TENNESSEE DEPARTMENT OF HEALTH

It's About Time!

Tennessee Department of Health Public Health Laboratories Newsletter

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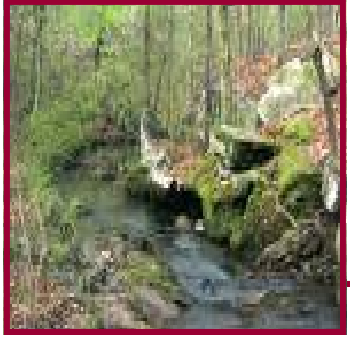
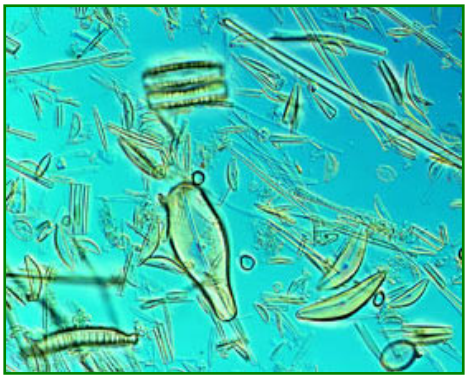
Inside this issue:

Algae growing on rocks along a Tennessee stream



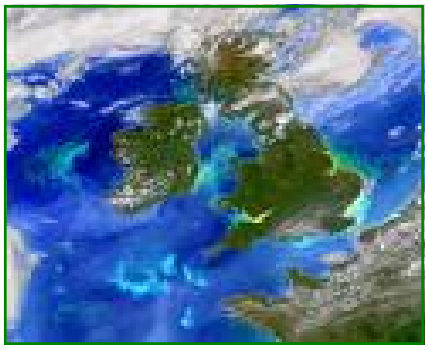
What IS Periphyton Anyway?

We know you haven't asked, but since this critter forms most of the Earth's atmospheric oxygen, we thought you'd like to know anyway.



A slide mount of diatoms, a type of periphyton

To begin to understand, you should know that periphyton is part of a greater whole, known as **algae**. Yes, it's that slimy green stuff that has caused many a Tennessean to utter colorful phrases as they perform a balance ballet while crossing rocks in a stream. These algae are actually very important organisms, not only locally, but globally. For instance, it has been estimated that **75% of all of the Earth's atmospheric oxygen is produced by algae…yes algae.**



Ocean growing phytoplankton blooms appear as bright aqua patches in these aerial photos

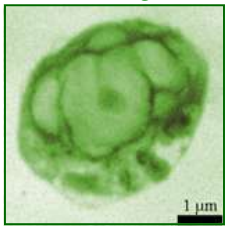
Algae are a group of primitive, mainly aquatic plants that lack true stems, roots and leaves. Algae growth is a natural occurrence in all water bodies including lakes, ponds, streams, rivers and oceans. Algae as a whole is usually broken down into two major groups consisting of periphyton and phytoplankton.

Periphyton are algae living on, or attached to, underwater rocks, vegetation or other substrates (structures). This is the culprit that makes stream rocks so slippery. Phytoplankton are free-living algae suspended in water. A large population of these can turn pond or stream water a green "pea soup" color. Tiny marine phytoplankton provide the main food source for many animals in the sea, including whales.

So why do Tennessee environmentalists want to study algae? Because periphyton are attached to the substrate and cannot move, their community structure is affected by the chemical and physical properties of the water in their environment. Like most organisms, certain species can thrive in polluted water while others prefer clean water. By studying the species that make up the community as a whole, aquatic biologists can get an idea of water quality within that system. Tennessee has been collecting this kind of data on macroinvertebrates (aquatic insects) for many years.

Tennessee pond with an algae bloom in progress

Algae cell



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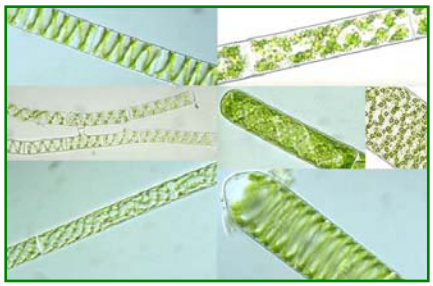
What IS Periphyton Anyway? (Continued)

This data forms the base for stream water quality determination. By combining the data on algae with the data on aquatic insects Tennessee hopes to have a more defined "three dimensional" evaluation of water quality.

