Nebraska Public Health Laboratory Newsletter

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NPHL Updates

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

This issue of the NPHL Newsletter has been a long time in coming due to the many unexpected activities that have occurred, some of which are described in more detail in this newsletter. Recently, two new laboratory tests became available through the NPHL. Our clinical microbiology laboratory partner at Nebraska Medicine verified a new FDA-approved molecular multiplex assay for the detection of GI pathogens in stool and NPHL has validated a new molecular assay for the confirmation of Gram-negative rod resistance to carbapenems. Dr. Caitlin Murphy provides an overview on the new GI panel; highlighting the need for isolates from this new and other similar culture-independent assays to perform molecular fingerprinting for epidemiological purposes.

Roxanne Alter reports on our experiences for the screening and confirmation of carbapenemase resistant Enterobacteriaceae (CRE). She provides a template that can be utilized to screen for CRE with the NPHL providing confirmation testing for our state lab partners.

Andi Brockman provides information on testing of mosquitoes in Nebraska for arboviruses. With the warm wet summer followed by extended days of dry periods, Nebraska has seen a bumper crop of mosquitoes and thus an increased potential for the transmission of arboviruses such as West Nile virus.

Finally, both Vicki Herrera and Karen Stiles describe on our experiences to provide diagnostic services in the care of patients with Ebola virus disease (EVD). NPHL became the first state laboratory in the U.S. to provide laboratory diagnostic services for the care of patients with EVD and the first laboratory in the U.S. to arrange for courier services to ship Ebola virus- infected specimens to a CDC reference laboratory.

Although difficult to determine what might be the next outbreak, laboratorians at the NPHL are available and welcome the opportunity to provide consultation services to our laboratory partners and others in Nebraska and around the U.S.

INSIDE THIS ISSUE:

Culture - Independent Testing for Gastrointestinal Pathogens

By Caitlin Murphy, PhD, Clinical Microbiology Fellow

As more rapid multiplexed, molecular testing platforms enter the market, the clinical laboratory must investigate the benefits of making the switch from traditional culture testing. Often, the use of these tests comes with an increased cost but provides a quicker turn-around time and increased sensitivity compared to culture. A new assay revolutionized by multitarget molecular testing is for the detection of gastrointestinal pathogens. One test that has recently been FDA-approved is the FilmArray® Gastrointestinal (GI) Pathogen Panel (BioFire Diagnostics [bioMerieux], Salt Lake City, UT). This panel can detect 12 bacterial, 4 parasitic, and 5 viral targets in one assay (**Table 1**) and replaces traditional stool culture in many instances. This approach also simplifies testing by eliminating the need for multiple testing orders to cover the same range of bacterial, parasitic, and viral targets.

With more laboratories evaluating multiplex PCR for GI testing, studies have documented the increased rate of pathogen detection and subsequent improvement in patient management. One study demonstrated that the Film-Array detected at least one organism in more than 50% of samples tested, compared to an 18.1% positivity rate when the same specimens were evaluated by conventional methods[1]. This type of comprehensive, rapid testing is also valuable for infection control practices since patients with infectious gastroenteritis are recommended to be placed under contact precautions. Using the FilmArray® GI Panel one acute care hospital demonstrated that 60% of patients were not placed on appropriate contact precautions due to traditional testing methods that were unable to detect one or more infectious agents[2]. Additionally, 20% of patients that could have been cleared from contact precautions remained under precautions in the absence of molecular testing.

The use of new molecular tests also introduces the laboratorian and the clinician to gastrointestinal pathogens that were not routinely detected for by nonmolecular methods such as culture. This is exemplified by the inclusion of multiple diarrheagenic *E. coli* on the FilmArray® GI Panel. Enterotoxigenic *E. coli* (ETEC) is a common cause of travelers' diarrhea with Enteropathogenic *E. coli* (EPEC) and Enteroaggregative *E. coli* (EAEC) common causes of infantile diarrhea, particularly of consequence in the developing world. In the developed world, shigatoxin-producing *E. coli* (STEC) and more recently EAEC are

(Culture Independent Testing, Continued on page 2)

(Culture-Independent Testing….Continued from page 1)

associated with foodborne outbreaks. Detection of STEC has always been a priority in clinical labs, but since implementing the GI Panel at Nebraska Medicine, the incidence of other diarrheagenic *E. coli* strains has been greater than anticipated.

