TENNESSEE DEPARTMENT OF HEALTH
It's About Time!

Tennessee Department of Health Public Health Laboratories Newsletter

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Commissioner of Health

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Inside this issue:

Genotyping Links TB Patients Nationally

Measuring Genetic Variation

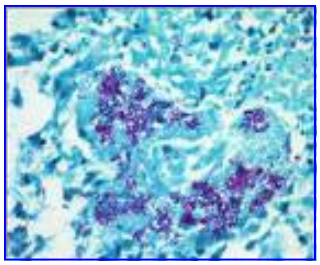
Since January of 2004, the Mycobacteriology Section has participated in a national genotyping program for *Mycobacterium tuberculosis* complex (M. tbc) isolates. Genotyping data provides a measurement of the genetic variations among a species. The Mycobacteriology Section sends an initial isolate from every tuberculosis patient in the state to the TB Genotyping Lab in Lansing, Michigan. As matches are seen among the genotyping data, cluster numbers are assigned. Cluster numbers are assigned according to the state that submitted it. The state cluster number assignment aids the Tuberculosis Elimination Program in linking tuberculosis patients together in an effort to stop the spread of the organism and potentially the disease.

Matching the Data

Genotyping data is received in three formats. Two are PCR-based tests and the other is an RFLP-based test. Spoligotyping, spacer oligonucleotide typing, and MIRU, mycobacterial interspersed repetitive unit-variable-number assay, are the two PCR-based tests. All isolates are processed with these two assays. The PCR assays produce an algorithmic number of 15 and 12 digits, respectively. For a cluster number to be assigned, these two numbers must be identical. Any future matches will also be assigned the same cluster number. In many cases this process is enough to identify two or more individual cases as being linked and investigative questionnaires can ascertain a connection between the individuals. Some cases are more difficult to link together. Another test is offered in these situations, the IS6110-based Restriction Fragment Length Polymorphism (RFLP) assay, which is only performed as part of the genotyping data under these circumstances.

Turn-Around-Times

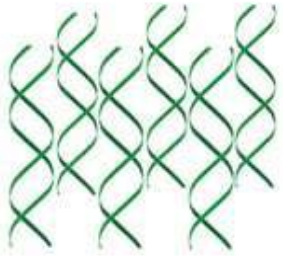
Spoligotyping and MIRU testing have an average 10-day turn-around-time from time of receipt and are performed on the liquid media submitted. The combination of these tests provides for earlier detection of outbreaks, and as the database grows, this tool has proven to be quite useful. RFLP is less often requested for several reasons; a few of which follow. 1) It has an extended turn-around-time due to the technology used. 2) Some strains do not produce a great deal of discrimination in the data generated and therefore provide little additional information. 3) Testing is performed from an isolate on solid media which may add additional time to obtain a result.



Acid-Fast Stain preparation of *M. tuberculosis* in lung

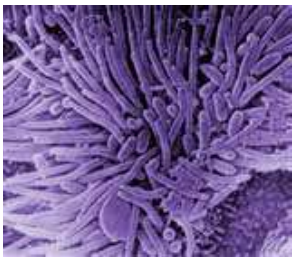
New Element Added in 2007

The newest element to this program was added at the beginning of 2007; state programs now receive a national PCR number in addition to their state cluster number. This allows the state program to work with bordering states. In addition, since citizens are only a plane ride away from either coast, it allows us to help track cases that may require such data. This addition has alleviated many of the issues encountered among state programs as well as allowed the CDC to track outbreaks across the country. National PCR cluster information has also allowed CDC to characterize outbreaks that were peculiar to a certain region or area of the country and watch the spreading patterns or, the more desired outcome, the lack of spreading patterns.



Contributed by Teresa Smith, Assistant Director, Clinical Division

Laboratory Information Systems Update



During the past month, Laboratory Services has transferred 5 tests to our newest Laboratory Information System, StarLIMS. We are now reporting the following assays via this system:

- *Bordetella*, both culture and PCR testing,
- Heterotrophic Plate Count (HPC, a water assay),
- TPPA (Treponema pallidum Particle Agglutination), and
- Arbovirus IFA.

