# Nebraska Public Health Laboratory Newsletter

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## The Impact of Molecular Testing on Public Health.

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

While we have heard for many years about the potential of molecular testing in the clinical laboratory, most new tests did not use prepackaged kits approved by the Food and Drug Administration (FDA) and required in house validation. Amplification tests that used PCR were practical only in the research or academic laboratory but when probe based tests became available for sexually transmitted diseases such as Chlamydia, they had a major impact. Within the past year, commercial amplification tests have been released that use molecular amplification technologies with kits approved by the FDA, most notably tests for Methicillin (oxacillin) resistant *Staphylococcus aureus* and Group B Streptococcus. These tests also have been developed for single use applications, meaning they don't have to be batched and can be run on a STAT basis when needed. It is now being predicted that molecular approaches will replace many of the tests used by the microbiology laboratory. Therefore, all microbiologist may want to gain at least a basic level of understanding in the field of molecular biology. Two articles in this issue of the newsletter were written to provide examples of the importance of molecular approaches.

Dr. Peter Iwen has reviewed one of the applications of molecular technology that can be used to identify virtually any type of bacteria. His laboratory has applied this technology to determine the species of bacteria that cannot be identified using standard biochemical tests or phenotypic assays. This is the same technology being used to identify new species of organism as illustrated by the recent identification of Mycobacterium named after our state.

Dr. Paul Fey has summarized the key principles and methods that are used in pulse field gel electrophoresis studies. These approaches are one of the best approaches to support epidemiologic investigations and trace the origin of a strain of bacteria that is capable of moving from patient to patient.

Taken together, these articles provide some of the framework needed to understand the basic technologies behind these tests. The information is a primer toward a working knowledge of molecular approaches. Both of these authors are more than happy to provide educational opportunities beginning with MRSA and bacterial susceptibility and will be participation as speakers in the next round of the Center for Biopreparedness Education (formerly Nebraska Center for Bioterrorism Education) 2005 Symposia (see details on page 3). Those laboratorians participating in the symposia will again be invited to join us for a laboratory advisory committee meeting during lunch on day two of the symposia. We look forward to seeing you at the upcoming meetings throughout the state.

## Identification of Microbial Pathogens Using Nucleic Acid Sequencing

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

For more than 100 years, Robert Koch's postulate that required in part the cultivation of a pathogen to show a disease/pathogen relationship, was seldom questioned and was considered the basic standard used in clinical diagnostics. Organism identification to taxon (species, genus) was subsequently accomplished by studying phenotypic characteristics such as Gram stain, morphology, culture requirements, and biochemical reactions along with a combination of intuition and stepwise analysis of the results. In today's laboratory, the ability to detect and identify pathogens has undergone major changes. The development of molecular methods that rely on the detection of genomic elements (DNA or RNA) with or without culture has led the way in this charge. Some of the main reasons for this change from phenotypic to molecular testing include such issues as the slow growth of pathogens, the detection of organisms that exhibit biochemical characteristics that do not fit patterns of known species, and the inability to detect non-cultivatable organisms. Although culture-based methods are still considered the gold standard for identification diagnosis, molecular methods have emerged as the confirmatory method for identification in many diagnostic applications.

The basic principle of any molecular test is the detection of a specific nucleotide sequence (signature sequence) within the organisms' genome which is then hybridized to a labeled complementary sequence followed by a detection mechanism. The first application of these methods in the clinical laboratory was in the development of labeled probes for culture confirmation testing. The original probes were designed to detect "problem" pathogens such as those that were historically difficult to identify using phenotypic methods. These original probes included tests for the culture confirmation of dimorphic fungal pathogens (*Blastomyces dermatitidis, Coccidioides immitis,* and *Histoplasma capsulatum*) and to identify the more common *Mycobacterium* species (*M. tuberculosis* complex and *M. avium* complex). Subsequently, direct detection probes were designed for high volume testing of STD pathogens e.g., *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and for the testing of pathogens that were difficult to grow and identify in the laboratory e.g., *Legionella pneumophilia* and Human papillomavirus.

