Nebraska Public Health Laboratory Newsletter

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NPHL Updates

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

The NPHL staff have been involved in a wide variety of activities over the past three months and several of their projects are highlighted in this issue. The activities range from new testing procedures for investigating infections with tuberculosis to a CDC sponsored training activity in the Far-East. We have summarized key information about another organism recently added to the Infectious Diseases Society of America's list of dangerous bugs. From the Chemical Terrorism Preparedness Laboratory, an overview of arsenic is provided to give you information on an issue that will be appearing in the news following a change in EPA regulations about the maximal contamination level of arsenic in drinking water. The Nebraska Laboratory Network- of which you all play an important role has also been busy adjusting to new regulations and documents for processing select agents as described in the article by Pete Iwen, Associate Director of the NPHL. In addition to these articles, we have created a new "Hot Topics" section in this issue to provide you with a quick update on important topics such as the Mumps virus outbreak that hit the state this past winter and fall, alerts about West Nile Virus, Select Agents, activities within the Nebraska LRN, and STATPack project. Through all of these activities in collaboration with the Nebraska Health and Human Services System as well as laboratorians in the Nebraska Laboratory Network we are collectively working to enhance the effectiveness of all public health efforts. We again thank you for your participation and support.

Arsenic Testing at the Nebraska Public Health Laboratory

By Dana El-Hajjar, MBA, Chemistry Specialist, NPHL

Arsenic (As, mw 75) is a highly poisonous metallic element that is named from the Greek word arsenikon. Arsenic containing minerals have been known for centuries. Arsenikon is synonymous with orpiment which is an orange to yellow arsenic sulfide mineral (As_2S_3 or arsenic trisulfide). Arsenic sulfide (As_4S_4) which is also known as realgar (from the Arabic word rahjalghar meaning "powder of the mine") (1), was described by Aristotle in the 4th Century BC.

The Chemical Terrorism Preparedness Laboratory of the NPHL recently received the methodology from the Centers for Disease Control and Prevention (CDC) to test arsenic levels in urine. Arsenic is measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrumentation coupled with a Dynamic Reaction Cell (DRC) instrument. In addition to the ICP-MS, DRC is required to remove potential interferences that have the same molecular weight as arsenic. The methodology involves passing acidified urine through a nebulizer and spray

(Arsenic, continued on page 5)

Quantification of Interferon Production for Investigating TB Exposure

By Jodi Garrett, MT(ASCP)SM, Microbiology Manager, NPHL

The NPHL has recently evaluated an FDA approved in-vitro test called the QuantiFERON® -TB Gold (QFT-G) assay (Cellistis Limited, Carnegie, Victoria, Australia) as an aid in investigating both latent and active *Mycobacterium tuberculosis (Mtb)* infections. One of the major uses is as a screen for tuberculosis in people who have been immunized with the attenuated BCG vaccine, since prior vaccination does not cause the QFT-G test to become positive.

In the assay, peripheral blood is tested for the production of interferon by *Mtb* stimulated lymphocytes. Two *Mtb* antigens are added to heparinized peripheral blood containing antigen presenting cells and T- lymphocytes. T-lymphocytes that were previously exposed to *Mtb,* produce interferon-γ (IFN-γ) in response to the addition of antigens. The quantity of IFN-γ produced is detected using a single-step enzyme-linked immunosorbant assay (ELISA).

In unvaccinated individuals without *Mtb* infection, clinical trials have shown a specificity of 99.8% for the QFT-G test as compared to 99.1% for the tuberculin skin test (TST). In culture-confirmed infected individuals, *Mtb* QFT-G testing demonstrated a sensitivity of 91.3%, compared to 79.2% with TST, while in BCG-vaccinated individuals, the specificities were 98.1% and 68.1%, respectively for QFT-G and TST. Studies in children and immunosuppressed patients, to include HIV-positive individuals, are currently on-going as the test is not approved to evaluate these groups. Studies showed that indeterminate test results were common (21%) among immunocompromised patients with negative TST results (1).

The QFT-G test was evaluated at the NPHL using peripheral blood from 15 TST positive individuals who were clinically negative for *Mtb* disease (all negative by chest x-ray), 16 TST-negative individuals, and six BCG-vaccinated individuals. Thirteen of the 15 TST-positive individuals without tuberculosis had a negative QFT-G test (two individuals tested positive). All 16 TST-negative cases and 5 of the 6 BCG-vaccinated individuals (one tested as indeterminate) were negative with the QFT-G assay. These data suggest the QFT-G assay could be used to reduce the number of individuals treated for a false-positive TST results.

