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 Updates

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

This issue of the NPHL newsletter contains two topics of specific importance to clinical laboratories as well as clinical partners regarding the role that all laboratories play in the submission of bacterial and viral isolates for epidemiologic surveillance purposes. The NPHL uses the bacterial isolates for multiple different functions including confirmation of antibiotic resistance and emergence of new antibiotic resistance patterns. The viral isolates are particularly important this time of season for determining which strains of influenza virus A are circulating in our population. The NPHL selects appropriate samples from different geographic areas of the state and submits them to the CDC as part of the World Health Organization's influenza surveillance program. The CDC performs subtyping tests on these specimens to determine whether they are the subtype in the vaccine or new subtypes introduced into our population. If they are found to be new and are present in multiple places across North America, these isolates will be included in next year's vaccine. The second important topic involves an issue in the news and that is the contamination of peanut butter by a specific strain of salmonella called *Salmonella* serotype Tennessee. The NPHL first identified this strain in November and after the CDC posted the molecular fingerprint, we were able to confirm that the Nebraska isolates were from the same outbreak. You will also be interested in the featured laboratorian who is Jolene Smith from Faith Regional Health Services in Norfolk. Taken together, these articles illustrate again that everyone is a public health laboratorian.

Shiga Toxin Testing Recommendations

By Jodi Garrett, MT(ASCP)SM, Microbiology Manager, NPHL and Paul D. Fey, PhD, Associate Director, NPHL

Due to recent outbreaks of gastroenteritis caused by Shiga toxin-producing *E. coli*, the Centers for Disease Control and Prevention (CDC) has recently made a recommendation to test all stool samples for the presence of Shiga toxin (for more details, see: Importance of Culture Confirmation of Shiga Toxin-producing *Escherichia coli* Infection as Illustrated by Outbreaks of Gastroenteritis --- New York and North Carolina, 2005, Morbidity and Mortality Weekly Report, September 29, 2006/55 (38);1042-1045, http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5538a3.htm). These recommendations have been made because the Shiga toxin assay provides better sensitivity than culture for the O157:H7 serotype. In addition the antigen test provides a more complete picture of Enterohemorrhagic *E. coli* (EHEC) infection by detecting Shiga toxin production which may result from *E. coli* serotypes other than the O157:H7.

EHEC is recognized as an important cause of endemic diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS). The most commonly reported serotype associated with outbreaks in the United States to date has been O157:H7, but more than 50 other non-O157:H7 EHEC serotypes have been reported to be associated with human disease, including HUS.

The traditional laboratory diagnosis of EHEC infection has been dependant on the recovery of *E. coli* O157:H7 in culture on sorbitol-MacConkey agar (SMAC) followed by immunologic confirmation. SMAC culture has a demonstrated sensitivity of 50%-80% for detection of *E. coli* O157:H7 and will miss the non-O157:H7 EHEC serotypes. One virulence trait of all EHEC stains is the ability to produce one or two potent cytotoxins called Shiga like toxins (SLT). Studies have shown that an EIA for EHEC Shiga toxin detects approximately 40% more EHEC O157:H7 than the conventional SMAC culture, and is also able to detect an additional 20% more Shiga toxin-producing *E. coli* that are non-O157-H7, depending on the prevalence.

From March 1, 1998, to October 31, 1998, Dr. Paul Fey requested and received specimens from nine institutions in Nebraska to test for the prevalence of non-O157:H7 Shiga toxin-producing *E. coli* in diarrheal stool samples. Of the 335 samples submitted, 14 of them were positive for Shiga toxin. Non-O157 serotypes account for about 50% of the EHEC strains recovered from the study. This clearly emphasizes the need for use of assays capable of detecting non-O157 serotypes. His article entitled, Prevalence of Non-O157:H7 Shiga Toxin-Producing *Escherichia coli* in Diarrheal Stool Samples from Nebraska, Emerging Infectious Diseases, Vol. 6, No. 5, Sep-Oct 2000 can be read online at http://www.cdc.gov/ncidod/eid/vol6no5/fey.htm.

