instrument
    - Min 8/year (2/quarter)
  - MDL_b: value calculated from the method blanks
    - No additional sample required, use your routine method blanks

45

How MDL Studies are Performed

- Annual Verification:
- At least once every 13 months, re-calculate MDL_s and MDL_b
  - Ideally, use all method blank results from last 24 months for MDL_b calculation
    - An option to use less data is included in the rule
- The verified MDL = the higher of the two numbers
  - Existing MDL may be left unchanged if specific criteria are met

46

MDL Calculations

- Samples used to calculate MDL should be performed throughout the year, not on a single date
  - Samples analyzed every quarter, but calculation performed only once a year
- Lab has the option to pool data from multiple instruments to calculate one MDL that represents multiple instruments

47

Where do I find the MDL Calculator?

1. Go to Fleming Training Center's website
   - https://www.tn.gov/environment/program-areas/wr-water-resources/fleming-training-center.html
2. On left side, click on "Course Books and Reference Material"
3. In the drop-down menu, click on "Waste Water Information"
4. Click on "Method Update Rule – Method Detection Level Math 2018"

Laboratory Reagent Blank

- LRB
- Also known as Method Blank
- Standard Methods 1020.B.5
  - A reagent blank (method blank) consists of reagent water and all reagents that normally are in contact with a sample during the entire analytical procedure (distillation, incubation, etc.)
- What tests does this apply to?
  - Ammonia, BOD/CBOD, Chlorine, Total Phosphorus, TSS
- How often?
  - Depends on method QA/QC

49

Laboratory Fortified Blank

- LFB
- Also known as a "Standard"
- Why is this important?
  - *Accuracy* refers to the closeness of test results to the correct (known) value
- Standard Methods 1020.B.6
  - A laboratory-fortified blank is a reagent water sample to which a known concentration of the analyte of interest has been added
  - Sample batch = 5% basis (see batch size for more information)
  - Use an added concentration of at least 10 times the MDL, or less than or equal to the midpoint of the calibration curve

50

Laboratory Fortified Blank

- Standard Methods 2020.B.2.e – TSS
  - Using stock solutions, prepare fortified concentrations so they are within the calibration curve
- Standard Methods 4020.B.2.e – Ammonia, BOD/CBOD, Chlorine, Phosphorus
  - Calculate percent recovery, plot control charts and determine control limits
  - **More control chart info later**
- What tests does this apply to (for most municipal WWTPs)?
  - Ammonia, BOD/CBOD, Chlorine, Total Phosphorus, TSS
  - There may be others

51

Laboratory Fortified Blank

- How often?
- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
  - *Influent and Effluent are 2 different samples*
  - If a permit stated that 3 analyses per week, we would allow for a LFB to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would allow twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

52

Laboratory Fortified Matrix and Duplicate

- LFM/LFMD
  - Also known as a "Spike" and "Spike duplicate"
  - Also called a Matrix Spike/Matrix Spike Duplicate (MS/MSD)
- Are there interferences in the effluent matrix?
- What tests does this apply to (for most municipal WWTPs)?
  - Ammonia and Total Phosphorus
  - There may be others

53

Laboratory Fortified Matrix and Duplicate

- Standard Methods 1020.B.7
  - A laboratory matrix (LFM) is an additional portion of a sample to which a known amount of the analyte of interest is added before sample preparation
  - The LFM is used to evaluate analyte recovery in a sample
  - Sample batch = 5% basis (At least once a month.)
  - Add a concentration of a known standard less than or equal to the midpoint of the calibration curve
  - Preferably the same concentration as the LFB (laboratory fortified blank)

54

Laboratory Fortified Matrix and Duplicate

- Standard Methods 4020.B.2.g
  - When appropriate for the analyte, include at least one LFM/LFMD … with each batch of 20 samples
  - Add a known concentration of analyte (ideally from a second source) to a randomly selected routine sample Calculate percent recovery and relative percent difference, plot control charts and determine control limits for spikes at different concentrations

55

Laboratory Fortified Matrix and Duplicate

- How often?
- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria:
  - *Influent and Effluent are 2 different samples*
  - If a permit stated that 3 analyses per week, we would allow for a spike to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would allow twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

56

Laboratory Fortified Matrix and Duplicate

- Calculate RPD between Spike and Spike Dup
- **2014 Update – Spike volume should be less than 1% of the volume.**
  - **Example: spike with 1 mL of 1000 mg/L into 100 mL sample will equal a 10 mg/L increase in ammonia concentration.**

57

Duplicate

- "Dup"
- Why is this important?
  - *Precision* refers to the closeness of two or more measurements to each other
- Not a part of the 12 Steps of QA- an addition from the State of TN or an approved method
- What tests does this apply to?
  - BOD/CBOD, Chlorine, pH, DO, TSS and Settleable Solids

58

Duplicate

- Standard Methods 1020.B.8
  - As a minimum, include one duplicate sample with each sample set or on a 5% basis
- Standard Methods 1020.B.12
  - Calculate the RPD (relative percent difference)
  - Equal to or less than 20% RPD

59

Duplicate – TSS & Settlable Solids

- Standard Methods 2020.B.2.f
  - Include at least one duplicate for each matrix type daily or with each batch of 20 or fewer samples
- Standard Methods 2540.A.2
  - To aid in quality assurance, analyze samples in duplicate.
  - Dry samples to constant weight if possible.
  - This entails multiple drying-cooling-weighing cycles for each determination… more info later in presentation.
- Standard Methods 2540.D.3.c
  - Analyze at least 10% of all samples in duplicate

60

Duplicate – Cl_2, pH and DO

- Standard Methods 4020.B.2.f
  - Randomly select routine samples to be analyzed twice
  - Process duplicate sample independently through the entire sample preparation and analysis
  - Include at least one duplicate for each matrix type daily or with each batch of 20

61

Duplicate – BOD/CBOD

- Standard Methods 5020.B.2.f
  - Randomly select routine samples to be analyzed twice
  - Process duplicate sample independently through the entire sample preparation and analysis
  - Include at least one duplicate for each matrix type daily or with each batch of 20

62

Duplicate

- How often?
- For samples that need to be analyzed on a 5% basis or once for every 20 samples follow these criteria: (10% would be once every 10 samples for TSS)
  - If a permit required 3 analyses per week, we would allow for a dup to be analyzed at least once per month.
    - Pick a date and be consistent, the 1st of every month or the 1st Thursday of every month. Mark your calendar!!
  - If a permit stated 5 analyses per week, we would allow twice a month.
    - Pick a date and be consistent, the 1st and 15th of every month or the 1st and 3rd Thursday of every month. Mark your calendar!!