The QFT-G test is appropriate to screen individuals who have received BCG vaccination and to evaluate health care workers who test positive by the TST. Cost savings are expected from a decreased need for treatment of skin-test positive individuals who test negative with the QFT-G assay. Additionally, repeat skin-testing should be limited in individuals who

(TB Exposure, continued on page 5)

Reporting the Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory: Revised APHIS/CDC Form 4

By Peter C. Iwen, PhD, Associate Director, NPHL

The Animal and Plant Health Inspection Service (APHIS) and the Centers for Disease Control and Prevention (CDC) recently revised the form for reporting the identification of a select agent or toxin from clinical specimens [Form 4, Reporting of the Identification of a Select Agent or Toxin, Exp 12/13/2008]. Major changes were made to simplify the reporting process. Guidance is given below to help laboratories fill out and submit this new form.

Section 1A (To be completed by all)

1. **Legal name of entity**. The legal entity refers to the reporting laboratory's official name.
2. **Entity registration number**. This number is recorded by the Nebraska Public Health Laboratory (NPHL), while all other laboratories record "Not applicable".

3-7. **Address.** This is self explanatory.

7-11. **Responsible official (RO) or facility director, title, telephone, fax, and e-mail.** This is generally the Laboratory Director but it may also be another responsible person such as the Medical Director or Biosafety Officer.

12-15. **Address of RO or facility director.** This is self explanatory.

Section 1B (Leave Blank)

16-24. **Name of federal law enforcement agent.** To be completed by a federal law enforcement agency when appropriate.

Section 2 (To be completed by all)

25. **Select agent or toxin being reported.** Include a scientific name.
26. **Date(s) agent was identified.** This can be recorded as the "date for notification of final result" for those laboratories sub mitting a clinical specimen or isolate to a reference lab for testing, or the date of identification for those laboratories doing confirmation testing.
27. **Agent ID number.** Record the specimen accession number or whichever number is used to identify the specimen/agent.
28. **Total quantity of select agent or toxin identified.** Record quantity if an environmental sample is tested otherwise record as, "Not applicable".
29. C**haracterization of select agent or toxin.** For laboratories submitting a specimen to a reference laboratory without testing, indicate the following: "Specimen submitted to [list the laboratory] for isolation and/or confirmation". For laboratories submitting an isolate to a reference laboratory for confirmation testing, indicate the following: "Isolate presumptively identified as [give presumptive ID] using the following characteristics [list the methods used] was submitted to [list the laboratory] for confirmation testing". For laboratories performing confirmation testing indicate the following: "Confirmation of species identification performed using the following criteria: [list the criteria used]". Attach additional sheets if necessary.
30. **Type of specimen.** For all laboratories handling the original specimen, check "Clinical/diagnostic sample". Check "Environmental sample" if from the environment or "Isolate" if the laboratory received an isolate for confirmation testing. Indicate "Other" for situations that do not meet criteria for the other categories and specify the sample type.
31. **Specimen type.** For laboratories handling the original specimen, indicate whether the sample is a "Fluid" or "Tissue" and specify sample type. Check "Isolate" if applicable or "Other" for situations not listed and then specify the specimen type.
32. **Source of sample.** In most cases the sample will be "Human", but it may also represent other sources as described.
33. **Is the source expected to provide additional specimens?** Usually check "No" or "Unknown". If additional specimens are expected, check "Yes" and give anticipated quantity and end date.
34. **Location where laboratory testing was conducted.** If no testing was done, list as "Not applicable", otherwise list the building and room where testing was performed.
35. **Biosafety level of laboratory.** For laboratories sending a specimen to a reference laboratory without testing, record as "Not applicable". For laboratories performing tests on the specimen and/or the isolate, list either BSL-2 or BSL-3 depending on the containment of the laboratory used.
36. **Was select agent or toxin isolated under conditions prescribed by the BMBL?** Generally answer as "Yes". Specimens can be handled under BSL-2 conditions while an identified isolate may require BSL-3 conditions where appropriate. In unusual circumstances where exposure to a select agent in culture may have occurred, indicate "No" and describe whether the appropriate medical surveillance has been instituted according to laboratory protocol.
37. **Has the sender of the sample been notified of the identification of the select agent or toxin?** Indicate "Yes". Laboratories performing confirmation testing should routinely notify the submitting laboratory of the identification of a select agent or toxin.

