



TENNESSEE
DEPARTMENT OF
HEALTH

It's About Time!

Tennessee Department of Health Public Health Laboratories Newsletter

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2008-09 Influenza (Flu) Season

What sort of flu season is expected this year? Flu seasons are unpredictable in a number of ways. Although epidemics of flu happen every year, the beginning, severity, and length of the epidemic depends on many factors, including the different types and strains of influenza viruses circulating and whether the viruses in the vaccine match flu viruses that are circulating.

CDC recommends a yearly flu vaccine as the first and most important step in protecting against this serious disease. While there are many different flu viruses, the flu vaccine is designed to protect against the three main flu strains that research indicates will cause the most illness during the flu season. The vaccine can protect you from getting sick from these three viruses or it can make your illness milder, if you get a different flu virus.

Flu activity typically does not reach its peak in the U.S. until January or February. Getting the flu vaccine soon after it becomes available each year is always a good idea, and the protection you get from vaccination will last throughout the flu season. However, flu activity can occur as late as May so getting a vaccine later in the season, including in December, January or even later, and even if flu activity has already started in your area, can still offer protection in most years.

Will new strains of flu circulate this season? Flu viruses are constantly changing so



it's typical for new strains of flu viruses to appear each year. For more information about how flu viruses change, visit "[How the Flu Virus Can Change.](http://www.cdc.gov)" at www.cdc.gov.

How effective is the flu vaccine? The effectiveness of the vaccine can vary and depends in part on the match between the viruses in the vaccine and flu viruses that are circulating in the community. If these are closely matched, vaccine effectiveness (VE) is higher. If they are not closely matched, VE can be reduced. During well-matched years, clinical trials have shown VE between 70% and 90% among healthy adults.

Immunization against flu protects not only the person vaccinated, but family, friends and co-workers as well!

From the CDC web site at http://www.cdc.gov/ecoli/qa_ecoli_sickness.htm

New Generation of HIV Test to Improve Sensitivity, Specificity and Diagnosis

Effective October 1, 2008, the Division of Laboratory Services Immunology Section has changed from testing for only antibody to HIV-1 to testing for antibodies to HIV-1 and HIV-2. While the EIA (enzyme immunoassay) method stays the same, the new test incorporates highly conserved recombinant and synthetic peptide sequences representing HIV-1 (Groups M and O) and HIV-2. This new generation of test promises to improve the sensitivity and specificity of detection of HIV antibodies for blood and plasma screening and act as an aid in the diagnosis of HIV infection. Early cases of HIV-1 and HIV-2 may be detected because this test detects both IgG and IgM. Since reactivity in the EIA test does not differentiate between HIV-1 and HIV-2 antibodies, confirmatory testing will consist of the usual Western Blot for HIV-1. In those rare cases where HIV-1 Western Blot produces negative or indeterminate results, a Western Blot for HIV-2 will be performed. If you have questions about these procedures, please contact Jerry Hindman, Immunology Manager at 615-262-6374 or by e-mail at jerry.hindman@state.tn.us



Emerging Pathogens Update 2008: A Tennessee Perspective on World Health

Laboratory Services traveled the state to present *Emerging Pathogens Update 2008: A Tennessee Perspective on World Health*. This day long seminar was presented in Memphis, Nashville, Johnson City, and Knoxville. The update was intended for clinical laboratorians on the front lines of infectious disease diagnosis. All 4 sessions were very well attended and the audience was excited and eager to learn about the constantly changing face of infectious diseases. One thing we all agreed we had in our favor: Job Security!

The first presentation was an update on *Staphylococcus aureus*, *Bordetella pertussis*, and a CDC program, the Active Bacterial Core Surveillance (ABC's). This was presented by General Bacteriology Supervisor Paula Bailey of the Laboratory in Nashville. Highlights of her presentation included: 1) CDC recommendations to add a vancomycin screen agar plate when testing *S. aureus*. The CDC algorithm for testing *S. aureus* can be found at http://www.cdc.gov/ncidod/dhqp/ar_visavrsa_algo.html; 2) Ten states in the US, including TN, are a part of the CDC's Emerging Infections Program. A component of this program is the ABC's. Eleven counties in TN actively participate in the ABC's program by submitting Group A Streptococcus, *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* organisms isolated from sterile sites. The isolates are characterized to help epidemiologists assess patterns of illness and to monitor these organisms for changes in infectivity or virulence.