The inclusion of viruses associated with gastroenteritis, such as sapovirus and astrovirus, marks the first time these agents have been detected. Both viruses are typically seen in the pediatric population, but have been known to cause gastrointestinal infections in immunocompromised and healthy adults. These viruses are similar to norovirus since they can be associated with outbreaks in institutional settings and cause self-limiting gastrointestinal infections. Reports, however, have indicated that these viruses may be associated with more mild symptoms. Reliable detection of an increased array of bacterial and viral pathogens causing gastroenteritis will no doubt expand our knowledge about the epidemiology and pathogenesis of these infectious agents.

If your laboratory has adopted one of these culture-independent tests or is planning to do so in the future, be aware that stool samples positive for *Vibrio*, shigatoxin-positive *E. coli*, *Salmonella*, *Shigella* (species other than *sonnei),* and *Cyclospora* (or culture isolates from these stools) should be forwarded to NPHL. As a part of patient care, NPHL recommends that follow-up culture be done at individual institutions for susceptibility testing on *Campylobacter* and *Shigella* species. This is a reminder also, that all stools positive for shiga-toxin by the EIA method can be submitted to NPHL in Cary-Blair transport media as a Category B biological substance. When original specimen is not available, subculture broth to MacConkey plate and ship as a Category A infectious substance.

References:

1. Spina, A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L, Tassiois P, Popescu GA, Rafila A, Eerola E, Batista J, Maass M, Aschbacher R, Olsen KE, Allerberger F. 2015. Spectrum of enteropathogens detected by the FilmArray GI Panel in a multicenter study of community-acquired gastroenteritis. Clin Microbiol Infect, 21(8): 719-28.
2. Rand KH, Tremblay EE, Hoidal M, Fisher LB, Grau KR, Karst SM. 2015 Multiplex gastrointestinal pathogen panels: implications for infection control.Diagn Microbiol Infect Dis, 82 (8):154-7.

Upcoming NPHL 2015 Events

BT Proficiency Test
LPX - Shipping Sept 8
Challenge Set - Sept 21
Summation Webcast - Oct 16

BT Training - Full Day Workshop
Omaha @NPHL - October 9
Onsite Training - November -December
(Call to schedule)

Upcoming NPHL 2016 Events

Chemical Terrorism Workshops - Jan/Feb
Packing & Shipping Training
Lincoln - March 29
North Platte - March 31
BT Proficiency Test
LPX & Challenge Set - TBA
Gram Stain Workshop - TBA

Table 1. Targets on the FilmArray® Gastrointestinal Pathogen Panel.

Other Bacteria	Diarrheagenic *E. coli/Shigella*	Parasites	Viruses
Campylobacter	Enteroaggregative *E. coli* (EAEC)	***Cryptosporidium***	Adenovirus F 40/41
Clostridium difficile toxin A/B	Enteropathogenic *E. coli* (EPEC)	***Cyclospora cayetanensis***	Astrovirus
Plesiomonas shigelloides	Enterotoxigenic *E. coli* (ETEC)	*Entamoeba histolytica*	Norovirus GI/GII
Salmonella	**Shiga-like toxin-producing *E. coli* (STEC)**	*Giardia lamblia*	Rotavirus A
Vibrio	***E. coli* O157**		Sapovirus
Vibrio cholera	*Shigella*/Enteroinvasive *E. coli* (EIEC)*		
Yersinia enterocolitica			

Organisms in bold will require submission of positive stool sample or recovered isolate to NPHL for epidemiological purposes.
* Only refer *Shigella* species that are not *S. sonnei*

Ebola - Nebraska Experiences

by Vicki Herrera, MS, NPHL Laboratory Manager

On 1 September 2014, the Nebraska Public Health Laboratory (NPHL) received notice to prepare for the possible transfer of a patient with Ebola virus disease (EVD) from West Africa to the Nebraska Biocontainment Unit (NBU). Since inception of the NBU, the Special Pathogens Branch of the NPHL had an agreement to coordinate laboratory testing of specimens for patients admitted to this unit. This report describes some of the challenges encountered as we became the first state public health laboratory (PHL) in the US to provide laboratory support for a patient with EVD.