There are currently two commercially available cartridge kits: BioStar® OIA® SHIGATOX test from Inverness Medical–BioStar Inc. and ImmunoCard STAT! EHEC kit from Meridian. There is also a Premier EHEC 96-well format assay available from Meridian Diagnostics. The American Proficiency Institute offers proficiency testing for Shiga toxin antigen; it is not currently available from the College of American Pathologists.

EHEC infections are a reportable disease in Nebraska and need to be reported to the Nebraska Health and Human Services System (NHHSS). Isolates submitted to the NPHL will be further characterized by pulsed-field gel electrophoresis (PFGE) to facilitate investigation of possible outbreaks. Therefore, to provide information to the NHHSS epidemiologic program, it is important to obtain an isolate from as many Shiga toxin positive stool samples as possible. If your laboratory is currently testing for the presence of Shiga toxin in stool samples, but is not isolating the organism, the positive stool sample can be sent to the NPHL for further strain isolation. To send positive stool samples to the NPHL, please send the stool sample (preserved in enteric transport media; stable ambient or refrigerated for up to 4 days) with a NPHL Special Microbiology Requisition. Fresh stool should not be sent. Mark the requisition under the "Confirmation/Identification: *Escherichia coli* O157:H7." A report will be sent back to your laboratory regarding the results. For further questions, please contact either the NPHL Client Services (866-290-1406), Jodi Garrett (402-552-3235) or Dr. Paul Fey (402-559-2122).

Meet the Laboratorian – Jolene Smith

Compiled by Josh Rowland, State Training Coordinator, NPHL

Jolene Smith, MT (ASCP)SM, is the Microbiology Supervisor at Faith Regional Health Services in Norfolk, Nebraska. Faith Regional Health Services is a 166-bed acute care facility founded in 1923 that serves over 100,000 people within a 75-mile radius in northeastern Nebraska. Jolene will be celebrating her 30th anniversary as a laboratorian.

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

In high school, I really enjoyed chemistry and biology courses, even though there were limited classes offered! I always was interested in the medical field and my high school guidance counselor encouraged me to look into medical technology.

Where did you attend medical technology school?

I attended Wayne State College and took my year of internship at St. Luke's Medical Center in Sioux City, Iowa. My specialty in microbiology was attained in 1991.

How long have you worked in your present location?

I have worked in the laboratory for several commercial companies such as Pathology Medical Services, Nichols Institute, Corning Clinical Laboratories, and Quest Diagnostics. For the past 30 years, I have been employed with Norfolk hospital laboratories which became part of Faith Regional Health Services in 2002. During my time with the hospital laboratories, I started as a generalist and subsequently I took the position of microbiology supervisor in 2002.

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

I feel my biggest area of expertise is in plate reading, which I love. Since I am in a hospital setting of about 100 beds, I have the opportunity to work on the bench and still do supervisory work. Using my knowledge every day to produce quality results that will benefit the patient is important to me. Also, when we became part of Faith Regional Health Services, I had the opportunity to do research on new testing methodologies which were brought on-site and to participate in writing new procedures which I have really enjoyed.

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

The ability to incorporate molecular methods into the microbiology laboratory in a cost effective way to provide physicians with quality results in an expedited manner. Also, the shortage of technologists can impact areas such as the microbiology laboratory which is highly dependent on the expertise of the technologist and less on automation than some other areas of the laboratory.

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

One of my biggest challenges is to keep up with the advancements made in technology and making sure the best, yet most cost effective products or systems available are being used. Keeping current with the ever-changing world of antibiotics and with laboratory regulating agencies such as CAP also poses a challenge. Additionally, it is important that technologists are given the quality training needed to keep up with these demands even though time is a limiting factor in the laboratory.

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

Don't get overwhelmed!! Unless you have a strong desire to work in one specialty, starting as a generalist is a great way to explore the different areas of the laboratory. There are many opportunities to grow so it is important to keep an open mind and do not be afraid to ask questions and rely on the expertise from others. I have learned much over the years by practicing this philosophy and I continue to learn today from other laboratory scientists such as Rosa Crook, the Microbiology Supervisor at BryanLGH in Lincoln.

