Nebraska Public Health Laboratory Newsletter

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NPHL Updates

By Steven Hinrichs, M.D., Director, NPHL

While we look forward to the summer months of outdoor activity, we also recognize that the warm weather brings a new set of public health concerns including shiga toxin producing *E. coli*, West Nile virus and Enterovirus. In addition to these familiar topics, this issue of the NPHL Newsletter contains summaries of new concepts. First, we extend our series of short basic science articles with a description of biofilms by national authority, Dr. Ken Bayles and discussion of taxonomy by our ABMM certified microbiologist, Dr. Peter Iwen. These articles are accompanied by an excellent synopsis of carbapenam beta lactamase resistance in *Enterobacteriaceae* written by Dr. Baha Abdalhamid our medical microbiology fellow. We are pleased to announce that our collaborative education program with UNMC and Creighton University Medical Center has been successful in obtaining funding by CDC and APHL to begin addressing the local and national shortage of PhD microbiologists. But the excitement doesn't only occur in microbiology; Jennifer Svoboda and her colleagues at the Regional West Medical Center laboratory in Scottsbluff are to be congratulated for their dedicated efforts to establish automated electronic messaging of reportable laboratory diseases to the State Epidemiologist. On the chemistry side, the NPHL has established a proficiency testing program so that our local emergency response personnel can document their competence in the identification of unknown powders, and we participated in a cross borders CST exercise in the Nebraska panhandle early April. Our summer edition introduces you to the career of Susan Simmons and then concludes with an update of efforts by the public health laboratory directors in our region to improve communication and cooperation through a meeting sponsored by the Mid America Alliance.

The Biological Significance of Bacterial Biofilm

By Ken Bayles, Ph.D., Professor, UNMC

Bacterial biofilm is one of the hottest topics in microbiology today. Although dentists have been dealing with biofilms for ages during routine dental exams where plaque (a form of biofilm) is scraped off neglected teeth, only recently has the bacteriology community learned of its significance for other types of infections. Having been trained to think of bacteria as independent single-celled organisms, it has been an epiphany to realize that they are, in fact, highly "social" organisms that live and multiply in large organized communities. Biofilms can even be thought of as multicellular organisms as they exhibit cell-to-cell communication and cellular differentiation during biofilm growth.

A bacterial biofilm is a collection of bacterial cells held together by an extracellular polymeric matrix composed of polysaccharide, protein, DNA, or any combination of these three molecules. It can contain a heterogeneous mixture of bacterial species, as often occurs in the lungs of cystic fibrosis (CF) patients, or can be comprised of a single species such as on the surface of an artificial hip. Like more complex eukaryotic organisms, biofilms develop into what appears to be specialized groups forming mushroom-like structures that maximize nutrient acquisition and waste dispersal. Because of the multilayered organization of biofilms, the cells that comprise the biofilm are extremely heterogeneous at the physiological level, likely due to gradients of oxygen, nutrients, and waste byproducts that are formed within this structure.

The unique nature of a bacterial biofilm makes it a formidable foe to the immune system as well as to modern medicine. Similar to a flock of birds or a school of fish, there is power in numbers that the immune system has a hard time dealing with. This is especially true for phagocytic cells as studies have shown that the biofilm matrix provides a barrier to phagocytosis compared to free-living planktonic cells (2). A diffusion barrier, however, is not responsible for the increased tolerance to antibiotics that biofilm cells exhibit. Although the mechanism by which biofilm tolerance occurs is unclear, several models have been proposed to offer an explanation (1, 3-5).

Unfortunately, biofilms are a major mode of growth in a variety of bacterial infections. More than 60% of all microbial infections are estimated to be caused by biofilms. As mentioned above, the best known examples of this include dental plaque, CF lung infections, and colonization of implantable devises. However, other forms of infections are also thought to involve biofilm formation including infectious endocarditis and middle ear infections. The presence of biofilms in these types of infections likely explains the high level of treatment failures that are associated with them.

In summary, despite the fact that biofilms play a major role during bacterial infection we are only now beginning to understand the complexities of these multicellular communities. This lack of understanding has hindered the development of effective therapeutic strategies to fight many types of bacterial infections. Fortunately, with increased interest in studying this fascinating bacterial lifestyle, there is reason to be optimistic that these new strategies are on the horizon.