Prior to arrival of the first patient, a Risk Assessment Team was formed, compromised of NPHL staff, hospital leadership, physicians, nurses, and other laboratorians. The role of this team was to define the essential laboratory tests needed to care for the patient, to determine which tests could be performed safely in the laboratory, to provide alternative tests for those that could not be performed safely, and to determine how the laboratories on campus could be integrated into the testing process. The team agreed on the following points:

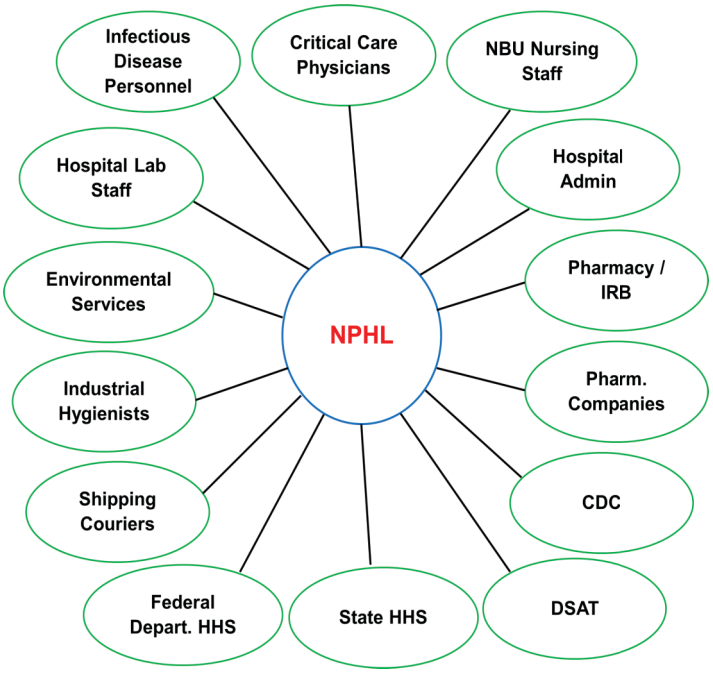
1. All point-of-care testing would be done in a BSL-3 containment laboratory.
2. No specimen tubes would be uncapped unless in a BSL-3 environment.
3. Centrifugation of tubes would only take place in a BSL-3 environment, and only in a centrifuge with sealed rotors.
4. All specimens would be double-bagged and placed into a hard-sided container for transport.
5. All specimens would be escorted on-site to the lab by at least 2 persons.
6. An off-site method of transport would be defined.
7. Protocols for experimental drug therapy would be defined.

The Risk Assessment Team devised a list of essential tests that could be performed safely and provided alternative testing for those that could not be performed safely. In the assessment process, a BSL-3 laboratory within the NBU was developed to be used for point-of-care testing and initial processing of specimens; the hospital core laboratory was recognized to provide closed-tube testing on automated chemistry, hematology, and immunoassay analyzers for some of the essential tests; and the BSL-3 laboratory at the NPHL was identified to provide for molecular testing, other infectious diseases testing, and archiving of clinical specimens for future studies and follow-up testing.

Our experiences showed the necessity of being flexible to the requests of the medical staff and the need to maintain direct lines of communication to prepare and care for a patient with EVD **(Table 1)**. Since most medical facilities in the US do not have direct access to a PHL, our experiences also showed the importance for PHL personnel to develop open lines of communication with other laboratory partners within their jurisdiction which is critical for the management of a patient with or under investigation for EVD.