Public Health Laboratories Bioterrorism Coordinator Irmgard Brown, presented an overview of Sentinel Laboratory Protocols for agents of bioterrorism. The key message to our clinical laboratory partners: Please clearly label an isolate as being suspected of a bioterrorism agent. Please do not label these generically, i.e. as "gram negative cocci"; provide as much information as you can regarding testing already performed on the organism. Remember there are 4 LRN laboratories in TN; Memphis, Jackson, Nashville, and Knoxville.

Molecular biologist Amy Woron and Dr Robyn Atkinson, Director of the Knoxville Regional Laboratory, partnered to present an overview of the "Sentinel Laboratories Role in Foodborne Illness Detection." This presentation explained that in the 21st century the characterization of an infectious organisms DNA is a vital piece of information required to classify sick individuals as part of an outbreak. It is essential for the detection of food-related illnesses that enteric organisms are forwarded to the state public health laboratory for further



characterization and DNA analysis. This includes all isolates of Salmonella, Shigella, and *Escherichia coli* (*E. coli*) O157 or any *E. coli* known to produce Shiga toxin. Not only is the submission of these isolates encouraged, **it is the law under the Tennessee Medical Laboratory Act (T.C.A. 53-4105), 1200-6-3.12 Referral of Cultures to the Department of Health.**

This presentation also highlighted the changing technology for the detection of Shiga toxin in a stool specimen. Since the late 1990's, enzyme immunoassays (EIA's) have been available to detect Shiga toxin. As laboratories switch to this methodology, the public health system loses access to the organisms that are causing illness. This inhibits public health's ability to characterize these organisms and public health can no longer molecularly link patients to an outbreak potentially causing many outbreaks to go unrecognized. Therefore, we highly encourage laboratories performing EIA for the detection of Shiga toxin to forward any positive specimens to the state laboratory for organism isolation and characterization.

Dr David Kirschke, Deputy State Epidemiologist, provided an overview of Tennessee's Notifiable Disease list and how epidemiologist use this information to recognize and investigate an outbreak in the state of Tennessee. Look for an article by Dr. Kirschke in the December issue.

State Training Coordinator, Sean O'Connell, presented an overview of packaging and shipping regulations focusing on the requirement to use professional judgment to classify dangerous or biohazardous shipments (see article next page). If you missed the opportunity to participate in this informative and interactive session, please contact Sean O'Connell at Sean.OConnell@state.tn.us. Please put "add to e-mail" list in the subject line in order to receive e-mail updates about future activities and new editions of the newsletter.

**Submitted by Dr. Robyn Atkinson, Director
Knoxville Regional Laboratory**



Knoxville Regional Laboratory Accepting E. coli O157 Isolates

The Knoxville Regional Laboratory is now able to accept *Escherichia coli* O157 isolates for confirmation and characterization from laboratories in East Tennessee. The KRL can also accept any specimens that signal positive for Shiga toxin via an enzyme immunoassay. We will isolate and characterize the Shiga toxin-producing organism.

Please contact us for shipping requirement and questions: (865) 549-5201.

Critical Judgment and the Classification of 6.2 Hazardous Materials for Shipment

The biggest task faced by a shipper these days is deciding how to class hazardous materials that need to be transported from one location to another. In 2006, the big three regulators harmonized shipping regulations. The Department of Transportation (DOT), the United States Postal Services (USPS) and the International Air Transportation Association (IATA) made the task of packaging and shipping Division 6.2 Hazardous Substances much easier. Materials being transported are divided into **Category A** (highly dangerous), **Category B** (less likely to cause harm), **Exempt Human** and **Not Subject** shipments. The difficulty comes in determining if a material is Category A or Category B. The task calls for the application of critical judgment. Critical judgment is the capacity to gauge the potential for harm to humans and the environment should the contents of the package be spilled or leaked while in transit. Critical judgment in this case is the product of education in the science and biology of the possibility for harm from microorganisms, professional experience in the laboratory and formal training in hazardous materials transport. Once a determination as to the appropriate class of a package has been made and that assessment communicated, the task of putting together packages can be handed over to other staff trained in the packaging of 6.2 hazardous material.

