TENNESSEE DEPARTMENT OF HEALTH
It's About Time!

Tennessee Department of Health Public Health Laboratories Newsletter

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Fall foliage at Nashville's Radnor Lake

Wildlife and Domestic Animals Infected with Trypanosoma cruzi in Tennessee

Chagas disease (also known as American typanosomiasis) is a protozoan infection in humans that affects 8 to 11 million people in Latin America and an emerging disease in the southeast, including Tennessee. *Trypanosoma cruzi,* the protozoan that causes Chagas disease, is transmitted by kissing bugs. As a zoonotic infection, *T. cruzi* occurs throughout the Americas from the Great Lakes of North America to southern Patagonia. Chagas disease is influenced by climate and its current distribution and intensity of disease may potentially increase in the future.

Clinically, Chagas disease has two phases, acute and chronic. The acute phase is characterized by fever, general discomfort, enlargement of the liver, spleen and lymph nodes, meningitis and encephalitis, and weakening of the heart muscle. During this phase, the mortality rate is 5-15%. The chronic phase may last for decades without symptoms but 20-30% of patients in this phase will develop symptoms after decades due to progressive destruction of muscle and nervous tissue of the heart. Severe cardiac lesions can lead to sudden death. Aside from cardiac pathology, some of the trypanosomes can cause destruction of intestinal tissue, causing intestinal megasyndromes (gross dilation of the intestinal tract).

In the U.S., *T. cruzi* prevalence has been reported for over 20 different wildlife species such as raccoons and opossums, and

Tennessee's Kissing Bug (*Triatoma sanguisuga*) can transmit *Trypanosoma cruzi*, the causative agent of Chagas disease.

In some counties as many as 63.6% of eastern Tennessee raccoons are infected with *T. cruzi*

seven autochthonous (domestic, without travel history) human cases have been documented since 1955. Autochthonous human and canine transmission of *T. cruzi* has been documented in Tennessee, but little is known about its ecology, including the prevalence of *T. cruzi* among wildlife and domestic animals in Tennessee. In order to better describe transmission of *T. cruzi* in Tennessee, the Tennessee Department of Health Vector-Borne Diseases Section, the Southeastern Cooperative Wildlife Disease Study in the College of Veterinary Medicine and Warnell School of Forestry and Natural Resources at the University of Georgia, the Departments of Biology at Middle Tennessee State University and Belmont University, the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services and the Tennessee Veterinary

Biotinidase deficiency is one of the tests performed in the

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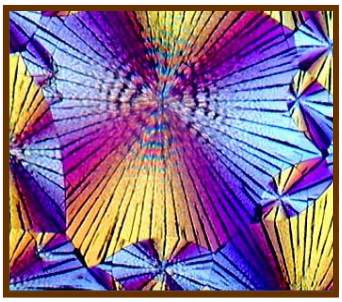
Public Health Laboratory Performs Biotinidase Deficiency Screening

Newborn Screening Laboratory. In normal patients the enzyme biotinidase recycles endogenous biotin and plays an important role in the processing of protein-bound dietary biotin(1). In biotinidase deficient patients the biotin-salvage pathway is blocked, therefore high concentrations of dietary biotin are required to prevent or treat symptoms of biotin deficiency(2,3). Early diagnosis and treatment with Biotin results in rapid and usually complete regression of symptoms, with hearing loss and optic atrophy being less reversible(4). However, all affected children have improved clinically with Biotin supplementation(1).

Biotinidase Deficiency has an incidence of greater than 1 in 75,000, with the highest incidence in Caucasians(4). Patients diagnosed with biotinidase deficiency can have profound or partial enzyme deficiency. Profound deficiency is defined as enzyme activity that is less than 10% of mean normal activity. Partial deficiency is between 10 to 30% of mean normal(1). Most patients with profound enzyme deficiency present early

A picture of crystallized biotin.



Newborn Screening Program promotes healthy babies.

in life whereas those with partial deficiency present later with skin problems and no neurologic findings. Because some patients remaining asymptomatic, controversy exists whether patients with partial deficiency need treatment with Biotin, however, this should not negate screening all infants for biotinidase deficiency as others may develop symptoms(5).

Because of the availability of laboratory testing, efficacy of treatment with Biotin, and relative low cost, biotinidase deficiency is ideal for inclusion in all newborn screening programs. The federal Maternal and Child Health Bureau commissioned the American College of Medical Genetics (ACMG) to outline a process of standardization for newborn screening. In 2006, this panel identified 29 conditions for mandatory screening. Biotinidase deficiency was included in this core panel(6). Prior to the ACMG recommendation, the Tennessee Department of Health Newborn Screening Program began screening for Biotinidase deficiency.