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Resistance in Gram Negative Rods: KPC

By Baha Abdalhamid, Ph.D., Clinical Microbiology Fellow, UNMC and Paul Fey, Ph.D., Associate Director, NPHL

Klebsiella pneumoniae carbapenemases (KPC) are class A and group 2f β-lactamases that can hydrolyze penicillins, cephalosporins, monobactams, and carbapenem. KPC β-lactamases are inhibited by clavulanic acid and are generally considered plasmid mediated. KPC-1 was first detected through the Intensive Care Antimicrobial Resistance Epidemiology (ICARE) program in North Carolina in 1996. Although predominant in *Klebsiella pneumoniae*, KPC β-lactamases have also been discovered in other gram-negative rods, especially in species from *Enterobacteriaceae*, such as *Escherichia coli*, *Enterobacter* spp., *Salmonella enterica*, *K. oxytoca*, and *Citrobacter freundii*. In addition, KPC-2 was isolated from *Pseudomonas aeruginosa* in Colombia in 2006.

KPC β-lactamases are transferable and are generally associated with genes encoding resistance for other antimicrobial agents including aminoglycosides, fluoroquinolones, and trimethoprim/sulfamethoxazole. To date, there are four KPC enzymes (KPC-1, 2, 3, and 4) isolated mostly from the East Coast of the USA including North Carolina, Maryland, New York, and Massachusetts. Several outbreaks by KPC-producing *Klebsiella pneumoniae* have been identified in New York City hospitals. In addition, KPC β-lactamases have been detected outside the US in Scotland, Colombia, Israel, China, and France.

KPC β-lactamases can be difficult to detect for several reasons. First, KPC β-lactamases are detected in *K. pneumoniae* and other *Enterobacteriaceae* which are well known to have mechanisms of β-lactam resistance such as porin mutations and encode other β-lactamases including ESBLs and AmpCs which can hinder the detection of KPCs. Second, the minimum inhibitory concentrations (MIC) of imipenem are significantly influenced by inoculum volume in which a lower inoculum has resulted in susceptible MICs for imipenem in a KPC producing organism. Third, there is inconsistency of KPC detection using automated systems, microbroth dilution, and E-test methods. The factors mentioned above suggest the need for methodologies that are able to accurately detect KPC producing organisms.

According to the Clinical and Laboratory Standards Institute (CLSI) recommendations, *Enterobacteriaceae* that are resistant to the expanded-spectrum cephalosporins and have a carbapenem MIC $\geq$ 2 μg/ml or a carbapenem intermediate or resistant zone of inhibition by disk diffusion may produce a KPC β-lactamase. For KPC detection, ertapenem is the most sensitive carbapenem while meropenem and imipenem are more specific than ertapenem. Some automated system panels have been modified to match the CLSI carbapenem MIC interpretive standards for detection of KPC β-lactamases. As examples, there are two new MicroScan (Siemens) panels, neg combo (NC) 50 and neg/urine combo (NUC) 51, with imipenem and meropenem concentrations of 1 μg/ml. In addition, NUC 51 panel has breakpoints for ertapenem. The definitive detection of a KPC encoding organism can be performed using PCR and/or a phenotypic test called the modified Hodge test (1). We are not aware of any KPC producing *Enterobacteriaceae* in Nebraska, however, if any laboratory suspects a KPC producing organism, NPHL would be happy to provide consultation for further testing.

KPC producing *Enterobacteriaceae* are generally susceptible to both colistin and tigecycline. CLSI interpretive criteria are available for colistin only for *Pseudomonas aeruginosa* but not for *Enterobacteriaceae*. In addition, there are no CLSI guidelines for tigecycline (tigecycline FDA breakpoints do exist). The accurate detection is imperative to effectively control the spread and emergence of KPC-producing *Enterobacteriaceae*.

For additional information regarding resistance in gram-negative rods: KPC, please contact Dr. Abdalhamid at 402-552-3305 or Dr. Fey at 402-559-2122.