38-43. **Name of the entity that sent sample.** For laboratories who received the original specimen, indicate, "Not applicable". For reference laboratories who received the specimen or isolate from another facility, indicate the sending facility legal name and record the sender's telephone number and address.

44-48. **Name of RO or facility director for the sending entity.** Only fill out this section if different than Section 1. This could be the supervisor or medical director of the laboratory handling the original specimen.

49-50. **Name of treating physician, veterinarian, botanist, or person most familiar with the case and telephone number.** Normally this is the primary care physician of the patient from whom the specimen was obtained.

(Select Agent, continued on page 3)

(Continued from page 2, Select Agent)

51. **If more than one case.** Generally this will be listed as "Not applicable" however, when multiple cases are involved, describe the date of the index case, the number of cases, and the inclusive reporting dates if known.

Section 3 (Leave blank)

52-58. This section is completed for select agents or toxins identified from proficiency testing. Contact personnel at the NPHL for advice on filling out this information when needed.

Section 4 (To be completed by all)

59. **Deposition of select agent or toxin.** The laboratory that receives the specimen for culture and subsequently sends an isolate for confirmation testing to a laboratory other than the NPHL or the laboratory that has done in-house confirmation testing should check "Transferred" and then call the NPHL personnel to make arrangements for transfer of the identified isolate [an APHIS/CDC Form 2 will need to be processed in consultation with personnel at NPHL]. **NOTE: NO TRANSFER IS TO OCCUR UNTIL FORM 2 HAS BEEN PROCESSED AND AN AUTHORIZATION NUMBER HAS BEEN ISSUED BY THE CDC.** The laboratory that receives a specimen for culture and subsequently sends a suspicious isolate for confirmation testing to the NPHL should check, "Destroyed on site" and indicate date specimen and culture material are destroyed and the method of destruction. A laboratory that receives an isolate for confirmation testing that is subsequently retained after confirmation should check, "Retained" and then give the name of the Principal Investigator and the date the select agent or toxin was transferred [only registered laboratories such as the NPHL can retain these isolates]. The laboratory that submits a specimen to a reference laboratory for testing should indicate "Other, Specimen submitted to reference laboratory for culture and confirmation testing".

60. **Signature**. The Laboratory Director or other individual as indicated in Section 1 of the form if generally the individual who signs the form however, it may be another person who can certify that the information provided is true and correct to the best of their knowledge.

When questions occur, please do not hesitate to contact personnel at the NPHL or Dr. Iwen at 402-559-7774 for additional information.

Table 1. Which laboratories must complete and submit the APHIS/CDC Form 4?[a,b]

Laboratories who:

- Handle the original specimen that contained a viable select agent or toxin prior to submission to a reference laboratory for testing.
- Conduct the initial plating of the specimen but submit the suspect isolate to reference laboratory for confirmation testing.
- Conduct the initial plating of the specimen and perform confirmation testing.[c,d]
- Confirm the identification following isolate submission[c,d,e] (Select agent confirmation is performed at the NPHL in most circumstances)

[a]In many instances, multiple laboratories may handled a specimen or isolate containing a select agent or toxin which thus requires multiple submissions of Form 4.

[b]The laboratory handling serum from a patient who ultimately is confirmed as positive for a select agent-caused disease by serological testing does not need to file Form 4. However, they are still responsible for reporting immediately the result to the county or State Health Department.

[c]The laboratory performing confirmation testing is responsible to contact the CDC for those agents that require immediate reporting (telephone 404-498-2255, facsimile 404-498-2265, or e-mail [Irsat@cdc.gov]).

[d]Agents that require immediate reporting to the CDC are listed in the instructions for Form 4.

[e]The UNMC/NPHL Special Pathogens Laboratory has the reagents and protocols available to confirm the identification of a select agent or toxin.

Table 2. Checklist to report a select agent or toxin after diagnosis and verification.