Although extensively used today, nucleic acid probing unfortunately has been shown to have limited selectivity and to lack sensitivity when testing from direct specimens. To overcome these problems, a process whereby the genomic target could be amplified using non-selective means was developed. The most widely used method for nucleic acid amplification is

*(Sequencing, continued on page 2)*

(Continued from page 1, Sequencing)

the polymerase chain reaction assay i.e., PCR. This assay includes a specific primer pair to amplify a unique genomic target nucleotide sequence for analysis. Following PCR, a variety of post-amplification methods are used to evaluate the product such as direct sequence analysis, use of genus or species specific probes, and utilization of restriction enzymatic analysis of the product, e.g., restriction fragment length polymorphism analysis (RFLP).

Even though all these post-amplification methods have been shown to be useful for the evaluation of microbes, sequence analysis is considered a particularly useful method for the identification of microbial species due to its wide range application to a variety of species. The basic steps involved in this technique are shown in **Table 1**. One drawback to this methodology is that access to sequencing facilities is not readily available for many laboratories, limiting the ability of most laboratories to conduct routine sequence analysis testing. To overcome this issue, commercial kits and some reference laboratories now offer low-cost sequencing for those instances where identification is required for diagnostic purposes.

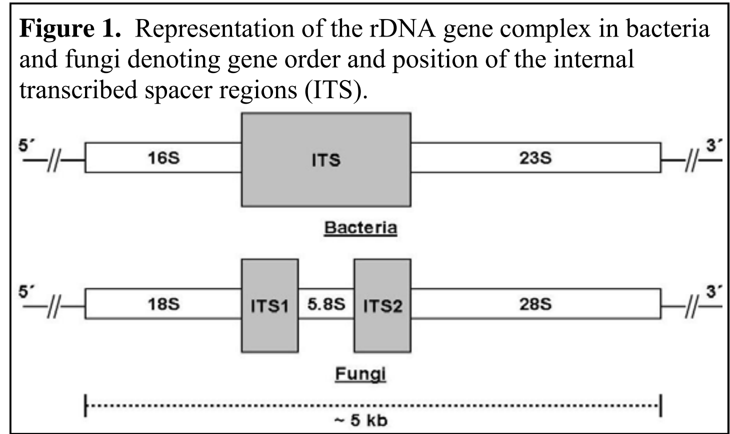
**Table 1.** Sequential steps for the molecular identification of microorganisms using nucleic acid sequencing.

1. DNA or RNA extraction
2. *In vitro* amplification
   e.g., PCR-based assays to detect specific DNA target
3. Sequence determination
   i.e., analyze the PCR product
4. Computer-aides sequence analysis
   e.g., BLAST search using the NCBI GenBank database[a]

[a] Basic Local Alignment Search Tool (BLAST) is a computational method for sequence comparison alignment which is available for public use (http://www.ncbi.nlm.nih.gov/).

Sequence-based identification requires the recognition of a molecular target that is large enough to allow discrimination of a wide variety of microbes. One such target area that has been recognized is the rDNA gene complex which is present in all microbial pathogens. In bacteria, this complex is composed of a 16S rRNA gene and a 23S rRNA gene separated by a genomic segment called the internal transcribed spacer (ITS). Within fungi there are three genes (18S, 5.8S, and 28S) with spacers located between the genes (ITS1 and ITS2). **Figure 1** shows a representation of the rDNA gene complex in bacteria and fungi denoting gene order and position of the spacers. Located in the rDNA gene complex are highly variable sequences that provide unique signatures for the identification of species and also conserved regions that contain genomic codes for the structural restrains that are present within organism groups. It has been shown that the ITS regions contain the most variability and that these regions are useful under most circumstances for species recognition. The availability of these variable sequence regions (ITS) surrounded by conserved sequences (16S/23S and 18S/5.8S/28S) allows for the utilization of an amplification system using universal (or consensus) bacterial or fungal primers. Once amplification has occurred using the consensus primers, the sequence is determined and comparison analysis of the unknown sequence to known sequences contained within a large database (such as the National Center for Biological Information (NCBI), GenBank databases) can be done to determine similarity and subsequently may lead to species identification.