The *Bordetella* assays are currently only performed at the Nashville location. HPC and TPPA are performed at Jackson, Nashville and Knoxville. Arbovirus IFA testing is performed at Nashville and Knoxville.

The assays listed joined the assay for Norovirus as the ground work for connecting and standardizing all three locations of Tennessee's Public Health Laboratories. While not all tests are performed at all three locations, the data that is accumulated from the locations will now be available in a central location.

The centralization of data will make any type of outbreak investigation or organism surveillance more timely and be of greater benefit to the citizens we serve.

Photomicrographs of *Treponema pallidum* (syphilis) left and *Bordetella pertussis* (whooping cough).

Teresa Smith,
Assistant Director, Clinical Division

Updated Newborn Screening Specimen Collection Training CD-ROM Available

TDH and NLTN Produce CD-ROM

The state of Tennessee requires newborn screening of metabolic and genetic disorders such as Phenylketonuria (PKU) and other amino acid disorders, Hemoglobinopathies, and over 50 other serious genetic disorders. Each of these disorders requires early detection to insure the child will lead as healthy and normal a life as possible. Working under a grant from the Tennessee Department of Health, the National Laboratory Training Network (NLTN) has produced an educational CD-ROM, "Let's Do It Right the First Time," to train local healthcare providers in the fundamentals of collecting and submitting blood specimens for newborn screening.

Specimen Collection

Prompt and correct specimen collection allows the laboratory to provide testing results quickly so that appropriate healthcare can commence for patients with abnormal results. The one-hour program consists of modules on genetic disorders, how to complete the Tennessee collection form, proper specimen collection technique, quality assurance, the follow-up process, and an introduction to the hearing testing program in Tennessee. The CD-ROM format provides convenient delivery of the educational material, as well as interactive exercises to increase confidence in the mastery of the material. Resource materials are included in a printable format so healthcare providers can access them easily after the conclusion of the training period.

Continuing Education Credits

The program includes a post-test and has been approved for continuing education credits by both the Tennessee Nurses Association and the American Society of Clinical Laboratory Science P.A.C.E.® program. For further information contact the NLTN Office at 615-262-6315 or seoffice@nltn.org.

The National Laboratory Training Network is a training system sponsored by the Association of Public Health Laboratories (APHL) and Centers for Disease Control and Prevention (CDC).

Continuing Education Corner

In January 2006, a new regulation went into effect concerning continuing education (CE) requirements for medical laboratory professionals in the state of Tennessee. To maintain a state license, a medical technologist or medical laboratory technician must earn at least 24 hours of Board approved continuing education in the two calendar years prior to the year of license renewal.

Recent Revisions to the Regulations
The entire regulation, which has been recently revised, may be found on pages 10-15 at the link at the end of this article. To date in 2007, Tennessee Department of Health Laboratory Services' Training Resources Section has coordinated over 30 contact hours of continuing education in the form of teleconferences, live presentations, online courses, webcasts, and videos. In addition, workshops have been hosted locally and statewide on pertinent topics such as pandemic influenza preparedness, packaging and shipping of biological substances, public health emergency preparedness, and recognition of agents of bioterrorism.

Laboratorians seeking educational courses offering CE credit have many other options. Opportunities for CE are presented in professional journals and on the internet through government and professional organizations, such as CDC and ASCP, respectively. Courses are available in several formats, many at low or no cost to the participant.

Susan L. McCool,
Training Coordinator,
Training Resources Section

http://www.state.tn.us/sos/rules/1200/1200-06/1200-06-01.pdf

New Employees and Promotions

New Hires	Section/Location	Start Date
Kristina Arden	Clerk 2, Reporting Office, Nashville	10-01-2007
Tracey Goman	Micro 2 Certified, Bacteriology, Nashville	10-01-2007
Patrick Leathers	Chemist1, Inorganics, Nashville	08-27-2007
Bill Moore	Chemist 1, Radiochemistry, Nashville	09-04-2007
Debra Smith	Micro 2 Certified, Bacteriology, Nashville	09-04-2007
Meredith Todd	Chemist 1, Organics, Nashville	09-17-2007
Dennis Turner	Chemist 1, Organics, Nashville	08-31-2007