(Ebola-Nebraska Experiences References, Continued on page 6)

Table 1. Communication lines at NPHL.
Abbreviations: HHS-Health and Human Services; IRB-Institutional Review Board; DSAT-Division of Select Agent and Toxins



Flexibility & Safety

Picture 1. Mobile BSC, Centrifuge with sealed rotors, hard sided transport container, PPE

Collection and Transport of High Consequence Pathogens to the Nebraska Public Health Lab

by Karen Stiles SM(ASCP)^{CM}, State Training Coordinator NPHL

Although unlikely, the possibility exists that a patient could present with a potential highly infectious pathogen at a local acute care hospital, other emergency care settings including urgent care clinics, or critical access hospitals. All Nebraska healthcare facilities play an important role in the prevention of exposures in this scenario, and MUST have a plan in place to recognize signs and symptoms, identify a patient as a person under investigation (PUI); immediately isolate; and inform local and state health departments. Immediate transfer of the patient may not be feasible, based on distance, bed availability, or other considerations. Therefore, frontline facilities may be expected to provide prolonged care for 12-24 hours and be required to collect specimens to be transported to NPHL for laboratory testing. The CDC has asked all states to adopt "*A Framework for a Tiered Approach,"* outlining the roles of different health care facilities. These roles include serving as treatment centers, assessment hospitals, and frontline health care facilities. While the focus has been on preparedness for Ebola, preparedness for other novel, highly pathogenic diseases such as Middle East Respiratory Syndrome (MERS) or avian influenza will also be enhanced through these activities.

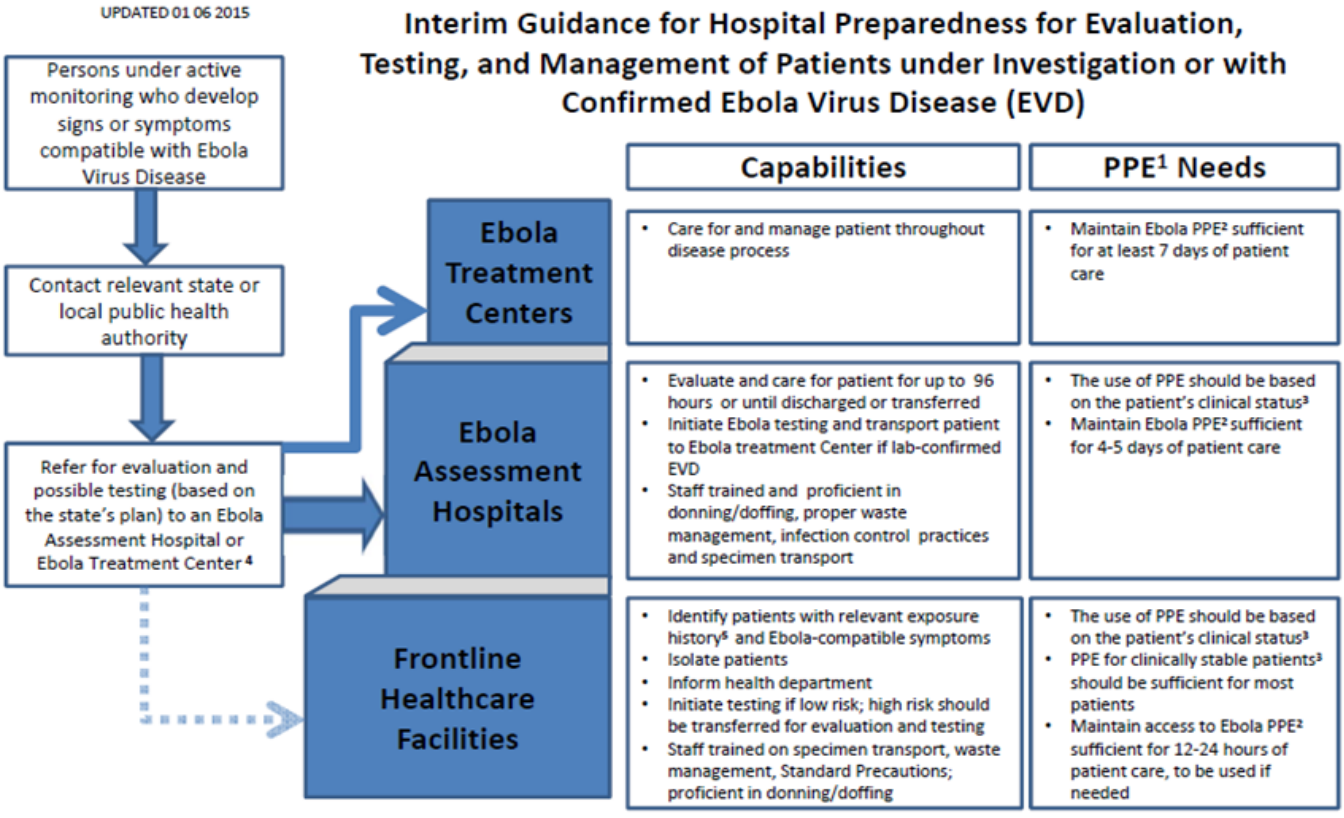
Assessment hospitals should be prepared to receive and isolate a PUI and care for the patient until a diagnosis can be confirmed or ruled out and until discharge or transfer is completed. These hospitals should be equipped with PPE sufficient for 96 hours for clinical care of patients. Enhanced PPE will be needed for ill patients and staff must be trained in correct PPE usage for a PUI with vomiting, copious diarrhea, or obvious bleeding. Prior training should include demonstrated proficiency in donning and doffing (putting on and taking off) PPE, proper waste management, infection control practices, and specimen packaging and transport.

