Nebraska Public Health Laboratory Newsletter

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NPHL Updates

By Steven Hinrichs, MD, Director, NPHL

This edition of our newsletter marks another milestone in the development of the NPHL as well as provides an update on the topical issues of respiratory infections and their prevention. Dr. Peter Iwen has assumed the duties of Editor-in-Chief of the newsletter and his role in the laboratory continues to grow. Pete is well known throughout Nebraska for his expertise in clinical microbiology and throughout the US as an expert in mycology. He has written numerous research articles and reviews on the topic of fungal infections and has developed molecular assays to assist in their diagnosis. In addition, we are pleased to introduce Karen Stiles as our new State Training Coordinator and newsletter editor. Karen brings a great familiarity with the state to her new role in addition to long experience as a microbiologist working in the clinical laboratory. Josh Rowland, our previous editor, has taken a position with the Association of Public Health Laboratories with the responsibility for developing educational opportunities nationally. This coming on the heels of Beth Schweitzer and Tricia Aden assuming important laboratory positions at the national levels is evidence for the quality of training and experience obtained in Nebraska.

Whether you call it "swine flu" or Influenza A 2009 H1N1v, the summer and fall have been a challenge for everyone. The article by Tony Sambol summarizes information we collected on the controversial performance of rapid flu antigen assays during this year. If we can answer any questions regarding testing procedures for the influenza virus, you are welcome to give us a call. But influenza is not the only new virus circulating as you will read in the article on Metapneumovirus. We also provide background on the topic of respiratory protection with a short summary of the terms and technology used in respirators and masks. These topics are intended to help you stay healthy.

INSIDE THIS ISSUE:

Rapid Influenza Diagnostic Tests Used as Screening Tools During an Outbreak of the 2009 Novel Influenza Virus: The Nebraska Experience

By Tony Sambol, MA, SM(NRM), CBSP, Assistant Director, NPHL

The NPHL performs influenza surveillance testing to support the Nebraska Department of Health and Human Services (NE-DHHS). For the 2008-09 season, surveillance testing at the NPHL was performed using a Food and Drug Administration (FDA)-cleared Luminex xTAG Respiratory Viral Panel (RVP) assay (Luminex Molecular Diagnostics, Toronto, Canada). This assay identifies 11 upper respiratory viruses to include adenovirus, respiratory syncytical viruses (RSV) A and B, parainfluenza viruses 1 - 3, human metapneumovirus, rhinovirus, and influenza A virus /H1, A/H3 virus, and influenza B virus. Year-round surveillance activity in Nebraska includes testing of specimens received from sentinel physician clinics, hospitals, and reference laboratories. These facilities utilize a variety of commercially available CLIA waived rapid influenza diagnostic tests (RIDTs) that distinguish and differentiate between influenza A and B viruses.

On April 22nd, the Centers for Disease Control and Prevention reported that a novel strain of influenza A virus (hereafter called the 2009 H1N1 variant strain [H1N1v]) was identified in California and traced to a point-source in Mexico**[1]**. In preparation for the anticipated increased testing demands, the NPHL and the Nebraska Department of Epidemiology decided to restrict samples to optimize testing. The testing algorithm included the evaluation of clinical specimens from patients meeting the following conditions: 1) RIDT-positive with travel history to Mexico or having an exposure to someone that had traveled there or 2) known travel history to Mexico or had an exposure to someone with travel history and were symptomatic but RIDT-negative.

During the 5-week outbreak period, 5,730 RIDTs were reported by local hospitals, reference labs, and physician clinics throughout the state. Of these, 255 were positive for influenza A virus, 150 were positive for influenza B virus, and 8 were reported as positive for influenza virus but not differentiated. From the RIDTs performed state-wide, 336 specimens underwent further diagnostic testing at the NPHL for H1N1v. They consisted of 234 (69.6%) RIDT-positive and 102 (30.4%) RIDT-negative specimens from the following test kits: Inverness Medical BinaxNOW Influenza A&B (150 specimens; 44.6 %), Meridian TruFlu (44 specimens; 13.1%), Quidel QuickVue Influenza A&B (92 specimens;

(RIDT, continued on page 2)

(RIDT, Continued from page 1)

27.4%), and Remel Xpect Flu A&B (50 specimens; 14.9%).