**Figure 1.** Representation of the rDNA gene complex in bacteria and fungi denoting gene order and position of the internal transcribed spacer regions (ITS).




Though public databases such as GenBank are useful, the lack of quality sequences and the absence of sequence information on a large number of species as well as the availability of computational tools to reliably analyze the results are drawbacks to this technology. Additionally, strain variability within species also has not been fully evaluated and has proven to be problematic when evaluating species.

Even with these challenges however, nucleic acid sequence analysis has proven to be a valuable asset for organism identification in a number of applications. Some of the most interesting applications of this technology are for the identifications of variant strains of know species, the identification of uncultivatable organisms in clinical samples and the recognition of new species.

**Identification of variant strains of known species**. The utilization of phenotypic identification methods classically requires a probability-based analysis to determine identity. In cases where identification probabilities are ≥98% with known species, the identification is generally considered acceptable. The lower the probability percentage however, the less accurate the identification becomes, frequently resulting in supplemental testing to resolve discrepancies among test results. It is not unusual for the laboratory to be unable to identify variant strains of known species using phenotypic methods. DNA sequencing now allows the laboratory a means to resolve those instances where phenotypic testing cannot differentiate among closely related organisms.

**Identification of non-cultivatable pathogens**. The etiological agents for a variety of diseases continue to elude current diagnostic testing. The inability to perform *in vitro* culture of microbial pathogens is not a new concept. For example, *Treponema pallidum* even though recognized as the cause of syphilis, continues to be non-cultivatable in the laboratory. Other organisms such as *Bartonella* species, *Legionella* species, *Ehrlichia* species, and *Helicobacter pylori* were only recently cultivatable once the nutritional requirements were recognized. Fortunately, DNA sequencing now allows for the direct detection of microbial genomic material in tissues suspected to contain a microbial pathogen. **Table 2** (see page 3) gives examples of human pathogens that were first examined in clinical material using a molecular approach. The bacterial pathogens of this group were all detected in tissue using universal bacterial primers followed by sequence analysis of the 16S rDNA gene complex. Expectations are that other microbial pathogens will be recognized in the future using this technology.

(Sequencing, continued on page 3)

*(Continued from page 2, Sequencing)*

**Table 2.** Human pathogens first identified in clinical specimens using molecular approaches.

| Disease | Causative agent |
|---|---|
| Non-A, non-B hepatitis | Hepatitis C virus |
| Bacillary angiomatosis | *Bartonella henselae* |
| Whipple's disease | *Tropheryma whipplei* |
| Hantavirus pulmonary syndrome | Sin nombre virus |
| Kaposi's Sarcoma | Human herpesvirus 8 |
| Disseminated infection in AIDS | *Mycobacterium genavense* |

**Identification of new species**. The recognition of a species that does not match known schemes for phenotypic identification may represent a previously unrecognized species. Sequencing of areas within the rDNA complex may be useful to suggest a new species when there is a < 98% of the sequence similarity with known species. The ability to separate a new species from an atypical strain of a known species is however, difficult. The first approach to recognition of a new species is to determine the phylogenetic position of the suspect new species compared to closely related known species. Phylogenetic trees using the 16S gene for bacteria and the 18S gene for fungi are commonly used for this type of analysis. A degree of high degree of phenotypic consistency and rDNA sequence similarity as well as, a significant degree of DNA-DNA hybridization, is suggestive of a new species. Similar approaches were recently used in a research laboratory at UNMC to describe a previously unrecognized species subsequently named *Mycobacterium nebraskense* (see separate article on page 5)

In closing, researchers at the UNMC in collaboration with the clinical lab scientists at The Nebraska Medical Center continue to study ways to apply molecular detection techniques to enhance disease diagnosis. One recent improvement was the development of a molecular assay in combination with a computational algorithm (called MycoAlign) for the identification of *Mycobacterium* species. This prototype system is currently undergoing evaluation at multiple off-site locations throughout the United States and at this time being considered for international distribution. Additionally, an algorithm and database for the identification of fungal pathogens is also being developed. Personnel at the Nebraska Public Health Laboratory will continue to conduct research in this area as the technology evolves.