In collaboration with state public health officials, healthcare facilities should be prepared to collect specimens for possible limited testing in their own laboratory setting as well as transferring specimens to the NPHL for molecular testing. Each facility must ensure there is no delay in the care of these patients by being prepared to test, manage, and treat alternative etiologies of febrile illness (e.g., malaria in travelers) as clinically indicated.

CDC funding has been awarded to the state of Nebraska to assist healthcare facilities with the above directives. This funding will allow the Nebraska DHHS and the NPHL to hire full time infection control and biosafety/biosecurity personnel that are available to consult with all healthcare facilities in the state. This funding also accelerates this capacity for (1) healthcare facility infection control assessment and response; (2) laboratory safety; and (3) global migration, border interventions, and migrant health. Funding allocated for laboratory purposes will be used to develop and implement measures to improve biological safety and security practices for dealing with current and emerging infectious diseases, such as MERS, Severe Acute Respiratory Syndrome (SARS), pandemic flu, and drug-resistant organisms (MDRO). The Hospital Preparedness Program (HPP) Ebola Preparedness and Response grant will concentrate on funding used for collection and transport of specimens from patients infected with these highly infectious pathogens.

Specimens potentially infected with a highly infectious pathogen such as Ebola virus, are shipped as a Category A Infectious Substance. Such specimens CANNOT be transported by the normal NPHL ground courier and most hospital

(Continued on page 7)