Submitted by Seton Bonney, Aquatic Biologist



Composite wet mount of algal filaments, types of periphyton

2009 Plan of Action: Bioterrorism Preparedness for Clinical Laboratories

Laboratory Services in Nashville will be presenting this informative wet workshop again in 2009. This class is for TN licensed MTs and MLTs who are working in a clinical laboratory. This year's dates are March 27, May 8, September 18, October 9 and November 20. To register go online to http://health.state.tn.us/lab/education.htm There is no charge for these workshops. Priority admission will be given to applicants who work in microbiology laboratories and who are responsible for bench work involving the rule-in/rule-out of organisms for bioterrorism preparedness.

Bordetella pertussis; Highly Communicable and Vaccine Preventable

Pertussis is a highly communicable, vaccine-preventable disease that lasts for many weeks and is typically manifested in children with paroxysmal spasms of severe coughing, whooping and posttussive vomiting. In the United States, 5,000 – 7,000 cases are reported each year. Incidence of pertussis has increased steadily since the 1980s. Pertussis is an endemic illness. In the United States epidemics occur every 3-5 years. The most recent epidemic occurred in 1996. There has been an overall increase in cases since 1990, with a disproportionate increase in adolescents and adults. Source: www.cdc.gov/ncidod/dbmd/diseaseinfo/pertussis_t.htm

Major complications are most common among infants and young children and include hypoxia, apnea, pneumonia, seizures, encephalopathy and malnutrition. Most deaths occur among unvaccinated children or children too young to be vaccinated. Transmission occurs through direct contact with discharges from respiratory mucous membranes of infected persons.

Laboratory Services in Nashville performs testing for *Bordetella pertussis*. This testing consists of a polymerase chain reaction (PCR) assay as well as culture. The Nashville laboratory has recently converted from a conventional PCR amplification with Enzyme-linked Oligosorbent Assay (ELOSA) detection to a real-time PCR assay. The real-time PCR assay has decreased our test time from 8 hours to less than 5 hours, including extraction. Real-time PCR is a closed tube system, reducing the possibility of contamination. Two major upgrades with the real-time assay are the ability to detect *Bordetella parapertussis* as well as *B. pertussis* and the inclusion of a human internal control target to assess sample quality. This assay was modified from a published protocol and its performance characteristics were determined by the Molecular Biology Unit of Laboratory Services. Michael Lehman (pictured) was the lead molecular microbiologist on this project.

Submitted by Amy Woron, Molecular Biologist Manager, Molecular Biology Section

A single cell of *Bordetella pertussis*

Laboratory Reporting and Isolate Submission: Cornerstones of Public Health Surveillance

Public health surveillance is the systematic and ongoing collection, analysis, interpretation and dissemination of data related to disease occurrence. Surveillance is situational awareness for local, state and federal public health officials. It is the responsibility of public health to create a link to public health practice because surveillance should be information for action. The Council of State and Territorial Epidemiologists (CSTE) and the Centers for Disease Control and Prevention (CDC) determine which diseases are important to report to help prevent or control spread of disease. Tennessee typically adopts and codifies the recommended list of diseases as reportable and may add some diseases of local interest. The most current list of notifiable diseases in Tennessee can be accessed on the Department of Health Web-site at: http://health.state.tn.us/ceds/notifiable.htm. Also listed are the communicable diseases and disease clusters or outbreaks should be reported.

Although disease reports come from several sources, including physicians and other healthcare providers, most communicable disease reports are received from laboratories after a positive result is obtained of a notifiable disease. The report is made to the local health department and is transmitted electronically to the state health department and then to CDC. Different levels of reporting urgency are indicated for the Category 1diseases such as botulism, anthrax, or disease outbreaks require immediate telephonic reporting followed by a written report using form PH-1600. This is to ensure potential communicable disease threats can be investigated and controlled promptly. Category 2 diseases require only a written report using form PH-1600; this data is used to monitor trends that may require public health interventions and sometimes to detect unrecognized outbreaks. Over time, it also serves to shape public health policy and funding at the local, state and federal levels.