- Report immediately to the CDC by telephone, facsimile, or e-mail when required.[a] **Note:** Only for laboratories performing confirmation testing.
- Report immediately to the county or to the Nebraska State Health Department.
- Dispose of the select agent or toxin to include specimen and cultured material[b,c] **Note:** Either by transfer to the NPHL or by onsite destruction.
- Obtain a copy of the APHIS/CDC Form 4 from the CDC web site.[d]
- Complete Sections 1A, 2, and 4 and sign/date form.
- Make 3 copies of the completed form.
- Send the original Form 4 to the CDC, one copy to the NPHL, and one copy is retained by the laboratory for three years.[e]

[a]The instructions to the APHIS/CDC Form 4 lists those agents that require immediate reporting to the CDC (telephone, 404-498-2255; facsimile, 404-498-2265; or e-mail at Irasat@cdc.gov)

[b]Only laboratories registered by the Select Agent Program may retain materials containing a known select agent or toxin.

[c]A subculture of a select agent identified by a reference laboratory other than the NPHL should be sent to the NPHL for banking. **Transfer of a known select agent or toxin will require additional paperwork. Personnel at the NPHL will coordinate this transfer.**

[d]Refer to the CDC website at http://www.selectagents.gov/cdForm.htm to obtain Form 4.

[e]Send the completed form to the CDC at the Centers for Disease Control and Prevention, Division of Select Agents and Toxins, 1600 Clifton Road NE, Mailstop E-79, Atlanta, GA 30333.

NPHL Goes to Thailand

By Josh Rowland, State Training Coordinator, NPHL

The avian influenza virus (H5N1) is a potential cause of pandemic disease if viral adaptation should impart sustained human-to-human transmission with a presentation of highly virulent features. Surveillance activities supported by laboratory procedures could prove vital to contain the disease should pandemic conditions arise. The United States government, through the Centers for Disease Control and Prevention (CDC), has thus offered financial and technical assistance to underdeveloped countries to build laboratory capacity to support surveillance activities. As the need to have world-wide surveillance for the virus continues to spread, the CDC recently requested volunteers from public health laboratories with experience in the laboratory procedures used to detect avian influenza virus, to assist in providing technical support.

Participants in the April avian influenza virus workshop in Bangkok included: Ms. Trinh Thi Xuan Mai from Nha Trang, Vietnam; Ms. Punnarai Veeraseatakul from Chiang Mai, Thailand; Ms. Triyani Soekarso from Jakarta, Indonesia, and Bob Wickert, Molecular Microbiologist, NPHL.

Bob Wickert, molecular microbiologist for the NPHL, applied to participate and was subsequently assigned to a team conducting laboratory workshops in Bangkok, Thailand. The original workshop he conducted was from April 24th to 28th. He returned to Bangkok and also participated in a workshop from July 17th to 21st. (See photo above) Laboratorians from the surrounding countries of Vietnam, Indonesia, Myanmar, Cambodia, India, Nepal, Laos, and the Philippines, along with staff from Thailand's provincial laboratories spent time learning principles of the real-time polymerase chain reaction (PCR) assay for avian influenza virus detection. The workshop included formal lectures given in English, as well as experiences in RNA extraction, real-time PCR assay set-up, result analysis, and trouble-shooting.

Bob shared that most of the participants were experienced laboratorians and some had prior experience with standard PCR assays. Therefore, the techniques were not difficult to teach and all participants had successful results. The workshop participants and volunteer staff were also given the opportunity to socialize in a welcome reception hosted by the Thai Ministry of Public Health. This included a fun night of eating, drinking, dancing and karaoke singing which will long be remembered as the highlight of the workshop. Bob felt that the pleasant manner of the Thai staff and their genuine hospitality will go a long way to promote cooperation between these Southeastern Asian countries as they provide surveillance for the avian influenza virus.

For more information about avian influenza virus testing, please contact Bob Wickert at rwickert@unmc.edu or 402-559-2123.

HOT TOPICS

By Tony Sambol, M.A., SM(NRM), Assistant Director, NPHL

Periodically we will highlight various topics in this section that are of importance to clinical laboratorians throughout the state.