Arbovirus Testing At NPHL – It Bites

by Andrea Brochman-Williams, MS, NPHL Technical Supervisor

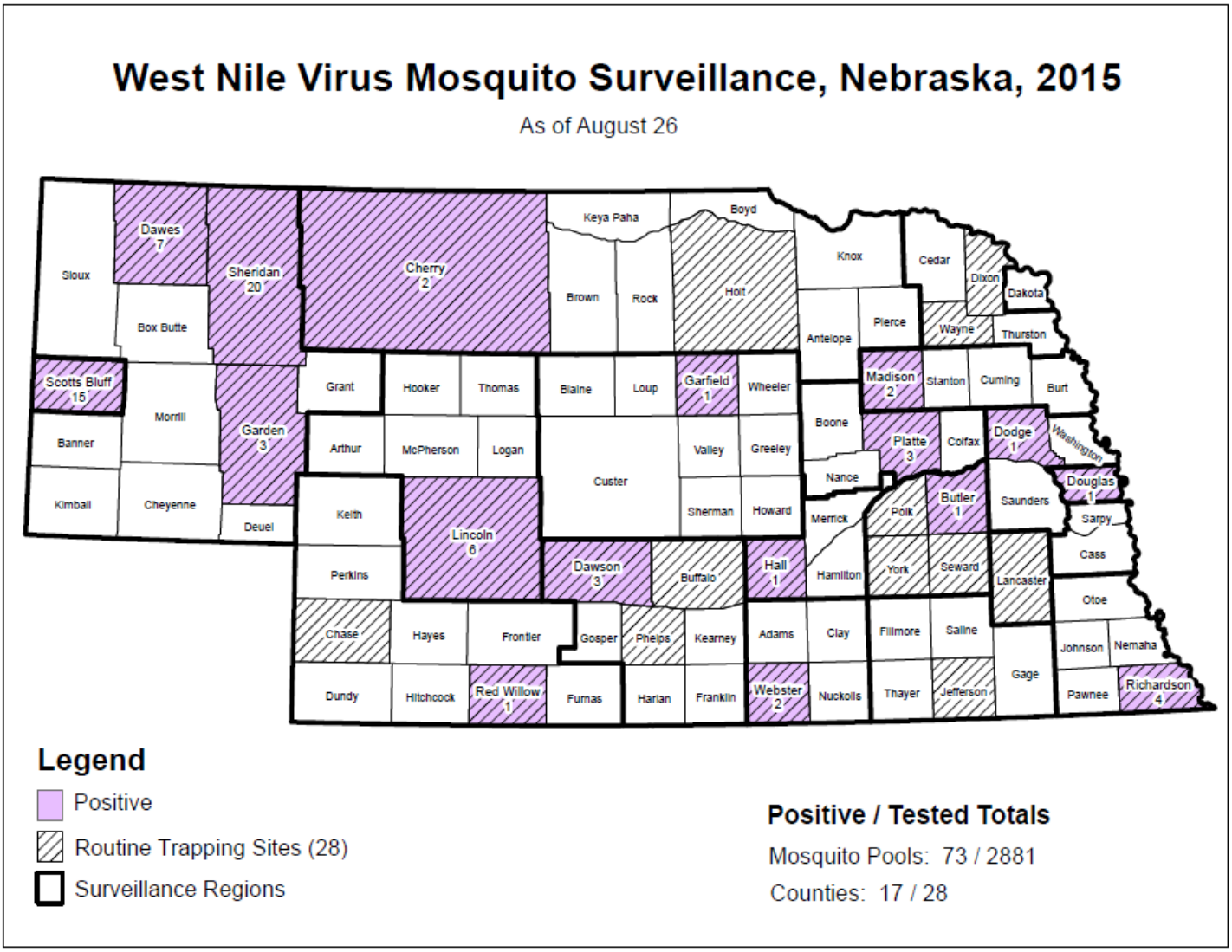
This time of year, clinical laboratory scientists at NPHL are busy making what is lovingly called "skeeter soup." Mosquitoes are trapped in 28 counties throughout the state and are sent to Lincoln where they are counted and sorted by county, collection site, collection date and species. Vials of pooled *Culex* mosquitoes are sent to NPHL where they are sonicated to release the nucleic acids. After shearing, the vials contain a black sludge (aka skeeter soup) that is centrifuged and then the supernatant collected for RNA extraction and subsequent testing by real-time PCR. In previous years, NPHL tested *Culex* mosquitoes for West Nile virus (NWV) but this year St. Louis Encephalitis virus (SLE), and Western Equine Encephalitis Virus (WEE) have been added to the test in a multiplex PCR assay.

The state of NE participates in a CDC program called ArboNET, a national surveillance system for the detection of arboviral diseases. Although NPHL has been testing mosquitos for WNV since 2000, the 2015 season has proven to be a busy one! The five year average for mosquito pools tested in June is 267 but this year 606 mosquito pools were tested. Likewise, in July, over 1,800 pools were tested.

As of August 26th, WNV has been detected in the following counties: Scotts Bluff, Dawes, Sheridan, Garden, Cherry, Lincoln, Red Willow, Dawson, Garfield, Hall, Webster, Platte, Butler, Dodge, Douglas, and Richardson. There have been 20 confirmed human cases of WNV disease so far this season. In addition, SLE was recently detected in mosquitoes from one western Nebraska county. No WEE has been detected.