Tennessee began testing in February 2004 using specimens of whole blood spotted on filter paper. The semi-quantitative colorimetric test procedure based on the Wolf method and using continuous flow analysis was selected. An upgrade to our instrumentation and test procedure has since occurred. The colorimetric assay is favored over tandem mass spectrometry (MS/MS) for screening. Although some symptomatic patients with Biotinidase deficiency will have elevated C5-OH (3-Hydroxyisovaleryl carnitine) by MS/MS(7), not all patients with Biotinidase deficiency have this elevation level. Therefore MS/MS is not the best method for screening(5).

Biotinidase deficiency screening has proven to be beneficial for newborns in Tennessee. Since the inception of routine testing there have been 18 diagnosed cases of Biotinidase deficiency: Eleven were found to have profound deficiency and seven patients were diagnosed with partial enzyme deficiency.

Submitted by Christine McKeever, Manager, Tandem Mass Spectrometry Newborn Screening Section

References

1. Wolf, B., Heard, G.S., Screening for Biotinidase Deficiency in Newborns: Worldwide Experience. Pediatrics 85, 512-517 (1990).
2. Heard, G.S., Secor-McVoy, J.R., Wolf, B. A Screening Method for Biotinidase Deficiency in Newborns. Clinical Chemisty 30, 125-127 (1984).
3. Wolf, B., Grier, R., Allen, R., Goodman, S.I., Kien, C.L. Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency. Clinica Chimica Acta 131, 273-281 (1983).
4. American College of Medical Genetics Expert Group, Newborn Screening: Toward a Uniform Screening Panel and System-Main Report. Genetics in Medicine 8, 103s-104s, (2006).
5. Kaye, C.I., Newborn Screening Fact Sheets. Pediatrics 118, e934-e963 (2006).
6. American College of Medical Genetics Expert Group, Newborn Screening: Toward a Uniform Screening Panel and System-Executive Summary. Pediatrics 117, 296-307 (2006).
7. American College of Medical Genetics, Newborn Screening Act Sheet (Absent/Reduced biotinidase activity) Biotinidase Deficiency, (2006).

Methodology Change for Biotinidase Testing

Beginning with Julian Date 213 or August 1, 2009, our Biotinidase assay method changed from the continuous flow method to the Microplate reagent kit manufactured by Astoria Pacific.

This new assay brings two changes:

1. The unit of measure will change from ERU (Enzyme Response Units) to MRU (Microplate Response Units).
2. Cutoffs will change for normal, partial deficient and deficient.

Our new cutoffs for Biotinidase are as follows:

Within Normal Limits	**≥ 27 MRU**
Partial Deficient	**≥ 13 MRU - < 27 MRU**
Deficient	**< 13 MRU**

If you have any questions, please call the Newborn Screening Laboratory at 615-262-6352 or the Women's Health and Genetics Follow-up Program at 615-262-6304.

TN Public Health Laboratories Director Smalley Awarded the Army Legion of Merit

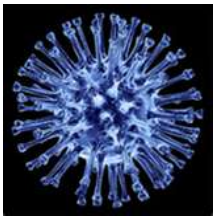
The Tennessee Public Health Laboratories Director, Dr. David L. Smalley, is also active in the US Army Reserve and has served the past three years as the Assistant Surgeon General for Force Management, Mobilization, Readiness, and Reserve Affairs in the Office of the Surgeon General, Falls Church, VA and as Deputy Commanding General for the Army Reserve Medical Command, Pinellas Park, FL. At the completion of his tenure at OTSG and MEDCOM, he was awarded the United States Armed Forces' Legion of Merit.

The Legion of Merit citation reads:

> "For exceptionally meritorious service while serving as Deputy Commanding General, Army Reserve Medical Command and Assistant Surgeon General. Brigadier General Smalley's tireless devotion to duty and ability to coordinate multiple agencies for the betterment of the Army medical community are a testament to his strategic perspective and strong leadership. The positive impact that BG Smalley made on the structure and operations of Army Reserve Medical will be felt for years to come."

Beginning October 1, 2009, BG Smalley is assigned as the Deputy Commanding General for Professional Services at the 807th Medical Deployment Support Command, Salt Lake City, UT.