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What's in a Name? The Genus *Citrobacter*

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

The ability to reliably identify bacterial pathogens in clinical specimens is a major function for the clinical microbiologist. Working with a biological system however does not always make this an easy task. Additionally, the classification of bacteria into species is an artificial process where boundaries are set by humans and thus the identification of a bacterium becomes a subjective process.

Currently, the most accurate method for species identification involves a polyphasic approach to classification including the integration of genotypic, phenotypic, and chemotaxonomic features of an organism. Complete genomic sequencing is considered the standard for species delineation but complete sequences are not available for most bacterial species. Therefore, techniques such as DNA-DNA hybridization analysis and direct sequence analysis of various target genes have been a reliable standard for species identification in the interim. Results show that the phenotypic and chemotaxonomic features generally agree with the genomic information for most bacterial species.

Classification of bacteria to species also includes the process of naming new species. In this recent period of genomic sequencing, the variety and number of new and reclassified species has become a challenge for the laboratorians. When new species are named or when old species are re-named, the naming must follow the rules of the *Bacteriological Code*. To be validated, a species name that is published in any peer-reviewed journal must subsequently be placed on a validation list in the *International Journal of Evolutionary and Systematic Bacteriology* (formerly *International Journal of Systematic Bacteriology*) (IJSEM) or the new species name must be published as a full report in the IJSEM.

Prior to the availability of genomic methods to identify species, many bacterial species unknowingly had multiple validated names published in the IJSEM for what eventually turned out to be the same species. Thus each name is valid according to the rules of bacterial nomenclature. One example of multiple names for the same species would be the names of *Pseudomonas maltophilia, Xanthomonas maltophilia*, and *Stentrophomonas maltophilia*, all species names considered valid for the same organism. This certainly adds to the confusion for the identification and reporting of bacterial species.

The new genomic techniques now available for species identification also lead to problems for the clinical microbiologists in the ability to differentiate and identify completely a microbial pathogen. The routine phenotypic methods are not sensitive enough for species identification in many cases, thus requiring genotypic methods not available in most laboratories. This has resulted in the use of "group" or "complex" names to include

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multiple species in a report. This article will be a part of an ongoing series to provide guidance for the clinical microbiologist in how to most accurately report the identity of a bacterial pathogen.

The Genus *Citrobacter*. The genus *Citrobacter* within the family *Enterobacteriaceae* is composed of 11 unique species that includes 12 valid names organized into 3 major subgroups (**Table**). Subgroup A consists of 8 species which have similar phenotypic and genotypic characteristics. These species in most cases cannot be reliably identified using the standard phenotypic methods employed in the laboratory. Therefore, the identification for any one of these species is most accurately reported as "*Citrobacter freundii* complex".

Table Current species within the genus *Citrobacter*.

Subcategory	*Citrobacter* species
A[a]	*braakii* *freundii* *gillenii* *murliniae* *rodenticum* *sedlakii* *werkmanii* *youngae*
B	*amalonaticus* *farmeri*
C	*koseri/diversus*[b]

[a]Collectively identified as the *Citrobacter freundii* complex.
[b]Both *C. koseri* and *C. diversus* are valid scientific names.

Subgroup B consists of 2 species, *C. amalonaticus* and *C. farmeri* (formerly called *C. amaloniaticus* biogroup 1). These two species can generally be separated by phenotypic methods commonly used in the laboratory however, in some cases where atypical strains are encountered, these species cannot be separated. When this occurs, the more accurate identification may be a combination of both species such as *C. amalonaticus/farmeri.*

Subgroup C also consists of two valid species *C. diversus* and *C. koseri.* Both of these species were originally published as valid names by the *IJSB* in 1980 [3]. Recent genotypic analysis of these two species now recognizes both as representing the same species. In 1993, a request was made to the *Judicial Commission on the International Committee on Systematic Bacteriology* to determine which species was correct and thus should be used to identify this species [1]. This Commission subsequently rejected C. *diversus* and elevated the name *C. koseri* as the accepted species [2]. This opinion has unfortunately caused some controversy within the scientific community. The rules of the *Bacteriological Code* say that the older name has precedence and should be the accepted valid name. Therefore, *C. diversus,* which was originally published as a name in 1932, should have precedence as the accepted name since *C koseri* was not published until 1970. Based on the opinion from the Judicial Commission, most automated systems now use *C. koseri* as the accepted name for this organism. Until the controversy surrounding these two names can be settled some automated systems suggest that the most accurate reporting should use both names i.e., *C. diversus/koseri* or *C. koseri/diversus.*

In conclusion, reference laboratories are available to provide sequence comparison analysis testing to validate the identification of the *Citrobacter* species or other microbial pathogens when necessary. Although the NPHL does not provide this service, molecular tools are available at UNMC to identify microbial pathogens for research purposes. Contact Dr. Iwen at 402-559-7774 for additional information on the availability of this service.

