Nebraska Public Health Laboratory Newsletter

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NPHL Updates

By Peter C. Iwen, PhD, D(ABMM), Associate Director, NPHL

Warm weather brings a new set of public health laboratory issues that we all need to be prepared to address. Specifically, as the outdoor barbeque season continues, an increased number of shigatoxin-positive *E. coli* cases, whether caused by the O157 strain or by the non-O157 strains, will inevitably be detected in our laboratories. Karen Stiles, the NPHL training coordinator, provides an article on how to test for these pathogens and guidance on submitting specimens to the NPHL for routine DNA fingerprinting. NPHL provides this testing as a part of the national surveillance program coordinated by the CDC.

Other issues that challenge the laboratorian are the increased request for testing to detect for lead poisoning and the potential to evaluate for measles virus exposure. Denise Timko, clinical scientist at our affiliated laboratory at The Nebraska Medical Center and consultant with NPHL provides an overview article on the lead testing program for public health in Nebraska. The volume of tests to screen blood for lead levels has expanded dramatically over the past year with continued expansion in testing expected.

Finally, with the escalation in the measles cases in the US, Dr. Amity Roberts, clinical microbiology fellow, provides an overview of the issues and the testing that is available through the public health laboratory to support state programs for the control of measles.

We hope your summer is going well and welcome the opportunity to provide laboratory support to help keep our population healthy.

INSIDE THIS ISSUE:

Lead Testing at NPHL

By Denise M. Timko, MT(ASCP)SC

Lead is one of the most serious metallic poisons with children particularly sensitive to lead poisoning. The exposure of children to lead-contained paint and plaster continues despite regulations, labeling laws, and attempts to educate the public. Severe poisoning in a child can cause lead encephalopathy, which has a high mortality rate. Children who survive frequently show evidence of permanent central nervous system damage. Lead interferes in the biosynthesis of hemoglobin, which results in anemia. Three precursors of hemoglobin that accumulate in lead poisoning are d-aminolevulinic acid, coproporphyrin III, and protoporphyrin IX.

Blood lead concentrations, which are defined to reflect adverse health effects, have been revised downward over the past 20 years. In 1991, the U.S. Centers for Disease Control and Prevention (CDC) described an elevated blood lead concentration to be $\geq$ 10 µg/dL. Now, the Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) recommends using a reference value based on the 97.5th percentile of the BLL (blood lead level) distribution among children 1 to 5 years old in the United States. This is currently 5 ug/dL. To identify children with elevated BLL, data generated by the National Health and Nutrition Examination Survey is used.[2]

Screening tests for elevated blood lead in children are routinely done in laboratories throughout the US using a variety of blood samples to include capillary, whole blood or blood spots. At the NPHL, capillary, venous whole blood and capillary samples collected on filter paper are tested using the Perkin Elmer ICPMS analyzer. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a reference method for trace element analysis. A typical instrument consists of a nebulizer, ICP torch and a mass spectrometer. The liquid solution to be analyzed is transferred by a pump to a nebulizer which converts the solution into an aerosol. The aerosol is carried by argon gas into the center of the ICP torch. The high temperature plasma vaporizes and ionizes the sample, directs ions into the mass spectrometer, where the specific isotopes of lead (208Pb) and internal standard (159Tb) are detected on the basis of the mass-to-charge (*m/z*) ratio by the quadrupole mass spectrometer.[1]

Lead testing at the NPHL is performed on Monday, Wednesday and Friday, with results reported on the following work day. This past year, over 11,000 samples were

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Measles Makes a Comeback

by Amity Robert, PhD, Clinical Microbiology Fellow

In 2000, the CDC declared that measles was eradicated from the US due to stringent vaccination strategies that led to high population immunity.[1] However, measles is still prevalent in many areas of the world particularly Europe, Africa, Southeast Asia, and the Western Pacific. Additionally, unfounded fear that vaccines may cause adverse side-effects has led many parents to elect not to vaccinate their children. The reduction in the number of vaccinations within the population as a whole has decreased the population immunity (herd immunity).