Tennessee Department of Health (TDOH) State Laboratory Testing Policy for H1N1 Influenza— Effective Date: September 1, 2009



TDOH State Laboratory will limit testing specimens for H1N1 to the following:

- Those specimens submitted by providers in the Tennessee Sentinel Provider Network (SPN)
- Those specimens submitted by providers in the Emerging Infections Program (EIP)
- Capacity for testing additional specimens is limited and exceptions will only be made for cases of public health significance. All special requests for testing by the state lab must be pre-approved on a case by case basis.

All specimens received by the state laboratory without pre-approval will be returned to the provider.

- **If you are east of the Tennessee River**,
- your exception request will go to the Knoxville Regional Laboratory. Contact the Knoxville Laboratory Director, Robyn Atkinson at 865-549-5217 for pre-approval.
- **If you are west of the Tennessee River**,
- your exception request will go to the Jackson Regional Laboratory. Contact the Jackson Laboratory Director, Dr. Oristyne Walker at 731-426-0686 for pre-approval.

If the exception request is pre-approved, results, positive or negative, will be reported via facsimile to the submitting provider identified on the exception request.

In the majority of cases, testing is unnecessary. Treatment should be based on clinical presentation and should not be delayed for a confirmatory test. If indicated, treatment is most effective if initiated within 48 hours of symptom onset. Rapid tests have limited sensitivity but, when positive, can distinguish between influenza A and B. This may help with clinical decision making. Specific groups in which testing is likely to be beneficial include: pregnant women, healthcare workers, and hospitalized patients, particularly those in intensive care units. Specific testing for H1N1 is available commercially. Providers are urged to contact their routine commercial laboratory providers to inquire about testing options.

The TDOH will post updates on our website on the status of commercial availability of H1N1 tests, a summary of rapid test data, and frequent updates including distribution of circulating serotypes in the state at:

http://health.state.tn.us/H1N1.htm#hcp .

Additional information may be found at:

■ **Rapid influenza testing:**
http://www.cdc.gov/h1n1flu/guidance/rapid_testing.htm
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5830a2.htm

■ **Overview of tests and specimen handling:**
http://www.cdc.gov/h1n1flu/guidance/rapid_testing.htm

■ **Information for clinicians on a wide range of H1N1 related topics:**
http://www.cdc.gov/h1n1flu/guidance/

State specific information
■ **Regional/local health depts.**
http://health.state.tn.us/localdepartments.htm

■ **State Laboratory (615-262-6300)**
■ **Communicable and Environmental Disease Services (615-741-7247)**

Division of Laboratory Services Influenza H1N1 Testing FAQ's

1. What tests will be performed?
The state Public Health Laboratory will only test patients that are under the Sentinel Provider Network (SPN); Emerging Infection Program (EIP) Hospitals and selected Pre-Approved Requests. All other tests for H1N1 Influenza must be done through clinical referrals to reference and commercial labs.

2. How will results be reported?
All reports will be faxed to the provider and a copy sent to the regional office.

3. What results will be reported?
We will report each of the PCR markers tested. This will include Seasonal A; Seasonal H1; Seasonal H3; Seasonal B; Novel (Swine) A; and Novel (Swine) H1.

4. Who will be sent influenza test collection supplies?
SPN participants will get supplies shipped to them. Shippers will be returned each time a batch of specimens are sent to Nashville. EIP hospitals will provide their own swabs and collection supplies. Supplies for Pre-Approved tests will be the responsibility of the provider requesting the test.

5. What supplies are sent to SPN participants?
SPN participants will get a set of shippers, swabs, requests, and viral transport media.

6. How do SPN participants get supplies?
SPN participants can order supplies using a supply requisition form and sending it to:
Jerry Hindman Fax 615-262-6393 jerry.hindman@tn.gov
or
Susan McCool Fax 615-262-6393 susan.mccool@tn.gov
SPN can also contact their Regional SPN Coordinator.

7. What is pre-approval?
Since CDC has asked Public Health Labs to limit testing to those that allow us to monitor the changes in the virus and not test all persons suspected of an infection, we have limited routine testing to SPN and EIP Hospitals. However, we recognize that there are some circumstances that warrant testing individuals. We have established a process that allows providers to call public health to request H1N1 testing outside the SPN and EIP networks. **No testing will be done without pre-approved clinical assessment for public health information.**

8. What are the criteria for pre-approval?
Pre-approval will be done based on specific information about the patient and the medical conditions. All providers east of the Tennessee River call Dr. Robyn Atkinson of the Knoxville Regional Laboratory at 865-549-5217. All providers west of the Tennessee River call Dr. Oristyne Walker of the Jackson Regional Office at 731-426-0686.