Promotions	Section/Location	Effective Date
Patricia Alicea	Manager, Aquatic Biologist, Nashville	10-01-2007
Teresa Smith	Assistant Director, Clinical Division, Nashville	07-16-2007

2007-2008 Influenza Season is Upon Us

The most active time of the year for influenza activity in the United States is usually from the beginning of October through mid-May with the highest number of cases reported December through February. Several health care providers have volunteered to help the Tennessee Department of Health (TDH) create a sentinel providers network (SPN) for surveillance of influenza. Each SPN participant has agreed to send at least one specimen per month from a patient presenting with an influenza-like illness (ILI) or with respiratory symptoms strongly suggestive of influenza infection. This surveillance program is not designed to test all patients suspected of having an influenza infection. The network is designed to test a representative sample of the state's population with an ILI.

Surveillance specimen collection kit.

Collection and Shipping Laboratory Services has already sent materials and instructions to the SPN participants for the collection and submission of specimens to culture and identify influenza virus in the state. A letter was included with each shipment identifying the items and quantity of each item sent to the SPN participant. The shipment also contains an instruction sheet to be used as a guide for collection and shipment of the specimens to Laboratory Services in Nashville. **If any SPN participant has not received influenza surveillance materials or materials in correct quantities**, please call Jerry Hindman or Bill Reimels at 615-262-6374 or 615-262-6350, respectively. To use the supplies as efficiently and economically as possible, the plan is to replace materials as they are received from the SPN participants.

Jerry Hindman, Manager
Serology and Virology Units

Vector-Borne Diseases Laboratory Discovers Potential New Virus Cycle

New Vector Borne Disease Lab
Dr. Abelardo Moncayo is the Director of the Vector-Borne Diseases (VBD) Laboratory, which is under the Communicable and Environmental Diseases Services Section of the Tennessee Department of Health. The VBD Laboratory is housed at Laboratory Services in Nashville. The mission of the VBD Laboratory is to enhance investigations and surveillance of vector-borne disease outbreaks.

Eastern Equine Encephalitis
Eastern equine encephalitis (EEE) in North America is a serious mosquito-borne disease of humans and equines with mortality rates of 35-90%, depending on the outbreak. This high mortality rate makes it one of the most severe human arboviral diseases in the United States. Eastern equine encephalitis virus (EEEV) is traditionally thought to be maintained in North America in an **enzootic** avian cycle primarily by *Culiseta melanura* mosquitoes in hardwood swamps. *Culiseta melanura* appears to be replaced by *Culex (Melanoconion) erraticus* mosquitoes at inland sites where EEEV transmission occurs in Tennessee and other south-central states.

Venezuelan Equine Encephalitis
Culex mosquitoes of the Melanoconion subgenus maintain Venezuelan equine encephalitis virus (VEEV) and possibly EEEV enzootically among rodents in

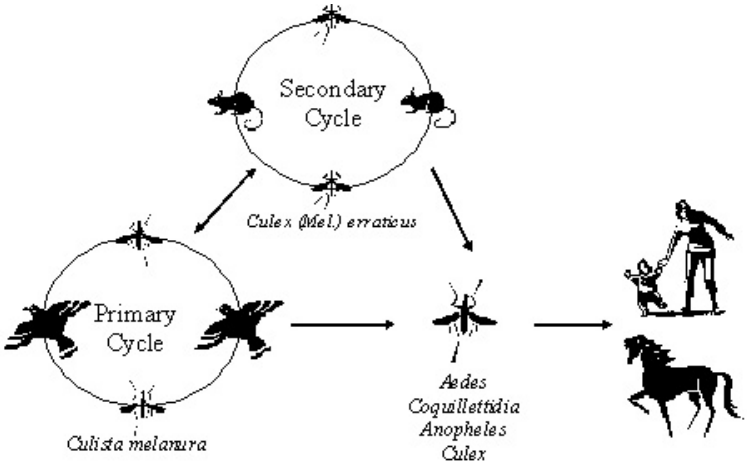
A hamster-baited Trinidad trap

Latin America. VBD Laboratory entomologists used hamster-baited Trinidad traps to determine if *Cx. (Mel.) erraticus* would be attracted to hamsters as are other species of *Melanoconion* in Latin America. The study site was a hardwood swamp, which was at the epicenter of a large equine **epizootic**. Vaccinated hamsters were placed in Trinidad traps and these traps were compared to CO2-baited CDC light traps in order to determine which mosquito species of those at this site were attracted to hamsters. Only *Cx. (Mel.) erraticus* were significantly attracted to hamster-baited Trinidad traps. There was no difference between the two traps with respect to their ability to capture *Cx. (Mel.) erraticus;* however, CDC light traps are not reliably able to predict the rate of mosquitoes feeding on hamsters in Trinidad traps.