The CDC released an update on measles for 2011.[1] During this time, 222 cases of measles occurred in 31 states within the US. The majority of patients (86%) had not been vaccinated. Additionally, 200 of these cases were either due to immigration into the US or foreign travel outside the US. Of these reports, 39% were >20 years old. Importantly, 14% were <12 months old, meaning they were too young to be vaccinated. The increased number of international travelers either to or from countries that continue to have a high rate of wild-type measles outbreaks increase the risk for exposure for susceptible individuals (unvaccinated, age <12 months, or adults whose immune response has waned).[4] For individuals traveling outside the US, recommendations are they be vaccinated with the measles/mumps/rubella (MMR) attenuated vaccine. It is also important to note that exposure can occur in airports.[2]

Measles virus is highly infectious, where 99% of unvaccinated individuals who become exposed to the virus will develop symptoms. Measles virus is transmitted through aerosolized droplets or through fomites that have become contaminated with nasal or throat secretions. The incubation period for measles, the time from exposure to clinical presentation, is 7 to 10 days.[3,5] Measles virus is a vaccine protective pathogen and humans are the only known host so that vaccination strategies may have a higher success rate at eradicating the virus from the population.

The most common measles vaccine series in the US is two doses of MMR. The first dose is administered at age 12-15 months, followed by a second dose at age 4-6 years.[4]

Adults born after 1957 who have not received the MMR vaccine should also be vaccinated. Currently, the NPHL can provide immune status screening by testing for measles virus specific antibody IgG levels in serum. This antibody persists for life. To determine if a person has had a recent infection, paired acute phase and convalescent phase sera samples would need to be collected and tested. To test for the presence of measles virus-specific IgM, which is indicative of recent infection or recent immunization, one serum sample at the acute phase of infection can be sent for testing.

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Electronic Lab Information Reporting Technology (ELIRT) Computer Training for Epidemiology Testing Order Entry Begins Fall 2012

Is your lab sending requests on paper requisitions for epidemiology testing? This September, NPHL will offer training on ELIRT. ELIRT is the web-based system that can be used by hospital and clinic laboratories to place test requests and view test results online. ELIRT also has functionality that allows for downloads of orders and results for easy data management. Please contact us to set up an onsite training.

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tested with positivity rate of 1.3% (>10 µg/dL) Of these specimens, 16 (0.1%) were above the critical range of 25 µg/dL. With the new standards (≥ 5 combine portion for elevated led levels) the positivity rate will be expected to rise.

References:

1. ELAN 6100 Hardware Guide, PerkinElmer SCIEX Instruments, 2003.
2. CDC Response to Advisory Committee on Childhood Lead Poisoning Prevention Recommendations in "Low Level Lead Exposure Harms Children: A Renewal Call of Primary Prevention" June 7th, 2012; http://www.cdc.gov/nceh/lead/acclpp/CDC_Response_Lead_Exposure_Recs.pdf

To Screen or Not to Screen Stools for Shigatoxin?

by Karen Stiles SM(ASCP)CM
State Training Coordinator NPHL

In the early 1990's, *E.coli* outbreaks involving taunted hamburger served by fast food restaurants in the Pacific Northwest were reported. Today, outbreaks of infection caused by shigatoxin-positive *E. coli (STEC)* are common with an estimated 100,000 cases reported each year in the US, including Nebraska. The CDC reported 2,138 hospitalizations and 68 deaths to FoodNet sites in 2011.[1]

The toxins produced by STEC are similar in structure and function to those produced by *Shigella dysenteriae* type I, hence the name, shigatoxin. STEC infections cause bloody, acute diarrhea, with approximately 8% of infected persons developing hemolytic uremic syndrome (HUS), depending on the strain virulence and the host factors. Historically, the O157 serotype of *E. coli* was most associated with disease; however the non-O157 strains are becoming more prevalent[1]. These strains produce only the Stx2 toxin which is more associated with HUS.[2]

STEC transmission occurs through consumption of foods, including undercooked ground beef, unpasteurized juice, raw milk, and raw produce. Ingestion of contaminated water or contact with animals or their environment can also cause infection. Direct person-to-person transmission has been observed in the daycare setting. Prompt and accurate diagnosis is important because appropriate treatment early in the course of disease might decrease renal damage and improve patient outcome.[1]

In October 2009, the CDC recommended that clinical laboratories test all stools not only for the presence of bacterial enteric pathogens (*Campylobacter*, *Salmonella*, and *Shigella*) but also for STEC. Testing should include both culture on selective medium and by a direct immunoassay to detect for the non-O157 shigatoxin-producing strains.[2] The additional cost however has lead to discussions among health care professionals on the rationale of following these CDC recommendations.

