Nebraska Public Health Laboratory Newsletter

A publication of the Nebraska Public Health Laboratory (NPHL) at the University of Nebraska Medical Center.

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NPHL: Continuing the Focus on Education

by Steven Hinrichs M.D., Director, NPHL

Readers of this issue of the newsletter will see that the focus of our activities continues to be on education. Tony Sambol and Josh Rowland have just returned from a tour of Nebraska where they gave presentations on two of the topics summarized in this issue, the NPHL's expanded chemical terrorism preparedness program and a methodology article from the West Nile Virus testing performed during last summers outbreak. In addition to the primary information they provide, the take-home message of these two articles is that federal and state money investments in biological and chemical terrorism preparedness have paid a dividend in helping us prepare for events of all types, including natural disasters.

We have received a great deal of positive feedback regarding the laboratory focused articles in past newsletters, therefore we have continued to identify topics that provide important background information for laboratorians. Our web site (www.nphl.org) is capable of monitoring the number of times past articles are accessed and you will be interested to know that educational articles are most frequently accessed. The current article by Bob Wickert continues the emphasis on education by providing key information about an important virus commonly transmitted in food that generates a significant amount of disease every year. People will be interested to know that the name has been changed from Norwalk agent to Norovirus and therefore test order forms and send-out test lists will need to be updated as well.

Finally, this issue will feature Rosa Crook in the "Meet the Laboratorian" article. To learn more about Rosa, please go to page five. If you know someone who should that you would like to be nominated for the feature, please e-mail Josh Rowland (jrowland@unmc.edu) with their names.

NPHL West Nile Virus Testing Methodology

by Tony Sambol, Assistant Director, NPHL
and Beth Schweitzer, Microbiology Specialist, NPHL

Due to the lack of an FDA approved assay, very few laboratories in the United States performed West Nile Virus Nile (WNV) antibody tests during the 2003 outbreak. Now that an approved assay is available and the virus has reached the Midwest some local laboratories are considering adding the test to their menu. To assist in this effort, the NPHL has reviewed the experience it gained last year in the testing of over 10,000 samples and the evaluation of two different test formats. One of the most important discoveries was the identification of a problem with interfering antibodies or other substances (referred to as Interfering Factors or IF) causing false positive results.

The following information is intended to provide background for laboratories in Nebraska that are considering bringing the test in house, and for other laboratories that may need more information to provide explanations of test results for their local providers.

West Nile Virus infections in the United States were first recognized in New York State during the summer of 1999. The virus has since moved across the US, with the first human case detected in Nebraska in 2002. The NPHL began testing for human WNV IgM antibody in 2003 using non-Food and Drug Administration (FDA) approved reagents from Focus Technologies. The NPHL only performed the IgM antibody screen in 2003. These reagents were subsequently incorporated into a test kit that has now received FDA approval. However, during the evaluation of the reagents used by the NPHL, it was discovered that a number of specimens that screened positive could not be confirmed using the confirmatory test called the Plaque Reduction Neutralization Assay (PRNT). This finding led to additional investigations and the realization that most of the false positives were caused by the presence of Interfering Factors (IF) in the patient serum. IF are represented by different types of antibodies including Heterophile antibodies, Rheumatoid factor (RF), Forssman antibodies (serum sickness) as well as other factors. Heterophile antibodies were first shown to be induced in the presence of Epstein Barr Virus (EBV) and are the detection target for most Infectious Mononucleosis assays. EBV infections are common and may be present in a latent state in more than 80% of humans. A second screening method using a "normal control" preparation that did not include WNV antigen was added to identify those WNV specimens with IF with the goal of reducing the number of false positives.