Any questions concerning sequence analysis testing can be directed to Dr. Peter Iwen at 402-559-7774.

**References**

**Iwen, P. C., S. H. Hinrichs, and M. E. Rupp.** 2002. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. Med. Mycol. **40:87-109.**

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## 2005 Symposia Series

The NPHL would like to invite you to attend one of the 2005 Center for Biopreparedness Education (formerly Nebraska Center for Bioterrorism Education) symposia:

- March 23rd & 24th - Lincoln, Cornhusker Hotel
- April 20th & 21st - Norfolk, Life Long Learning Center
- May 24th & 25th - Kearney, Holiday Inn
- June 16th & 17th - Scottsbluff/Gering, Gering Civic Center

The NPHL will present on day two of each of the programs. Presentations include: "Nebraska Public Health Laboratory Chemical Terrorism Preparedness Activities", "Molecular Techniques for Microbiology". Presentations will be given by Dr. Peter Iwen, Dr. Paul Fey, Tony Sambol, and Josh Rowland

For more details, including registration information, go to www.nphl.org/training.html for a symposia brochure.

## Revisiting the 2002 Group B Streptococcal Disease Guidelines

*By Jodi Garrett, MT(ASCP)SM, Microbiology Manager, NPHL and Josh Rowland, MBA, MT(ASCP), State Training Coordinator, NPHL*

In 1996, the Centers for Disease Control and Prevention (CDC) released guidelines for the prevention of perinatal Group B Streptococcus (GBS) disease (Prevention of Perinatal Group B Streptococcal Disease: A Public Health Perspective. MMWR 1996; 45[RR-7]:1-24). In 2002, the CDC released a new set of guidelines (Prevention of Perinatal Group B Streptococcal Disease Revised Guidelines from CDC. MMWR 2002; 51[RR-11]:1-28) in reaction to evidence and expert opinion which indicated that more effective methods exist to prevent perinatal GBS disease. The new guidelines specify that culture-based screening is substantially more effective than the risk-based approach. The guidelines also recommend that laboratories perform antimicrobial susceptibility testing for women at high risk for penicillin anaphylaxis. In addition, it explained that laboratories are advised to report the presence of GBS in the urine specimens from pregnant women.

The following information was taken from the August 2002 MMWR.

**GBS Colonization**

The gastrointestinal tract serves as the natural reservoir for GBS and is the likely source of vaginal colonization. Approximately 10% to 30% of pregnant women are colonized with GBS in the vagina or rectum. Maternal intrapartum GBS colonization is a major risk factor for early-onset disease in infants, and vertical transmission of GBS from mother to fetus primarily occurs after the onset of labor or membrane rupture. Culture screening of both the vagina and rectum for GBS late in gestation during prenatal care can detect women who are likely to be colonized with GBS at the time of delivery and are thus at higher risk of perinatal transmission of the organism. Heavy colonization, defined as culture of GBS from direct plating rather than only from selective broth, is associated with higher risk for early-onset disease. GBS identified in clean-catch urine specimens is considered a surrogate for heavy maternal colonization and also is associated with a higher risk for early-onset GBS disease; it has been included among indications for intrapartum antibiotic prophylaxis.