Study Conclusion
Culex. (Mel.) erraticus is competent for EEEV and is found in great abundance at EEE epizootic inland sites. Rodents are able to elicit a viremia high enough to potentially infect mosquitoes. In this study it was found that *Cx. (Mel.) erraticus* are readily attracted to rodents at EEEV epizootic hardwood swamps. These observations suggest the potential of a secondary silent EEEV cycle that may be occurring in inland swamps in the south-central U.S.

Abelardo Moncayo, Ph.D., Director
Vector-Borne Diseases Laboratory

Potential Secondary Silent EEEV Life Cycle



Vocabulary Corner

Endemic: Present in a community at all times, but in relatively low frequency. Something that is endemic is typically restricted or peculiar to a locality or region.

Enzootic: Endemic in animals. An enzootic disease is constantly present in an animal population, but usually only effects a small number of animals at any one time.

Epizootic: An epidemic outbreak of disease in an animal population, often with the implication that it may extend to humans.

APHL/CDC Fellows in Residence at Vector-Borne Diseases Laboratory

The Tennessee Department of Health Laboratory Services has begun hosting fellows from the Association of Public Health Laboratories (APHL) and the CDC. These fellows are mentored by Dr. Abelardo Moncayo at the Vector-Borne Diseases (VBD) Laboratory.

Present Fellows

In 2006, our first APHL fellow, Sudeshna Mukherjee, joined the VBD Laboratory to work on projects to study the epidemiology of eastern equine encephalitis virus and West Nile virus. Ms. Mukherjee is a M.S. graduate of Drexel University in Philadelphia, PA. In 2007, Sara Cohen joined the VBD Laboratory to work on mosquito and tick-borne disease projects. Ms. Cohen obtained her B.S. from Cornell University at Ithaca, NY.

At the VBD Laboratory, field and laboratory studies interface as we use a combination of ecological and molecular approaches to understand the epidemiology of arthropod-borne diseases at various levels of hierarchy. Under the leadership of Dr. David Smalley, APHL fellows have the opportunity to experience work done in other sections of the State Laboratory. Dr. Timothy Jones, Deputy State Epidemiologist, provides opportunities at CEDS for fellows to participate in outbreak investigations. These opportunities give APHL fellows a truly unique experience that prepares them for working in public health laboratories, research, and other careers in public health.

2008 Emerging Infectious Diseases (EID) Laboratory Fellowships

The Emerging Infectious Diseases (EID) Laboratory Fellowship Program, sponsored by APHL and CDC, trains and prepares scientists for careers in public health laboratories and supports public health initiatives related to infectious disease research. The EID Advanced Laboratory Training Fellowship is a one-year program designed for bachelor's or master's level scientists, with emphasis on the practical application of technologies, methodologies and practices related to emerging infectious diseases. The EID Laboratory Research Fellowship is a two-year program designed for doctoral level (Ph D, MD or DVM) scientists to conduct high-priority infectious diseases research.

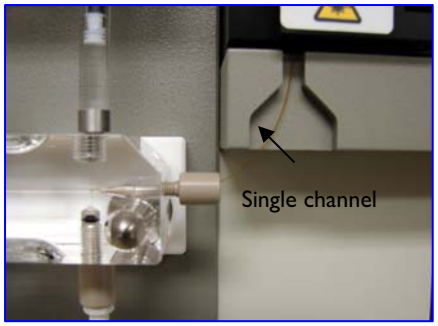
To Make Application

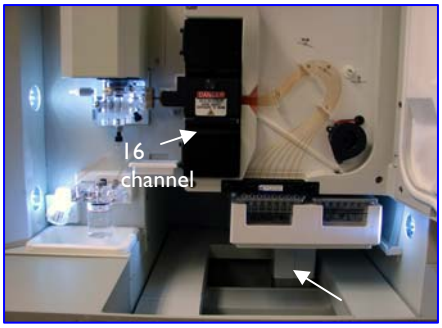
Interested individuals are invited to contact Heather Roney, the Fellowship Program Manager of APHL for more information at the contact information below.