The Focus Technologies Flavivirus (West Nile Virus) ELISA IgM Reagent Pack, used in both tests performed at NPHL, consisted of analyte specific reagents. The Focus Technologies reagents were used for qualitative detection of IgM antibodies in serum and cerebral spinal fluid (CSF). The test was performed on the Diamedix MAGO® Plus Automated Enzyme Immunoassay (EIA) Analyzer. The original optical density (OD) reading from the instrument of each test well was recorded and divided by the mean of the calibrator OD to generate an index value. Test result index values used in the evaluation were originally defined by Focus Technologies as $\leq$0.9, IgM negative; >0.9 to $\leq$1.1, IgM equivocal; and >1.1 IgM positive. Data analysis was performed on specimens obtained between August 1 and October 31. During that time 10,887 specimens were tested, of which 10,371 (95.3%) were sera and 516 (4.7%) were CSF. Of the specimens tested, 2,282 (21%) were determined to be above the positive cut-off level of >1.1. These specimens were separated for study on the basis of OD's and index values with special attention paid to those samples with an

(Continued on page 2, WNV Methodology)

(WNV Methodology, Continued from page 1)
index value in the range of >1.1 to ≤3.5. The hypothesis to be tested was that this range would encompass the majority of samples with IF. This hypothesis was based on the fact that a number of specimens did not confirm positive by PRNT testing that was performed on the specimens in this range. The PRNT testing was performed at the Centers for Disease Control and Prevention's (CDC) branch at Fort Collins, CO.

Due to the discrepancies found by the CDC's PRNT testing, the IF test was performed on all screen positive samples in the range from >1.1 and ≤3.5 using the Focus Technologies reagents. The IF screening test was performed on 794 (34.8%) specimens. 770 (97%) of the 794 specimens tested were serum and 24 (3%) were CSF. 54 (6.8%) of the sera tested were reported as indeterminate due to the presence of IF while only one (1) of the CSFs tested was positive for IF.

The NPHL also performed IF testing on an additional 126 positive specimens that had test result values >3.5. All of these specimens gave negative results in the IF screen.

This study showed that IF was responsible for 6.9% of the indeterminate results in the "low-positive" range. Consequently, those specimens would have been reported out as false-positives if a second screening method would not have been performed. As with all screening tests, confirmatory methods are needed such as PRNT or Heterophile antibody testing to determine the true nature of specimens.

When interpreting WNV IgM testing results that are positive but have low index values of >1.1 to ≤3.5, providers and laboratorians should be aware of false positives due to IF and that additional testing may be required when results do not correlate to the patient's condition. Additionally, antibodies detected by the WNV assay may cross react with other flaviviruses, including St. Louis Encephalitis Virus. Cross reactivity has also been observed with some Enterovirus infections.

For questions about WNV testing methodology, please call or email Tony Sambol (402-559-3032, asambol@unmc.edu) or Beth Schweitzer (402-559-6098, bschweitzer@unmc.edu).

Chemical Terrorism Section Expands at the NPHL

by Douglas F. Stickle, PhD, Assistant Professor, UNMC

The Centers for Disease Control and Prevention (CDC) has established a nationwide program to provide 41 state public health laboratories with the analytical technology needed for detection and quantitation of agents that might be used in chemical terrorism. While some of this analytical technology is available in the private sector, many of the chemicals being tested are controlled or select agents and testing must be performed in a specially credentialed laboratory. As part of this program, the Chemical Terrorism Section was developed within the NPHL.

Known as the CDC's "Focus Area D", the chemical terrorism preparedness program hinges on development of public health laboratory capability to rapidly detect the presence of chemical agents in human specimens such as blood, urine using two types of mass spectrometers. An Inductively-Coupled Plasma-Mass Spectrometer (ICP-MS) and a Gas Chromatography-Mass Spectrometer (GC-MS) will be utilized in the program. In both types, the mass spectrometry portion is able to measure distinct charged (ionized) molecules on the basis of their mass-to-charge ratio (*m/z*), with a discriminating capability of less than 1 atomic mass unit (1 amu, equivalent to the mass of a hydrogen atom). The mode of ionization of the molecules for introduction into the mass spectrometer varies for each spectrometer. An analyte molecule in GC-MS is ionized by electron-impact ionization (EI, using an electron source filament), or by chemically-induced ionization (CI, usually by reaction with methane). GC-MS is widely used in forensic toxicology for identification of unknown compounds. The ICP-MS ionizes atoms for elemental analysis (e.g., arsenic, heavy metals) and the GC-MS analyzer is used for compound analysis, such as cyanide.