9. What form is used?
There are three forms specific for each program:
- Emerging Infection Program (EIP)
- Sentinel Provider Network (SNP)
- Pre-Approval – will be faxed to approved providers

10. What is the mailing address?

EIP and SNP
Division of Laboratory Services
630 Hart Lane
Nashville, TN 37216-2006

Pre-approved Specimens only
All providers east of the Tennessee River will ship to:
Knoxville Regional Laboratory
1522 Cherokee Trail
Knoxville, TN 27950-9019.

All providers west of the Tennessee River will ship to:
Jackson Regional Laboratory
295 Sumnar Avenue
Jackson, TN 38302-0849.

11. What laboratory do I send my specimens to?

EIP and SNP
Division of Laboratory Services
630 Hart Lane
Nashville, TN 37216-2006

Pre-approved Specimens only
All providers east of the Tennessee River will ship to:
Knoxville Regional Laboratory
1522 Cherokee Trail
Knoxville, TN 27950-9019.

All providers west of the Tennessee River will ship to:
Jackson Regional Laboratory
295 Sumnar Avenue
Jackson, TN 38302-0849.

12. How do I collect a specimen?
The following should be collected as soon as possible after illness onset: nasopharyngeal swab, nasal aspirate, or a combined nasopharyngeal swab with oropharyngeal swab. If these specimens cannot be collected, a nasal swab or oropharyngeal swab is acceptable (see CDC specimen collection recommendations at: http://www.cdc.gov/h1n1flu/specimencollection.htm). A dacron or polyester swab with plastic or metal shafts must be used. **Cotton swabs or swabs with a wooden shaft cannot be used for PCR testing.**

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Division of Laboratory Services Influenza H1N1 Testing FAQ's Continued

13. How do I ship a specimen?

Ship overnight delivery with specimen kept cold using cold ice packs. Send as a Biological Specimen, Category B.

14. Who are the Sentinel Physicians?

The Sentinel Provider Network (SNP) consists of 65 Physician practices evenly located across Tennessee.

- Sentinel Providers collect specimens from patients who meet Influenza-like illness (ILI) case definition.
- They collect up to a maximum of 10 specimens/week/provider
- Ship specimens at the end of the collection day
- PCR testing is conducted after the receipt of specimens

15. Who are the EIP hospitals?

There are 18 hospitals in Middle Tennessee that have been recruited to send in specimens on patients admitted to the hospital with influenza.

16. What commercial labs are testing for H1N1?

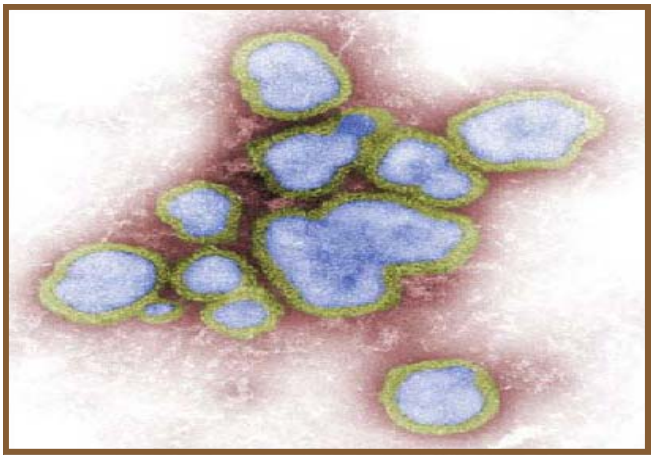
Quest – performs Rapid Culture, uses Focus Labs for PCR
ARUP – developed a PCR and is seeking FDA EUA status
Spectrum – working with Focus Labs to obtain their test kit
AEL – will send tests to Focus Labs
LabCorp – no formal response

17.How long does it take to get results?

Once received by the State Laboratory, the results should be reported no later than 3 – 5 days.

