

Tennessee Department of Health Public Health Laboratories Newsletter

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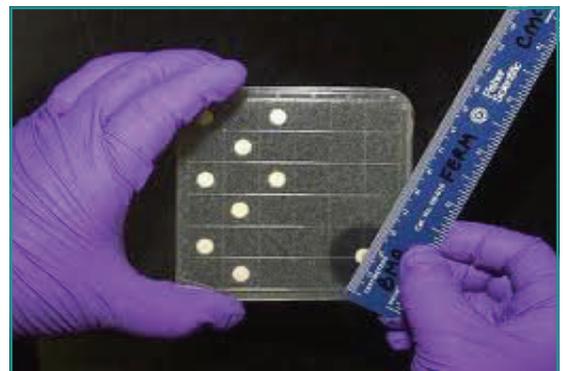
Summer in Tennessee



The Good and the Bad of Non-Culture Specimen Testing

As hard as scientists try, no microbiological laboratory diagnostic test is perfect. The culture of infectious organisms is the gold-standard upon which all new tests are based. However, culture has its inherent drawbacks; not all organisms thrive on synthetic media and the numbers of organisms present in a clinical sample may not be of sufficient density to yield a recognizable colony. Since the mid-1980's, manufacturer's have worked in earnest to help clinical laboratories overcome these issues. Clinical diagnostic tests that seek antibodies to certain organisms or tests that seek the presence of a particular antigen related to an organism most recently, tests that determine the presence of an organisms DNA in a clinical sample have been applied to the field of clinical

appealing since the results are considered 100% reliable. Either the organism is present and DNA is detected or the organism is not present and no DNA is detected. In a perfect world this would be correct, but even PCR assays have a limit of detection and very rarely will the assays detect a single organism's DNA. So, while



Measuring drug sensitivities of a culture

a positive result may indicate the organism is present, a negative result does not indicate the organism is absent. It simply was unable to be detected.

A second downside to these types of non-culture tests is that the clinical and public health community lose access to isolates of infectious organisms. This prevents further study of these organisms for antimicrobial resistance testing, prevents the detection of enhanced virulence factors, and prevents public health from monitoring the spread of a particular clone of a bacterial species within the population. Tracking of organisms across communities and across states during an outbreak has become the corner stone for infectious disease surveillance. As a result, some surveillance systems have been restructured. For example, the



Non-culture based PCR analysis

microbiology. Even though these types of tests have the advantage of speed, which helps physicians treat a patient in a faster and maybe more appropriate manner, these tests are neither 100% sensitive nor specific, especially when compared to the gold standard of isolating the causative organism. With the advent of PCR, the drive to default to DNA based testing is

2010 Enteric Bacteriology Statewide

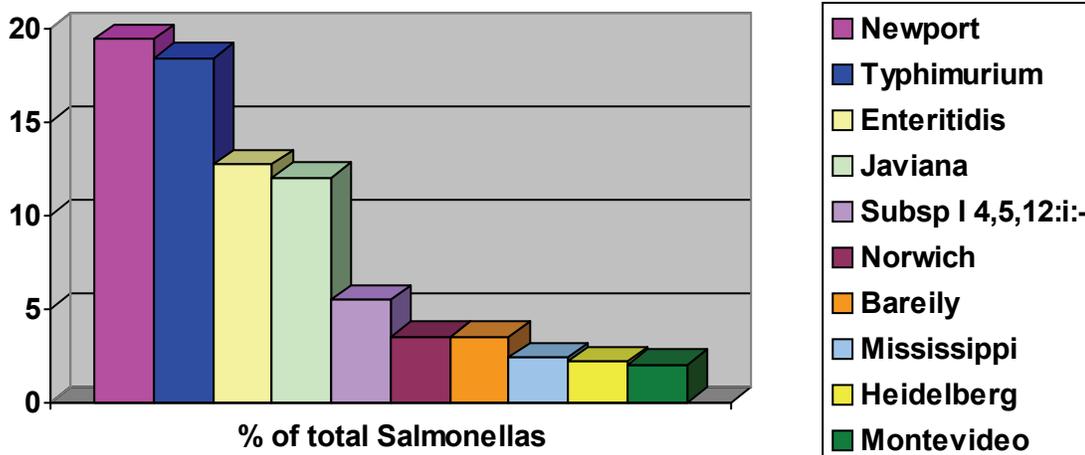
The year 2010 was a very busy time in the state of Tennessee for identifying enteric pathogens. A total of 1470 main enteric pathogens were identified by the combined efforts of the three state public health laboratories in Nashville, Knoxville and Jackson. The two charts listed below show a quick summary of the pathogens identified. The first chart lists the seven main enteric pathogens and the totals identified for each pathogen. The second graph shows, by percentage, a summary of the top ten Salmonella serotypes identified in the state during the year. Please continue to send in those samples so that we may quickly identify possible enteric outbreaks.