The gas chromatography portion allow the analyzers to separate the compounds of interest from other components in the original sample matrix before its introduction into the ionizing chamber. Both types of instrumentation allow for the identification and quantitation of a select group of analytes that could be used in a chemical terrorism event. Training and procedures for the analysis of this select group of analytes will be provided by the CDC. A nationwide training program will ensure uniform mass spectrometry capabilities in state public health laboratories throughout the U.S., and will provide networked support services if needed. The ICP-MS and GC-MS instruments are now on site at the NPHL with training and method validation in progress. Once training and validation has taken place the Chemical Terrorism Section of the NPHL will be able to test for 13 elements including arsenic and cyanide. The NPHL anticipates testing protocols for other chemical agents will be made available from the CDC in the future.

Chemical Terrorism Laboratory Preparedness training seminars are currently being offered at the Nebraska Center for Bioterrorism Education's (NCBE) 2004 Symposia. Information regarding the NCBE's Symposia can be found by going to http://www.nphl.org/training.html. Questions about the Chemical Terrorism Section can be directed to Dr. Doug Stickle (dstickle@unmc.edu or 402-559-8785).

Bioterrorism Symposia, 2004

The NPHL would like to invite you to the final session of the 2004 Bioterrorism Symposia series presented by the Nebraska Center for Bioterrorism Education. The final symposium will be in the following location:

- Kearney – August 25, 26 (Wednesday and Thursday)

The NPHL will present on day two of each of the programs. Presentations include: "Biosecurity Preparedness and Meeting Other Public Health Threats", "Chemical Terrorism Laboratory Preparedness", "Food Safety and Antibiotic Resistance", and a presentation on VISA/VRSA. Following the presentations, you are invited to join a panel discussion on antimicrobial resistance/antimicrobial susceptibility testing issues. Those involved with the panel discussion will be Dr. Steven Hinrichs, Dr. Paul Fey, and Dr. Peter Iwen.
For more details, including registration information, go to www.nphl.org for a Symposia brochure.

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Federal Policy Changes Impact Foreign Healthcare Workers

by Brenda Kouba, MT(ASCP), UNMC and Josh Rowland, State Training Coordinator, NPHL

There has been a major change in the Federal Policy (from the United States Department of Homeland Security (DHS)) that affects the hiring of foreign healthcare workers in the United States. The new policy called the Homeland Security Rules, passed in 2003, mandates that all laboratories must meet the regulation by July 25, 2004.

The final regulation requires Medical Technologists and Medical Laboratory Technicians, (as well as Nurses, Physical Therapists, Occupational Therapists, Physician Assistants, Audiologists, and Speech-Language Pathologists) who are not U.S. citizens, to obtain a special certificate to provide healthcare services in this country. The new rules are intended to ensure that foreign healthcare workers meet professional training and standards necessary to provide diagnostic services and patient care in U.S. hospitals and clinical facilities. The final rules approved by the DHS apply to foreign healthcare workers seeking either temporary or permanent occupational visas (Green Cards). Previously, the rules applied only to foreign healthcare professionals seeking permanent occupational visas. The new rules also will apply to healthcare professionals from Mexico and Canada who were exempt under the terms of the North Atlantic Free Trade Agreement (NAFTA).

The Commission on Graduates of Foreign Nursing Schools (CGFNS) was designated by the U.S. Congress in 1996 to manage a healthcare worker visa certification program. CGFNS established the International Commission on Healthcare Professions (ICHP), to manage the application process for this program. ICHP calls its application program "*VisaScreen*".

The "*VisaScreen*" program reviews a foreign healthcare worker's education to ensure it is equivalent to that available in the U.S, evaluates all current and previous licenses to practice received by the foreign healthcare worker, and assesses English language proficiency.

The new rules require that a "*VisaScreen*" certificate be presented whenever a foreign healthcare professional enters the U.S. to work, applies for an extension of stay, or when there is a change in immigration status or employment. Although the new rules went into effect on September 23, 2003, they include a transition period for foreign healthcare workers to minimize disruption in the current U.S. healthcare employment market. Temporary workers, including those previously covered under the NAFTA agreement, will be admitted to the US with or without a "*VisaScreen*" certificate until July 25, 2004, but for a period of not more than one year.