Testing at the NPHL showed that the distribution of influenza viruses detected among RIDT and RVP influenza-positive specimens was roughly equivalent for seasonal influenza viruses (A/H1, A/H3 and B) and the H1N1v strain. While the combine sensitivity of all RIDTs was 97.69%, the overall specificity (i.e. true positives) of the RIDTs for influenza viruses was low at 48.05%. Of the 102 RITD-positive and RVP influenza-negative specimens, 55 were negative for any virus while 47 specimens were positive for other upper respiratory viruses by the RVP test: rhinovirus (28), adenovirus (5), parainfluenza viruses (13), and RSV (1).

In conclusion, an overall marked difference in the performance between types of RIDTs was varied. The Xpect Flu kit was associated with more discrepant results (74%) than the other RIDTs (BinaxNOW 29%; TruFlu 34%; and QuickVue 16%). Additionally, a difference in the rates of discrepancies by type of test facility was observed. Hospitals had a higher proportion of discrepant results compared to doctor offices (40% vs. 19%). Some facilities using one type of kit had 100% agreement with the Luminex xTAG RVP while other facilities using the same kit had less than 25% agreement. Factors that could account for the low levels of specificity and the agreement rates include variations in: 1) specimen collection and transport, 2) specimen collection material used, 3) testing techniques, and 4) subjective interpretation of the lateral-flow immunodiagnostic solid-phase RIDT results to observe whether or not a "line" is visible, to indicate a positive test.

While being in the midst of a second and possibly facing an impending third wave of the H1N1v strain in the coming months, medical personnel are encouraged to contact their sales representative or technical support for utilization of the RIDT assays. A proposed webinar by the National Laboratory Training Network will also be available in the near future. By taking these steps, it is hoped that the RIDTs can become an effective tool to screen for influenza infection.

References

1. Centers for Disease Control and Prevention. Outbreak of swine-like origin influenza A (H1N1v) virus infection - Mexico, March-April 2009. *MMWR Morb Mortal Wkly Rep 2009;* **58**: 467-70.

2010 NPHL Upcoming events:

Challenge Sets

Special Pathogens "Train the Trainer" Workshop

Chemical-Exposure Event Training Workshop

APHL Upcoming events:
APHL Annual Meeting and State Environmental Laboratory Conference

Cincinnati OH - June 6-9, 2010

Omaha NE - June 5-8, 2011

Respiratory Infection Control: Respirators vs. Surgical Masks

By Michael Lore, MS

The recent onset of a novel influenza virus strain has refocused attention on personal respiratory protection. This is of particular interest in hospital settings where controlling the spread of disease is important. The need to understand the limitations and use of respiratory protection devices to minimize exposure of potentially pathogenic laboratory specimens is important for laboratory workers.

Exposure to droplets on hands and environmental surfaces is thought to account for the majority of infections by the influenza virus. However, inhalation is also an important route of pathogen entry into the human body. As a precautionary measure, recommendations are that laboratory personnel reduce their exposure to airborne pathogens through the use of respiratory devices. Respirators are an effective protection measure against airborne particulate exposures when properly selected and worn. However, a common mistake seen in the workplace is the use of the wrong filter mask.

Image 1

In a hospital setting, there are typically two types of disposable respiratory protection devices available: the surgical mask and the filtering facepiece respirator. The surgical mask **(Image 1)** is primarily designed to protect others from the wearer's oral and nasal pathogens. Droplets that can be visually seen are produced through respiratory events such as talking, coughing or sneezing. These droplets are easily captured through the surgical mask's filter barrier. However, this type of mask is not intended to protect the wearer from micro-droplets or from very small particles like viruses.