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

Definitely the computerization and the application of advanced analyzers, which did not exist when I started my career. Although challenging for me to adapt, both have had a tremendous positive impact on the laboratory.

What do you like most about your job?

No two days or cultures are the same and I like "investigating the unusual". The ability to help a physician in the management of their patient - effectively can really make my day! Also, I find a high sense of enjoyment working with my fellow employees, who are like a second family to me!

Syphilis Testing Update

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

The NPHL has recently implemented new technology for screening of serum samples for evidence of infection by *Treponema pallidum*, the cause of human syphilis. As with many other large volume tests in the laboratory it is important to find means to automate the assay and keep costs at their absolute minimum while still providing the best information possible. The new test incorporates recombinant proteins in an enzyme linked immunoassay (EIA) format similar to other antibody detection methodologies. The use of a treponemal antigen is important because the previous screening assay incorporated a non-treponemal antigen in a test called the Rapid Plasma Reagin (RPR). An algorithm developed by Dr. Victoria Pope (Chief of the Syphilis Serology Reference Laboratory, Division of STD Prevention, National Center for HIV, STD and TB Prevention, Centers for Disease Control and Prevention, Atlanta) serves as the basis for our new protocol. The detection of a positive EIA screen is followed by a qualitative RPR and then, if positive a quantitative RPR. In the past, a screening RPR was followed by a fluorescent treponemal antibody (FTA) absorption test which is labor intensive and subject to interpretation by the reader. In the new algorithm the FTA is reserved for cases where the screening test is positive but the follow up RPR is negative. This process reduces the total number of FTAs that need to be performed, reducing the overall cost of the analysis. As in any test there are special situations such as a patient with latent disease or early non-symptomatic disease where antibodies may not be present

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Salmonella Serotypes in Nebraska for 2006

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

Two hundred nineteen *Salmonella* isolates were submitted for serotyping to the NPHL from laboratories throughout Nebraska in 2006 (the number includes only one isolate per patient). The top 5 *Salmonella* serotypes detected in the state during this time were serotype Typhimurium (Group B, 15.5%), Enteritidis (Group D, 13.2%), Typhimurium 5 null (Group B, 12.3%), Newport (Group C2, 7.3%), and Heidelberg (Group B, 4.6%) (see **Table 1**).

Table 1. Most frequently detected *Salmonella* serotypes identified from Nebraska in 2006.

Rank	Serotype	Serogroup	Number	(%)[a]	National Rank	(%)[b]
1.	Typhimurium	B	34	(15.5)	1.	(16.4)
2.	Enteritidis	D	29	(13.2)	2.	(14.1)
3.	Typhimurium 5 null[c]	B	27	(12.3)	6.	(2.8)
4.	Newport	C2	16	(7.3)	3.	(9.3)
5.	Heidelberg	B	10	(4.6)	5.	(4.9)
6.	Montevideo	C1	9	(4.1)	7.	(2.4)
6.	Muenchen	C2	9	(4.1)	9.	(2.1)
8.	Oranienburg	C1	5	(2.3)	14.	(1.4)
8.	Saintpaul	B	5	(2.3)	10.	(1.9)
8.	Thompson	C1	5	(2.3)	15.	(1.4)

[a]Based on a total of 219 isolates that were serotyped.
[b]Reported through the CDC Public Health Laboratory Information System (PHLIS) and recorded by the National *Salmonella* Surveillance System, 2004.
[c]Formerly called serotype Typhimurium var Copenhagen.