Submitted by Sheri Roberts,
Supervisor Enteric Bacteriology

Organism Totals Identified by Region

Total Identified by Lab	
Organism	Total Identified in 2010
<i>Campylobacter</i>	48
<i>Escherichia coli non-</i>	49
<i>Escherichia coli O157</i>	49
<i>Salmonella</i>	1105
<i>Shiella</i>	253
<i>Vibrio</i>	3
<i>Yersinia</i>	12

Top Ten Salmonella Serotypes by Percentages



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Register at <http://health.state.tn.us/Lab/workshops.htm>

Staphylococcus aureus; the "Superbug" that Keeps on Changing

Staphylococcus aureus infections are noted frequently in the news these days and perhaps most familiar to the public is methicillin-resistant *Staphylococcus aureus* (MRSA), which is now responsible for more deaths in the United States each year than AIDS. It has been nicknamed the "superbug" by the media because of its resistance to several commonly used antibiotics. MRSA can be treated effectively with antibiotics, but it requires the use of more powerful and toxic antibiotics such as vancomycin. Enter VISA and VRSA to the scene.

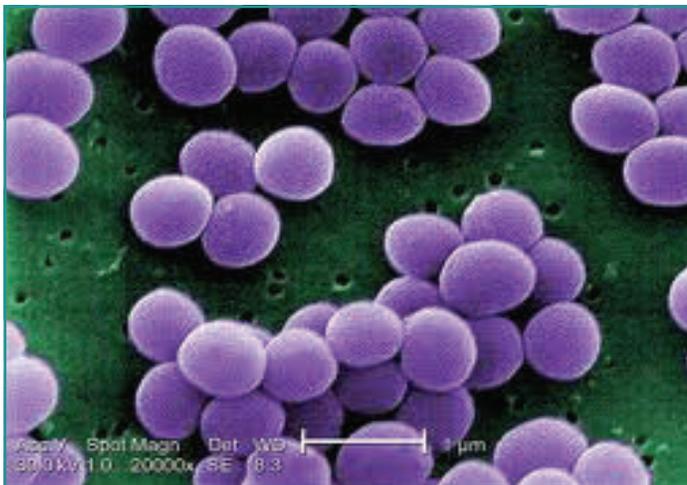
Vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) are specific staph bacteria that have developed resistance to the antimicrobial agent vancomycin. Persons who develop this type of staph infection may have underlying health conditions (such as diabetes and kidney disease), devices going into their bodies (such as catheters), previous infections with methicillin-resistant *Staphylococcus aureus*, and recent exposure to vancomycin and other antimicrobial agents. Vancomycin-intermediate *Staphylococcus aureus* has a minimum inhibitory concentration (MIC) of 4 – 8 ug/ml. The Vancomycin-resistant *Staphylococcus aureus* (VRSA) has a minimum inhibitory concentration (MIC) of > 16 ug/ml.

VRSA isolates are detected by reference broth microdilution, agar dilution, Etest, MicroScan overnight and Synergies plus, BD Phoenix system, TREK Sensititre MIC plate, disk diffusion, VRSA screen test for VITEK 2 and vancomycin screen agar plates (brain heart infusion (BHI) agar containing 6 ug/ml of vancomycin).

A sample algorithm for detecting VISA/VRSA specifically for the laboratory may be found at

www.cdc.gov/ncidod/dhqp/ar_visavrso_algo.html

As of October 2010 all VISA and VRSA are treatable with



several FDA approved drugs. The clinical laboratory has an important role in the prompt recognition, isolation and proper management of VRSA.

All *Staphylococcus aureus* non-sensitive forms are Category 1B diseases requiring immediate telephonic notification (615-741-7247 next business day), followed by a written report within one week. VISA and VRSA are communicable and dangerous to the public and are to be reported to the local health department by all persons knowing of or suspecting a case. In addition, cultures of VISA and VRSA are required to be submitted to the TDH Laboratory for additional testing and surveillance. The culture must be accompanied with the patient's full name, address, age and sex, physician's name and address and anatomic source of infection.