Dr. Peter Gilligan, Editor for the *Journal of Clinical Microbiology* recently published an article to address the issue on whether all stools should be tested for STEC.[3] He described a number of factors that needed to be considered before testing commences such as test quality, cost, clinical relevance for the patient population, staffing and technical expertise of technologists. Dr. Gilligan had asked two prominent facilities to explain the rationale for their decision to adapt or not adapt the CDC recommendations. The two sides of the debate were presented by Mario Marcon, PhD from Nationwide Children's Hospital in Columbus, Ohio and Deanna Kiska PhD and Scott Riddell PhD of Upstate University Hospital in Syracuse, NY.

Dr. Marcon made a strong case for routine testing of all stools by both a selective culture method for O157 STEC and by a non-culture method for shigatoxins. He addressed four major questions:
1) Does the frequency of STEC infection due to O157 and non-O157 justify the routine use of antigen detection as well as culture? Surveillance figures showed that total cases due to non-O157 have increased; 2) Does the severity of STEC infection justify the routine use of antigen detection and culture? Although STEC infection is generally a self-limited disease associated with relatively mild diarrhea, 2% to 10% of infected individuals develop HUS, with higher rates in children. Up to 10% of children with HUS develop chronic renal failure. Thus, both O157 and non-O157 may be associated with significant morbidity and mortality in children and adults; 3) Given that antigen detection will detect both O157 and non-O157 STEC, aren't antigen tests good enough as stand-alone tests? Data presented showed that both tests should be used, particularly when first implemented by the laboratory; and 4) Can't most cases of STEC infection be detected if only selective situations are submitted, such as on physician request, bloody stools, during summer months, or on children <5 yrs of age? None of the criteria have acceptably high sensitivity. Studies have shown that only 20% of specimens positive for STEC were visibly positive for blood and history of blood in the stool may not be communicated to the laboratory. Additionally, the median age of patients positive with non-O157 was 12 years. Other studies showed only 3% of specimens submitted for bacterial pathogens in 2009 were requested for STEC detection by EIA.

The counter-point discussion by Kiska and Riddell agreed with the CDC regarding culture but did not support universal screening for shigatoxin. They reported that selective testing is a clinically sound and cost-effective approach based on their experience. The low STEC prevalence in the test population and questionable beneficial impact of universal shigatoxin screening was also indicated. They argued that a diagnosis of HUS can be made on clinical grounds and positive results can be supportive but not required for the initial assessment. Currently, there is no method to prevent the onset of HUS and renal damage can be ameliorated through parenteral volume expansion.

Kiska and Riddell believe the combination of screening criterion is likely to capture the majority of STEC cases. An algorithm was developed to assist physicians to determine the likelihood of infection. The key to the algorithm is education of the health-care providers of the appropriate clinical and epidemiological characteristics of STEC to identify patients for testing.[3]

Both sides of the debate agreed that laboratories must do research prior to making a final decision to test or not to test routinely for shigatoxin producing *E. coli* in stool. Evaluation of both methods in parallel for 3 to 6 months (especially in summer) are necessary comparisons to allow for an educated decision.[3]

The Nebraska Public Health Laboratory recommends that the EIA to detect shigatoxin producing *E. coli* should be done on ALL stools. The prevalence of non-O157 is increasing in Nebraska. Similar to our results published in 2000[4], non-O157 shigatoxin producing *E.coli* are more common in Nebraska than serotype O157. From July 2011 to June 2012, 27 O157:H7 isolates were obtained whereas 40 (including serotypes O26, O103, O111, O121 and O145) were reported. Criteria also showed that limitations do not work and cannot be accurately predicted. The cost on a national basis of HUS is high. Data from the USDA Economic Research Service show a single HUS case without severe renal disease was over $47,000 in 2003. For a case of end-stage renal disease was estimated at $5.2 million in non-fatal cases and $6.2 million in fatal cases.[5] Savings on a

(E.coli, continued on page 4)

Listeria monocytogenes Outbreak 2011

by Amity Roberts, PhD, Clinical Microbiology Fellow

The US experienced the largest *Listeria monocytogenes* outbreak ever recorded during July through October of 2011. The CDC reported 139 cases of listeriosis linked to consumption of contaminated pre-cut Rocky Ford-cantaloupes from Jensen Farms in Colorado. The outbreak affected 28 states, with 29 deaths and 1 confirmed miscarriage. Six of these cases were reported in Nebraska: Douglas county (2), Lancaster county (3), and Hitchcock county (1). All individuals affected in NE were > 70 years old. Pulse-field gel electrophoresis (PFGE) was utilized to determine the DNA fingerprint patterns of recovered *L. monocytogenes* isolates. One of the unique features for this outbreak was that 4 different strains of *L. monocytogenes* were identified by PFGE. This is an unusually high number of strains associated with a single outbreak source. Within NE, the 6 identified isolates were represented by 3 of the PFGE patterns.