63

Initial Calibration Verification & Continuing Calibration Verification

- ICV
  - Standard Methods 1020.B.11.b
    - Perform initial calibration using at least three concentrations of standards for linear curves
  - Calibrate meter (DO, pH or ISE) or verify balance, thermometer and colorimeter/spectrophotometer

64

Initial Calibration Verification & Continuing Calibration Verification

- CCV
- Standard Methods 1020.B.11.c
  - Analysts periodically use a calibration standard to confirm that the instrument performance has not changed significantly since initial calibration.
  - Verify calibration by analyzing one standard at a concentration near or at the mid-point of the calibration range.
- Verify the calibration (especially if preset by manufacturer) at beginning of day, after every 10 readings and at the end of the batch
  - Daily (day of)

65

ICV - TSS

- 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 technicians initials
    - Recertify reference weights as specified in the certificate of calibration or at least every 5 years.

66

ICV – Temperature

- Standard Methods 9020.B.4.a
  - Annually, or preferably, semiannually check accuracy of all working temperature sensing devices, such as liquid-in-glass thermometers, thermocouples and temperature-recording instruments at the use temperature against a certified National Institute of Standards and Technology (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
    - State of TN recommends annual verification

67

ICV –Ammonia, BOD/CBOD, Chlorine, pH, DO, Phosphorus

- Standard Methods 4020.B.2.a
  - Calibrate initially with at least one blank and three calibration standards
  - The appropriate linear correlation coefficient for standard concentration-to-instrument response should be greater than or equal to 0.995.
  - The back-calculated and true concentrations should agree within ± 10%.

68

CCV –Ammonia, BOD/CBOD, Chlorine, pH, DO, Phosphorus

- Standard Methods 4020.B.2.b
  - Verify calibration by periodically analyzing a calibration standard and calibration blank during a run – typically after each batch of 10 samples and at the end of the run.
  - For the calibration verification to be valid, check standards must be within 10% of its true value

69

ICV –Probes for Ammonia

- Standard Methods 4500-NH_3 D.4.a.
  - Prepare a series of standard solutions covering the concentrations of 1000, 100, 10, 1 and 0.1 mg NH_3-N/L
- Standard Methods 4500-NH_3 D.4.b.
  - Calibrate from lowest to highest concentration.
  - Wait until the reading has stabilized (at least 2-3 min) before recording millivolts for standards and samples containing ≤ 1 mg NH_3-N/L.
- Standard Methods 4500-NH_3 D.4.c.
  - If the electrode is functioning properly a tenfold change of NH_3-N concentration produces a potential change of about 59 mV.

70

ICV/CCV –Chlorine

- Prepare a set of Chlorine Standards or Potassium Permanganate ($KMnO_4$) standards in accordance with **"Guidance for Secondary Standards use in Calibration"** monthly
- Initial (ICV) – Chlorine Standards or Potassium Permanganate ($KMnO_4$) standards monthly
- Continuing (CCV) – Chlorine Standards, Potassium Permanganate ($KMnO_4$) standards or gel standards daily (day of)

71

ICV/CCV –Chlorine

- Secondary standards (gel standards) are specifically designed to verify the instrument's calibration and to check the instrument's performance
- They are not intended to be used to create calibration curves or to calibrate the instrument
- Because the DPD reagent cannot be mixed with the gel standards, the quality and the reaction time of the reagent cannot be assessed
- For these reasons gel standards cannot take the place of primary standards

72

ICV/CCV – Phosphorus

- ICV/CCV – does not go through digestion
- LFM and LFB – does go through digestion

73

Accuracy Control Charts

- Standard Methods 1020 B.13.a
  - The accuracy chart for QC samples (e.g., reagent blanks, LFBs, calibration check standards and LFMs) is constructed from the average and standard deviation of a specified number of measurements of the analyte of interest.
  - The accuracy chart includes upper and lower warning levels (WL) and upper and lower control levels (CL).
  - Common practice is to use ±2*s* and ±3*s* limits for the WL and CL, respectively, where *s* represents standard deviation.

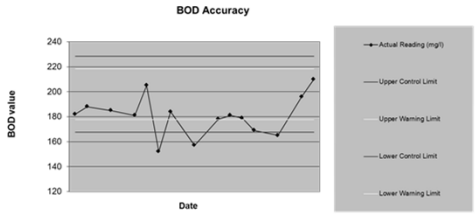
74

BOD Accuracy Control Charts

Date	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
8/25	185	198	-13	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
9/1	157	198	-41	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
9/8	165	198	-33	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

75

BOD Accuracy Control Charts



76

Precision Control Charts

- Standard Methods 1020 B.13.b
  - The precision chart also is constructed on the average and standard deviation of a specified number of measurements (e.g., %RSD [relative standard deviation] or RPD) for a replicate or duplicate analyses of the analyte of interest.

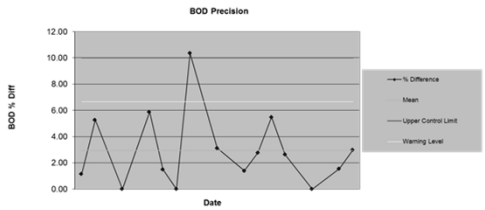
77

BOD Precision Control Charts

Date	#1 (mg/l)	#2 (mg/l)	Avg.	Diff. (Range)	% Diff.	2 X SD	3 X SD	Mean	Max Variat.	2/3 Max Var.
8/22	87	86	86.5	1.0	1.16	5.63	8.45	2.94	10.00	6.67
8/23	78	74	76.0	4.0	5.26	5.63	8.45	2.94	10.00	6.67
8/25	62	62	62.0	0.0	0.00	5.63	8.45	2.94	10.00	6.67
8/27	70	66	68.0	4.0	5.88	5.63	8.45	2.94	10.00	6.67
8/28	67	66	66.5	1.0	1.50	5.63	8.45	2.94	10.00	6.67
8/29	76	76	76.0	0.0	0.00	5.63	8.45	2.94	10.00	6.67
8/30	61	55	58.0	6.0	10.34	5.63	8.45	2.94	10.00	6.67
9/1	65	63	64.0	2.0	3.13	5.63	8.45	2.94	10.00	6.67
9/3	72	71	71.5	1.0	1.40	5.63	8.45	2.94	10.00	6.67
9/4	73	71	72.0	2.0	2.78	5.63	8.45	2.94	10.00	6.67
9/5	75	71	73.0	4.0	5.48	5.63	8.45	2.94	10.00	6.67
9/6	77	75	76.0	2.0	2.63	5.63	8.45	2.94	10.00	6.67
9/8	83	83	83.0	0.0	0.00	5.63	8.45	2.94	10.00	6.67
9/10	65	64	64.5	1.0	1.55	5.63	8.45	2.94	10.00	6.67
9/11	66	68	67.0	2.0	2.99	5.63	8.45	2.94	10.00	6.67
					2.94	mean				
					2.82	St.Dev				