Heather Roney
APHL Fellowship Program Manager
8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Phone: 240.485.2778
Fax: 240.485.2700
Email: heather.roney@aphl.org

New Genetic Sequencer 16 Times Faster Than Old Sequencer

The Molecular Biology laboratory recently installed an Applied BioSystems (ABI) 3130xl genetic sequencer to replace the ABI 310 genetic sequencer.





Both systems provide fragment analysis and sequencing by capillary electrophoresis. The most obvious difference between the two versions is the number of capillaries. The ABI 3130xl has 16 capillaries compared to the single capillary of the 310. What this means to us is that we can provide results on 96 reactions in 18 hours instead of 12 days. The increased capacity will allow the Molecular Biology laboratory to participate in MLVA (Multi-locus VNTR (Variable number of tandem repeats) Analysis as part of the next-generation subtyping methodologies of PulseNet.

Amy M. Woron, Manager
Molecular Biology Unit

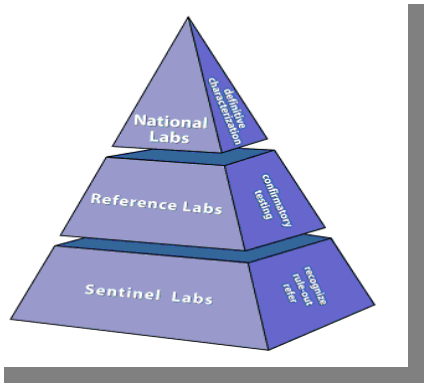
Pictured left is the ABI 301 single-channel sequencer which has been replaced by the new ABI 3130XL 16-channel sequencer.

Refer Rule-out Isolates and Specimens to the Nearest LRN Reference Labora-

All laboratories in Tennessee capable of analyzing or referring specimens or samples that may contain microbial agents or biological toxins function as **sentinels** in the nation's Laboratory Response Network (LRN).

Sentinel Labs Varied
These include environmental, food, veterinary, agriculture, public health, and clinical laboratories in Tennessee. Because of their routine activities, all of these laboratories have the potential to handle materials that may contain agents that threaten the public's health. While all are sentinel, these laboratories may differ in the level of testing that they can provide. For successful operation of the LRN, each LRN sentinel must collaborate with the Tennessee local LRN reference laboratory located in Knoxville, Nashville, Jackson, or Memphis.

Immediate Referrals
When sentinel clinical laboratories are unable to rule out possible bioterrorism agents using standard ASM

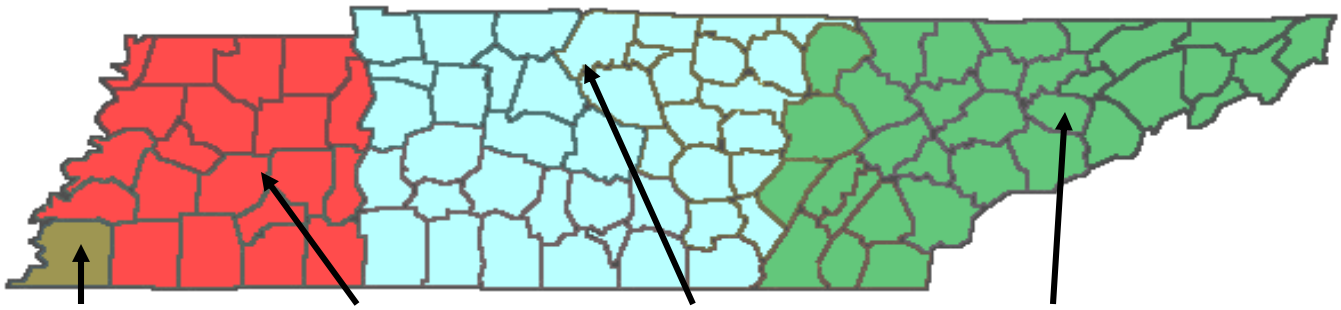


(American Society for Microbiology) protocols, they must immediately refer suspicious isolates or specimens to closest collaborating LRN reference laboratory. At the LRN reference laboratory, additional analyses are conducted using standardized, validated confirmatory assays made available through the CDC.