Emerging Infection Program (EIP) Hospitals

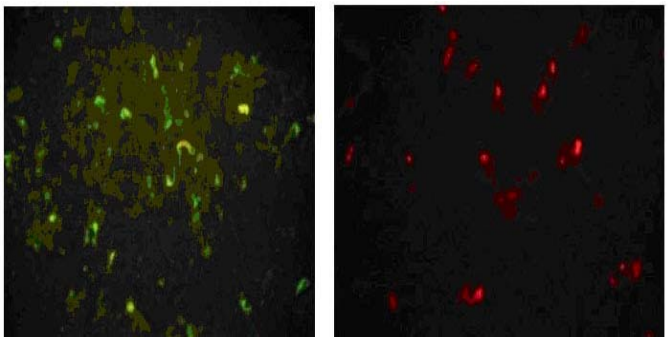
Baptist Hospital- Nashville
Centennial Medical Center – Nashville and Ashland City
Hendersonville Medical Center – Hendersonville
Horizons Medical Center – Dickson
Metro-General Hospital - Nashville
Middle Tennessee Medical Center - Murfreesboro
Northcrest Medical Center – Springfield
Saint Thomas Hospital – Nashville
Skyline Medical Center – Nashville
Southern Hills Medical Center – Nashville
Stonecrest Medical Center– Smyrna
Summit Medical Center – Nashville
Sumner Regional Medical Center – Gallatin
University Medical Center – Lebanon
Vanderbilt University Medical Center - Nashville
Veteran's Administration Medical Center – Murfreesboro
Veteran's Administration Medical Center – Nashville
Williamson Medical Center – Franklin



This digitally-colorized, negative stained transmission electron micrograph (TEM) depicts influenza A virions. There are three types of influenza viruses: A, B, and C. Human influenza A and B viruses cause seasonal epidemics almost every year. Influenza type C infections cause a mild respiratory illness and are not thought to cause epidemics.
Source: CDC Image Library at http://phil.cdc.gov/phil/home.asp

Wildlife and Domestic Animals Infected with Trypanosoma cruzi in Tennessee (Continued)

Medical Association partnered to conduct a collaborative study funded through a grant from the Southeastern Center for Emerging Biological Threats at Emory University. From 2005 to 2007, the USDA-Wildlife Services collected serum samples from raccoons as part of an Oral Rabies Vaccination Program in eastern Tennessee. Serum samples from 704 raccoons (*Procyon lotor*) from 10 counties in the Ridge and Valley and Blue Ridge Mountains ecoregions of eastern Tennessee were tested for antibodies reactive to *T. cruzi* using indirect fluorescent antibody (IFA) assay.



Indirect Immunofluorescence assay (IFA) test results of animal sera showing positive (green *T. cruzi*) and negative (red *T. cruzi*) results.

A total of 206 (29.3%) samples were seropositive, with 9 counties yielding positive samples (range of 14.6-63.6%). A significantly greater number of raccoons from rural habitats (35.1%) were found to be positive for *T. cruzi* exposure compared to raccoons from suburban habitats (23.1%). Deciduous forests were the most common site from where raccoons were trapped and the most common site of positive raccoons in rural areas (42%). Interestingly, age was positively associated with seropositivity. Raccoons older than 1 year of age (adults) were 40.1% seropositive compared to 12.2% of those less than 1 year of age (juveniles; $P<0.001$). This study demonstrates that *T. cruzi* is well established in portions of the raccoon populations in eastern Tennessee.

Previous canine (*Canis familiaris*) serosurveys have been limited either by small sample size or confined geographic reporting areas. In our study we report a seroprevalence of 6.4% among 860 canines from 31 counties and five ecoregions throughout the state of Tennessee via Indirect Immunofluorescence assay (IFA). This study is the first canine serosurvey for *T. cruzi* in Tennessee and to our knowledge is the largest *T. cruzi* canine serosurvey conducted to date in the US. Statistically significant associations between seropositivity and age, weight, and outdoor living were noted. Differences in seropositivity were not noted based on AKC group, sex, habitat, landcover, and ecoregion. Greater attention should be given to possible *T. cruzi* transmission in Tennessee and veterinarians should consider Chagas disease as a differential diagnosis with compatible signs. In a human case of *T. cruzi* that occurred in an infant in Tennessee in 1998, the family dog was both IFA positive and RIPA positive for anti-*T. cruzi* antibodies. Since infected dogs can facilitate transmission of *T. cruzi* to their owners, a better understanding of the epidemiology of *T. cruzi* in canine populations could help to reduce the risk of *. cruzi* transmission to humans. Potential changes in the prevalence of Chagas in wildlife and domestic canines could increase risks of human autochthonous transmission.

Submitted by Abelardo Moncayo, Ph.D.
Director, Vector-Borne Diseases Section

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

Large, older outdoor dogs have a greater risk for being infected with T. cruzi.