The second type of respiratory mask typically used in laboratory situations is the filtering facepiece respirator **(Image 2)**. These respirators contain an electrically charged filter medium. This special charge is embedded into the filter medium of the mask and works by attracting very small particles, much like a magnet. These masks have a National Institute for Occupational Safety & Health (NIOSH)

Image 2

(Masks, Continued on page 3)

(Masks, Continued from page 2)

certification which means that they have undergone rigorous testing to verify their air-filtering ability. NIOSH approved masks are labeled according to their resistance to oil-based aerosols and particle collection efficiency. The most commonly recommended filtering facepiece respirator in the health care setting is the N95 mask where the "N" means "not resistant to oil aerosols" and "95" means that it will trap 95% of particles 300 nm or larger. Although the N95 filters are not certified at particle sizes smaller than 300 nm, they do provide adequate protection below 300 nm. As a reference, the sizes of airborne pathogens are highly variable but typically, most bacteria are larger than 300 nm while most viruses are smaller.

Sneezing or coughing leads to the generation of large droplets (4000+ nm) that can be easily captured by a respiratory mask but what about very small particles? A single influenza virus particle average about 100 nm in size, too small to be trapped by a mask. Or is it? Shown in the figure below is a graph of an N95 filtering facepiece respirator tested in our lab against a polydisperse (broad size range distribution) salt solution. A polydisperse aerosol is used to test the mask's ability to capture particle sizes from 30-400 nm. The graph demonstrates the typical penetration curve of an N95 filter with few particles (300 nm size) passing through the filter mask (0.4%, circle), well below the NIOSH standard of 5%. As the size of the particle increases, fewer were able to pass through the filter. Note, however, that small particles were able to penetrate through the filter; however, they were still well below the 5% limit established by NIOSH.

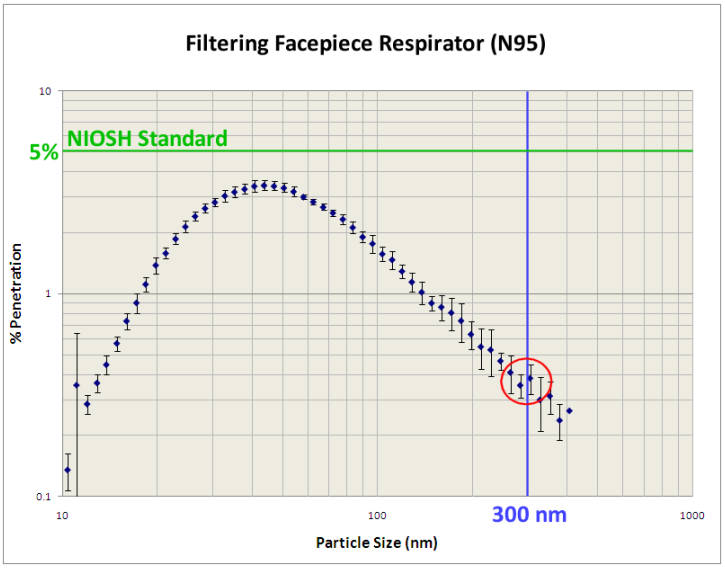

Figure 1. The NIOSH Standard line represents the 95% NIOSH limit for particle penetration at 300 nm. If the particle curve exceeds the line, the filter is less than 95% efficient at capturing particles. If the particle curve remains below the line (as above) the filter is greater than 95% efficient at removing airborne particles.

When fitted properly, the N95 filtering facepiece respirator has been proven to stop 95% of airborne particulates down to the 10nm range. A current research project at UNMC is to investigate whether significant disease can occur from the number of particles that do in fact penetrate the respirator.