78

BOD Precision Control Charts



79

Control Charts

- **2014 Update** - Create and maintain control charts if you have 20-30 data points within 90 days.
  - If you do not meet the above criteria, follow QC Acceptance Criteria below.
    - Blanks < MDL
    - LFB ± 15%
    - ICV/CCV ± 10%
    - LFM/LFMD ± 20%
    - RPD < 20%
    - Reporting limit = MDL

80

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)

81

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.

82

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

83

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

84

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

85

QC Frequency

- Usually lumped in with the definition of a "batch" and should be in the SOP of some kind

86

Standard Operating Procedure

- Here's that "13th Step"… your SOP
- All procedures must be documented in some type of SOP
- It can be very simple but must provide the information necessary for someone who is not familiar with the test to perform it
  - Step by step instructions on how and where to collect the samples, how to run the test, and how to report values.
- It must include the QC Acceptance Criteria, the definition of a "Batch" and the minimum frequency of QC checks

87

Standard Operating Procedure (SOP)

- Describes the analytical method
- Sufficient detail that someone unfamiliar with the method could perform it and get satisfactory results
- Can include pictures (Ex: where samples are collected)
  - Pictures can be very helpful
- It must include the QC Acceptance Criteria, the definition of a "Batch" and the minimum frequency of QC checks

88

Standard Operating Procedure (SOP)

- Should include :
  - Title of reference
  - Method #
  - Summary
  - Definitions
  - Interferences
  - Safety considerations
  - Equipment and supplies
  - Preservation and storage requirements
  - QC information
  - Etc…

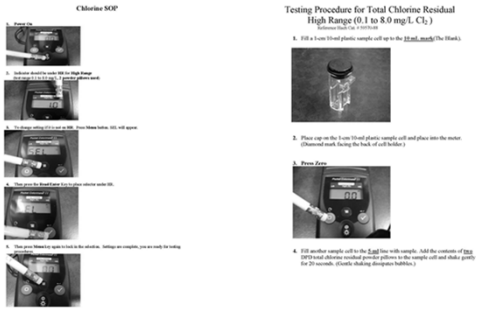
89

Standard Operating Procedure (SOP)

- Annually:
  - Review/Update
    - Make any necessary adjustments
    - Changes to facility?
    - Changes to staff?
  - Document new Revision
  - Training
    - Have all analysts review/read
    - Have analysts sign off that they have done refresher
    - Documentation

90

Standard Operating Procedure



Chlorine SOP

1. **Press On**

2. **Indicator should be under HR for High Range (0.0 mg/L to 8.0 mg/L Cl2 [Chlorine] above need).**

3. To change setting HR is not at HR. Press **Menu** button. HR will appear.

4. Then press the **Read Enter** key to place mister under HR.

5. Then press **Menu** key again to lock in the selection. Settings are complete, you are ready for testing procedure.

Testing Procedure for Total Chlorine Residual High Range (0.1 to 8.0 mg/L Cl_2)

Reference Hach Cat. # 50170-01

1. Fill a 3-cm 10-ml plastic sample cell up to the **10 ml. mark**(The Blank).

2. Place cap on the 3-cm 10-ml plastic sample cell and place into the meter. (Diamond mark facing the back of cell holder.)

3. **Press Zero**

4. Fill another sample cell to the **5 ml** line with sample. Add the contents of **two** DPD total chlorine residual powder pillows to the sample cell and shake gently for 20 seconds. (Gently shaking dissipates bubbles.)

Standard Operating Procedure

5. Place sample cell in meter and cover instrument with cap. Let sample stand 3 to 6 minutes, then **press Read/Enter**.

- What are some key points that are missing from this SOP?

QA/QC for Chlorine

DOC - Demonstration of Capability – Each operator analyzes 4 standards and signs form documenting reading and understanding SOP for specific instrument used.

MDL – Method Detection Limit
Run 7 samples annually at low levels.

Method Blank
Analyze Deionized or distilled water as a sample 2/month.

LFB – Lab Fortified Blank
Analyze a known standard of 0.5 mg/L concentration 2/month.

DUP – Duplicates
Analyze a sample twice (use fresh sample for duplicate) run 2/month.

ICAL/CCV - Initial Calibration and Continuing Calibration Verification:
Prepare potassium permanganate or chlorine standards 1 month. Verify meter daily with secondary standards. (Std Standards)

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

93

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

94

Any Questions?

95

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
Certification Exam
Need To Know Information

Understanding Areas

Process = Addresses the biological, chemical and mechanical actions, which may be occurring in any of the subject areas. It certainly includes all the many biological and chemical actions but it may also include how a piece of machinery or equipment works

Operation = Addresses activities in which an operator has the ability to exercise judgment and take action to affect the processes. Included within this definition is plant design and plant construction.

Maintenance = Includes both routine and emergency maintenance. Included within this definition is troubleshooting and other problem identification activities.

General = Includes any basic knowledge questions which address the subject in a broad nature. (e.g. What is the purpose of pretreatment?

Procedure = Is primarily designed for lab questions but can be used whenever an industry-recognized procedure for accomplishing a task is identified. (e.g. testing reduced pressure backflow preventers)

Data Interpretation = Is used for questions where data is received, analyzed and acted upon

Public Health = Includes any questions relating to the subject's impact on public health. (e.g. Which of the following diseases is transmitted by inadequately treated water?). It includes laws, rules and regulations, quality control and compliance

Math = Includes any applicable math activity associated with the subject area. It includes basic operations, square/cubes, fractions/percents, graphs, tables, formulas, conversion, etc.

Science = Includes any basic science, biology, chemistry and microbiology concept or principle which relates to the subject

Safety = Includes any recommended or required safety practices as well a the "common sense" practices (e.g. What does the beeping sound on the tow motor mean?). It includes identification and description of categories, procedures and violations

Rules = Includes any applicable state or federal rule or reporting which addresses the subject

Grade 1 Wastewater Treatment Operator
Need-To-Know Criteria (Subject Areas)

The following list of categories suggests topics of information which are important to know in order to be a successful and proficient Grade 1 Wastewater Treatment Operator. The list may not be all inclusive, and knowledge of additional topics may be of benefit to the operator.