Mumps Virus. Testing of serum samples from individuals across Nebraska has shown that most Nebraskans have been successfully immunized. Many hospitals checked the immune status of their employees as recommended by the Nebraska Health and Human Services System (NHHSS). According to NHHSS, there were 361 acute cases of Mumps infection in Nebraska between February and July of this year. The question on everyone's mind was whether the outbreak resulted from a failure of the vaccine or the failure to vaccinate? In an effort to better document the level of immunity in young adults in Nebraska, Josh Rowland was asked by the CDC and NHHSS to assist them and Terry Krohn, Director of the Two Rivers Public Health Department in a study of students at the University of Nebraska at Kearney (UNK). Over a period of two days, over 500 blood specimens were collected from UNK students for the evaluation of antibodies to the mumps virus. The results of this study are slated to be published in an upcoming Morbidity and Mortality Weekly Report. The successful completion of the project in a very short time period was a strong demonstration of the importance of building working relationships for responding to public health events.

West Nile Virus. Although early data this summer indicated that very few cases were developing, the WNV has roared back to life in late August. In addition to testing for the virus in mosquitoes the NPHL began seeing a significant upsurge in requests for serological testing of CSF and serum from human patients exhibiting neurological symptoms consistent with viral infection. Going into publication, the number of cases of WNV infection is lower than that observed last year, with 131 human infections and one death. WNV has been confirmed in 34 out of Nebraska's 93 counties. Therefore, we expect the number of human cases to grow before our first frost. The state-wide WNV surveillance efforts this year are coordinated by Annette Bredthauer, DVM, the NHHSS Public Health Veterinarian. For more WNV information go to http://www.hhs.state.ne.us/wnv/.

Special Pathogens. The NPHL continues to receive isolates of *Francisella tularensis* from clinical laboratories around the state emphasizing the importance of being aware that some suspect organisms may require at a minimum the use of Biological Safety Level-2 (BSL-2) containment with the added protection of a biological safety cabinet with proper personal protective equipment to include masks, gowns, and gloves. Since special training is required to handle and screen for these special pathogens, the NPHL offers *Sentinel Laboratory* wet-workshop training. This training outlines procedures that can be used to "recognize, rule-out, or refer" these organisms. One

(HOT TOPICS, continued on page 6)

(Continued from page 1, Arsenic)
chamber where the urine is ionized using high temperature (6000-8000°K) argon gas (hotter than the temperature of the sun). The ions, along with the argon gas, enter the mass spectrometer and pass through the DRC where the arsenic is separated from the interferences and enters the mass spectrometer where it is detected (based on mass to charge ratio) and quantitated.

Arsenic Levels in Nebraska

Arsenic and arsenic-containing compounds are found naturally in the environment with the main cause of arsenic poisoning from the ingestion of contaminated drinking water. In 2004, the U.S. Environmental Protection Agency (EPA) lowered the maximum contaminant level (MCL) in water from 50 parts per billion (ppb) to 10 ppb and required water systems to comply with this standard by January 23, 2006 (2). The EPA estimated that approximately 4,000 Public Water Systems (PWS) of the 74,000 PWS in the United States had to make changes to comply with this new regulation (2). According to the Nebraska Health and Human Services System there are currently 81 PWS in Nebraska that have arsenic concentrations above 10 ppb. These water systems serve nearly 100,000 people and are mostly found in the Panhandle and the western Sandhills regions of the state (3).

Arsenic Usage

Arsenic has several uses in agriculture, industry, and medicine. Historically, arsenic was used in cosmetics and as a pigment in paint and during the Victorian era was used as a cure-all medicine to treat everything from skin warts to fever and diabetes. Arsenic is now used in the manufacture of fungicides, insecticides, pesticides, and herbicides and in the semiconductor and transistors industry. In medicine, arsenic is used in several drugs. For example, arsenic trioxide (As_2O_3) is used to treat acute promyelocytic leukemia.

Arsenic as a Murder Weapon

Throughout history, arsenic has attained notoriety as a method for committing murder. Since the symptoms of arsenic poisoning can be confused with those of many other illnesses, it was difficult to detect arsenic after death which provided a practical way of murdering someone without getting caught. It was not until the development of a chemical test to detect for arsenic called the Marsh Test, that arsenic was proven to be used as a poison. Another less sensitive test was subsequently identified that could be used to detect arsenic, called the Reinsch test (1). Arsenic was such a common method for murder among the ruling class that it became known as the "Poison of Kings" and the "King of Poisons", and became referred to as the "Inheritance Powder". Several arsenic compounds are tasteless and colorless and have the appearance of white sugar which makes them undetectable by the victim(4). Napoleon Bonaparte is believed to have died of arsenic poisoning (1). Today, arsenic-containing compounds are considered a potential means for chemical terrorism. As a chemical warfare agent, dichloro(2-chlorovinyl)arsine (Lewisite) was first produced in 1918 to be used in World War I, however, the war ended prior to its use.