*(GBS, Continued on page 5)*

## Pulsed-Field Gel Electrophoresis (PFGE): The Molecular Epidemiologists Tool

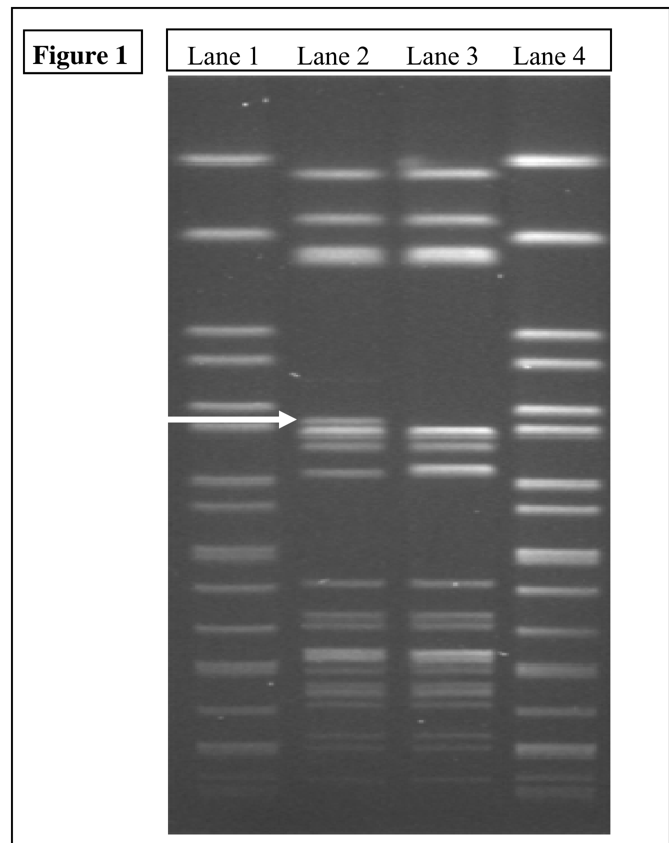
*By Paul D. Fey, PhD, Associate Director, NPHL*

The study of epidemiology related to bacterial outbreaks has come a long way since Mary Mallon (Typhoid Mary) was sent to a tuberculosis sanatorium at North Brother Island (East River of New York City) because city officials did not know what to do with her. However, it was just these types of unfortunate situations which spurred epidemiologists and microbiologists to develop techniques to answer the basic question posed to a molecular epidemiologist which is whether "strain A is related to strain B." The main hypothesis of molecular epidemiology is the following: within the "epidemiologic window," the isolates being considered will have either indistinguishable characteristics (i.e. the same genetic fingerprint) and thus be considered clonally related (same strain), or the isolates will have distinguishable characteristics and be considered different strains. Currently, multiple molecular techniques have been developed to assess this question. Although it is believed that DNA sequencing of highly variable genes will become the method of choice for molecular epidemiologists in the future, currently the "gold standard" technique used in this discipline is pulsed-field gel electrophoresis (PFGE). PFGE is currently used by the Nebraska Public Health Laboratory (NPHL) for molecular epidemiology of foodborne and nosocomial pathogens.

PFGE is essentially the comparison of large genomic DNA fragments after digestion with a restriction enzyme. Since the bacterial chromosome is typically a circular molecule, this digestion yields several linear molecules of DNA. The basic concept of interpretation of this experiment is the following: if one is comparing two strains that are clonal (i.e. the same strain), the sites at which the restriction enzymes act on the DNA and the length between these sites would be identical. Therefore, after digestion of the DNA and electrophoresis through an agarose gel, if the DNA banding patterns between any two isolates is identical, then these isolates are considered the same strain. Conversely, if two isolates are not the same strain, then the sites at which the restriction enzymes act on the DNA and the length between these sites would be different; thus their DNA banding patterns will be different.

The preparation of genomic DNA suitable for PFGE begins by lysing bacteria that are encased in agarose blocks. After multiple washes, the DNA within the agarose is digested with restriction enzymes and electrophoresed using PFGE. PFGE differs from conventional agarose electrophoresis in that the orientation of the electric field across the gel is periodically changed in contrast to being unidirectional and constant in standard electrophoresis. The variability in the electric field allows PFGE to resolve the very large fragments (>600 kb) associated with this analysis.