Category of Information: Processes

Collection Systems
- Types
- Inflow, Infiltration, Exfiltration
- Sources of wastewater
- Pre-treatment
- Pump stations

Flow
- Measurement
- Flow equalization

Screening
- Bar screens (hand-cleaned and mechanically cleaned)
- Static screen
- Microscreen
- Rotating screens

Grinding
- Grinders
- Comminutors
- Barminutors

Grit Removal
- Hand-cleaned grit chambers
- Mechanically-cleaned grit chambers
- Aerated grit chambers
- Vortex

Clarification
- Primary (Rectangular; Circular)
- Secondary

Attached Growth/Fixed Film
- Trickling Filters
  - Standard Rate
  - High Rate
  - Roughing
- Rotating Biological Contactor (RBC)
- Activated Biofilter (ABF)

Lagoons
- Aerobic
- Facultative
- Anaerobic
- Aerated
- Discharging
- Total Containment

Activated Sludge
- Conventional
- Step Feed
- Contact Stabilization
- Extended aeration
- Tapered aeration
- Oxidation ditch
- Types of aeration
- Sequencing Batch Reactor (SBR)
- Membrane Biological Reactor (MBR)

Solids handling
- Sludge conditioning
- Sludge thickening
- Anaerobic digesters
- Aerobic digesters
- Dewatering
- Drying beds
- Land application
- Composting
- 503 Regulations

Disinfection/ Use of Chlorine
- Chlorination
- Dechlorination
- UV
- Other methods

Advanced Treatment
- Nitrogen removal
- Biological

Effluent Disposal
- Discharge
- Direct Use
- Irrigation
- Underground Disposal
- NPDES Permit

Cross- Connection
- Prevention
- Types of devices

Category of Information: Support Systems

Motors
- Single Phase
- Poly Phase
- Variable Speed

Drives
- Coupled
- Direct (Shaft; Gear)
- Speed Reducer (Fixed; Variable)
- Right Angle

Pumps
- Air Lift
- Centrifugal
- Positive Displacement
  - Piston Plunger
  - Progressive Cavity
  - Diaphragm
- Screw
- Turbine
- Metering
- Ejector

Blowers and Compressors
- Centrifugal
- Positive Displacement (Rotary; Piston)

Generators – AC & DC

Engines – Gasoline, Diesel, Gas

Joints
- Flanged
- Compression
- Dresser
- Victualic
- Fused
- Threaded

Valves
- Ball
- Check
- Globe
- Gate
- Plug
- Petcock
- Pressure Control
- Vacuum Relief
- Mud
- Butterfly
- Multiport
- Telescoping
- Sluice Gate
- Air Release
- Foot
- Altitude

Fittings
- Coupling
- Union
- Plug/Caps
- Corporation (Ferrell; Cock)
- Curb Stop
- Special

Measuring/ Control
- Signal Generators
  - Kennison Nozzle
  - Magnetic Flowmeter
  - Parshall Flume
  - Proportional Weir
  - Rectangular Weir
  - Venturi
  - Propeller Meter
  - Ultrasonic
  - Pitot Tube
- Signal Transmitters
  - Electric
  - Pneumatic
  - Hydraulic
  - Mechanical
  - Telemetry
- Signal Receivers
  - Counters
  - Indicators
  - Log Scale Indicators
  - Totalizers
  - Recorders
  - Combination Recorders
- Meters
  - Hydraulic – Rotameter
  - Electrical – Amp
  - Electrical – Watt
  - Electrical – Watt – Hour
  - Electrical – Multi.
  - Electrical – VOM
  - Electrical – Megger
  - Mechanical – RPM
- Alarms
- Controls
  - Pneumatic
  - Float
  - Hydraulic
  - Electrical
  - Telemetry
  - Timers

Chemical Feeders
- Solids
- Liquids
- Evaporators
- Gas
- Slurry

Category of Information: Support Systems (continued)

Odor Control
- Biofilters
- Chemical Additives
- Scrubbers

Rolling Stock
- Service Vehicles
- Trucks
- Lawn Mowers
- Loaders
- Portable Pumps
- Generators

HVAC
- Fans

Safety Equipment
- Personal Protection Gear
- Traffic Control (Warning Devices; Barricades)
- Hazard Detectors
- First Aid/Hygiene

Category of Information: Lab Tests

Routine Tests
- B.O.D.
- Ammonia
- Chlorine Residual
- Coliform (Fecal)
- Dissolved oxygen
- E. coli
- Total Kjedahl nitrogen
- pH
- Phosphorus
- Solids
  - Settleable
  - Suspended
  - Total dissolved
  - Total
  - Volatile

Control Tests
- Centrifuge
- Alkalinity
- C.O.D.
- Nutrients
- Micro exam
- Nitrate - nitrite
- Oil and Grease
- Surfactants
- Temperature
- Total organic carbon
- Turbidity
- Volatile acids
- Settleability

Special Tests:
- Metals
- Chlorinated organics
- Cyanide
- Phenol
- Sulfate - sulfide
- Biomonitoring

Category of Information: General Information

Units of expression
- Define units
- Convert units

Sources and characteristics
- Characterize
- Quality/quantity
- Physical/chemical/biological
- Effects
- Pre-aeration
- Chemical pre-treatment
- Grease control

Electrical (Basic)

Hydraulics (Basic)

Maps/plans – Interpretation and use

WWT Processes
- Sketch sequence
- Describe processes
- Explain why treated
- Goals of WWT (protect public; protect environment)

Grade 2 Wastewater Treatment Operator
Need-To-Know Criteria (Subject Areas)

The following list of categories suggests topics of information which are important to know in order to be a successful and proficient Grade 2 Wastewater Treatment Operator. The list may not be all inclusive, and knowledge of additional topics may be of benefit to the operator.