Information pertaining to the new regulation can be found at www.ascp.org on the left side of the page. Detailed information and applications can also be found on the International Commission on Healthcare Professions website, www.cgfns.org.

Norovirus: An Infectious Cause of Montezuma's Revenge

by Bob Wickert, Molecular Microbiologist, NPHL

Cruise ship passengers may have believed they were the latest victims of the Aztec emperor's curse when multiple outbreaks of gastroenteritis hit the industry especially hard last year. Modern medicine has determined an infection with Norovirus, previously known as Norwalk virus, was the true cause of the travelers' malady. The NPHL has developed molecular tests capable of detecting this virus. Why would a public health laboratory in a land locked state hundreds of miles from an ocean need to offer this test? This article details more specific facts about Norovirus.

What are Noroviruses?

Noroviruses are a family of viruses that cause gastroenteritis (*i.e.* stomach flu) but are not related to influenza viruses which cause respiratory illness. Noroviruses are found all over the world, infecting all age groups. Current data suggest the viruses are the most common cause of diarrheal-type illness in humans. Interestingly, less than 10% of Norovirus outbreaks are associated with vacations, while most are associated with restaurants or catered events, health care facilities such as nursing homes, and schools or day cares.

Noroviruses were originally named "Norwalk viruses," after a outbreak of gastroenteritis in a school in Norwalk, Ohio, in 1968. The virus has also been referred to as a Calicivirus. Caliciviruses are a large group of viruses that are small and round in structure. Subsequent work by molecular virologists has confirmed that the virus belongs to the Calicivirus Family but is distinct and thus the International Committee on Taxonomy of Viruses has renamed the organism Norovirus. There are four distinct genotypes of Noroviruses that differ slightly in their genetic makeup but are all capable of causing disease.

What are the symptoms?

Infections with Norovirus generally present suddenly with nausea, vomiting, diarrhea, and stomach cramping. Some people experience a low-grade fever, chills headache, muscle aches, and fatigue. Symptoms usually begin 24 to 48 hours after ingestion of the virus and subside within 1 to 4 days. Generally, there are no long term health effects, although dehydration may require medical attention.

How do people become infected?

Noroviruses are present in the stool or vomit of infected people. One can become infected in several ways, including: eating food or drinking liquids that are contaminated with the virus, touching contaminated surfaces or objects and then placing one's fingers in the mouth, or direct contact with an infected person (sharing food or utensils with someone who is ill). It is important to remember that this illness is very contagious and good hand washing and proper hygiene prevents the virus from spreading. In addition, it is recommended that people not prepare food for others until three days after symptoms have passed.

Can you get Norovirus again?

Yes. Little is known about immunity to Norovirus infections; however, people can be infected repeatedly, most likely due to a combination of a lack of lasting immunity and the large diversity of circulating Norovirus strains.

How is Norovirus detected?

Norovirus cannot be cultured, thus, other methods must be used to detect this pathogen. For years, electron microscopy was the only method available, however, laboratories now use Polymerase Chain Reaction (PCR) amplification specific for the virus's genetic material. For this type of testing, a portion of stool or vomitus (1 mL minimum) should be collected during the acute phase and sent frozen in appropriate packaging. Testing can also be performed on food suspected of being contami-

(Continued on page 5, Norovirus)

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Third Case of VRSA Isolated in United States
Prompting Changes in Screening Methods

by Paul Fey, PhD, Associate Director NPHL

In the April 23, 2004 issue of the Morbidity and Mortality Weekly Report, the Centers for Disease Control and Prevention (CDC) reported the third known case of *vanA*-mediated vancomycin-resistant *Staphylococcus aureus* (VRSA) in the United States (New York). This isolate, which was obtained from the urine of a patient that resided at a long-term health care facility, had no genetic relatedness to the first two VRSA isolated in 2003 from Michigan and Pennsylvania. Unfortunately, this isolate, in a similar manner to the VRSA isolate from Pennsylvania, was not detected using automated susceptibility testing systems (i.e. Vitek® or Microscan®) and was only detected using E-test, broth microdilution, agar dilution, or vancomycin screen agar (agar containing 6 μg/ml vancomycin). Due to the public health implications of VRSA isolation, the CDC has recommended that clinical microbiology laboratories add a vancomycin screen agar plate (BHIA with 6 μg/ml vancomycin) to their primary testing procedure (http://www.cdc.gov/ncidod/hip/vanco/vanco.htm). An updated flow chart for VRSA detection and reporting is shown in figure 1 (adapted from CDC flow chart). If you have any questions regarding VRSA/VISA testing, please call Dr. Paul Fey at (402) 559-2122.

