Nebraska Public Health Laboratory Newsletter

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NPHL Updates

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

With spring come new beginnings which are also evident in the activities of the NPHL. We said goodbye to a long-time technologist, Rhonda Noel-Hurst, who is pursuing new activities and hello to our newest employee, Sue Peters. Sue worked as a medical technologist in our partner laboratory at The Nebraska Medical Center and has now joined the public health laboratory as a lead technologist in the Laboratory Response Network section.

Additionally, we have initiated recent changes in the laboratory to include validation of WNV environmental testing on new instrumentation, the transition of *Salmonella* serotyping from the conventional agglutination assay to a molecular based test (using a Bioplex assay developed by the CDC), and the validation of an antimicrobial susceptibility test assay for *Neisseria gonorrhoeae,* which is described further in this Newsletter. Other topics provided are reports on both norovirus and influenza testing and an update on best practices for tuberculosis diagnostics provided by Karen Stiles, our State Training Coordinator.

Finally, we highlight that 2013 represents the 100th anniversary of legislative approval for the state public health laboratory in Nebraska. Multiple activities are planned to recognize this milestone, with discussions on the historical aspects and the future response of the laboratory to be presented at the Annual Preparedness Symposia at various locations in Nebraska this spring and summer. As always, we continue to serve the people in Nebraska and welcome the opportunity to collaborate with our colleagues from other laboratories.

The State Public Health Laboratory In Nebraska:
The First Hundred Years
(Annual Center for Preparedness Symposium)

May 7th Norfolk - Divot's Convention Center
June 11th Lincoln - Embassy Suites
July 16th Kearney - Holiday Inn Convention Center

INSIDE THIS ISSUE:

Influenza 2013 Update

By Robin Williams MPH, Epidemiology Coordinator, NDHHS

The Office of Epidemiology in the Division of Public Health at the Nebraska Department of Health and Human Services (NDHHS) collects, compiles, and analyzes information on influenza activity year round in Nebraska. Subsequently, a weekly report is produced from October through mid-May. The Nebraska influenza surveillance system is a collaborative effort between NDHHS and many partners in the state including, local health departments, public health and clinical laboratories, vital statistics offices, healthcare providers, clinics, schools, and emergency departments. Information in five categories is collected from different data sources to:

- Identify areas of influenza activity
- Track influenza-related illness
- Determine the influenza viruses circulating
- Detect changes in influenza viruses
- Measure the impact influenza deaths have in the US

The Nebraska Weekly Influenza Report is available at: http://dhhs.ne.gov/publichealth/Documents/Report.pdf.

This influenza season began earlier and has been more severe than previous influenza seasons. The first cases appeared in mid-October and activity quickly increased. Activity peaked during the last week of December but continued to circulate widely until mid-March.

The predominant virus circulating this season was influenza A (H3N2). The H3 viruses tend to cause more serious illness in all age groups. While the H3N2 strain remained the predominant virus overall, the proportion of influenza B viruses did increase during the latter part of the season. Twenty deaths have been associated with influenza in Nebraska with one child and the rest adults. Since influenza-associated adult deaths are not reportable by law, this number may be under-reported. Children 0 to 4-years-old and adults >65 had the highest number of hospitalizations for influenza-like-illness this year which are the age groups most affected by influenza each season.

The single best way to protect against seasonal influenza and its potential severe consequences is to receive a seasonal vaccine each year. Recent CDC vaccine effectiveness studies showed this year's vaccine was moderately (56%) effective. Thus out of those vaccinated, 56% were less likely to go to a doctor to get treated for influenza. Studies showed limited protection (9%) for those >65 due to this season's influenza A H3N2.

Best Practices in the TB Laboratory Diagnostics

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

Approximately 1.7 billion people worldwide have tuberculosis (TB) with an estimated 2.7 million deaths each year. This represents about 10-15 million US citizens with latent TB and an additional 10-12,000 cases of active disease per year. Since the infective dose of *Mycobacterium bovis/ tuberculosis* (MTB) is low (ID50<10 bacilli), laboratory workers who process and test TB specimens are 3-5 times more likely to develop latent TB than other laboratory staff.[1] This represents an estimated 8-30% of laboratorians seroconverting during their career.[2] To reduce the risk of a laboratory acquired MTB infection, strict adherence to biosafety practices in the laboratory setting is essential. This article provides a review on best practices for safety in the mycobacteria laboratory.