Submitted by Henrietta D. Hardin, Manager, General Bacteriology

R E L C Y C O M R E H T P A C
 L A M R E H T O S I D Z N Y W
 E A T E B R E T A W N I S A B
 L E N O R O V I R U S T E C P
 I A N T I M O N Y F I L T E R
 N L A C H A T N E C R O P S Y
 T E L E C T R O F I S H I N G
 S E P O T O S I O I D A R B I
 E D A Q X G B C O L I F O R M
 W I P S T R E A M S U R V E Y
 A S R M O A L L E N O M L A S
 P O G S V P E R M I T T I N G
 F N I E A H P Y H O D U E S P
 G S C I N T I L L A T I O N I
 E D I M I L I N A F L U S M U

Laboratory Operations Word Search

Try your hand at discovering all of the words in this word search. They are taken from microbiology, chemistry and aquatic biology.

- | | |
|----------------|---------------|
| ANTIMONY | NOROVIRUS |
| BASINWATER | PAPR |
| BETA | PERMITTING |
| CHROMATOGRAPH | PFGE |
| COLIFORM | PSEUDOHYPHAE |
| CYSTICFIBROSIS | RADIOISOTOPES |
| EDISON | SALMONELLA |
| ELECTROFISHING | SCINTILLATION |
| FILTER | STEC |
| ISOTHERMAL | STREAMSURVEY |
| LACHAT | SULFANILIMIDE |
| MRSA | THERMOCYCLER |
| NECROPSY | WESTNILE |

Submitted by Mona Baggett, Manager Laboratory Support Services

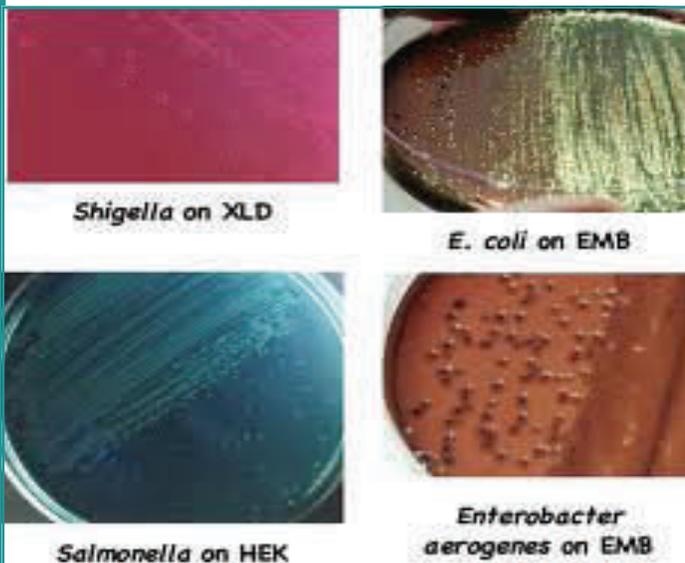
The Good and the Bad of Non-culture Specimen Testing (Continued from Page 1)

technology of nucleic acid amplified tests (NAAT's) was applied successfully to the diagnosis of *Neisseria gonorrhoeae* and the numbers of clinical and public health laboratories utilizing these tests increased during the 1990's and early 2000's (Sexually Transmitted Diseases 34:1, 41-46). The turn around time for results decreased from 3-4 days to 1-2 days giving physicians the opportunity for earlier treatment. By 2004, 78.7% of all clinical and public health labs surveyed were using NAAT's for testing. Only 8.5% of labs were still performing culture. In anticipation of this dramatic shift in culturing practices for this organism, CDC implemented the Gonococcal Isolate Surveillance Project (GISP). The GISP has been in existence since 1986 and its purpose is to collect approximately 6000 urethral isolates a year to monitor trends in antimicrobial resistance. This is the only system the US has to monitor resistance associated with this organism. (MMWR April 13, 2007 page 332-336.) If every infectious organism had to be tracked by a special surveillance system reliant on culture results, valuable information could be lost, changes in organism character-



GC isolated from a culture

Differential culture media



istics could be delayed, and state and federal public health systems would be overwhelmed.