An example of the use of critical judgment in classifying a sample can be found when you consider the shipment of *Mycobacterium tuberculosis* or *Mtb*. *Mtb* is a highly infectious organism, the causative agent of TB in humans and animals. Some strains of *Mtb* are multi-drug resistant. Consider that *Mtb* is an organism that has a very low infectious dose (in other words, it doesn't take much to begin the disease process) and that *Mtb* is generally passed by the respiratory

route. These are the classic capabilities of a dangerous organism. Now, how would you class a sputum that needs to be tested for acid-fast bacilli and grown characteristics? How would you class a pure culture of *Mtb*? Or a culture of multi-drug resistant *Mtb*? Most probably you would class a sputum specimen as a Category B substance. Sputum contains a relatively low concentration of bacteria and each bacilli is contained within a viscous substance (mucous) that encapsulates it. In addition, it is relatively difficult to create an aerosol from sputum, except of course when it comes straight from a patient. Now consider a pure culture of *Mtb*. A culture of any organism represents a very highly concentrated group of freshly grown, potent bacteria. In the case of *Mtb* they are highly infective. And if the culture is in a liquid medium the danger of highly infectious aerosols is exponentially increased. *Mtb* (cultures only) are without question classified as Category A. They are also listed on the *Examples of Category A Infectious Substances*, published in the DOT publication entitled *Transporting Infectious Substances Safely*. The Category A list is made up of many exotic viruses and cultures of organisms that are highly pathogenic. This list provides guidance only and is not all inclusive.

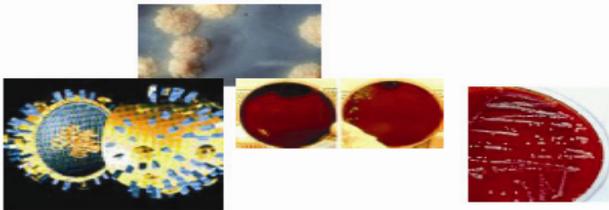
This simple to use guide (PHH-50-0079-0706) to packaging and shipping infectious substances is available by request and at no charge by contacting the U.S. Department of Transportation Pipeline and Hazardous Materials Safety Administration at 400 Seventh Street, SW, Washington, DC, 20590-0001 or by fax at 202-366-7342, e-mail at training@dot.gov or by telephone at 202-366-2301.

Submitted by Sean O'Connell, State Training Coordinator and Laboratory CDC Select Agent Responsible Official

Examples of Shipping Categories

Category A

Infectious substance capable of causing fatal disease in healthy humans. These include cultures of *M. tuberculosis*, *Y. pestis*, *Francisella* or viruses such as Ebola, Nipah or VEE



Category B

Infectious substance not generally capable of causing life-threatening disease in otherwise healthy humans, including clinical patient cultures and specimens sent for routine identification and testing of non-A suspected pathogens and therapeutic blood, vaccines, and antitoxins.



Exempt Human

A human sample transported for routine testing not related to the diagnosis of an infectious disease.



Not Subject

Foods and water for routine purity testing, Newborn Screening specimens and fecal occult blood tests.