Figure 1

MIC method plus vancomycin screen agar (BHIA with 6 μg/ml of vancomycin)[1]

- Vancomycin MIC ≤ 2 μg/ml and no growth on VA screen plate → Report as vancomycin susceptible
- Vancomycin MIC ≤ 2 mg/ml and growth on VA screen plate (rare)
- Vancomycin MIC ≥ 4 mg/ml and growth on VA screen plate

→ Possible VISA/VRSA

1. Check purity/confirm ID
2. Repeat primary susceptibility tests
   A. MIC method plus van. screen agar plus
   B. Vancomycin E-test (should be ≥ 6 μg/ml if VISA/VRSA).
   C. Agar dilution MIC or microbroth dilution (if available).

→ Send Isolate to NPHL for Conformation

[1] Disk diffusion methodology alone does not reliably detect VISA/VRSA. If disk diffusion is used as the primary testing method, BHIA vancomycin screen agar can be used to detect vancomycin-resistance.

New PROTOCOLS/PROCEDURES Page on www.nphl.org

by Josh Rowland, State Training Coordinator, NPHL

A new page has been developed for the NPHL website (www.nphl.org) that will be a repository for protocols and procedures. Bioterrorism agent reference procedures will be the primary type of document on the new page. The procedures listed are meant to be used by Sentinel (Nebraska Laboratory Network *Level-A* and *Level-B*) Laboratories. The procedures were developed by the Centers for Disease Control and Prevention (CDC) and the American Society of Microbiology (ASM). The procedures described function to rule out or presumptively identify bioterrorism agents from clinical specimens. Those isolates that cannot be ruled out should be referred to the NPHL for confirmatory testing. Procedures currently on the webpage include:

From the CDC
- *Bacillus anthracis, Brucella* spp., *Clostridium botulinum* toxin, *Francisella tularensis, and Yersinia pestis*

From the ASM
- *Burkholderia mallei, Burkholderia pseudomallei*
- *Coxiella burnetii,* Staphylococcal Enterotoxin B, and
- Unknown Viruses

It should be noted that clinical laboratories should not attempt to perform toxin analysis, or culture specimens suspected of containing an unknown virus or *Coxiella burnetii,* these specimens should be referred to the NPHL. In addition, if a Smallpox Virus, Filovirus, Arenavirus, Alphavirus, or Crimean-Congo Hemorrhagic Fever Virus is suspected, contact public health officials (see CONTACT US on www.nphl.org) before specimen collection.

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nated.

Why test for Norovirus?

While no vaccine can prevent infection and no drug can cure Norovirus, the value of testing lies in identifying the cause of illness and taking measures to prevent its spread.