The two most common serotypes detected in Nebraska were similar in percentage to the two most common detected in the U.S.; however, the ranking for the following 8 isolates showed a substantial difference when comparing between the Nebraska and the national data (1). Especially notable were the #4 ranking for serotype Javiana nationally (5.0%) with the rare detection of this isolate in Nebraska (0.4%) and the #3 ranking of serotype Typhimurium 5 null in Nebraska compared with the #6 ranking nationally. Serotype Typhimurium 5 null has been shown to have increased resistance to antimicrobial agents when compared to other *Salmonella* serotypes. Resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline are especially troublesome with strains of this serotype. One strain, referred to as the Definitive Phage Type 104 (DT104) was originally detected in the US causing an outbreak in Nebraska during 1996 and continues to be recognized from isolates submitted to the NPHL (2).

To date, 43 different *Salmonella* serotypes have been identified as isolates detected in Nebraska during 2006. Of these, 9 serotypes were identified that had been rarely observed nationally between the years 1994 and 2004 (the most recent data available from the CDC). **(Table 2)**. One serotype called S.I 9:Lz28 following testing at the CDC, had not previously been detected in the U.S.

Table 2. *Salmonella* serotypes detected from isolates submitted to the NPHL that have been rarely detected following national surveillance activities. [a]

Serotype[b]	National yearly average[c]
Aberdeen	5
Arechawaleta	6
Idikan	2
Kingabwa	3
Wadsworth	6
S.I 9:Lz28:-	ND[d]
S.IIIa 47:z4 z23:-	<1
S.IIIa 48:z4 z24:-	1
S.IIIb 50:k:z	1

[a]Reported through the Public Health Laboratory Information System (PHLIS) and presented in the National *Salmonella* Surveillance Summary, 2004.
[b]All were reported in one case each and all were confirmed by the CDC.
[c]A yearly average of cases reported nationally for the period 1994 through 2004.
[d]Not previously detected nationally.

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Overall, 206 of the *Salmonella* serotype isolates were detected from stool with 10 detected from blood, 2 from sputum, and one from a right knee specimen **(Table 3)**. The blood isolates represented 7 different serotypes with serotype Typhi detected in one sample.

Table 3. Specimen source of *Salmonella* isolates submitted to the NPHL for serotyping in 2006.

Source	Number[a]
Stool	206
Blood	10[b]
Sputum	2[c]
Knee	1[d]

[a]Includes only one isolate per patient for a total of 219 isolates.
[b]Includes the following *Salmonella* serotypes (number): Anatum (1), Bareilly (1), Dublin (1), Heidelberg (2), Typhi (1), Typhimurium (1), and Typhimurium 5 null (3)
[c]Includes one isolate each of serotype Idikam and Schwarzengrund.
[d]Isolate identified as serotype Gaminara.

The recent (February 2007) multi-state outbreak of *Salmonella* serotype Tennessee associated with peanut butter contamination, was also represented in the results of testing at the NPHL during the Fall of 2006 [http://www.cdc.gov/incidod/dbmd/diseaseinfo/salmonellosis_2007?outbreak_notice.htm]. Four isolates submitted to the NPHL (2 from Nebraska, 1 from Kansas, and 1 from Iowa) were identified as serotype Tennessee (Group C1) before an outbreak from peanut butter was recognized. Once a national outbreak was identified following an epidemiological investigation, the isolates were fingerprinted using pulse field gel electrophoresis (PFGE) to determine if they were associated with the outbreak. The isolates tested at the NPHL had an identical PFGE fingerprint to those associated with the outbreak thus indicating they were a part of the clonal spread of this strain. No additional cases have been identified in Nebraska as of April 1, 2007. The NPHL continues to serotype isolates sent to the laboratory to recognize those that may be associated with the present outbreak and any other potential outbreaks that may occur.

For additional information concerning the *Salmonella* Serotyping Program at the NPHL, contact Beth Schweitzer at 402-559-6098 or Dr. Iwen at 402-559-7774. Information concerning susceptibility testing of the *Salmonella* will be presented in a future newsletter.

References

1. CDC. *Salmonella* Surveillance Annual Summary, 2004. Atlanta, Georgia; U.S. Department of Health and Human Services, CDC, 2005.
2. CDC. Multidrug-resistant *Salmonella* serotype Typhimurium, United States, 1996. MMWR, Morbidity and Mortality Weekly Report. 1997, 46: 308-10.