**Figure 1** shows a PFGE gel of *Escherichia coli* O157:H7. Note that lanes 1 and 4 have indistinguishable banding patterns and are thus considered the same strain. However, lanes 2 and 3 have significantly divergent banding patterns from lanes 1 and 4 and thus are considered separate strains. In addition, note that although the DNA banding patterns in lanes 2 and 3 are very similar, there is an additional band found in lane 2 (identified by the white arrow). Therefore, one would interpret the data as stating that the isolates in lanes 1 and 4 are indistinguishable by PFGE (and divergent from the isolates in lanes 2 and 3) whereas the isolates in lanes two and three are highly related strains but distinguishable by PFGE.




One remarkable aspect of performing molecular epidemiology testing by PFGE is that all state public health laboratories as well as the Centers for Disease Control and Prevention (CDC) perform these protocols using the same methodology. Therefore, using common software and normalization protocols, the NPHL can assess whether certain foodborne pathogens (*E. coli* O157:H7, *Salmonella enterica* serotype Typhimurium, etc.) are not only indistinguishable from certain isolates within Nebraska but also from any other isolate in the United States as well as Canada. This system and database, which was developed at the CDC, is called "PulseNet." Currently, the NPHL can perform PFGE testing on a variety of foodborne (*E. coli, Salmonella, Campylobacter, Listeria,* etc.) as well as nosocomial pathogens (*Staphylococcus aureus, Enterococcus faecium, Klebsiella pneumoniae, Pseudomonas aeruginosa,* etc.).

Please call either Robert Wickert at 402-559-2123 or Dr. Paul D. Fey at 402-559-2122 with questions concerning PFGE testing.

**Selected reviews on PFGE**

**Goering, R.V.** Pulsed-field gel electrophoresis. In: Persing, D.H., Tenover, F.C., Versalovic, J., Tang, Y-W., Unger, E.R., Relman, D.A., and White, T.J., editors. Molecular Microbiology; Diagnostic Principles and Practice. Washington, D.C.: American Society for Microbiology; 2004: **185-196.**

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**Swaminathan B., Barrett, T.J., Hunter, S.B., Tauxe, R.V., and CDC PulseNet Task force.** 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg. Infect. Dis. **7:382-389.**

(Continued from page 3, GBS)

**GBS Laboratory Screening Guidelines**

Numerous studies have documented that the accuracy of prenatal screening cultures in identifying intrapartum colonization status can be enhanced by careful attention to the timing of cultures, the anatomic sites swabbed, and the precise microbiologic methods used for culture and detection of organisms. Collection of cultures between 35 and 37 weeks gestation is recommended to improve the sensitivity and specificity of detection of women who remain colonized at the time of delivery. Swabbing both the lower vagina and rectum (i.e., through the anal sphincter) increases the yield substantially compared with sampling the cervix or sampling the vagina without also swabbing the rectum. Studies have indicated that when women in the outpatient clinic setting collect their own screening specimens, with appropriate instruction, GBS yield is similar to when specimens are collected by a health-care provider. Although swabbing both sites is recommended and use of two swabs can be justified, both swabs should be placed in a single broth culture medium because the site of isolation is not important for clinical management and laboratory costs can thereby be minimized. Because vaginal and rectal swabs are likely to yield diverse bacteria, use of selective enrichment broth is recommended to maximize the isolation of GBS and avoid overgrowth of other organisms. When direct agar plating is used instead of selective enrichment broth, as many as 50% of women who are GBS carriers have false-negative culture results.

**Procedure for collecting clinical specimens for culture of GBS at 35–37 weeks gestation**

- Swab the lower vagina (vaginal introitus), followed by the rectum (i.e., insert swab through the anal sphincter) using the same swab or two different swabs. Cultures should be collected in the outpatient setting by the healthcare provider or the patient herself, with appropriate instruction. Cervical cultures are not recommended and a speculum should not be used for culture collection.
- Place the swab(s) into a nonnutritive transport medium. Appropriate transport systems (e.g., Amies or Stuart's without charcoal) are commercially available. If vaginal and rectal swabs were collected separately, both swabs can be placed into the same container of medium. Transport media will maintain GBS viability for up to 4 days at room temperature or under refrigeration. Specimen labels should clearly identify that specimens are for GBS culture. If susceptibility testing is ordered for penicillin-allergic women (Box 2), specimen labels should also identify the patient as penicillin allergic and should specify that susceptibility testing for clindamycin and erythromycin should be performed if GBS is isolated.