Laboratories are also required to submit certain isolates of public health significance to the state public health laboratory for confirmation, typing and sometimes antibiotic sensitivity testing. The list of isolates required to be submitted can be found beginning on page 46 of the Tennessee Rule 1200-6-3, which is also accessible on the Web at www.tennessee.gov/sos/rules/1200/1200-06/1200-06-03.pdf

Information obtained from these isolates is critical to the identification, investigation and control of infectious diseases. Some isolates are DNA fingerprinted, which allows detection of previously unrecognized outbreak using molecular epidemiology to link seeming unrelated cases. Several high profile nation-wide outbreaks have been discovered using these methods, including Salmonella associated with peanut butter and *E coli* O157 associated with frozen pizza. Public health owes a great debt of gratitude to laboratories across Tennessee for helping us prevent and control communicable disease in Tennessee.

Contributed by David Kirschke, M.D.
Communicable and Environmental Disease Services
Tennessee Department of Health

Chemical Terrorism; Public Health's Response

David Whybrew, Chemical Terrorism Response Coordinator for the TDH Division of Laboratory Services, has instituted a series of informational meetings. Coordinated by the TDH regional hospital coordinators, with representatives from each Tennessee sentinel hospital, the meeting is to discuss the roles of the sentinel labs, the CDC and the State of Tennessee in responding to a chemical event. The discussion includes an explanation of CDC's collection, shipping and chain of custody of clinical specimens procedure to be used following a chemical terrorism event. Facility representatives will receive a CD with the program's PowerPoint slides, references and a certificate of completion, to enable them to provide continuing education hours for other participants in his/her facility.

A Look Inside the TN Public Health Laboratory System

An early version of the Tennessee Department of Health (TDH) Division Laboratory Services began in 1877 when a Cholera epidemic spread across the state from Memphis to Knoxville. As a consequence, the Tennessee Legislature established the first Board of Health in an effort to protect the state from the further rapid spread of epidemics. The first state bacteriology laboratory was located in downtown Nashville in 1900. The location changed in 1954 to the Cordell Hull Building, named after Nobel Prize winning Tennessean Cordell Hull. The laboratory occupied several floors of the ten-story high-rise. Its oddest feature may have been the rooftop barnyard housing the sheep and rabbits used for blood products needed in culture media, as well as the chickens whose eggs were used for live studies of viruses. In 1985, the state completely gutted and renovated the former Dr. R. S. Gass Tuberculosis Hospital into a state-of-the-art laboratory specifically fitted for clinical human and environmental laboratory operations. The building was renamed the TDH Division of Laboratory Services R.S.Gass Facility. The Public Health Laboratory (PHL) system in Tennessee also has regional laboratories, the Jackson Regional Laboratory and the Knoxville Regional Laboratory and works closely with the Memphis and Shelby County Health Department laboratory.

Laboratory Services in Nashville began working with pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) technologies in 1999 as a component of the microbiology department. Staffing consisted of one technologist and one supervisor. In 2004 the Molecular Biology Section was formed as an independent entity. Molecular Biology now employs five full-time technologists, all of whom are gel- and analysis certified in PFGE for *E. coli, Listeria, Salmonella, Shigella and Campylobacter.* Molecular Biology has grown into three distinct areas: PFGE, PCR and Sequencing. In an effort to maintain proficiency by all staff, the department splits technologists' time between the three sections, although each employee has an area specialty. Under the direction of Dr. Robyn Atkinson, the Knoxville Regional Laboratory began performing PFGE on January 1, 2009

Amy Woron, the manager of Molecular Biology, has a Masters of Science degree from the State University of New York at Albany. She is currently working on her PhD at Tennessee State University. Prior to coming to Tennessee in August of 2004, she worked at the Wadsworth Center, New York State PHL. Sheri Roberts began working at the state lab in 1999 in the Virology department. She transferred to the Microbiology Biology section in 2000 and began performing PFGE in 2002. Sheri was promoted to molecular biology supervisor in 2004. Christina Moore began her career at the state lab in October 2006, fresh out of medical technology (MT) school. Christina specializes in sequencing and participated in the multiple-locus variable-number tandem repeat analysis (MLVA) training in September 2008. Michael Lehman joined the department in February 2007 and specializes in PCR assay development. He is working on his Masters in Public Health at East Tennessee State University, as well as taking a Clinical Molecular Biology online course from Michigan State University. Since the state of Tennessee requires that clinical medical laboratory personnel be licensed, all Molecular Biology personnel have state MT or MT Supervisor licenses.