Prior Notification
As soon as the Sentinel laboratory recognizes that they have a suspicious isolate or specimen that requires referral, the sentinel laboratory should arrange for transfer to the closest Tennessee LRN reference laboratory. They must call their regional state laboratory prior to sending a specimen for rule out and confirmation and provide the following information:

1. When and how the specimen is transported to the LRN reference laboratory,
2. List of ASM procedures performed with corresponding results,
3. Patient/isolate information and history.

Contributed by James A. Gibson, Director, Clinical Division
And
Irmgard Brown, Coordinator Bioterrorism Laboratory



Listed are the four Regional LRN Reference Laboratories and the counties they support. Sentinel laboratories should refer all rule-out isolates or specimens to the closest LRN

Nashville Central Laboratory Services
630 Hart Lane
Nashville, TN 37243
Contact person: Irmgard Brown, Paula Bailey, or Henrietta Hardin at 615-262-6300

Counties Served: Bedford, Cannon, Cheatham, Clay, Coffee, Davidson, DeKalb, Dickson, Franklin, Giles, Grundy, Hickman, Houston, Humphreys, Jackson, Lawrence, Lewis, Lincoln, Macon, Marion, Marshall, Maury, Montgomery, Moore, Overton, Perry, Pickett, Putnam, Robertson, Rutherford, Sequatchie, Smith, Stewart, Sumner, Trousdale, Van Buren, Warren, Wayne, White, Williamson, Wilson

Refer Rule-out Isolates and Specimens to the Nearest LRN Reference Laboratory (Cont'd)

Jackson Regional Laboratory **295 Summar Drive** **Jackson, TN 38301** **Contact person: Peggy Pate or Deanne Sharpe** **731-426-0686**	**Counties Served:** Benton, Carroll, Chester, Crockett, Decatur, Dyer, Fayette, Gibson, Hardeman, Hardin, Haywood, Henderson, Henry, Lake, Lauderdale, Madison, McNairy, Obion, Tipton, Weakley
Knoxville Regional Laboratory **1522 Cherokee Trail** **Knoxville, TN 37920** **Contact Person: Mike McWilliams** **865-549-5201**	**Counties Served:** Anderson, Bledsoe, Blount, Bradley, Carter, Campbell, Claiborne, Cocke, Cumberland, Fentress, Grainer, Green, Hamblen, Hamilton, Hancock, Hawkins, Jefferson, Johnson, Knox, Loudon, McMinn, Meigs, Monroe, Morgan, Polk, Rhea, Roane, Scott, Sullivan, Unicoi, Union, Washington
Memphis & Shelby County Health Department **814 Jefferson Avenue** **Memphis, TN 38105** **Contact person: Stephen Gooch 901-544-7583**	**Counties Served:** Shelby

MRSA Toolkit Available for Athletic Directors, Coaches, School Health Teams

MRSA or Methicillin–resistant *staph aureus* is a type of infection that is resistant to many antibiotics. MRSA skin infections are generally spread by skin-to-skin contact or by direct contact with the drainage from an infected wound. Research indicates 85% of all serious cases of the infection are associated with health care settings, while the remaining 15% of reported infections are considered community-associated. Community-associated MRSA can be spread by contact with contaminated surfaces or items such as sports equipment or personal hygiene items.

In Tennessee, there were more than 1,800 invasive cases of MRSA in both 2005 and 2006. An invasive case occurs when the infection is found in organs other than the skin. As of September 30, 2007, there were 1,400 diagnosed cases of MRSA in Tennessee. For your toolkit or more information go to the Tennessee Department of Health web site at

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

Newborn Screening Fee Increase

Effective December 28, 2007, the state public health laboratory will increase the fee for Newborn Screening to $75.00 for conducting any one or all of the following tests on newborn blood samples submitted to the laboratory: Biotinidase Deficiency, Congenital Adrenal Hyperplasia (CAH), Congenital Hypothyroidism, Galactosemia, Hemoglobinopathies, Homocystinuria, Maple Syrup Urine Disease (MSUD), Medium Chain Acyl/CoA Dehydrogenase (MCAD) Deficiency, Phenylketonuria (PKU), and other metabolic/genetic tests as designated by the Department of Health. In early 2008, the Division of Laboratory Services will add Cystic Fibrosis as a screening test to the Newborn Screen. These $75.00 fee includes the cost of testing and follow up services.