**Procedure for processing clinical specimens for culture of GBS**

- Remove swab(s) from transport medium.* Inoculate swab(s) into a recommended selective broth medium, such as Todd-Hewitt broth supplemented with either gentamicin (8 µg/ml) and nalidixic acid (15 µg/ml), or with colistin (10 µg/ml) and nalidixic acid (15 µg/ml). Examples of appropriate commercially available options include Trans-Vag broth supplemented with 5% defibrinated sheep blood or LIM broth.
- Incubate inoculated selective broth for 18–24 hours at 35º–37ºC in ambient air or 5% $CO_2$. Subculture the broth to a sheep blood agar plate (e.g., tryptic soy agar with 5% defibrinated sheep blood).
- Inspect and identify organisms suggestive of GBS (i.e., narrow zone of beta hemolysis, gram-positive cocci, catalase negative). Note that hemolysis may be difficult to observe, so typical colonies without hemolysis should also be further tested. If GBS is not identified after incubation for 18–24 hours, reincubate and inspect at 48 hours to identify suspected organisms.

Various streptococcus grouping latex agglutination tests or other tests for GBS antigen detection (e.g., genetic probe) may be used for specific identification, or the CAMP test may be employed for presumptive identification.

* Before inoculation step, some laboratories may choose to roll swab(s) on a single sheep blood agar plate or CNA sheep blood agar plate. This should be done only in addition to, and not instead of, inoculation into selective broth. The plate should be streaked for isolation, incubated at 35–37ºC in ambient air or 5% $CO_2$ for 18–24 hours and inspected for organisms suggestive of GBS as described above. If suspected colonies are confirmed as GBS, the broth can be discarded, thus shortening the time to obtaining culture results.

**Procedure for clindamycin and erythromycin disk susceptibility testing of isolates, when ordered for penicillin-allergic patients**

- Use a cotton swab to make a suspension from 18–24-hour growth of the organism in saline or Mueller-Hinton broth to match a 0.5 McFarland turbidity standard.
- Within 15 minutes of adjusting the turbidity, dip a sterile cotton swab into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. Use the swab to inoculate the entire surface of a Mueller-Hinton sheep blood agar plate. After the plate is dry, use sterile forceps to place a clindamycin (2 µg) disk onto half of the plate and an erythromycin (15 µg) disk onto the other half.
- Incubate at 35ºC in 5% $CO_2$ for 20–24 hours.

Measure the diameter of the zone of inhibition using a ruler or calipers. Interpret according to NCCLS guidelines for *Streptococcus* species other than *S. pneumoniae* (2002 breakpoints: clindamycin: >19 mm = susceptible, 16–18 = intermediate, <15 = resistant; erythromycin: >21 mm = susceptible, 16–20 = intermediate, <15 = resistant).

**Molecular Testing**

In 2004 a molecular based GBS assay was approved by the Food and Drug Administration (FDA) for use on the Smart Cycler analyzer. This test utilizes polymerase chain reaction (PCR) for the amplification of a cfb gene sequence of GBS and fluorogenic target-specific hybridization for the detection of amplified DNA. The NPHL is currently validating this assay for use in the Microbiology Department.

Questions about GBS laboratory testing can be directed to Jodi Garrett at 402-552-3532 or Josh Rowland at 402-559-6070.

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## Meet the Laboratorian - Keith Beardshear

*Compiled by Josh Rowland, MBA, MT(ASCP),*
*State Training Coordinator, NPHL*

Keith Beardshear is the Laboratory Supervisor of the United States Public Health Service (USPHS) Indian Hospital and associated clinic in Winnebago Nebraska. Keith's work at the Indian Hospital is truly unique in Nebraska as his work directly benefits Nebraska's Native American population.