The most recent CDC recommendations suggest that TB testing should be conducted in a biosafety level 3 (BSL-3) containment laboratory.[2] However, the authors of this recommendation also recognized that this level of containment is not universally available in all laboratories. Thus, the guidelines provide alternatives for laboratory containment ONLY IF three conditions are documented: 1) a risk assessment has determined that work with MTB can be conducted safely in a separate, closed BLS-2 laboratory where exhaust air is 100% vented to the outside of the building, 2) BSL-3 practices and procedures are implemented; and 3) the laboratory director is fully aware of the risks associated with testing and approves the practice.

Risk Assessment. Traditionally, a classic risk assessment is based on the risk-group classification of the highest risk organism expected, such as MTB which is classified as risk group 3. More recently, microbiologists have begun to rely less on rigid classification of the high-risk organism and more on the different levels of containment to match the risk within the laboratory. Since not all TB laboratories are alike, this approach might include a risk assessment based on factors such as test volume, the probability of bacilli in patient specimens, the incidence of multidrug-resistant MTB, the type of testing performed and whether or not aerosol-generating procedures are indicated.[3]

Overall, the risk assessment process consists of five steps: 1) identify the hazard(s); 2) recognize activities that might cause exposure; 3) consider competencies and experience of personnel; 4) evaluate and prioritize risk and 5) develop and implement, evaluate controls to minimize risk of exposure.[2] To assist with the process, the Association of Public Health Laboratories (APHL) published a self-assessment tool, that consists of 94 questions for review with several questions labeled as "critical."[1] A "No" answer indicates a significant gap in the safety of laboratory testing with multiple negative answers to non-critical questions also indicating significant deficiencies. APHL recommends the risk-assessment be done at least yearly, or more frequently when new staff are trained or new equipment to the TB laboratory is implemented.

Facilities. For TB facilities, the new CDC recommendations state that the BSL-2 laboratory should be separate and isolated from the main microbiology laboratory.[2] This closed BSL-2 laboratory should consist of a separate area with self-closing and double-door access, a biological safety cabinet (BSC) to process all concentrated or decontaminated specimens, an aerosol proof centrifuge with safety-shield rotors, a sink area for staining, a microscope and a computer for reporting. The layout of the room should be planned for ease and safety, minimizing movement in the work area. An example of a TB laboratory arrangement is seen in Figure 1.

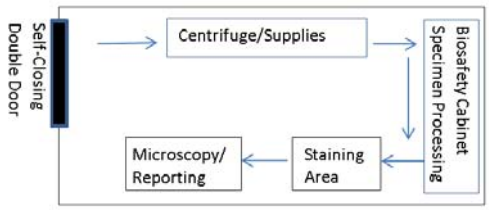

Figure 1

Specimen Setup. Within this laboratory, BSL-3 practices and procedures are recommended when working with TB specimens. These practices include personal protective equipment (PPE) such as a solid-front, disposable gown with snug (knit) cuffs, gloves long enough to externally overlap the sleeves of the gown, and donning a respirator, such as a N95 respirator (surgical masks are not effective) where the individual has successfully complete a medical evaluation and respirator fit test or alternatively wearing a powered air purifying respirator (PAPR).[2] Other BSL-3 practices include chemical decontamination of all waste prior to removal from the BSC, removing all outer protective clothing and thorough washing of hands before exiting the laboratory.

Since specimens for TB testing are generally received and accessioned in the main microbiology laboratory, handling needs to be done with the knowledge that tubercle bacilli from a patient may be present. As a general practice, all specimens should be collected in a leakproof container and transported to the laboratory in a clear sealable leakproof plastic bag. Any evidence of gross contamination within the bag should prompt the laboratory to reject the specimen according to the facility's protocol and request a new specimen. The outside of the collection container for TB specimens is disinfected with a tuberculocidal agent, regardless of the presence of visible contamination.

All specimens for routine bacterial cultures in the general microbiology laboratory should be processed within a BSC. This includes the fixation of smears for Gram stain since these samples may contain viable tubercle bacilli. Once heat fixed, the Gram stain smear may be removed from the BSC and stained on an open bench.

A specimen slated for TB workup can be set up in the general microbiology area only if it is collected from a sterile site and centrifugation or decontamination is not performed. Smears for TB staining (e.g. acid fast and auramine O) must be heat-fixed on an electric slide warmer with the temperature set between 65-75°C for 2h within the BSC. The temperature of the warmer should be recorded for each day of use.