***Vibrio cholerae*, an early microscopic image, circa 1860s and *V. cholerae* as visualized today.**

Jeannette Dill, the primary PFGE technologist, earned her Bachelor of Science degree in Microbiology from Austin Peay University in Clarksville, TN. She holds an MT supervisor license from the State of Tennessee. She worked in the General Bacteriology section of the laboratory for 12 years before transferring to Molecular Biology and is the department's resident "PulseNut" [sic]. Since her transfer to Molecular Biology in 2005, Jeannette has attended BioNumerics training at CDC, two PulseNet general meetings and a regional meeting. She has participated on the planning committee for both the PulseNet 2008 General Meeting and 2008 Southeast Regional meeting. Now performing the majority of the gel analysis, uploading to CDC and CDC Team monitoring and posting for Tennessee, she communicates on a regular basis with CEDS (Communicable and Environmental Disease Services) regarding local and national clusters. Tennessee epidemiologists contact her when they need comparisons or statistical analysis from our local database or from the national database. Recently she worked on developing a standardized protocol for Group B streptococci (GBS) using the standard H9812. The protocol was presented at the American Society for Microbiology General Meeting in Boston, MA in June 2008.

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A Look Inside the TN Public Health Laboratory System (Continued)

The State of Tennessee has a mandatory submission law in place for *Escherichia coli, Listeria, Salmonella and Shigella. Campylobacter* is considered a reportable disease, but is not yet under mandatory submission. For reporting forms and the most up-to-date list go to:

http://health.state.tn.us/CEDS/index/htm

Submitted by Amy Woron, Molecular Biologist, Manager
&
Sheri Roberts, Microbiologist, Supervisor
Molecular Biology and Enteric Bacteriology

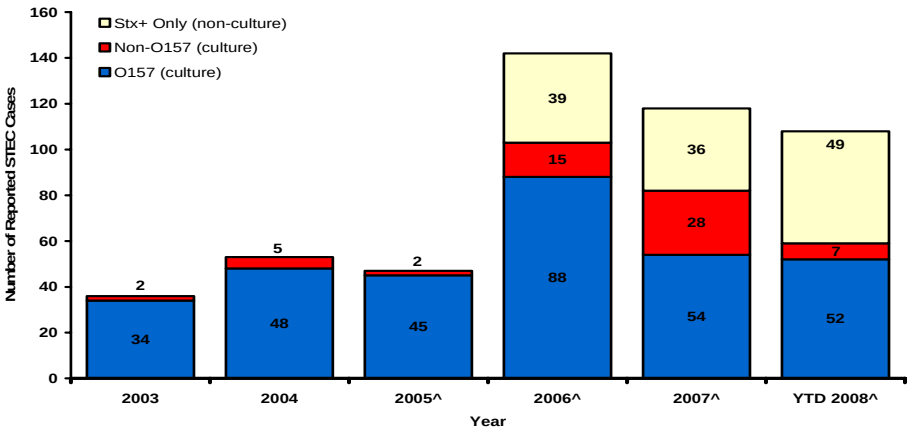
Laboratory Practices & Shiga-toxin Producing Escherichia coli (STEC)

Shiga-toxin producing *Escherichia coli* (STEC) infections are an important cause of enteric disease and *E. coli* O157 is the most common STEC isolated and reported in the US. Clinical laboratory identification, including traditional bacterial culture and biochemistry methods, play an important role in STEC surveillance and outbreak investigation. In recent years, non-culture-based methods such as EIA and PCR have become available and at the same time non-O157 STEC infections have been increasingly recognized in Tennessee (Figure 1). All STEC infections became reportable nationwide in 2000.

A 2007 web-based and telephone survey of clinical laboratories in 10 FoodNet sites, including Tennessee, helped describe laboratory practices for the identification and reporting of STEC. Of the 135 selected laboratories in Tennessee, one hundred and 132 responded. Fifty-six of these reported testing on site for *E coli* O157/STEC.