What's in a Name? The Taxonomic Overview of the Genus *Elizabethkingia*

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

With the utilization of genomic sequencing, a wide variety and number of new and reclassified bacterial species has been generated. This naming of a new or renaming old bacterial species is a highly formal process and the rules of the *Bacteriological Code* are followed. As part of this process, a new species name must either be placed on a validation list in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) or must be published as a full report in the IJSEM to become valid. The challenge for clinical microbiologists is to keep current on this changing nomenclature. This series on bacterial taxonomy was begun with the last newsletter with a goal to provide guidance for the clinical microbiologist to keep accurate in the reporting of a bacterial pathogen. The inaugural article in this series described species within the genus *Citrobacter*. This report describes a new genus composed of two new closely related species, *Elizabethkingia meningoseptica* (originally identified as *Flavobacterium meningosepticum*) and *E. miricola* .

Historically, the genus *Flavobacterium* was created in 1923 for those gram-negative non-sporulating yellow-pigmented rods that weakly produced acid from carbohydrates. Since this original classification, several *Flavobacterium* species have been reclassified into new or other genera, such that species within this genus are now seldom detected as causes of human disease. One new genus that was included in this reclassification was *Chryseobacterium* **[3]**. This genus included a newly named species called *Chryseobacterium meningosepticum* which replaced the previously named species *Flavobacterium meningosepticum*.

Recent studies have now revealed that the genus *Chryseobacterium* was genetically heterogeneous. Two of the previous 10 species recognized in this genus can be readily differentiated from the other *Chryseobacterium* species by both 16S rRNA sequence comparison analysis and DNA-DNA hybridization studies **[1]**. These two species were subsequently placed into a new genus called *Elizabethkingia*, named in honor of Elizabeth King, the individual who in 1959 described bacteria associated with infant meningitis **[2].** These two closely related species were subsequently validated in 2005 and became known as *Elizabethkingia meningoseptica* (epithet name referring to the association of this bacterium to both meningitis and to septicemia) and *Elizabethkingia miricola* (epithet name derived from the words "mir" which means peace and "incola" which means inhabitant; where the combined name refers to an inhabitant of the MIR space station where the isolate was first detected) **[1]**.

The major characteristics for both the *Elizabethkingia* and *Chryseobacterium* species is the production of oxidase, the ability to produce indole, and the presence of a non-fermenting gram-negative rod that grows on MacConkey agar. The major phenotypic characteristic to separate the *Elizabethkingia* species from *Chryseobacterium indologenes* (the most common species causing human dis-

(Elizabethkingia ,Continued on page 4)

(Elizabethkingia ,Continued from page 3)

ease in this genus) is by the lack of a yellow pigment in culture (see **Table**). Although most commercial identification systems still include *C. meningosepticum* in their databases, the identification of *C. meningosepticum* by a commercial test should now be reported as either *E. meningoseptica* or *E. miricola* depending on the organisms ability to hydrolyze urea (*E. miricola* is positive).

Reference laboratories are available to provide sequence comparison analysis testing to help validate the identification of the *Elizabethkingia* 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. For additional information of the availability of this service, contact Dr. Iwen at 402-559-7774.

References

1. Kim, KK, MK Kim, JH Lim, HY Park, and ST Lee. 2005. Transfer of *Chryseobacterium meninosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. Int. J. Syst. Evol. Microbiol. **55**: 1287-1293.

2. King, EO. 1959. Studies on a group of previously unclassified bacteria associated with meningitis in infants. Am. J. Clin. Path. **31**: 241-247.

3. Vandamme, P, JF Bernardt, P Segers, K Kersters, and B Holmes. 1994. New perspectives in the classification of the flavobacteria; description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nob., and *Empedobacter* non. rev. Int. J. Syst. Bacteriol. **44**: 827-831.

Table. Major phenotypic characteristics to differentiate among the oxidase-positive, indole-positive nonfermenting gram-negative rods that grow on MacConkey agar.[a]

Characteristics	*Elizabethkingia meningoseptica*	*Elizabethkingia miricola*	*Chryseobacterium indologenes*
Yellow pigment	N	N	P
Gelatin hydrolysis	P	P	P
Esculin hydrolysis	P	P	P
Urea hydrolysis	N	P	N

Abbreviations: N, negative; P, positive.