Category of Information: Processes

Collection Systems
- Types
- Inflow, Infiltration, Exfiltration
- Sources of wastewater
- Pre-treatment
- Pump stations

Flow
- Measurement
- Flow equalization

Screening
- Bar screens (hand-cleaned)
- Bar screens (mechanically cleaned)
- Microscreen

Grinding
- Grinders
- Comminutors
- Barminutors

Grit Removal
- Hand-cleaned grit chambers
- Mechanically-cleaned grit chambers
- Aerated grit chambers
- Vortex

Clarification
- Primary (Rectangular; Circular)
- Secondary
- SBR Clarification

Attached Growth/Fixed Film
- Trickling Filters
    - Standard Rate
    - High Rate
    - Roughing
- Rotating Biological Contactor (RBC)
- Activated Biofilter (ABF)

Lagoons
- Aerobic
- Facultative
- Anaerobic
- Aerated
- Discharging
- Total Containment

Activated Sludge
- Conventional
- Step Feed
- Contact Stabilization
- Extended aeration
- Tapered aeration
- Complete mix
- Pure oxygen
- Oxidation ditch
- Types of aeration
- Sequencing Batch Reactor (SBR)
- Membrane Biological Reactor (MBR)

Solids handling
- Sludge conditioning
- Sludge thickening
- Anaerobic digesters
- Aerobic digesters
- Dewatering
- Incineration
- Drying beds
- Land application
- Composting
- 503 Regulations

Disinfection/ Use of Chlorine
- Chlorination
- Dechlorination
- UV
- Other methods

Advanced Treatment
- Filtration
- Nitrogen removal
- Phosphorus removal
- Biological

Effluent Disposal
- Discharge
- Direct Use
- Irrigation
- Underground Disposal
- NPDES Permit

Cross- Connection
- Prevention
- Types of devices

Category of Information: Support Systems

Motors
- Single Phase
- Poly Phase
- Variable Speed

Drives
- Coupled
- Direct (Shaft; Gear)
- Speed Reducer (Fixed; Variable)
- Right Angle

Pumps
- Air Lift
- Centrifugal
- Positive Displacement
    - Piston Plunger
    - Progressive Cavity
    - Diaphragm
- Screw
- Turbine
- Metering
- Ejector

Blowers and Compressors
- Centrifugal
- Positive Displacement (Rotary; Piston)

Generators – AC & DC

Engines – Gasoline, Diesel & Gas

Joints
- Flanged
- Compression
- Dresser
- Victualic
- Fused
- Threaded

Valves
- Ball
- Check
- Globe
- Gate
- Plug
- Petcock
- Pressure Control
- Vacuum Relief
- Mud
- Butterfly
- Multiport
- Telescoping
- Sluice Gate
- Air Release
- Foot
- Altitude

Fittings
- Coupling
- Union
- Plug/Caps
- Corporation (Ferrell; Cock)
- Curb Stop
- Special

Measuring/ Control
- Signal Generators
    - Kennison Nozzle
    - Magnetic Flowmeter
    - Parshall Flume
    - Proportional Weir
    - Rectangular Weir
    - Venturi
    - Propeller Meter
    - Ultrasonic
    - Pitot Tube
- Signal Transmitters
    - Electric
    - Pneumatic
    - Hydraulic
    - Mechanical
    - Telemetry
- Signal Receivers
    - Counters
    - Indicators
    - Log Scale Indicators
    - Totalizers
    - Recorders
    - Combination Recorders
- Meters
    - Hydraulic – Rotameter
    - Electrical – Amp
    - Electrical – Watt
    - Electrical – Watt – Hour
    - Electrical – Multi.
    - Electrical – VOM
    - Electrical – Megger
    - Mechanical – RPM
- Alarms
- Controls
    - Pneumatic
    - Float
    - Hydraulic
    - Electrical
    - Telemetry
    - Timers

Category of Information: Support Systems (continued)

Chemical Feeders
- Solids
- Liquids
- Evaporators
- Gas
- Slurry

Odor Control
- Biofilters
- Chemical Additives
- Scrubbers

Rolling Stock
- Service Vehicles
- Trucks
- Lawn Mowers
- Loaders
- Portable Pumps
- Generators

HVAC
- Heat Exchangers
- Dehumidifiers
- Fans
- Compressors

Safety Equipment
- Personal Protection Gear
- Traffic Control (Warning Devices; Barricades)
- Hazard Detectors
- First Aid/Hygiene

Category of Information: Lab Tests

Routine Tests
- B.O.D.
- Ammonia
- Chlorine Residual
- Coliform (Fecal)
- Dissolved oxygen
- E. coli
- Total Kjedahl nitrogen
- pH
- Phosphorus
- Solids
  - Settleable
  - Suspended
  - Total dissolved
  - Total
  - Volatile

Control Tests
- Centrifuge
- Alkalinity
- C.O.D.
- Color
- Conductance
- Nutrients
- Micro exam
- Nitrate - nitrite
- Oil and Grease
- Surfactants
- Temperature
- Total organic carbon
- Turbidity
- Volatile acids
- Settleability

Special Tests:
- Metals
- Chlorinated organics
- Cyanide
- Phenol
- Sulfate - sulfide
- Biomonitoring

Category of Information: General Information

Units of expression
- Define units
- Convert units

Sources and characteristics
- Characterize
- Quality/quantity
- Physical/chemical/biological
- Effects
- Pre-aeration
- Chemical pre-treatment
- Grease control

Electrical (Basic)

Hydraulics (Basic)

Category of Information: General Information(continued)

Maps/plans – Interpretation and use

WWT Processes

- Sketch sequence
- Describe processes
- Explain why treated
- Goals of WWT (protect public; protect environment)

Grade 3 Wastewater Treatment Operator
Need-To-Know Criteria

The following list of categories suggests topics of information which are important to know in order to be a successful and proficient Grade 3 Wastewater Treatment Operator. The list may not be all inclusive, and knowledge of additional topics may be of benefit to the operator

Category of Information: Processes

Collection Systems
- Types
- Inflow, Infiltration, Exfiltration
- Pre-treatment
- Sources of wastewater
- Pump stations

Flow
- Measurement
- Flow equalization

Screening
- Bar screens (hand-cleaned)
- Bar screens (mechanically cleaned)
- Static screen
- Microscreen

Grinding
- Grinders
- Comminutors
- Barminutors

Grit Removal
- Hand-cleaned grit chambers
- Mechanically-cleaned grit chambers
- Aerated grit chambers

Clarification
- Primary (Rectangular; Circular)
- Secondary
- SBR Clarification

Trickling Filters
- Standard Rate
- High Rate
- Roughing
- Rotating Biological Contactor (RBC)Rotating Biological Contactor
- Activated Biofilter (ABF)