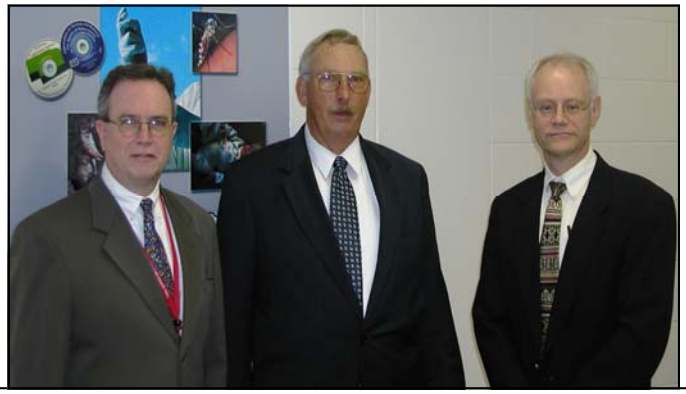
If physicians or laboratorians suspect that an outbreak of Norovirus is occurring they should contact their County Health Officials or Dr. Tom Safranek the State Epidemiologist. Researchers at the Centers for Disease Control and Prevention in Atlanta have developed a molecular test to characterize an outbreak strain and determine its extent. Therefore, a specimen that is positive at the NPHL may be submitted to the CDC at the request of epidemiology. Through comparison of the nucleotide sequences of PCR products, a genetic "fingerprint" of sorts, it is possible to confirm whether the strain detected in a food sample or food handler is identical to that of ill patients. Using this approach, a link can be established between ill patients from different geographical locations and a specific distributed food. For more information about Norovirus, please visit the CDC website (http://www.cdc.gov/ncidod/dvrd/revb/gastro/norovirus.htm).

Dr. Duane Boline, Kansas Public Health Laboratory Director, Visits NPHL

by Steven Hinrichs M.D., Director, NPHL

Dr. Duane Boline, Director of the Division of Health and Environmental Laboratories for the State of Kansas, visited NPHL to participate in a review of the chemical terrorism preparedness activities on May 20 and 21. The visit, hosted by Dr. Doug Stickle, Associate Director for the Chemical Terrorism Section at the NPHL, noted that, "Dr. Boline is an expert in analytical spectrometry and has established chemical testing laboratories in both the private and public sectors, and therefore is very knowledgeable about many of the challenges we are facing". In meetings with Dr. Stickle and other members of the laboratory, Dr. Boline discussed his experience with the various assays being introduced by the CDC for the chemical terrorism preparedness program. During his seminar, Dr. Boline emphasized the importance of understanding the relative risk of the various chemical agents to laboratory workers. In addition to his seminar regarding the laboratory response to terrorist acts involving chemicals, Dr. Boline toured the microbiology and special pathogens laboratory sections. Dr. Boline said, "this is a great opportunity for our states to work together and I am happy to share what we have learned with my colleagues in Nebraska".

Pictured below are Dr. Duane Boline (center) with Dr. Douglas Stickle (left) and Dr. Steven Hinrichs (right).



Meet the Laboratorian - Rosa Crook

Many microbiologists in the state know Rosa Crook, the Microbiology Supervisor at BryanLGH in Lincoln. For those of you who have not met her, we were fortunate to obtain the following interview of this highly regarded medical technologist.

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

When I started college I knew I wanted to go into the medical field. Originally I had considered hospital pharmacy or nursing and knew very little about medical technology. At Kearney State College I learned about the field and decided that was what I really wanted to do.

Where did you attend medical technology school?

I attended Kearney State Teacher's College before it was part of the University System. From there I went to Lincoln General Hospital and took my year internship there.

How long have you worked in your present location?

I have worked as a medical technologist since 1960. Since my husband's career was that of an Air Force officer, I worked in numerous laboratories before returning to Lincoln. I worked in laboratories from Bangor, Maine to Vancouver, Washington and from North Bay Ontario, Canada to Gulfport, Mississippi primarily as a generalist or in chemistry.

My husband and I returned to Lincoln 31 years ago and I started working in microbiology at Lincoln General Hospital. During that time we were Pathology Medical Services, Nichols Institute, Corning Clinical Laboratories, and Quest Diagnostic Laboratories. In 2002 our Microbiology transitioned to BryanLGH hospital as part of their laboratory.

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

My biggest challenge has been trying to keep current with different antibiotics that are now available and name changes that have occurred with different organisms. It has also been challenging to keep up with all of the new technological advances in laboratory medicine. These are challenges but are also what make microbiology so interesting.

What is your advice to a first-year medical technologist?

Since I started in this profession over forty years ago there is very little that hasn't changed. Automation and computerization have made it possible to do large amounts of work with fewer techs, which is necessary when technical personnel are not plentiful and turn-around-time is so critical for patient care, both personally and economically.

What do you like most about your job?

The constant variety in day to day testing, looking for the unusual, and the satisfaction of providing rapid turn around time in the identification of unusual organisms.

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