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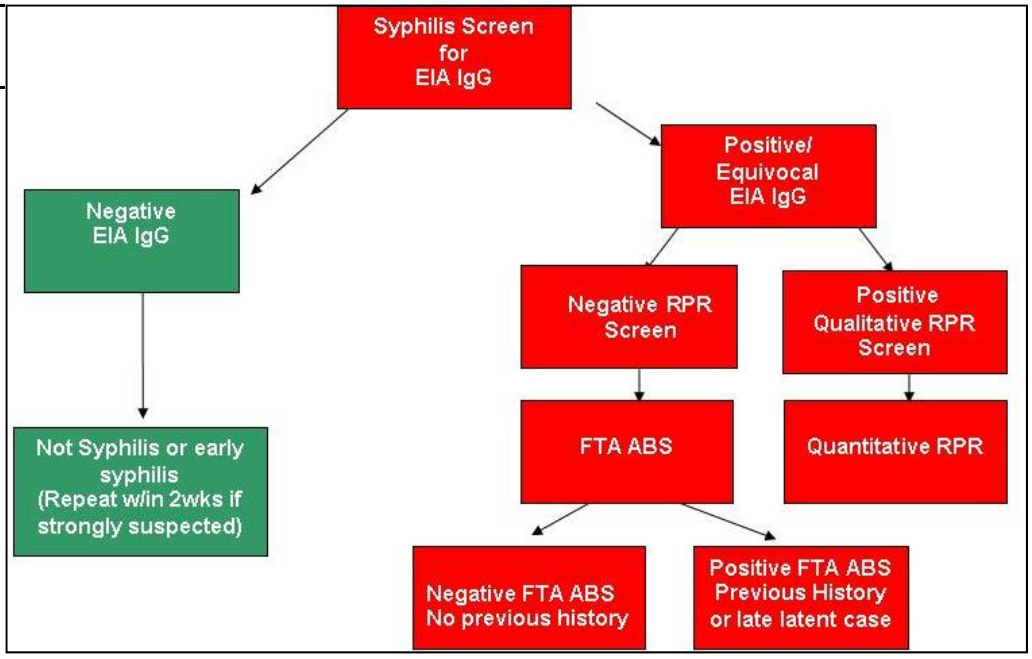
and it may be necessary to consult with the clinician to obtain an accurate interpretation of the laboratory findings. For quick reference, a copy of the protocol is inserted below and it can be reprinted from our website at http://www.nphl.org/testdirectory.html.

Syphilis Screening Algorithm, January 2007



Isolate Banking at NPHL

By Beth Schweitzer, MS, MT(ASCP)SM, Microbiology Specialist, NPHL

The banking of clinical isolates for epidemiological purposes has been ongoing in Nebraska for many years. In 2006, 878 isolates were submitted to the NPHL for banking (**Table 1**). The main goal of the banking program at the NPHL is to allow further testing to aid in outbreak investigations and to monitor the drug resistance of specific organisms. The NPHL uses some of these organisms to participate in the National Antimicrobial Resistance Monitoring System (NARMS) Enteric Bacteria program, in collaboration with the Centers for Disease Control and Prevention (CDC), U.S. Food and Drug Administration (Center for Veterinary Medicine), U.S. Department of Agriculture (Food Safety and Inspection Service and Agricultural Research Services) and state health departments. NARMS data have been collected continually since 1996 to provide useful information about patterns of emerging resistance, which in turn can guide mitigation efforts. NARMS data is also used as an asset to outbreak investigations. Since antimicrobial use in food-producing animals may result in antimicrobial resistance which can be transmitted to humans through the food supply, antimicrobial resistance data from humans are important for the development of public health regulatory policy for the use of drugs in food-producing animals.