Listeria monocytogenes is a catalase positive, slightly beta-hemolytic, Gram-positive rod that is an intracellular bacterial pathogen. The organism is motile at room temperature and grows at refrigerated temperatures (4°C). Ideal specimens for detection of this organism are blood and cerebrospinal fluid.

Listeria monocytogenes causes a food-borne illness that presents as invasive listeriosis (bacteremia, meningitis, or encephalitis) or febrile gastroenteritis (fever and diarrhea). Foods associated with listeriosis are milk, soft cheeses, turkey franks, and processed (deli) meats. The present outbreak is the first outbreak of *L. monocytogenes* associated with contaminated melons. Up to two months may occur before symptoms develop post-consumption of contaminated foods making the epidemiological investigating more challenging. Additionally, non-food related infections can also occur in the form of cutaneous listeriosis, endocarditis, keratitis, endophthalmitis, and intravenous catheter infections often associated with exposure to animal tissue or farm animals since these animals often carry *L. monocytogenes* within their gut.

Those most at risk for development of invasive listeriosis after consumption of contaminated foods are pregnant females, the immunocompromised, and the elderly. Symptoms can vary dependent on health status. Invasive listeriosis involves extraintestinal spread of the organism. Pregnant females are more likely to experience mild, flu-like illness (fatigue, headaches, chills, muscle aches, nausea and vomiting), which can lead to miscarriage, stillbirth, pre-term delivery, or severe infection in the newborn infant. In healthy individuals with a competent immune response, invasive listeriosis can occur; however, these individuals are more likely to develop intestinal limited diarrhea and fever. Listeriosis can readily be treated with a number of antibiotics.

Personnel at the NPHL have experience in the detection and identification of *L. monocytogenes* and are certified by the CDC to perform PFGE testing as part of the national PulseNet program for DNA fingerprinting of food-borne pathogens (http://www.cdc.gov/pulsenet/). All isolates of *L. monocytogenes* should be submitted to the NPHL for DNA fingerprinting. Questions concerning the submission of these isolates can be directed to Karen Stiles at kstiles@unmc.edu.

References

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(E.coli, continued from page 3)

laboratory level cannot compare.

Nebraska Communicable Disease Regulations, Title 173 requires that all shigatoxin positive stools, as well as any O157:H7 isolates cultured on selective media such as sorbitol-MacConkey agar (SMAC), cefixime tellurite-sorbitol MacConkey agar (CT-SMAC), CHROMagarO157 or isolates identified by automated identification systems (phenotypic) be forwarded to the NPHL. It is important to note that selective media will not detect verotoxigenic strains other than sorbitol negative O157. Therefore, if a positive shiga toxin stool does not have a corresponding O157 isolate, it is possible there is a non-O157 or a sorbitol-positive O157 may be present. If no corresponding O157 is found or if culture is not performed, all shigatoxin positive stools should be sent as soon as possible to the NPHL to isolate the shigatoxin producing organism.

Once isolated at NPHL, the organism is further characterized by pulse-field gel electrophoresis (PFGE). This is an essential tool to detecting, investigating and controlling STEC outbreaks and has the potential to reduce laboratory errors.[6]

Preserved stool specimens in Cary Blair or other transport media can be shipped to the NPHL using a UN3373 Biological Substance, Category B shipper. Sorbitol negative isolates on SMAC, CT-SMAC or CHROMagarO157 must be shipped as a Category A Infectious Substances UN2814 certified shipper. If preserved stool or a sorbitol negative isolate is not available, the primary MacConkey plate or a subculture of the positive GN broth can be sent in a Category A container. Sending the original positive GN broth is no longer recommended. Please refer to www.nphl.org for detailed guidelines on packaging and shipping.