Lagoons
- Aerobic
- Faultative
- Anaerobic
- Aerated
- Discharging

Activated Sludge
- Conventional
- Step Feed
- Contact Stabilization
- Extended aeration
- Tapered aeration
- Complete mix
- Pure oxygen
- Oxidation ditch
- Types of aeration
- SBR

Solids handling
- Sludge conditioning
- Sludge thickening
- Anaerobic digestors
- Aerobic digestors
- Dewatering
- Incineration
- Drying beds
- Land application
- Composting
- 503 Regulations

Disinfection/ Use of Chlorine
- Chlorination
- Dechlorination
- UV
- UV
- Other methods

Advanced Treatment
- Filtration
- Nitrogen removal
- Phosphorus removal
- Biological

Effluent Disposal
- Discharge
- Direct Use
- Irrigation
- Underground Disposal
- NPDES Permit

Cross- Connection
- Prevention

- Types of devices

Category of Information: Support Systems

Motors
- Single Phase
- Poly Phase
- Variable Speed

Drives
- Coupled
- Direct (Shaft; Gear)
- Speed Reducer (Fixed; Variable)
- Right Angle

Pumps
- Air Lift
- Centrifugal
- Positive Displacement
  - Piston Plunger
  - Progressive Cavity
  - Diaphragm
- Screw
- Turbine
- Metering
- Ejector

Blowers and Compressors
- Centrifugal
- Positive Displacement (Rotary; Piston)

Generators – AC & DC

Engines – Gasoline, Diesel & Gas

Joints
- Flanged
- Compression
- Dresser
- Victualic
- Fused
- Threaded

Valves
- Ball
- Check
- Globe
- Gate
- Plug
- Petcock
- Pressure Control
- Vacuum Relief
- Mud
- Butterfly
- Multiport
- Telescoping
- Sluice Gate
- Air Release
- Foot
- Altitude

Fittings
- Coupling
- Union
- Plug/Caps
- Corporation (Ferrell; Cock)
- Curb Stop
- Special

Cathodic Protection Devices
- Anode Rod/Bags
- Cathode Rod/Bags
- Rectifiers
- Potentiometers

Measuring/ Control
- Signal Generators
  - Kennison Nozzle
  - Magnetic Flowmeter
  - Parshall Flume
  - Proportional Weir
  - Rectangular Weir
  - Venturi
  - Propeller Meter
  - Ultrasonic
  - Pitot Tube
- Signal Transmitters
  - Electric
  - Pneumatic
  - Hydraulic
  - Mechanical
  - Telemetry
- Signal Receivers
  - Counters
  - Indicators
  - Log Scale Indicators
  - Totalizers
  - Recorders
  - Combination Recorders
- Meters
  - Hydraulic – Rotameter
  - Electrical – Amp
  - Electrical – Watt
  - Electrical – Watt – Hour
  - Electrical – Multi.
  - Electrical – VOM
  - Electrical – Megger
  - Mechanical – RPM
- Alarms
- Controls
  - Pneumatic
  - Float
  - Hydraulic
  - Electrical
  - Telemetry
  - Timers

Category of Information: Support Systems (continued)

Chemical Feeders
- Solids
- Liquids
- Evaporators
- Gas
- Slurry

Rolling Stock
- Service Vehicles
- Trucks
- Tractors
- Trailers
- Lawn Mowers
- Loaders
- Portable Pumps
- Generators

HVAC
- Heat Exchangers
- Dehumidifiers
- Fans
- Compressors
- Condensers
- Boilers

Safety Equipment
- Personal Protection Gear
- Traffic Control (Warning Devices; Barricades)
- Hazard Detectors
- First Aid/Hygiene

Category of Information: Lab Tests

Routine Tests
- B.O.D.
- Ammonia
- Chlorine Residual
- Coliform (Fecal)
- E. coli
- Dissolved oxygen
- Kjedahl nitrogen
- pH
- Phosphorus
- Solids
  - Settleable
  - Suspended
  - Total dissolved
  - Total
  - Volatile

Control Tests
- Centrifuge
- Alkalinity
- C.O.D.
- Color
- Conductance
- Nutrients
- Micro exam
- Nitrate - nitrite
- Oil and Grease
- Surfactants
- Temperature
- Total organic carbon
- Turbidity
- Volatile acids
- Settleability

Special Tests:
- Metals
- Chlorinated organics
- Cyanide
- Phenol
- Sulfate - sulfite
- Biomonitoring

Category of Information: General Information

Units of expression
- Define units
- Convert units

Electrical (Basic)

Hydraulics (Basic)

Maps/plans – Interpretation and use

Sources and characteristics
- Characterize
- Quality/quantity
- Physical/chemical/biological
- Effects
- Pre-aeration
- Chemical pre-treatment
- Grease control

Category of Information: General Information (continued)

WWT Processes
- Sketch sequence
- Describe processes
- Explain why treated
- Goals of WWT (protect public; protect environment)

Grade 4 Wastewater Treatment Operator
Need-To-Know Criteria (Subject Areas)

The following list of categories suggests topics of information which are important to know in order to be a successful and proficient Grade 4 Wastewater Treatment Operator. The list may not be all inclusive, and knowledge of additional topics may be of benefit to the operator.

Category of Information: Processes

Collection Systems
- Types
- Inflow, Infiltration, Exfiltration
- Pre-treatment
- Sources of wastewater
- Pump stations

Flow
- Measurement
- Flow equalization

Screening
- Bar screens (hand-cleaned)
- Bar screens (mechanically cleaned)
- Static screen
- Microscreen

Grinding
- Grinders
- Comminutors
- Barminutors

Grit Removal
- Hand-cleaned grit chambers
- Mechanically-cleaned grit chambers
- Aerated grit chambers
- Vortex

Clarification
- Primary (Rectangular; Circular)
- Secondary
- SBR Clarification

Attached Growth/Fixed Film
- Trickling Filters
  - Standard Rate
  - High Rate
  - Roughing
- Rotating Biological Contactor (RBC)
- Activated Biofilter (ABF)

Lagoons
- Aerobic
- Facultative
- Anaerobic
- Aerated
- Discharging

Activated Sludge
- Conventional
- Step Feed
- Contact Stabilization
- Extended aeration
- Tapered aeration
- Complete mix
- Pure oxygen
- Oxidation ditch
- Types of aeration
- Sequencing Batch Reactor (SBR)
- Membrane Biological Reactor (MBR)

Solids handling
- Sludge conditioning
- Sludge thickening
- Anaerobic digesters
- Aerobic digesters
- Dewatering
- Incineration
- Drying beds
- Land application
- Composting
- 503 Regulations