PFGE as a Tool for Determining Relatedness

By the early 1970's, molecular biologists were separating DNA fragments by electrophoresis. The ability to manipulate and analyze DNA is essential in the field of molecular biology. Linear electrophoresis was first used as a method to characterize an organism by analyzing a large number of very small fragments of DNA. This methodology was very labor intensive, time consuming and expensive. Pulsed field gel electrophoresis (PFGE) was described by Schwartz and Cantor in 1984 as a way to separate very large DNA fragments. PFGE allows for better separation of large DNA fragments due to the 120° angle change in electric current. Smaller pieces are better able to change direction more quickly, resulting in clear separation of the DNA fragments. As it turns out, the analysis of a small number of large genomic fragments by PFGE is useful for determining relatedness between strains of bacteria. As highlighted in our Vol. 2, Issue 2 newsletter, Chad Black, DVM, utilized PFGE methodology to research the relatedness of multiple drug resistant *Staphylococcus pseudintermedius* veterinary isolates. The following is an excerpt from Dr. Black's upcoming publication:

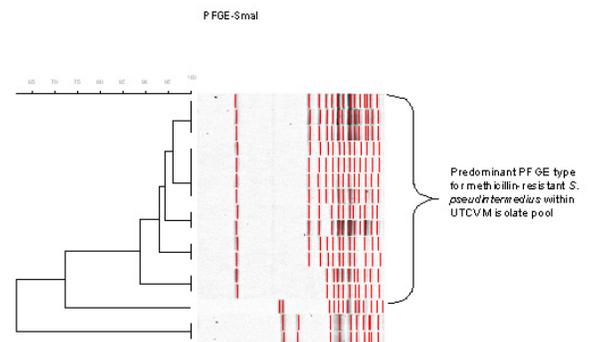
Identification of a predominant pulsed-field gel electrophoresis cluster in clinical isolates of American-derived *mecA*-containing methicillin-resistant *Staphylococcus pseudintermedius*. C. C. Black^{1*}, S. M. Solyman¹, D. A. Bemis¹, A. M. Woron², S. A. Kania¹. ¹Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN. ²Tennessee Department of Health Division of Laboratory Services, Nashville, TN.

Methicillin resistance encoded by the *mecA* gene is increasingly observed in *S. pseudintermedius*. *mecA* encodes penicillin-binding protein and the presence of this gene has been associated with multidrug resistance. We initially determined that the standardized pulsed-field gel electrophoresis (PFGE) protocol developed by the Centers for Disease Control for their MRSA USA typing system could produce interpretable banding patterns with *S. pseudintermedius* isolates. Our aim, using this technique,

was to determine the relatedness of resistant *mecA* bearing isolates and to compare them to methicillin susceptible isolates. *SmaI* macrorestriction fragment profiling was performed on 60 canine pyoderma isolates submitted to the University of Tennessee College of Veterinary Medicine (UTCVM) Bacteriology Service between 2006 and 2008.

All samples were confirmed as *S. pseudintermedius* using conventional identification techniques and PCR. Slightly over half of the samples were *mecA* positive and classified as either intermediately or highly oxacillin resistant using the disc diffusion method. A dendrogram of percent similarity, calculated with Dice coefficients from the PFGE data using a cutoff of 80%, revealed a single major cluster of 31 resistant isolates with 20 subtypes. All isolates within the cluster share an identical multi-locus sequence type (MLST). This suggests methicillin resistance in *S. pseudintermedius* within the area serviced by UTCVM Bacteriology Service originated from a single source which has persisted and expanded for several years.

Fig. 1 – Dendrogram and band marking for all isolates.



Submitted by Chad Black, DVM, UTCVM and Amy Woron, Molecular Biologist, Laboratory Services

Old Batches of NBS Filter Paper Expired 09/30/2008

Be aware that the Newborn Screening forms PH 1582 (green in color) with the revision date of 04/06 are now expired. These forms should not be used for the collection of newborn screening specimens collected after September 30, 2008. Any specimens collected after this date on these forms will be reported as Unsatisfactory – Filter paper expired. These forms once expired should be discarded and not used.

To request a supply of in-date collection forms (Rev 08/07 with expiration date 09/2010, yellow in color), please contact your local health department to fill your order. Please limit the number of forms requested to no more than a three month supply. Store in a dark and dry location and store the forms on edge rather than lying flat. Never store the forms with added weight on top, as this can cause a compression to the absorptive properties of the specimen collection end of the form.

If you have further questions, please contact Christine McKeever at Chris.McKeever@State.TN.US or 615-262-6352 or Thomas Childs at Thomas.Childs@State.TN.US or 615-262-6446.

Thank You for Your Cooperation!