Arsenic and Health Effects

Long-term exposure to arsenic has been linked to cancer of the bladder, lungs, skin, kidneys, nasal passages, liver and prostate (5). Additionally, other health effects that have been documented include skin lesions, swollen nodes, cardiovascular disease, certain neurological disorders, diabetes, hearing loss, and hematological disorders (anemia and leucopenia) (5). Arsenic exerts its toxicity by inactivating up to 200 enzymes, most of them involved in the cellular energy pathways and in DNA replication and repair hence causing DNA damage (4).

Arsenic exposure today occurs mainly from drinking contaminated water, which is why the EPA has tightened regulations on arsenic levels in water. Other exposure routes include inhalation, and absorption through the skin. Arsenic may be present in foods such as fish and algae in the relatively non-toxic organic form. Exposures to these organic compounds increases the arsenic levels in the blood following ingestion but are excreted rapidly through the urine. Arsenic, when present at high levels and unable to be excreted, tends to accumulate in various organs of the body and in the keratin-rich tissues, such as the nails, hair, and skin where it binds the thiol or sulfhydryl groups in tissue proteins (4).

Arsenic has had a continuing impact on human history over the ages. One reference to its effect survives in dermatology and pathology as actinic keratosis, an eruption of the skin originally due to arsenic poisoning and now used to describe skin damage to the sun. The ability to measure arsenic in urine is an important component of the chemical terrorism preparedness program at the NPHL. For more information about arsenic and testing for arsenic, please contact Dana El-Hajjar at 402-559-9421 or delhajja@unmc.edu.

References

1. Bentley R., Chasteen, T. 2002. Arsenic curiosa and humanity. *Chem. Educator*, 7 (2): 51-60.
2. http://www.epa.gov/safewater/arsenic/
3. Gosselin, D. Arsenic in Nebraska's groundwater and public water supplies. *University of Nebraska-Lincoln Conservation and Survey Division, Earth Science Notes* No 7. April 2004.
4. Ratnaike, R. 2003. Acute and chronic arsenic toxicity. *Postgrad Med J* , 79: 391-396.
5. http://www.atsdr.cdc.gov/HEC/CSEM/arsenic/index.html

(Continued from page 1, TB Exposure)
have consistently positive TSTs.

Specimen collection consists of two-5 ml green-top heparinized tubes (sodium heparin or lithium heparin). Other anticoagulants (EDTA, citrate dextrose) interfere with the assay and should not be used. The blood must be incubated with the test antigens within 12 hours after collection while the lymphocytes are viable. After the blood is incubated with antigens for 16-24 hours, the plasma is harvested at which time the processed specimens may be frozen prior to testing. A test is considered positive for an IFN-γ response to either antigen that is significantly above the non-stimulated IFN-γ level.

Questions regarding this new test may be directed to either Steven Hinrichs, M.D. (402-559-7203) or Jodi Garrett (402-552-3235). Further information can be found at www.cellestis.com and on the Centers for Disease Control and Prevention's website (www.cdc.gov).

References:

1. *Guidelines for Using the QuantiFERON®-TB Gold Test for Detecting Mycobacterium tuberculosis Infection, United States*, Morbidity and Mortality Weekly Report (MMWR), Recommendations and Reports, December 16th, 2005/54 (RR15); 49-55 (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5415a4.htm).

Acinetobacter baumannii: A Dangerous Pathogen Emerges

By Peter C. Iwen, PhD, Associate Director, NPHL

Acinetobacter baumannii is considered the most common oxidase-negative non-fermenting gram-negative rod encounter in the clinical laboratory [1]. This organism is widely distributed in nature and in the hospital environment and generally considered an opportunistic pathogen in debilitated patients. Recently, the Infectious Diseases Society of America included this microbe on a hit list of the six top priority dangerous drug-resistant microbes (see **Table 1**) due to the propensity of this organism to develop drug-resistance and to the lack of development of new drugs to treat infections caused by resistant *A. baumannii* [2]. Resistance of *A. baumannii* to carbapenems (Imipenem), which is the drug of choice to treat serious infections caused by this species, are fast becoming more common in the laboratory. The NPHL has lately observed multi-drug resistant *A. baumannii* from multiple laboratories within Nebraska.