Disinfection/ Use of Chlorine
- Chlorination
- Dechlorination
- UV
- Other methods

Advanced Treatment
- Filtration
- Nitrogen removal
- Phosphorus removal
- Biological

Effluent Disposal
- Discharge
- Direct Use
- Irrigation
- Underground Disposal
- NPDES Permit

Cross- Connection
- Prevention

- Types of devices

Category of Information: Support Systems

Motors
- Single Phase
- Poly Phase
- Variable Speed

Drives
- Coupled
- Direct (Shaft; Gear)
- Speed Reducer (Fixed; Variable)
- Right Angle

Pumps
- Air Lift
- Centrifugal
- Positive Displacement
  - Piston Plunger
  - Progressive Cavity
  - Diaphragm
- Screw
- Turbine
- Metering
- Ejector

Blowers and Compressors
- Centrifugal
- Positive Displacement (Rotary; Piston)

Generators – AC & DC

Engines – Gasoline, Diesel & Gas

Joints
- Flanged
- Compression
- Dresser
- Victualic
- Fused
- Threaded

Valves
- Ball
- Check
- Globe
- Gate
- Plug
- Petcock
- Pressure Control
- Vacuum Relief
- Mud
- Butterfly
- Multiport
- Telescoping
- Sluice Gate
- Air Release
- Foot
- Altitude

Fittings
- Coupling
- Union
- Plug/Caps
- Corporation (Ferrell; Cock)
- Curb Stop
- Special

Cathodic Protection Devices
- Anode Rod/Bags
- Cathode Rod/Bags
- Rectifiers
- Potentiometers

Measuring/ Control
- Signal Generators
  - Kennison Nozzle
  - Magnetic Flowmeter
  - Parshall Flume
  - Proportional Weir
  - Rectangular Weir
  - Venturi
  - Propeller Meter
  - Ultrasonic
  - Pitot Tube
- Signal Transmitters
  - Electric
  - Pneumatic
  - Hydraulic
  - Mechanical
  - Telemetry
- Signal Receivers
  - Counters
  - Indicators
  - Log Scale Indicators
  - Totalizers
  - Recorders
  - Combination Recorders
- Meters
  - Hydraulic – Rotameter
  - Electrical – Amp
  - Electrical – Watt
  - Electrical – Watt – Hour
  - Electrical – Multi.
  - Electrical – VOM
  - Electrical – Megger
  - Mechanical – RPM
- Alarms
- Controls
  - Pneumatic
  - Float
  - Hydraulic
  - Electrical
  - Telemetry
  - Timers

Category of Information: Support Systems (continued)

Chemical Feeders
- Solids
- Liquids
- Evaporators
- Gas
- Slurry

Odor Control
- Biofilters
- Chemical Additives
- Scrubbers

Rolling Stock
- Service Vehicles
- Fork Lifts
- Trucks
- Tractors
- Trailers
- Lawn Mowers
- Loaders
- Portable Pumps
- Generators

HVAC
- Heat Exchangers
- Dehumidifiers
- Fans
- Compressors
- Condensers
- Boilers

Safety Equipment
- Personal Protection Gear
- Traffic Control (Warning Devices; Barricades)
- Hazard Detectors
- First Aid/Hygiene

Category of Information: Lab Tests

Routine Tests
- B.O.D.
- Ammonia
- Chlorine Residual
- Coliform (Fecal)
- E. coli
- Dissolved oxygen
- Total Kjedahl nitrogen
- pH
- Phosphorus
- Solids
  - Settleable
  - Suspended
  - Total dissolved
  - Total
  - Volatile

Control Tests
- Centrifuge
- Alkalinity
- C.O.D.
- Color
- Conductance
- Nutrients
- Micro exam
- Nitrate - nitrite
- Oil and Grease
- Surfactants
- Temperature
- Total organic carbon
- Turbidity
- Volatile acids
- Settleability
- Specific Oxygen Uptake Rate (SOUR)

Special Tests:
- Metals
- Chlorinated organics
- Cyanide
- Phenol
- Sulfate - sulfide
- Biomonitoring

Category of Information: General Information

Units of expression
- Define units
- Convert units

Electrical (Basic)

Hydraulics (Basic)

Sources and characteristics
- Characterize
- Quality/quantity
- Physical/chemical/biological
- Effects
- Pre-aeration
- Chemical pre-treatment
- Grease control

Maps/plans – Interpretation and use

WWT Processes
- Sketch sequence
- Describe processes
- Explain why treated
- Goals of WWT (protect public; protect environment)

Suggested Primary Wastewater Treatment Exam References

The following are approved as reference sources for the wastewater treatment examinations. Operators should use the latest edition of these reference sources to prepare for the exam. These references are not the only reference an operator should use in studying for the exam, however these are the primary references used in developing the exam.

Biological Natural System

- ****CSUS Operation of Wastewater Treatment Plants Volume II**
- ****CSUS Operation of Wastewater Treatment Plants Volume I**
- Price Joanne Kirkpatrick **Applied Math for Wastewater Plant Operators** Technomic Publishing Company Inc. Lancaster PA 1991
- *Design Criteria for Sewage Works State of Tennessee Department of Environment and Conservation, Division of Water Resources Nashville TN Latest Revision*
- *National Pollutant Discharge Elimination System (NPDES) Permit State of Tennessee Department of Environment and Conservation Division of Water Resources, Latest Revision*
- *Rules Governing Water and Wastewater Operator Certification, State of Tennessee, Department of Environment and Conservation, Board of Certification for Water and Wastewater Operators, Nashville, Latest Revision*
- *State of Tennessee Water Quality Standards (General Water Quality Criteria and Permits, Effluent Limitations and Standards), State of Tennessee, Department of Environment and Conservation, Division of Water Resources, Nashville, Latest Revision.*

Wastewater Treatment 1

- ****CSUS Operation of Wastewater Treatment Plants Volume II**
- ****CSUS Operation of Wastewater Treatment Plants Volume I**
- **CSUS Advanced Waste Treatment**
- *National Pollutant Discharge Elimination System (NPDES) Permit State of Tennessee Department of Environment and Conservation Division of Water Resources, Nashville, TN Latest Revision*
- Price Joanne Kirkpatrick Applied Math for Wastewater Plant Operators Technomic Publishing Company Inc. Lancaster PA 1991
- *Design Criteria for Sewage Works State of Tennessee Department of Environment and Conservation Division of Water Resources Nashville TN Latest Revision*
- *Rules Governing Water and Wastewater Operator Certification, State of Tennessee, Department of Environment and Conservation, Board of Certification for Water and Wastewater Operators, Nashville, Latest Revision*
- *State of Tennessee Water Quality Standards (General Water Quality Criteria and Permits, Effluent Limitations and Standards), State of Tennessee, Department of Environment and Conservation, Division of Water Resources, Nashville, Latest Revision.*