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Scottsbluff's RWMC Laboratory Moves to ELR

By Jennifer Svoboda, MT(ASCP), LIS Support Analyst, RWMC

Regional West Medical Center (RWMC) in Scottsbluff, NE has joined national efforts in establishing electronic health information through its leadership of a Regional Health Information Organization or RHIO. The knowledge gained in those efforts set the stage for the work by our laboratory team to begin transmitting electronic messages of reportable conditions and organisms using standard HL-7 formats. HL-7 is the electronic language of communication by medical computers and it is used by our Laboratory Information Management System (LIMS) developed by McKesson to transmit orders and reports in our hospital. We take very seriously the responsibility of submitting laboratory test results that meet the state requirements for reporting to public health authorities such as hepatitis C and HIV. However, we spend a considerable amount of our employee's time finding and documenting these cases. There had been a number of publications that indicated significant cost savings could be achieved if the manual process could be automated. This would potentially eliminate the need for technologists to remember to fill out paper reports or web based formats.

After extensive discussions and research, we employed a software program developed at the University of Nebraska Medical Center (UNMC) called PHIELD to automatically search lab reports and electronically package those that meet specific qualifications for transmission to the state. We also worked in collaboration with our county health officials, particularly Bill Wineman at Scotts Bluff County Health Department who provided constant encouragement. The system is particularly valuable because the reports that we send to the State Epidemiologist Dr. Tom Safranek can also be seen by people at the Scotts Bluff County Health Department using the National Electronic Disease Surveillance System (NEDSS) program. I was invited to give a presentation on the process and steps that needed to be accomplished at national meeting of laboratory information specialists and we received many questions. PHIELD is essentially an electronic filter or net that finds whatever it is programmed to catch and hold. While the PHIELD software was critical to the overall process, we also received outstanding support from Jim Svoboda (no relation), John Glock at UNMC, and John Hall at the Nebraska Department of Health and Human Services (DHHS). Their help was especially useful in the selection of specific codes from LOINC and SNOMED tables so that our reports would match those submitted by other laboratories around the state. After we established the process, John Hall at DHHS monitored our progress for three months before we moved our reports from a test mode into regular production.

Since moving into production, the amount of time we spend on reporting has dropped significantly and I am also more confident that we aren't missing important information that public health needs to monitor disease in our part of the state.

Meet the Laboratorian – Susan Simmons

Compiled by Josh Rowland, State Training Coordinator, NPHL

Susan Simmons, MT(ASCP), is the Microbiology Supervisor at Regional West Medical Center (RWMC) in Scottsbluff, Nebraska. RWMC is a 180 bed facility that is also one of three Level II Trauma Centers in Nebraska. Founded in 1924, RWMC serves a large area that includes the Nebraska panhandle and eastern Wyoming. Susan celebrated her 30th anniversary as a laboratorian on February 1st this year.

How did you become interested in pursuing a career in laboratory science?

While attending Junior High in Gering, Nebraska, my friend's mother thought the medical laboratory might be a good profession for my friend and I, instead of the popular career choices at the time such as secretarial or teaching. We visited the laboratory at what was then called the West Nebraska General Hospital (now called Regional West Medical Center) on a school field trip and it looked pretty interesting. We were both interested in science and math. As it ended up, we both became Clinical Laboratory Scientists, my friend a blood banker, and myself a microbiologist.

Where did you attend medical technology school?

I had to choose between UNMC in Omaha or the University of Colorado in Denver. I chose CU as I got married and my previous husband accepted a job in Denver.

How long have you worked in your present location?