A third downside to non-culture based testing is the possibility of false-positives thereby contributing to the

investigation of a "pseudo-outbreak" when there is no culture to confirm the results. And, on the flip-side, there may be the possibility that a false-negative result could delay the detection of an outbreak. For example, a September 29, 2006 Morbidity and Mortality Weekly Report (MMWR) described an incident in which a clinical laboratory reported a Shiga toxin-positive enzyme immunoassay (EIA) result for an infant with non-bloody diarrhea who attended a day care center. The state public health laboratory and the CDC were unable to grow a Shiga toxin-producing *Escherichia coli* (STEC) from the stool on culture media nor were they able to detect the presence of the Shiga toxin genes in the stool sample. Ultimately after extensive laboratory testing of this patient's stool sample and others from the day care, another infectious process was to blame for the diarrheal illnesses. The initial test result was ultimately declared a false-positive Shiga toxin EIA test result. This situation raised concerns that EIA testing for Shiga toxin may produce false-positive results. In order to prevent such future events, testing guidelines for clinical laboratories were created to highlight the potential of false-positive test results when performing a Shiga toxin EIA (Clinical STEC guidelines MMWR R&R) October 2009.

By taking all of this information into consideration, public health greatly encourages that results of non-culture assays be utilized in combination with culture confirmation, if available, in addition to investigating the patient's clinical or epidemiological history as an aid for an ultimate diagnosis.

Submitted by Dr. Robyn Atkinson, HCLD, Director, TDH Knoxville Regional Laboratory

Nashville Welcomes Two Newcomers to Public Health

Employee Name	Position Title	Hire Date	Location
Sandra Buchanan	Data Entry Operator	May 2, 2011	Nashville Central Laboratory
Barbara Osborne	Data Entry Operator	May 2, 2011	Nashville Central Laboratory
Marka E. Smith	Aquatic Biologist	July 11, 2011	Nashville Central Laboratory

Aquatic Macroinvertebrates Make Good Water Quality Indicators

Aquatic invertebrates live in the bottom parts of our waters. They are also called benthic macroinvertebrates, (benthic = bottom, macro = large, invertebrate = animal without a backbone) and make good indicators of water quality. Macroinvertebrates are found in various aquatic habitats such as streams, rivers, lakes, ponds and marshes throughout Tennessee. These aquatic macroinvertebrates help maintain the health of aquatic ecosystems by eating bacteria and dead, decaying plants and animals. Water quality determines what types of organisms can survive in a body of water. The term "water quality" is used to describe the condition of the water, including its chemical, physical and biological characteristics; usually with respect to its suitability for a particular purpose such as drinking water, safety of human contact or health of the ecosystem (Diersing 2009). Water quality parameters include dissolved oxygen, pH, conductivity, levels of algal growth, and various pollutants which may be present. Some macroinvertebrates such as stoneflies, mayflies and water pennies require a high level of dissolved oxygen, and their abundance is an indication of good water quality.



Other macroinvertebrates can survive at a lower dissolved oxygen level because they can come to the surface to get oxygen. This is accomplished through a breathing tube or by carrying a bubble of air with them around their bodies or under their wings. Several species of macroinvertebrates, such as aquatic worms and leeches, are indicators of waterbodies with low dissolved oxygen levels. Low dissolved oxygen levels are often associated with polluted waters, while higher levels indicate good quality water.

Aquatic macroinvertebrates make good water quality indicators because of their lifecycle complexity, sensitivity to changes, limited mobility, duration of exposure, and ease of collection. The life cycle of a macroinvertebrate goes from egg to adult form and they can undergo either complete or incomplete metamorphosis. Complete metamorphosis has 4 stages, egg, larvae, pupa and adult. Organisms which undergo complete metamorphosis include true flies, beetles and caddisflies. Many of these organisms are aquatic for the egg and larval stages, but not in the adult stage. Incomplete metamorphosis has 3 stages, egg, nymph and adult. Organisms which undergo incomplete metamorphosis include stoneflies, mayflies, dragonflies and true bugs. Many of these organisms, such as dragonflies, do not live

in an aquatic ecosystem as adults. Other species, such as true bugs which include the backswimmers, water scorpions and the water striders, are examples of macroinvertebrates which spend their entire lives in the water. The length of the life cycle of a macroinvertebrate can vary from less than 2 weeks for some midges and mosquitos and two years or longer for some stoneflies, dragonflies and dobsonflies.

Aquatic macroinvertebrates are an important part of the food chain found in and around a body of water. In most streams, the energy stored by plants is available to animal life either in the form of leaves that fall in the water or in the form of algae that grows on the stream bottom. The algae and leaves are eaten by macroinvertebrates. The macroinvertebrates are a source of energy for larger animals such as fish, which in turn, are a source of energy for other animals including man.