To find more information on the West Nile virus surveillance program in NE, visit the Nebraska DHHS website at http://dhhs.ne.gov/publichealth/Pages/wnv.aspx. Information regarding human cases, bird testing, and mosquito testing is available.

NPHL is fortunate to have such a great group of skeeter beaters to help NE fight the bite!



http://dhhs.ne.gov/publichealth/Documents/Mosquito15.pdf

Validation and Performance of an Algorithm for the Detection of Carbapenemase-Producing Enterobacteriaceae

by Roxanne Alter, MS, MLS(ASCP)^{CM}, Center for Staph Research

Carbapenemases (β-lactamases) are enzymes that can inactive β-lactam antibiotics including carbapenems (eg., ertapenem, doripenem, imipenem, and meropenem). Carbapenems are broad-spectrum β-lactam agents used for the treatment of serious infections, especially for multidrug-resistant Gram negative bacteria. They are viewed as a "drug of last resort" and resistance is a significant clinical and public health concern. Carbapenemases can also inactivate other β-lactams such as penicillins, β-lactams- β-lactamases inhibitor combinations, and cephalosporins, so that isolates producing a carbapenemase can be resistant to all β-lactam drugs. Carbapenemase-producing Gram negative bacteria have been identified with increasing frequency within the continental U.S.

A recent report from NPHL describes the validation of an algorithm for the detection of a carbapenemase-producing Enterobacteriaceae (CPE) based on prospective analysis of carbapenenam-non-susceptible clinical isolates[1]. Thirty-two reference isolates of *Klebsiella pneumoniae* both positive and negative for carbapenemases and 40 clinical isolates resistant to one or more carbapenams were prospectively tested using the proposed algorithm.

During these studies, a 4-test algorithm was designed to detect CPE which included the Modified Hodge Test (MHT), chromID CARBA agar (biomerieux), (KPC + MBL) Confirm ID Kit (Rosco Diagnostica), and a lab developed test (LDT) real time polymerase chain reaction (RT-PCR) assay. The sensitivity and specificity of the assays for carbapenemase detection using reference isolates were 73.3% and 50% (PPV 84.6% and NPV 33.3%) for MHT; 100% and 100% for ChromID agar, Confirm ID and RT-PCR. Prospective analysis of the carbapenem-non-susceptible clinical isolates (18 urine, 18 wound/body fluid, 2 blood, 2 respiratory) showed specificity for ***bla***$_{KPC}$ detection was 86.4% for MHT, 97.4% for Confirm ID and 100% for ChromID and RT-PCR. These results showed that the ChromID agar, Confirm ID kit and the RT-PCR assay provided reliable results for the detection of CPE.

Suggested Carbapenemase Detection Algorithm (requires 2-day period):

1. Organism is flagged by automated system based on an elevated ertapenem and/or meropenem MIC.
2. Organism ID and susceptibilities are reported to the clinician and the isolate is referred for further carbapenemase testing.
3. Phenotypic testing is performed which might included the Modified Hodge Test, chromID CARBA agar or the confirm ID disk diffusion test.
4. Suspect isolate is transported (Category B) to the NPHL for molecular testing to include RT-PCR for the detection of bla_{kpc} and bla_{NDM-1} genes.
5. NPHL reports RT-PCR testing to the NPHL Director, Director of Microbiology and Director /manager of of submitting laboratory.
6. The bacterial isolate is frozen for storage at -70 ^{0} C and cataloged for future purposes.
7. When a CRE is confirmed, it will be reported to hospital infection control personnel. The patient's chart will be annotated to reflect the presence of a carbapenemase-producing Enterobacteriaceae, appropriate enhanced contact precautions are taken and surveillance around the patient commences.

NPHL is willing to accept suspicious isolates from laboratories in Nebraska following approval for CRE confirmation testing. Questions on this process can be directed to Dr. Peter Iwen at piwen@unmc.edu.

References:

1. Southern, T, et al.2015. Validation and performance of an algorithm for the detection of carbapenemase-producing Enterobacteriaceae; Association of Public Health Laboratories Annual Meeting, Indianapolis, ID; Abstract # P-08.

NEED TO CONTACT NPHL?