I have been at RWMC for 30 years as of February 1. I have worked in microbiology almost exclusively and feel very fortunate to have had the opportunity to do so.

Are there any specific areas of microbiology that you have expertise or interest?

I would never qualify myself as an expert in the field, as the field changes so much and there is just so much to know. I have a passion for fungi, and try to learn about the bacterial mechanisms of antibiotic resistance, which I consider a challenge. Bugs are so smart.

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

The future challenges I see deal with the scarcity of dedicated microbiologists, or clinical laboratorians as a whole. Many of us "older" microbiologists are looking forward to vacationing more and working less (some call that retirement, I see it as a life priority adjustment). Molecular testing offers an alternative to the gold standard of cultures, and am excited to see that technology becoming more affordable and being implemented in more clinical laboratories.

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

My biggest challenge is finding a way to instill the passion I have for the field into the newer CLS's. It is also a challenge to keep up with the technology and information with the ever changing world of microbes. Education budgets are tight, staffing is sparse, and challenges are encountered implementing the changes so that providers get the information they need for best patient care decisions.

What advice would you give to a first year medical technologist?

Never stop learning. Pick a subject and focus on it until the subject becomes second nature. Each question that is asked provides a learning opportunity for you.

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

The biggest changes in the microbiology laboratory that I have seen involve automation and the move towards molecular testing. Thirty years ago I started identification of bacteria using the Kligler's series of biochemicals. Then we became more sophisticated with API and code books, and systems that perform identifications (ID) and antimicrobial susceptibility testing (AST) in micro-titer wells in a matter of hours. I believe that knowing biochemical reactions for most microorganisms that we see in the laboratory is important, although I understand the need for rapid ID and AST turnaround times. Microbiologists should be able to look at the organism on the agar plate and at least have an idea whether the identification is correct. When molecular testing becomes the standard, this art will be lost.

What do you like most about your job?

That's always a hard question. Coworkers and the opportunity to make a difference are always mentioned (notice I didn't say for the money, although CLS's are starting to be recognized monetarily for their efforts). But for me, I think it is the interaction with the physicians and providers. Being able to discuss a particularly difficult case and hoping I provided necessary information resulting in a good patient outcome has always been rewarding.

Is an Exercise Coming to Your Facility?

By Josh Rowland, State Training Coordinator, NPHL

Recently, the NPHL had the opportunity to participate in a full scale terrorism exercise. The exercise, organized by the Wyoming 84th Civil Support Team (CST) and the Center for National Response based in West Virginia, took place in the Nebraska Panhandle and spanned many Nebraska counties and involved the State of Wyoming as well as the Nebraska 72nd CST. The primary focus of the exercise was to test communication and interagency cooperation among the many fire, police, hazmat entities within their city, county, and state organizations. The NPHL, along with the Wyoming Public Health Laboratory (WPHL) were invited to participate in order to make the medical/public health aspects of the exercise more realistic and to help create an exercise that involved clinical laboratories in the region.

Although the drill incorporated a variety biological, chemical, and radiologic elements, the NPHL, working in conjunction with the WPHL decided to only incorporate the biological and chemical portion into the laboratory portion of the exercise. Two of the larger clinical laboratories in the exercise region were included in the drill. Regional West Medical Center laboratory in Scottsbluff were presented with a chemical terrorism event scenario, while the Box Butte General Hospital laboratory was asked to address a biological event scenario. Both

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Strategic Meeting of the 10 Mid-America Alliance State Public Health Laboratory Directors

By Traci Camilli, MAA Public Health Advisor

Due to the continual pressure for efficiency, state public health laboratories need to be creative in developing approaches for public health preparedness. Identifying with this need, the 10 state public health laboratory directors, along with other state health department leaders within the Mid-America Alliance (MAA) (CO, IA, KS, MO, MT, ND, NE, SD, UT and WY), traveled to Rapid City, SD in March 2008 for a meeting. They discussed projects that have regional implications including continuity of operation plans, surge capacity, and specimen routing by couriers. They also drafted a "Laboratory Incident Command System (LICS)" training manual and made final comments on an intra-state laboratory memorandum of understanding (MOU) which will allow for information and specimen exchange between the states.