Aquatic macroinvertebrates differ in their sensitivity to water pollution. Some aquatic macroinvertebrates cannot survive in polluted water, while others can survive or even thrive in polluted water. In a healthy stream, the macroinvertebrate community will include a variety of pollution-sensitive macroinvertebrates, mayflies, stoneflies, and caddisflies. In an unhealthy stream, there may be only a few types of non-sensitive macroinvertebrates present.

Aquatic macroinvertebrates provide information about the quality of a stream over long periods of time. It may be difficult to identify stream pollution with water analysis such as pH and dissolved oxygen which can only provide information at the time of sampling. Even the presence of fish may not provide information about a pollution problem because fish can move away to avoid polluted water and then return when conditions improve. However, most aquatic macroinvertebrates cannot move to avoid pollution. A macroinvertebrate sample may provide information about pollution that is not present at the time of sample collection.

Aquatic macroinvertebrates are relatively easy to collect. Useful aquatic macroinvertebrate data is easy to collect without expensive equipment. The data obtained by taking a macroinvertebrate survey can serve to indicate the need for additional data collection on water samples.

Diersing, Nancy (May 2009). "[Water Quality: Frequently Asked Questions](#)". PDA. NOAA.

<http://floridakeys.noaa.gov/pdfs/wqfaq.pdf>.

Retrieved 2009-08-24.

**Submitted by Patricia Alicea, Manager
Aquatic Biology Laboratory**

Nashville Inorganic Laboratory Purchases New Mercury Analyzer

Mercury is a naturally occurring element that is found in the environment and exists in several forms; in its elemental or metallic state, in inorganic compounds, or in organic compounds. Mercury is found in coal, in chemical applications, and in pharmaceutical compounds. This element and its compounds affects both humans and wildlife. Mercury is known to be toxic and the Environmental Protection Agency (EPA) partners with state and local governments to reduce its impact. One of the key analyses performed in the Environmental Laboratories is the determination levels of mercury in water, soil, and fish. The Nashville Inorganic Chemistry laboratory has purchased a new instrument, the SMS 100, to aid in the analysis for mercury. The SMS 100 is a dedicated, automated analyzer for the determination of total mercury in solid samples. The instrument utilizes the principle of thermal decomposition, amalgamation and cold vapor atomic absorption as described in the Environmental Protection Agency's Method 7473. The sample is heated in the instrument's furnace under an oxygen rich atmosphere where the decomposition products, including mercury, are released and carried to a catalytic section. The catalyst traps any halogens, nitrogen oxides, and sulfur. The remaining vapor is then swept to the amalgamation cell where the mercury is trapped. The amalgamation cell is heated and the mercury vapor released and carried to the absorbance cell where detection of the mercury takes place. This approach has benefits, namely no lengthy sample preparation and lower detection limits. The SMS 100 eliminates digestion techniques and the necessity to work with strong, corrosive acids and



oxidizing chemicals. The sample, after any necessary grinding or drying, can be introduced to the instrument for analysis. Not including calibration and quality control analysis, a result for a solid sample can be obtained in five to ten minutes. The SMS 100 also provides a high level of sensitivity by using a 25 cm optical path length cell. A detection limit of 5 nanograms of mercury is achievable and the working range of the instrument covers from 5 nanograms to 600 nanograms. The SMS 100 is a welcome addition to the suite of analytical techniques offered by the Nashville Metals Analysis Unit.

**Submitted by Craig Edwards, Manager
Inorganic Chemistry**



Some Birth Defects Attributed to Mercury Poisoning from Ingestion of Contaminated Seafood

Mercury poisoning is a disease caused by exposure to mercury or its compounds. Mercury (chemical symbol Hg) is a heavy metal occurring in several forms, all of which can produce toxic effects in high enough doses. Toxic effects include damage to the brain, kidney, and lungs. Common symptoms of mercury poisoning include peripheral neuropathy (presenting as itching, burning or pain), skin discoloration (pink cheeks, fingertips and toes), swelling, and desquamation (shedding of skin). The consumption of fish is by far the most significant source of ingestion-related mercury exposure in humans and animals, although plants and livestock also contain mercury due to bioaccumulation of mercury from soil, water and atmosphere, and due to biomagnification by ingesting other mercury-containing organisms. Mercury and its compounds are particularly toxic to fetuses and infants. Women who have been exposed to mercury in pregnancy have sometimes given birth to children with serious birth defects.