[a]Bacterial species within this group that are closely related, but do not readily grow on MacConkey agar include *Empedobacter brevis, Weeksella virosa, Bergeyella zoohelcum,* and *Balneatrix alpica*.

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Checklist Updates from the Laboratory Accreditation Program Audioconference

By Lois Carmody, BSMT(ASCP) & Karen Stiles, MT(ASCP)SM

The Commission of Laboratory Accreditation of the College of American Pathologists (CAP) sponsored an audioconference on the topic of CAP Checklist Updates. This presentation was given by Stephen J. Sarewitz, MD, FCAP. The objectives of the audioconference were to:

- List important topics from the Lab General, Hematology, and Microbiology checklists,
- Describe current and recent updates to the LAP inspection checklists including a rational for the changes, and
- Use the checklists to improve laboratory quality.

The following is a brief synopsis of his presentation.

CAP Administrative Requirements addresses the "Terms of Accreditation" by asking if the lab has a policy that addresses compliance with these terms; if the lab notifies CAP when being investigated by a government entity or adverse media attention related to lab performance; or if the lab notifies CAP when there is a change in lab testing menu, change in location, ownership, and directorship.

Competency assessment requirements for CAP differ from CLIA. CAP requires competency documentation for all waived tests. CAP also requires competency assessment in specimen collection (if collected by laboratory personnel) and critical result reporting, which CLIA does not. Centers for Medicare and Medicaid Services (CMS) has recommended CAP develop procedures to systematically evaluate compliance. In the first year of new personnel, competency must be assessed no later than 6 months after an individual starts a testing procedure, and then assessed again at one year. From then on, it can be done annually. In the future, laboratories can expect inspectors to spend more time reviewing personnel records. Bottom line as Dr. Sarewitz said, is to be certain the personnel files contain documentation for education, training, and certification under CLIA regulations.

New for 2009 is the two identifiers requirement for all primary containers of in-patient and out-patient specimens (attached at time of collection). The largest change anticipated in 2010 may be the format of the checklist. The familiar "question" format will be changed to statements. Rather than asking "Does the lab have a quality management plan?", a statement will be made "The lab has a quality management plan" in which the inspector will evaluate. The purpose of the change is that people find statements easier to interpret. There will also be changes to font and formatting. Additional fields will appear intended to make the checklist more user friendly and provide examples.

Future plans beyond 2010 will include a new numbering system using invariant numbers with alphabetical prefixes. "Single checklist" databases will be developed. This will enable checklists to be customized to fit the organization of each laboratory by linking common sections for PT, QC, instrument maintenance list, to the checklist items specifically applicable to each section of the laboratory.

Dr. Sarewitz elaborated on the contents of each specialty's procedure manual, that contents must reflect CLIA as applicable. NPHL will reserve these topics for future newsletter articles. Further details on Dr. Sarewitz's teleconference can be found at **www.cap.org**

Meet our new State Training Coordinator – Karen Stiles

NPHL welcomes Karen Stiles as your new State Training Coordinator. Originally from Fremont, Nebraska, she now resides in Gretna with her husband, Mark and daughters Nicole and Jacquelyn.

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

At a very early age, my mom implanted the idea of laboratory science. I was one of those weird kids who tinkered with a chemistry set. Of course, being from the farm, my whole environment was immersed in science, from canning vegetables to the corn and soybean fields. The chicken house was a whole science class in itself!

Where did you attend medical technology school?

My undergraduate years were spent at the University of Nebraska, Lincoln. I was then accepted into the Medical Technology Program at the UNMC.

How long have you worked in your present location?

I have been with NPHL only 8 months and feel there is still so much to learn! Prior to accepting this position, I worked for over 2 years with the NPHL staff as a trainer for the **S**ecure **T**elecommunications **A**pplication **T**erminal **P**ackage ™ in Kansas and Oklahoma. The STATPack™ is a remote consultation device that Nebraska, Kansas and Oklahoma Public Health Laboratories use to communicate and share images with sentinel laboratories throughout each state.