Day one saw the group focus on efforts to better understand the importance of initiating the incident command structure (ICS) and aligning it with established laboratory operations. The group identified the need to educate both laboratory and hospital employees on the basics of ICS. For example, do the laboratory employees report to someone within the ICS or continue to report to the point person in the laboratory? Other issues discussed, concerned the laboratory's role as essential support to the health department and whether the laboratory director should remain within the lab or be at the Emergency Operations Center (EOC) during an event when ICS is activated? If the expectation is for the laboratory director on site at the EOC, then the right people need to be identified and trained within the laboratory to operate the laboratory while the director is performing another role.

This dialogue generated a planning process for a LICS. The group developed a mission statement and wrote job action sheets consistent with ICS terminology for the following positions: a laboratory incident commander, a public information officer, a liaison and a safety/security officer. The group outlined three chapters for the LICS training manual:

- Chapter 1: Definition of Key Players: Understand the different players and evaluate combinations or singularity of roles.
- Chapter 2: Job Action Sheets: Understand how all the roles fold into the overall ICS structure.
- Chapter 3: Documents/Tools/Forms: Identify the correct forms needed before, during and following a disaster for reimbursement, liabilities, etc.

In addition to understanding systems like ICS, additional questions were identified for future solutions, such as, how or what can be done to develop a regional or national system where public health laboratories share information and resources; how can public health laboratories improve conditions by working smarter, not harder; and how do we include state and national laboratories into an integrated system.

During the second day of the meeting the group shared each state's action plan to address these questions and offered assistance and support to other states that may have not been at the same point in the process. In one example, Montana requested a FTE for its laboratory to help set up electronic laboratory disease reporting. To help with this request, Nebraska offered to help train that individual to be compliant with CDC and public health information standards. The sharing of responsibilities clearly emphasized the importance behind regional collaboration. This historic gathering in Rapid City marked the beginning of an era of regional collaboration for the good of all regardless of state boundaries. For additional information on this meeting or this work group, please visit www.midamericaalliance.org.

Disclaimer: The findings and conclusions in this article are those of the author and do not necessarily represent the views of the Centers of Disease Control and Prevention (CDC).

Nebraska FTIR Proficiency Testing Program

By Dana El-Hajjar, MBA, BS, Technical Director, Chemistry, NPHL

Background

The Nebraska Fourier Transform Infrared Spectroscopy (FTIR) Proficiency Testing (PT) program was started in 2004 as a means of assuring capabilities of laboratories in Nebraska to test for unknown substances in environmental samples. The program was established to maintain proficiency on the FTIR Microscopy laboratory units, but has since expanded to include agencies that have the field versions of FTIR as well. It was recognized that FTIR units had been used by 1st responders and hazmat teams prior to documenting their proficiency with the instrumentation. This gap was addressed by the PT program. In March 2008 the proficiency testing program was opened to laboratories and agencies outside Nebraska that may be involved in testing of unknown substances using FTIR analysis.

How it Works

Three PT challenges are planned for each year. Samples are to be identified using FTIR analysis and reported within two weeks. The samples are mailed to laboratories in each challenge. Results will be mailed to laboratories, and event summaries will be posted on the NPHL web page after every PT challenge.

Who Can Enroll

This program is open to any laboratory or agency that tests unknown substances using FTIR analysis. This includes public health laboratories, civil support teams, environmental laboratories, HAZMAT units, fire departments, health departments, and etc.

How to Join

If you are interested in participating in this proficiency testing program please send an e-mail to: ftir-pt@nphl.org, or call us at (402) 559-9421. More information about the PT Program can be found on our website at www.nphl.org.

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laboratories successfully responded to and addressed multiple challenges during their individual events. While the exercise only lasted a short time, the lessons learned by all, including the NPHL and WPHL have been invaluable.

As the public health laboratory (PHL) community continues to prepare for events such as those incorporated into this exercise, we are reminded that laboratory response may rely heavily on those clinical laboratories on the front line, especially early-on in the event. One of the primary goals of PHLs is to be sure that clinical laboratories are able to respond to such events. Exercises like the one in Panhandle are a great way to further prepare the laboratory community.

For more information about this exercise or if you are interested in conducting a laboratory exercise, please contact Josh Rowland at 402-559-6070.

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