Specimens worked up for MTB that require centrifugation or decontamination should be moved from the main microbiology laboratory to either a certified BSL-3 containment laboratory or to a separate approved BSL-2 laboratory as described earlier. Centrifugation of the specimen must be

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done in an aerosol-proof centrifuge with a safety-shield rotor. After centrifugation, the sealed rotor is moved to the BSC within the TB laboratory prior to opening. During specimen processing using BSL-3 practices, all procedures to fill and/or decant the centrifuge tubes, vortexing or sonicating, preparation of cultures and preparation of smears must also be performed in the BSC.

Extreme care must be taken to prevent cross-contamination of one specimen with another which may lead to false-positive results. Signs of cross-contamination include an increased positivity rate, isolation of the same species from several specimens processed in the same batch, culture positive specimens from a patient who is not highly suspected of having TB, culture growth of a smear-negative specimen processed next to a smear-positive specimen or isolation of several cultures with specific drug resistance patterns. Measures to eliminate cross-contamination include processing all previously known or highly suspected positives and proficiency samples individually, aliquoting fresh reagents in small quantities, separate specimen tubes in the test tube rack, no more than one tube top open at any given time, the use of pipettes long enough to reach tube bottom without touching the tube sides when sampling the pellet, the avoidance of splashing while adding reagents, no reuse pipettes or multi-dispenser, the changing of gloves frequently, waiting 5m after mixing/vortexing before opening tube and never allow tube cap to be placed sample side down on the working surface.[7] Cross contamination episodes should be investigated thoroughly and corrective measures taken. Molecular typing (fingerprinting) techniques through NPHL are available to evaluate for containment.[2]

Culture Process. Following incubation of a TB culture, manipulation of growth from culture (e.g. visual growth on slants, flagged positive MIGIT tube or BacT/Alert TB bottle) should always be done in the BSC using BSL-3 practices. Manipulation of viable growth in the BSC leads to the potential of aerosols production. To reduce the risk of exposure, a disinfectant soaked paper towel or gauze on the work surface within the TB lab BSC is recommended. Additionally, use of disposable inoculating loops are recommended and incinerator devices such as a Bunson burner and the use of needles, syringes, and other sharp objects should be avoided.

TB cultures can be evaluated on an open bench within the TB laboratory only when the tasks involved are solely observational and do not present a risk of aerosol creation. Therefore, closed, non-glass containers of cultures can be taken out of the BSC for spectrophotometer or observational readings. Cultures must be transported securely in racks or other safety carriers, mask and gloves must still be worn and procedures must be in place to address possible breakage.[2] Positive cultures should only be manipulated in an approved BSL-3 laboratory.

Training. Strict adherence to procedures with enhanced training is required for all technologists working within the TB laboratory[7]. Some procedures to consider when working in the BSC include slow arm movement from clean to dirty areas; not blocking the grill, side or back vents; stocking with only supplies needed for the immediate task; and arranging for disposing of items at the completion of a particular task. Additionally, individuals should be trained in the proper use of PPE, waste disposal, spill clean-up, reporting of illnesses and exposures, as well as packaging and shipping when the need arrives.[2] Personnel should also be conscious of airflow of the BSC and have a visual or audible method to verify that the air within the BSC is negative to adjacent areas of the laboratory. Recertification of the BSC must also be done annually by trained personnel.[7]

All waste materials in the TB laboratory should be chemically disinfected (using a tuberculocidal disinfect and following manufacturer's recommendations) before removal from the BSC. The surface of culture containers that have been processed with the specimen should be wiped with the disinfection before removal from the BSC for incubation. At the completion of the work, the BSC work surface is decontaminated with the tuberculocidal agent.

Although ultraviolet (UV) lights within the TB laboratory for surface decontamination are discouraged, this method may be used if steps are in place to frequently clean the bulb with an alcohol-soaked gauze and radiation intensity of the bulb are checked periodically.[7]

Laboratorians who handle suspected TB specimens should be tested annually with the purified protein derivative (PPD) skin test or one of the interferon assays. Personnel must be aware of certain changes in their health and individuals who are receiving chemotherapy or are immunosuppressed due to treatment of chronic diseases should not work with potential TB-containing specimens.