Wastewater Treatment 2

- ****CSUS Operation of Wastewater Treatment Plants Volume I**
- ****CSUS Operation of Wastewater Treatment Plants Volume II**
- **CSUS Advanced Waste Treatment**
- ***Design Criteria for Sewage Works State of Tennessee Department of Health and Environment Division of Water Resources, Nashville TN, Latest Revision***
- Price Joanne Kirkpatrick Applied Math for Wastewater Plant Operators Technomic Publishing Company Inc. Lancaster PA 1991
- *National Pollutant Discharge Elimination System (NPDES) Permit State of Tennessee Department of Environment and Conservation Division of Water Resources, Nashville TN, Latest Revision*
- *Rules Governing Water and Wastewater Operator Certification, State of Tennessee, Department of Environment and Conservation, Board of Certification for Water and Wastewater Operators, Nashville, Latest Revision*
- *State of Tennessee Water Quality Standards (General Water Quality Criteria and Permits, Effluent Limitations and Standards), State of Tennessee, Department of Environment and Conservation, Division of Water Resources, Nashville, Latest Revision.*

Wastewater Treatment 3

- ****CSUS Operation of Wastewater Treatment Plants Volume II**

- ****CSUS Operation of Wastewater Treatment Plants Volume I**
- ****CSUS Advanced Waste Treatment**
- **WEF MOP 11**
- **Price Joanne Kirkpatrick Applied Math for Wastewater Plant Operators Technomic Publishing Company Inc. Lancaster PA 1991**
- *Design Criteria for Sewage Works State of Tennessee Department of Health and Environment Division of Water Resources, Nashville TN, Latest Revision*
- *National Pollutant Discharge Elimination System (NPDES) Permit State of Tennessee Department of Environment and Conservation Division of Water Resources, Nashville, Latest Revision*
- *Rules Governing Water and Wastewater Operator Certification, State of Tennessee, Department of Environment and Conservation, Board of Certification for Water and Wastewater Operators, Nashville, Latest Revision*
- *State of Tennessee Water Quality Standards (General Water Quality Criteria and Permits, Effluent Limitations and Standards), State of Tennessee, Department of Environment and Conservation, Division of Water Resources, Nashville, Latest Revision.*

Wastewater Treatment 4

- ****CSUS Operation of Wastewater Treatment Plants Volume II**
- ****CSUS Operation of Wastewater Treatment Plants Volume I**
- ****CSUS Advanced Waste Treatment**
- **WEF MOP 11**
- **Standard Methods**
- *Design Criteria for Sewage Works State of Tennessee Department of Health and Environment Division of Water Resources, Nashville TN, Latest Revision*
- *National Pollutant Discharge Elimination System (NPDES) Permit State of Tennessee Department of Environment and Conservation Division of Water Resources, Nashville, TN Latest Revision*
- *Rules Governing Water and Wastewater Operator Certification, State of Tennessee, Department of Environment and Conservation, Board of Certification for Water and Wastewater Operators, Nashville, TN, Latest Revision*
- *State of Tennessee Water Quality Standards, State of Tennessee (General Water Quality Criteria and Permits, Effluent Limitations and Standards)w, Department of Environment and Conservation, Division of Water Resources, Nashville, TN, Latest Revision.*

There are 2-3 primary references for each of exam. The ** denotes that 20+ of the exam items are linked to the noted reference.

Bold items have at least three items linked to them. Any references that are not bolded have only 1-2 items linked to them. The Tennessee State references are included, however, there is a sixth reference if the sixth had at least three items linked to it; in some cases this was a tie of 2-3 references with just a few items each. State of Tennessee references are italicized.

Suggested Wastewater Treatment Exam References

The following are approved as reference sources for the ABC wastewater treatment examinations. Operators should use the latest edition of these reference sources to prepare for the exam.

Textbooks

California State University, Sacramento (CSUS) Foundation, Office of Water Programs (www.owp.csus.edu)

- Operation of Wastewater Treatment Plants, Volume I and II
- Advanced Waste Treatment
- Manage for Success

National Environmental Training Center for Small Communities (NETCSC)

- Protecting Your Community's Assets: A Guide for Small Wastewater Systems
  A PDF version of this guide is available from: www.nesc.wvu.edu/training.cfm

Water Environment Federation (www.wef.org)

- Operation of Municipal Wastewater Treatment Plants - Manual of Practice No. 11
- Activated Sludge - Manual of Practice OM-9

Regulations

- National Pollutant Discharge Elimination System (NPDES) Permit, State of Tennessee, Department of Environment and Conservation, Division of Water Resources, Nashville, TN, 1977, Latest Revision.

- Design Criteria for Sewage Works, State of Tennessee, Department of Environment and Conservation, Division of Water Resources, Nashville, Latest Revision.

- Rules Governing Water and Wastewater Operator Certification, State of Tennessee, Department of Environment and Conservation, Board of Certification for Water and Wastewater Operators, Nashville, Chapter 400-49-01, Latest Revision.

- State of Tennessee General Water Quality Criteria, and Permits, and Effluent Limitations and Standards State of Tennessee, Department of Environment and Conservation, Division of Water Resources, Nashville, TN, latest versions, Chapter 400-40-03 and Chapter 400-40-05.

- State of Tennessee Cross-Connection Control Manual, State of Tennessee Department of Environment and Conservation, Division of Water Resources, Latest Revision.

- Water and Wastewater Operator Certification Act T.C.A. Section 68-221-901. T.C.A. Section 68-221-915.
- Protection of Environment, (40 CFR Part 136), U.S. Environmental Protection Agency,

Study Guides

- Applied Math for Wastewater Plant Operators. Price, Joanne. 2000. Boca Raton, FL: CRC Press (www.crcpress.com)

- WEF/ABC Wastewater Operators' Guide to Preparing for the Certification Examination ,Water Environment Federation, (www.wef.org)

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

<u>Sampling – p.</u>

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

<u>Chemical Oxygen Demand (COD) – p.</u>

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)

<u>Solids – p.</u>

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

<u>pH – p.</u>

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.

<u>Bacteriological Analysis – p.</u>

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