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402-559-2440

(Ebola-Nebraska Experiences References, Continued from page 3)

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5. CDC. Guidance for U.S. Laboratories for Managing and Testing Routine Clinical Specimens When There is a Concern About Ebola Virus Disease, CDC Website, March 19, 2015.

Meet the Laboratorian - Donnet Knapp

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

This edition features Donnet Knapp, MT(ASCP)SM, Microbiology Team Lead at CHI Health St. Elizabeth Regional Medical Center in Lincoln. Donnet has been at St Elizabeth's as a microbiologist and team lead for over 11 years. She covers a wide variety of responsibilities in her position including mentoring students and evaluating new instrumentation and tests.

What got you interested in pursuing a career in laboratory science?

I really liked Biology and Chemistry Lab in high school, and knew I wanted to work in the health care setting. I discovered the medical technology program while looking through the Allied Science brochure from UNMC, and thought the program looked fun and glamorous (ha ha)!

Where did you receive your formal training?

I attended UNL for my first three years, then did my clinical internship at Nebraska Methodist Hospital.

Are there any specific areas of clinical laboratory science in which you have special interest or expertise?

Microbiology, of course, and customer service. My 7 years in customer service made me a better microbiologist because of the communication skills and troubleshooting skills learned in that position. Customer service training also gave me the confidence to go outside the laboratory and use other resources such as pharmacy and the nursing educators. These educators can help initiate change. I also worked with a pharmacist and respiratory therapist to help publish a paper in Hospital Pharmacy Journal in 2014, entitled "Establishing a Quality Control Program: Ensuring Safety From Contamination for Recycled Metered -Dose Inhalers"

My special interest is in antimicrobial stewardship and rapid testing in the lab to get the patient on the right treatment as soon as possible. This can provide the best possible outcome, which reduces length of stay, and prevents readmission. I also have been fortunate to surround myself with great people who have my same objective – providing the best care possible to our patients.

What is unique about working at your facility?

The fact that the hospital is a healing ministry, and being closely connected to the patients. Also we have established Core Values Guiding and Behavioral Standards that we strive to uphold. I enjoy being involved in various committees outside the laboratory (e.g. Guiding Coalition, Pharmacy and Therapeutics, Sepsis Team, and Pandemic Preparedness to name a few).

What do you see as future challenges for the field of medical technology?

Staffing shortages, achieving expertise when many labs have gone away from specializing and keeping up with the technological changes.

What is the biggest challenge you face in your job today?

The variable work hours, pay and keeping up with changes.

What advice would you give to a first year clinical laboratory scientist?

Be patient and know you will make mistakes, so learn from them and use them to become better. Do not be afraid to ask questions. Find a mentor and surround yourself with people you want to be like. Listen to the older techs; they have a lot of wisdom and experience that you can learn from. Last, stay out of the office politics and get along with people, even those you don't necessarily have the best relationship with.

What do you think is the single biggest change in the laboratory since you started?

DNA-based technology has changed the microbiology world entirely. This has reduced the turn-around times substantially. It is mind-blowing to think that when I was a student, 6-12 weeks were required to diagnose MTB, which can now be done in a matter of hours.

What do you like most about your job?

The people and the personal reward of helping diagnose patients and potentially saving lives.

(Collection and Transport, Continued from page 4)

laboratories are only trained to ship Category B, Biological Substances. Therefore, NPHL, with the assistance of Nebraska DHHS, has developed a specimen transport plan utilizing officials from government departements, including local health departments and state patrol, which are not subject to Hazardous Materials Regulations (CFR 171.1 D5). Triple packaging is required according to CFR173.196, to provide the highest level of safety for the courier. NeDHHS has allocated funding to purchase Category A shipping containers and transport kits. Kits will be delivered by NPHL to provide training on site and discuss a memorandum of understanding (MOU) agreement.

Transport kits will be housed at each hospital laboratory and staff will trained to package and ship Category A specimens (DOT Hazardous Materials Division 6.2 certification training is highly recommended). Packaging instructions are included with kits. If necessary, NPHL will arrange qualified couriers, including local health department or state patrol to transport.

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IN THIS ISSUE

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