In situations where viable cultures suspected to be or confirmed MTB are transported off-site for testing, special packaging to reduce or eliminate the risk of exposure during transport are required. These cultures must be triple packaged and classified as a Category A and shipped as a UN2814 Infectious Substance. The laboratorian that does the packaging must be trained and certified by the Department of Transportation (DOT) in Packaging & Shipping of Division 6.2 Hazard Materials. Paperwork is required with a Category A shipments, regardless if offered to ground or air courier. Air couriers such as FedEx will require Shippers Declaration (completed with edit check software) and ground couriers must have a DOT Shippers Declaration Statement or similar. Ground couriers must also have Emergency Response Information (ERI) Guide 158 or the appropriate MSDS included with the paperwork. Clinical specimens that are sent for MTB testing must also be triple packaged, but are classified as Category B and can be shipped as a Biological Substance UN3373. Older terminology for Category B, Diagnostics Substances, is no longer an acceptable label. Contact State Training Coordinator (kstiles@unmc.edu) for further Packaging & Shipping information.

In conclusion, all laboratories processing specimens for MTB must conduct a risk-based assessment of their processing and determine best practices for their facility. This assessment will also define what precautions are required and the level of engineering necessary for the tasks performed. The laboratory director is responsible to ensure that all employees receive safety training and adhere to biosafety practices within the laboratory. This importance of biosafety must be instilled in all laboratory employees as protection of the laboratory worker will ultimately depend on them.[4]

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Neisseria gonorrhoeae Antimicrobial Susceptibility Testing at NPHL

by Amity Roberts, PhD, Clinical Microbiology Fellow

In August 2012, the Centers for Disease Control and Prevention (CDC) released a report, indicating that the current state of empiric treatment for *Neisseria gonorrhoeae* infections has changed.[1] In this document, cefixime as first-line treatment of gonococcal (GC) infections is no longer recommended due to increasing minimal inhibitory concentration (MIC) levels to this drug. Importantly, the report recommended that individuals with potential gonorrhea refractory to treatment, undergo culture to attempt recovery the GC isolate and then perform antimicrobial susceptibility testing (AST). AST-guided treatment substantially increases the probability of treatment success.

The caveat to performing AST for *N. gonorrhoeae* is that many laboratories no longer culture this organism. The majority of GC screening is performed with nucleic acid amplification testing (NAAT). NAAT's are highly sensitive tests and performed directly on specimens collected in specific NAAT-transport buffers. The NAAT buffers utilized are not conducive to the survival of the organism and therefore culture cannot be performed from specimens collected in these transport solutions.

Since isolate specific AST data is limited, most empiric therapy is based on the results of the Gonococcal Isolate Surveillance Project (GISP).[2] GISP is a program that monitors antimicrobial susceptibility trends for *N. gonorrhoeae* across the US in 28 sentinel and regional laboratories. Each month, the first 25 to 30 *N. gonorrhoeae* isolates recovered from culture of male urethral specimens at an affiliated STD clinic undergo AST. The AST results provides data for both regional and national *N. gonorrhoeae* antibiograms. Although AST provides generalized data on susceptibility patterns for *N. gonorrhoeae*, the AST results do not represent the whole region but the specific city in which that testing center is located. Additionally, GISP does not analyze isolates from female sources. The Omaha area is not included in this database.

In light of the new CDC recommendations, the NPHL now offers AST for cases of suspected GC treatment failure. If a clinician suspects a treatment failure, the clinician should order a culture, gonorrhoea screen (GCSCR). It is advisable to notify NPHL so specialized media for AST can be acquired. The specimen is directly inoculate at the time of collection onto either a JEMBEC or Gono-Pak transport plate. Since *N. gonorrhoeae* is highly sensitive to changes in CO_2 and temperature, the inoculated plate is transported to the clinical microbiology laboratory as soon as possible after collection. Susceptibility testing is performed on all isolates following the Clinical Laboratory Standards Institute (CLSI) recommendations. The following antimicrobials are tested: ceftriaxone (E-test), cefixime (E-test), ciprofloxacin (KB-disk), penicillin (KB-disk), spectinomycin (KB -disk), and tetracycline (KB-disk). AST interpretations are available for the preceding antimicrobials. Additional questions concerning AST for *N. gonorrhoeae* can be directed to Dr. Iwen at (402)559-7774.

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Upcoming 2013 NPHL Events

Bioterrorism Preparedness Recognize, Rule Out or Refer Workshop

Lincoln - UNL East Campus
Tuesday July 30
Wednesday August 7
Omaha - Nebraska Public Health Lab
Wednesday October 16
For details/registration, contact
kstiles@unmc.edu

2013 Nebraska Challenge Set

Packaging/Shipping exercise
Fall TBA

Norovirus Testing in Nebraska

By Manjiri Joshi, MPH, Epidemiology Surveillance Response NDHHS and Cathy Gebhart, PhD, Technical Director, Molecular Diagnostics, UNMC

Norovirus is contagious and spreads quickly and easily either by person-to-person spread or environmental or food-borne contamination. This virus is a major cause of gastrointestinal illness particularly in closed and crowded environments such as nursing homes, schools or cruise ships where people may consume food and water handled by infected individuals. Infected individuals often develop diarrhea, nausea, or vomiting that generally lasts 1-2 days. Infections may occur more than once and can be serious in young children, the elderly, and people with chronic conditions.