Pictured, right, is a newborn screening blood collection form. The baby is given a heel stick and the drops of blood are collected on a filter-paper collection device. The drops are then tested for over 65 different genetic inborn errors, many of which are treatable when discovered early. Over 83, 000 babies were born in Tennessee last year.

Aquatic Biologists Participate in the National Lake Study

In the summer of 2007, the U. S. EPA Office of Water conducted the National Lake Survey. The purpose of this survey was *to generate statistically-valid reports on the condition of our Nation's water resources and identify key stressors to these systems.* The goal of the survey was to answer two (2) key questions concerning the quality of the Nation's lakes, ponds and reservoirs. The first goal was to determine "*what percent of the Nation's lakes are in good, fair, and poor condition for key indicators of trophic status, ecological health, and recreational value*. The second goal was to determine *the relative importance of key stressors such as nutrients."*

Specialized Training By EPA

EPA conducted training for the National Lake Survey during May 2007 at Red Top Mountain State Park in Georgia. EPA was assisted in training activities by Tetra Tech, an environmental consulting firm that provides model training programs to the EPA. From Laboratory Services, Aquatic Biology section, Pat Alicea and Carrie Perry attended the training sessions. EPA provided all of the participants at the training sessions with the specific equipment that was required for sampling.

12 Lakes Studied in Tennessee

For Tennessee, the National Lake Survey consisted of 12 lakes across the state. The sites began in middle Tennessee with Cheatham Lake (or Cumberland River), Woodhaven (Montgomery Bell), and Elaine (Shelbyville). West Tennessee was next with Pickwick (Alabama), Cedar (Jackson), and Barrs Chapel (near Paris). Douglas Lake was the only one sampled in east Tennessee. In Chattanooga, Nickajack and Ocoee (Parksville) were sampled. Sampling was finally finished in Cookeville with Burgess Falls, Thousand Oaks, and Catherine.

Types of Samples Taken

At each site, the sampling began by finding the center of the lake (the index site) and collecting various samples. A lake profile was made by measuring the temperature, dissolved oxygen, and pH at various depths. A secchi disk was used to determine the euphotic zone (clarity), and water was collected from this zone with an integrated sampling device (PVC pipe construction) for various testing (chemical, phytoplankton, microcystin, and chlorophyll). Zooplankton was collected with two nets, and a corer was used for a sediment sample for mercury and diatoms.

Keith Gaddes (LS), Brandon Chance, and Darry Sparks (TN Dept of Environment and Conservation) using a corer device to extract a sediment sample to be analyzed for sediment diatoms and mercury.

P-Hab Sites

After the initial sampling, ten (10) sites equally spaced around the lake were sampled. These were the physical habitat sites (P-Hab site). At each site, a form looking at the lake shore in that area was completed and a macroinvertebrate (bug) sample was collected. At the last site, the bacteria sample was collected and a final form was filled out based on the overall lake. The final form allowed for the reporting of other things that were not seen at the specific P-Hab sites. Once back to the shoreline, the bacteria and chlorophyll samples were filtered and placed on ice. The bug sample was composited into several containers. Some of the samples were then shipped off immediately to EPA's Lab, while others were held for a batch shipment. The paperwork was then checked for completeness and neatness before being mailed.

Final Report Due in Fall 2009

The survey lasted from June to August 2007, and required the assistance of other biologists from various environmental field offices. Carrie Perry was the lead biologist and present at all lake samplings. An audit was also performed by a member of Tetra Tech by watching the sampling event and making sure that all protocols were followed. The final report for the National Lake Survey is scheduled for release by the EPA in Fall 2009.

Carrie Perry,
Aquatic Biologist
and
Bob Read, Director
Environmental Laboratories