All but one of these 56 reported using culture-based methods. Nine (16%) used non-culture based methods for *E coli* O157/STEC identification. Four out of these 9 labs used non-culture-based methods for the identification of non-O157 STEC. A single Tennessee laboratory reported using culture and non-culture methods simultaneously, as recommended by the 2000 CDC guidelines published in MMWR, Vol. 55, No 38. All 9 labs in Tennessee using non-culture methods indicated that they send positive specimens for further testing to the state public health laboratory. Eight more clinical labs indicated adding non-culture testing for *E coli* O157/STEC in the near future.

Number of Reported STEC Cases Identified by Culture and Non-culture Methods, Tennessee, 2003-2008.



Detection and characterization of non-O157 STEC is dependent on clinical laboratories using both culture and non-culture based methods or submitting Shiga-toxin positive specimens to their state public health laboratory for further testing. — *by Samir Hanna, MD, MSPH*

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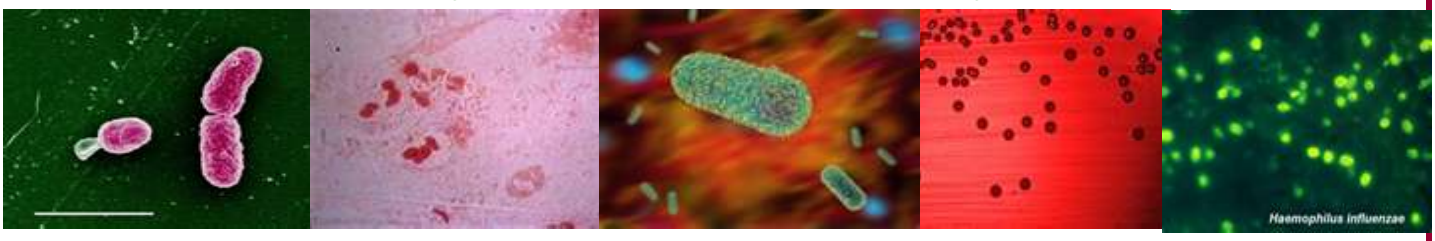
Haemophilus influenzae Surveillance in Tennessee for 2004-2008



Active Bacterial Core surveillance (ABCs) is a core component of CDC's Emerging Infections Programs Network (EIP), a collaboration between CDC, state health departments and universities. ABCs is an active laboratory- and population-based surveillance system for invasive bacterial pathogens of public health importance. This surveillance currently operates among 10 EIP sites across the United States, representing a population of over 38 million persons. For each case of invasive disease in the surveillance population, select isolates are sent to the Tennessee Department of Health EIP site for additional laboratory evaluation.

Invasive *Haemophilus influenzae* disease is one organism under surveillance for the ABCs. This surveillance is to evaluate progress in the elimination of serotype B disease; to detect possible emergence of disease due to other capsular types; to determine possible preventable reservoirs of the bacteria. Laboratory characterization includes *H. influenzae* serotyping (a-f), classified by characterization of their capsule. Non-typeable *Haemophilus influenzae* strains are the most common isolated and are part of the normal flora of the human upper respiratory tract. Capsule type *b* (**Hib**) is the most clinically significant because of its virulence. A *Haemophilus influenzae* type b or Hib case is defined as isolation of *H. influenzae* from a normally sterile site in a resident of the state.

The pathogenesis of *H. influenzae* infections is not completely understood, although the presence of the **type b polysaccharide capsule** is known to be the major factor in virulence. Before the introduction of effective vaccines, *Haemophilus influenzae* type b (Hib) was the leading cause of bacterial meningitis and other invasive bacterial disease among children aged <5 years. Therefore, accurate laboratory information is essential to correctly identify the serotype of the causative Hi isolate and to assess progress toward the elimination of Hib invasive disease.

Laboratory discrepancies might occur with some antisera. Polyvalent antisera that includes types a-f, is useful to help determine possible cross reactions or rough isolates when compared to control saline. Isolates of *Haemophilus influenzae* from sterile sites is a reportable disease. These isolates may be submitted to the Tennessee Department of Health Laboratory for typing and determine the incidence of invasive disease in Tennessee.

Submitted by Henrietta Hardin, Manager, General and Environmental Bacteriology