Proper hand hygiene is the best practice to prevent the spread of infection. Persons in high-risk occupations (food handler, daycare, health care with direct patient contact) should wait until at least 48 h after diarrhea ceases to return to work.

In March, a new norovirus (GII.4) was identified in Sydney, Australia. Since then, this virus has been associated with >80% of the outbreak strains typed in southern Australia and New Zealand. This Sydney strain has replaced the previously dominant GII.4 New Orleans (2009) variant in these countries. On the basis of CaliciNet (a network of states that submit norovirus specimens for genetic sequencing), it is known that this virus is circulating in the United States and associated with 14% of all reported outbreaks within the past 2 months.

During September 2012 to January 2013, 21 norovirus outbreaks have been reported affecting 813 individuals in Nebraska. Of these, 17 (81%) occurred in nursing homes with 2 (9.5%) in conferences or group meetings, and 1 each (5%) in a veterans home and wedding reception. An epidemiological investigation demonstrated that each nursing home outbreak was the result of person-to-person transmission, and none of the other outbreaks were found to be foodborne associated.

Qualitative diagnostic testing at the Molecular Diagnostic Laboratory section of the NPHL is specific for norovirus RNA, genogroup I and II extracted from stool specimens. The test method is a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) with the norovirus RNA polymerase region/capsid junction as the test target. The test also includes an internal control that detects nucleic acid extraction failure as well as the presence of RT-PCR inhibitors. The testing is carried out on the ABI 7500 real-time instrument.

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Meet the Laboratorian - Kathy Talmon

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

The Nebraska Public Health Laboratory celebrates 100 years of service in 2013. This edition's laboratorian has been an integral part of our state's public health laboratory since 1976. Kathy Talmon currently works in the TB laboratory at TNMC and shares her history and experiences of working for the state laboratory.

"About my junior year in high school, I realized that teaching was not for me as I did not want to spend the rest of my life in school. In the late 60's, the career choices for females were not as vast as they are now. In fact, in the newspaper, jobs were listed under male and female. I loved science so when I was introduced to medical technology as a possibility at the UNMC career day that became my choice. I attended Chadron State College for my formal studies and did my medical technology internship at Lincoln General Hospital. In the early 70's, there were 3 medical technology schools in Lincoln and 5 schools in Omaha. Things have certainly changed."

"I have been a MT/CLS for almost 40 years and have always worked in Microbiology. After 5 years of work, I took and passed the specialty exam. I worked at the Nebraska State Health Lab for over 20 years when located in Lincoln. The clinical laboratory area did a variety of testing at that time. Public health was the emphasis so the testing included STD's, TB, HIV, enteric pathogens, newborn screening, rabies, and assorted serology tests for Rocky Mountain Spotted Fever, typhus, and brucellosis. We also did outbreak or special investigations where hundreds of patient specimens would be sent to the lab to check for enteric pathogens, parasites, diphtheria, or other organisms."

Kathy continued to share her story of the first years in the lab, preparing and filtering Gram stain reagents, pouring culture media and biochemical media used in the identification process.

The early Bacteriological Laboratory used a more detailed and elaborate form of our LRN guidelines to workup hazardous organisms. A CDC resource nicknamed the "King's Round Table of Microorganisms," published by Elizabeth King for identification of unusual pathogenic organisms was used. It provided King's key and "Round Table" charts showing results for OF basal media testing metabolism of sugars and other reactions. All identification was performed manually, so many biochemicals were set for those more difficult organisms to identify.

Kathy's most memorable outbreak investigations involved members of a Laotian family who were infected with as many as 9 different types of parasites and another migratory population outbreak which brought in 100-200 specimens for ova and parasite testing. The latter took days to complete.

When asked about safety efforts made early on, she shared that even then, with the assistance of the CDC, their building was designed with safety in mind. The TB section was divided into 3 suites which separated the different levels of work, each had a more negative airflow. The TB work was completed in using a 6 foot long biosafety cabinet which offered the most up-to-date features of the time.

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IN THIS ISSUE

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