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NPHL Newsletter Has Gone Electronic

by Karen Stiles SM(ASCP)CM
State Training Coordinator NPHL

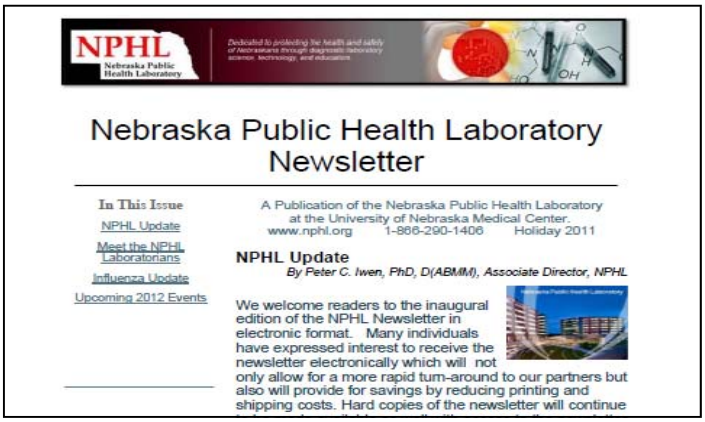
Late last year, the first edition of the NPHL newsletter was released electronically. The electronic version will have features to allow the reader to scan the article title and summary in one glance. If interested, the reader can click on the "read more" link. Additional links to reference articles, related websites and state wide information are also included.

Another distinct advantage to the electronic newsletter is the availability of color images and the ability to allow for expanded use of digital imaging. These images will help to enhance the reporting and make the newsletter more attractive to the readers.

Although, both the electronic and hard copy versions of the newsletter will continue to be published to reach as many readers as possible, readers are encouraged to use the electronic version. Contact Karen Stiles at kstiles@unmc.edu to sign up!

Figure 1. NPHL staff and students. Front - Kim, Dana, Vicki and Amy Back - Karen, Mai, Tony, Rhonda, Andy and Alyssa; David was not present.

Figure 2. NPHL administrative and management staff. From left - Scott, Tony, Steve, John, Pete, Amity, Amy, Jody & Brian



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NPHL Update
By Peter C. Iwen, PhD, D(ABMM), Associate Director, NPHL

We welcome readers to the inaugural edition of the NPHL Newsletter in electronic format. Many individuals have expressed interest to receive the newsletter electronically which will not only allow for a more rapid turn-around to our partners but also will provide for savings by reducing printing and shipping costs. Hard copies of the newsletter will continue

Meet the Laboratorians – NPHL

Compiled by Karen Stiles SM(ASCP)CM
State Training Coordinator NPHL

A famous quote by an unknown source once articulated, "Better to see the *face* than to hear the *name.*" As state training coordinator, I have ample chance to personally interact with many of our readers. Meeting individuals face-to-face is a great benefit of my job and is essential to providing required training. Equally, my associate director, Tony Sambol, is a familiar face to many of the laboratorians.

However, most laboratorians rarely get an opportunity to have a face-to-fact interaction with the NPHL staff. When calling or paging the NPHL, you likely speak to someone you never had the opportunity to meet. The 24/7 pager is manned by a core staff of highly-trained laboratorians, Tony Sambol, Vicki Herrera, Amy Kerby and Rhonda Noel-Hurst. All of these individuals have a variety of backgrounds in laboratory training. Tony was originally hired as a bioterrorism expert but has evolved in his duties to manage the basic operations of the NPHL. Vicki provides expertise in the molecular testing aspects of the laboratory with her experiences in the Molecular Diagnostics Laboratory at The Nebraska Medical Center (TNMC). Amy has experience in the commercial field with food microbiology from her work at ConAgra, and Rhonda has basic training as a medical technologist and has worked a number of years in research at UNMC. Both have been extensively trained in the national testing protocols as a part of the Laboratory Response Network (LRN) and the CDC PulseNet programs.

Other staff in the preparedness section at NPHL include Kim Rothgeb, administrative assistant; Dana El-hajjar, chemical terrorism specialist and Nebraska Fourier Transform Infrared Spectoscopy (FTIR) manager; David Moran, mass spectrophotometer specialist and Andi Brochman-Williams, laboratory technologist for *Salmonella* serotyping and environmental testing for West Nile virus. Last, but not least other individuals who provide the leadership and support for the NPHL include Scott Campbell and John Glock, computer programmers; Steve Hinrichs and Pete Iwen, our directors; Amity Roberts, post-doctoral microbiology fellow; Amy Armbrust, microbiology supervisor at our supporting laboratory at NMC; Jody Garrett, Clinical Pathology Director; and Brian Lenz, NPHL Laboratory Liaison. In addition to the staff, we have foreign nationalists and graduate students also training in our laboratories.

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IN THIS ISSUE

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