This bacterium is one of 17 recognized species within the genus *Acinetobacter*. Many of the species within this genus are difficult to separate reliably by phenotypic methods alone and frequently are placed into groups or complexes based biochemical test results. It is therefore not uncommon to identify an isolate as *A. baumannii/calcoaceticus* complex or *A. baumannii/ haemolyticus* All of these species have the ability to oxidize glucose and are therefore described as the saccharolytic species of *Acinetobacter* in contrast to the asaccharolytic species which is most commonly *A. lwoffii*. **Table 2** identifies some simple phenotypic observations that can be used to separate these common species following automated system identification. *A. baumannii* is considered by far the most common species isolated from human specimens followed by *A. lwoffii* and *A. haemolyticus* [1].

To monitor for the emergence of resistance within *A. baumannii* in Nebraska, laboratories are being asked to submit to the NPHL any multi-drug resistant isolates of this species that are encountered. These isolates can be submitted through the current courier system that is now used for the submission of other clinical isolates for epidemiological evaluation. Any results following the evaluation of these isolates will be communicated with the laboratories in a future newsletter. Contact Peter Iwen at 559-7774 for further information concerning this issue.

References

1. Schreckenberger, P.C., et al. 2003. *Acinetobacter, Achromobacter, Chryseobacterium, Moraxella*, and other nonfermentative gram-negative rods, pg 749-779. In P.R. Murray et al. (ed.), *Manual of Clinical Microbiology*, Volume 1. 8th Edition, ASM Press, Washington, DC.
2. Talbot, G.H., et al. 2006. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect Dis. 42: 657-668.

REMINDER

NPHL Newsletter articles are archived on www.nphl.org

1. Click "NEWSLETTERS" on the blue menu bar.
2. Newsletters are listed in reverse chronological order.
3. Newsletters are available in their entirety or as individual articles.
4. Click "Get Acrobat Reader" if you do not already have the ability to read PDF files.

Table 1. IDSA "hit list" of dangerous "bugs".

Methicillin-resistant *Staphylococcus aureus*
Escherichia coli and *Klebsiella* spp.[a]
Acinetobacter baumannii
Aspergillus species[b]
Vancomycin-resistant *Enterococcus faecium*
Pseudomonas aeruginosa

[a] Includes those isolates referred to as extended spectrum beta-lactamase producing gram-negative rods (ESBLs).
[b] Mould associated with life-threatening infections in immunocompromised patients.

Table 2. Major species within the genus *Acinetobacter*.[a]

Acinetobacter species	Growth at 44°C	Glucose Oxidizer	Hemolysis (SBA[d])
baumannii[b,c]	Positive	Positive	Negative
calcoaceticus	Negative	Positive	Negative
haemolyticus	Negative	Positive	Positive
lwoffii	Negative	Negative	Negative

[a] Major genus characteristics include the inability to ferment glucose (non-fermenter), lack of oxidase production (oxidase-negative), and non-motility.
[b] Specific phenotypic characteristics include the appearance as cocci or coccobacilli on Gram stain, the ability to grow on MacConkey agar, and resistance to penicillin.
[c] Formerly called *A. calcoaceticus* var *anitratus* and frequently not separated from other species due to the similarity in phenotypic test results i.e., *A. calcoaceticus/baumannii* complex or *A. baumannii/haemolyticus*.
[d] Sheep Blood Agar

(Continued from page 4, HOT TOPICS)

highlight of this "hands-on" experience is that laboratorians have the opportunity to work with an attenuated strain of *Bacillus anthracis* to help them recognize the identifying characteristics. All laboratories that have not sent personnel to one of the NPHL's wet-workshops are strongly encouraged to call Josh Rowland at 402-559-6070 to set up a training opportunity.

STATPack. The **S**ecure **T**elecommunications **A**pplication **T**erminal **Pack**age used for remote consultation was recently added to the hospital laboratories in Imperial and York and soon will be added to the hospital laboratory in O'Neill, bringing the total number of operating sites to 17. These efforts coincide with the recent deployment of STATPACK in Oklahoma and announcement by Kansas to install the system. Our next step is to begin offering microbiology "grand-rounds" educational material using the STATPACK with presentations prepared by Rhonda Noel and the NPHL staff. The start date of this activity is slated for this fall.