In addition to testing for antimicrobial susceptibility, many bacterial isolates are also tested for their potential association with a new outbreak of disease. The pulsed-field gel electrophoresis (PFGE) assay is performed on all *E. coli* 0157:H7 and *Listeria* species within 96 hours of submission for fingerprinting purposes. The results of this testing are immediately compared with data from other isolates from across the country for possible relatedness. All *Salmonella* isolates submitted for banking have serogrouping and serotyping performed as well as antimicrobial susceptibility testing. Serotyping is performed for epidemiological reasons while the susceptibility testing monitors for the development of drug resistance that is seen in certain *Salmonella* serotypes. Starting in the fall of 2006, the NPHL began sending all *Salmonella* isolates to the Minnesota State Public Health Laboratory for PFGE testing. Minnesota is a regional testing center for PFGE. The results of this testing are entered into the CDC PulseNet System. PulseNet is a real-time database of all PFGE results from across the country that is used for epidemiological purposes. The sooner laboratories provide isolates to the NPHL, the sooner an unusual organism can be detected leading to notification of the epidemiologist and subsequent outbreak investigation. Therefore isolates should not be stored at the community level, but should be transferred as soon as possible to the NPHL.

Haemophilus influenza and *Neisseria meningitidis* from sterile body sites are serotyped and if required are submitted to the CDC for further testing for detection of specific serotypes. PFGE can be performed on any submitted isolate if needed for an outbreak investigation.

The banking of isolates also allows the NPHL to participate in cutting edge research in the testing of new methodologies with the CDC. In the fall of 2006 the NPHL was 1 of 4 beta-test sites across the country and Canada to evaluate a micro-sphere immunoassay method for *Salmonella* serotyping being developed by the CDC. In 2007, the NPHL was chosen as a test site to validate a real-time PCR assay to identify *Campylobacter* species.

The participation of the clinical laboratories across the state is a vital part of this process for epidemiological testing and for the validation of new technologies. The prompt submission of bankable isolates is vital to allow the epidemiological process to begin and to help improve the discovery and investigation of any potential outbreaks **(Table 2)**.

For further information please contact Beth Schweitzer at the NPHL 402-559-6098.

Table 1: Isolates submitted to the NPHL for banking, 2006	
Campylobacter spp.	342
Salmonella serogroups	238
Shigella sonnei	100
Shiga toxin positive *E. coli*[a]	62
Streptococcus pneumoniae[b]	30
MRSA	10
Haemophilus influenzae[b]	10
Group A *Streptococcus*[b]	6
Neisseria meningitidis[b]	5
Group B *Streptococcus*[b]	4
Vancomycin-resistant *Enterococcus faecium*, synercid resistant	3
Bordetella pertussis	3
Aeromonas spp.	3
Listeria monocytogenes	2
Other[c]	1 (each)

[a] Includes all serotypes of Shiga toxin producing *E. coli* (i.e. 0157:H7, and 0157:NM)
[b] From sterile body sites only.
[c] *Plesiomonas shigelloides, Shigella boydii, Vibrio parahaemolyticus, Yersinia entercolitica,* and Vancomycin intermediate *Staphylococcus aureus.*

Table 2. Isolates to be routinely submitted to the NPHL for banking.[a,b]
Acinetobacter baumannii (multi-drug resistant)
Campylobacter spp.[c]
Escherichia coli 0157:H7
Haemophilis influnzae[c] (from sterile body sites)
Listeria monocytogenes
Mycobacterium tuberculosis complex
Neisseria meningitidis[c] (from sterile body sites)
Salmonella spp.
Shiga toxin + stool culture filtrate
Shigella spp.
Staphylococcus aureus (vancomycin non-susceptible)
Streptococcus pneumoniae[c] (from sterile body sites)

[a]Isolates for submission to the CDC are first forwarded to the NPHL to facilitate shipping, handling, and result reporting.
[b]Banking may include any organism with an unusual susceptibility pattern or outbreak association per request of the Epidemiology Division of NHHSS.
[c]The viability of this organism decreases over time and it should be sub-cultured to fresh sheep blood agar (*Campylobacter* and *Strep. pneumoniae*) or chocolate agar (*N. meningitidis*) if >3 days prior to submission.

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IN THIS ISSUE

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