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

Despite working in microbiology over 25 years, I have remained in the basic area of bacteriology and susceptibility testing. Through the years, however, I have always been involved to some degree in teaching. Whether it was to teach students individually on the bench or in a classroom setting, or present topics such as ESBL testing or high level aminoglycoside resistance in *Enterococcus* to a team of infectious disease residents, I loved the challenge sharing the field of microbiology.

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

Never give up. The vast amounts of information they will be exposed to can be formidable. Soak up what they can. More importantly, go back and review, for it will make more sense as time goes on. Continue to study, attend workshops if possible and participate in all areas of continuing education. Don't hesitate to ask questions! Continuing education will be the key to their success.

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

It is a privilege to have a job that allows me to pursue what I enjoy most in this profession. I realize that continuing education is unfortunately put on the back burner at many facilities because of lack of funding and staff shortages. My challenge will be to fill that void and make continuing education available to everyone.

Human Metapneumovirus in Nebraska

By Baha Abdalhamid, MD, PhD, D(ABMM)

Discoveries continue to occur in the world of microbiology. This time, it's a new virus called the human metapneumovirus (hMPV). This virus was first discovered in the Netherland in 2001 from children and adults with acute respiratory infections. Subsequently, hMPV was diagnosed in patients with acute respiratory symptoms in the USA, Canada, Australia, and the United Kingdom. This RNA virus is classified in the *Paramyxoviridae* family, which also contains parainfluenza, mumps and measles viruses.

Human metapneumonvirus has been found worldwide with high prevalence of the viral antibodies in all age groups. The sporadic infection by this virus can occur yearround with a peak incidence during the late winter to early spring, overlapping with that of the respiratory syncytial virus (RSV). The incubation period is 3-5 days and the virus is transmitted by close contact with contaminated secretions such as large-particle aerosols. The rates of hMPV infections are similar in males and females with the peak incidence in children younger than 5 years. The highest risk of the lower respiratory tract infections by hMPV is in the first 6 months of life which suggests that young age is a risk factor for severe disease. The virus causes infections in adults and the elderly but severe diseases is less likely to occur. Human metapneumovirus has been recognized as second most common cause of viral respiratory tract infections in children after RSV and can cause both upper and lower respiratory tract infections such as common cold, bronchiolitis, croup, and pneumonia.

The recommended specimens to detect hMPV include nasal washes, nasopharyngeal swabs, and BAL specimens transported in viral transport media at 4°C. Specimens should by processed immediately in the laboratory or should be stored at -70°C in case of delay.

Since the virus replicates poorly in most conventional cell cultures, an RT-PCR method using hMPV specific primers is the best method for detection. The Clinical Microbiology Laboratory at The Nebraska Medical Center is performing the FDA-approved respiratory viral panel (RVP) test to detect the most common causes of the viral respiratory tract infections including hMPV. After extraction of nucleic acids, RT-PCR is performed to amplify the viral nucleic acids followed by a step to clean the amplicon product. The cleaned product is then re-amplified using labeled nucleotides. The labeled amplicons are subsequently hybridized to beads with Luminex™ technology used for detection. The turnaround time for this test is 24 hours.

A total of 350 specimens (excluding H1N1v) during the 2008-09 viral respiratory season were tested by RVP. Within this series, 10 cases of hMPV were found (6 females and 4 males) as well as 54 rhinovirus, 13 adenovirus, 20 parainfluenza viruses, 26 influenza viruses, and 22 RSV. Of the 10 hMPV cases, 8 were >5 years old. The incidence rate of hMPV was 2.9% compared to 6.3% for RSV.

This data reveals that hMPV is is an important pathogen in our environment. Further analysis regarding hMPV typing and categorization is ongoing and laboratory scientists need to be aware of this virus to properly advise medical practitioners.

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IN THIS ISSUE

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