Chemical Terrorism Preparedness Laboratory. The Chemical Terrorism Preparedness Laboratory (CPTL) is currently performing validation for testing of clinical specimens for nerve and mustard agent metabolites. In preparation for this work and related activities NPHL scientists hosted a partnership meeting with the Civil Support Team and the FBI. See the included "Arsenic Testing at the Nebraska Public Health Laboratory" for more information about activities of the CTPL.

Meet the Laboratorian - Lois Carmody

Compiled by Josh Rowland, State Training Coordinator, NPHL

Lois Carmody, BSMT(ASCP), is the Resource Specialist in Microbiology at the Alegent Core Laboratory in Omaha, Nebraska. The Alegent Core Laboratory does all microbiology testing for Alegent Health System Hospitals and Clinics in Eastern Nebraska and Western Iowa. Lois recently celebrated her 40th anniversary as a laboratorian.

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

While attending high school in Mondamin, Iowa I was interested in Chemistry, Biology, History, and basketball. My interest in laboratory science started after the principal, who knew about this career field, said that I might enjoy the science and medicine field.

Where did you attend medical technology school?

I attend the College of Saint Mary in Omaha. After my internship at Bergan Mercy Hospital in Omaha, I accepted a job there as a generalist.

How long have you worked in your present location?

I have been working for 40 years, first as a generalist and currently in microbiology. Prior to Alegent Health merging with Bergan Mercy hospital, I worked my entire career at Bergan Mercy. I now work at the Alegent Health Core Laboratory as a microbiologist.

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

As a resource person in microbiology, my biggest challenge is to continue to encourage and train generalist technologists in microbiological procedures that they will be performing in the laboratory. Another challenge for laboratories is to use the most current technology available in a cost effective way.

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

As a new technologist, there are many opportunities in the laboratory field. The laboratory is a valuable part in patient care. One can work on front-line of laboratory testing in hospitals, use the career to specialize in a certain field of laboratory medicine. The laboratory technology education serves as a wonderful basis for continuing career development in such areas as medical school, pharmacy, nursing, public health, and others.

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

The biggest change in the laboratory has been advanced technological developments for the diagnosis of a variety of diseases. For example, learning about molecular microbiology is one of the current challenges for workers in the microbiology laboratory.

What do you like most about your job?

The best part of my job is the people with whom I work and my job activities I perform in the Department of Microbiology to include the day-to-day reading and interpreting of cultures. I also enjoy working with infectious disease physicians by providing information that helps them manage their patients. Finally, I enjoy helping other technologists in the department with their questions.

NEED TO CONTACT NPHL?

CUSTOMER SERVICE

866-290-1406 *(TOLL FREE)*

OR

402-559-2440

BIOTERRORISM/SPECIAL PATHOGENS

402-559-3032

TRAINING/EDUCATION

402-559-6070

CHEMICAL TERRORISM LABORATORY PREPAREDNESS

402-559-6070

www.nphl.org

Bioterrorism/Chemical Terrorism Procedures on www.nphl.org

By Josh Rowland, State Training Coordinator, NPHL

Bioterrorism and chemical terrorism procedures, along with related information are available on our website (www.nphl.org). The procedures were developed by the CDC and the ASM. The bioterrorism procedures listed are meant to be used by **Sentinel** (Nebraska Laboratory Network *Level-A* and *Level-B*) laboratories. The procedures described function to "recognize, rule-out or refer" bioterrorism agents or naturally occurring special pathogens from clinical specimens. Those isolates that cannot be ruled-out and are hence presumptively identified as bioterrorism agents or special pathogens should be referred to the NPHL for confirmatory testing.

Select Agent information is also available in this section of the website (See the *Reporting the Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory: Revised APHIS/CDC Form 4* article on page 1).

The chemical terrorism information on the web site is intended for all clinical laboratories in Nebraska. This information, developed by the CDC, details how clinical specimens (blood and urine) should be collect from patients in a real or suspected chemical incident. In addition to specimen collection information, packaging guidelines and supporting documentation including a chain-of-custody and shipping manifest forms are included. These reference documents are meant to direct laboratorians during a suspected event when collecting human specimens.

Look for NPHL to offer chemical terrorism laboratory preparedness training sessions in the future. Please contact Josh Rowland (402-559-6070, jrowland@unmc.edu) if you have questions.

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