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

I was raised on a small farm in northeast Nebraska. From that perspective, it was easy to observe the natural life cycles of both plants and animals. Biology became second nature to me. On a similar note, chemistry applications are readily found in the agricultural setting. In that early crucible of learning, it became natural to apply those experiences with the basic sciences we are taught in both the high school and college settings.

**Where did you attend med tech school?**

I received my medical technology training while serving in the United States Air Force (USAF). I did my year internship at Wilford Hall USAF Medical Center, located in San Antonio, Texas. At that time (late 70's) Wilford Hall was a 1000 bed referral hospital for USAF military personnel. After finishing the MT internship, I continued to work in the hematology department of the laboratory until of my tour of duty was completed.

**How long have you worked in your present location?**

I have been employed at the Winnebago USPHS Indian Hospital since 1981. Our hospital is one of hundreds of Indian Health Service facilities that serve the Native Americans Population. Indian Health Service is part of the United States Public Health Service which, in turn, falls under the auspices of the Department of Health and Human Services. Our hospital and clinic serves a population base of approximately 14,000 individuals.

**What is unique about working at the Winnebago USPHS Indian Hospital?**

The Winnebago USPHS Indian Hospital/Clinic is geographically located on the Winnebago Indian Reservation in northeast Nebraska. We serve the Native American population from two adjacent reservations, the Omaha and Winnebago. We also provide medical services for Native Americans who live in urban centers such as Omaha, Lincoln, Norfolk, and Sioux City. Both the Omaha and Winnebago Tribes are rich in native culture and tradition. Each has unique cultural aspects derived from tribal customs. The Omaha Tribe still lives on some of its original homeland. The Winnebago Tribe was deported from its native land of Wisconsin and traveled to the current reservation location. They purchased land from the Omaha Tribe. It is here where many Winnebago descendants remain today. If you have not experienced a pow wow consider attending during the late summer months. The regalia are colorful and the dancing to the rhythm of the drumbeat is something that should be experienced. The two reservations are not as isolated as some. They are located approximately 80 miles north of Omaha and 20 -25 miles south of Sioux City, Iowa. Much of the reservation land consists of farms nestled between the rolling hills. The eastern edges of the reservations overlook the Missouri River.

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

Meeting laboratory program objectives has become increasingly difficult, primarily due to an ongoing technical staff shortage that we face at Winnebago. It has not been easy for us to recruit qualified individuals.

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

Follow the advice of experienced and seasoned technologists. They can guide you through the first few months and years of your career. In my opinion, experience is the greatest of all teachers, so follow the wisdom of those whose skill and understanding has been developed in a given area of medical laboratory expertise.

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

Without a doubt, the advances in instrumentation and test methodology stand at the top of my list. When I began as a technologist, the transition from manual testing to automation was starting to gain momentum. Yet, at that time, a major part of our curriculum was to manually perform chemistry and hematology tests. Mouth pipetting was still a common practice. Glassware had to be washed and reused. We became proficient with a variety of pipetting techniques and at performing manual hematology cell counts. SMAC analyzers were chemistry workhorses in larger laboratories. Alkaline picrate permanently stained many countertops and floors. Coulter had progressed to the Model-S hematology analyzer. Microorganisms were being renamed, based more on genetics than morphology and biochemical reactions. Wang was a big name in laboratory computers when I was new to the field. I marvel at the genius of the researchers and engineers, who have developed laboratory medicine to the point it has reached today, yet wonder how much more it will advance in another 20 years.

**What do you like most about your job?**

There are really two aspects that I most enjoy in working in the smaller lab setting. First, I like the variety of challenges that we face from day-to-day. No two days are alike. We need to be self-reliant in many aspects with the ability to solve a variety of problems. Secondly, it is rewarding to perform testing on patients who we know as individuals. There is a stark difference between working in a large lab where patients are associated with a series of tubes and numbers, as opposed to more individualized testing within the smaller rural laboratory. We function primarily as a "stat lab", generating test results while patients are waiting. Giving providers test result information during the actual clinic visit has undoubtedly enhanced patient care here at the Winnebago USPHS Indian Hospital.

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## *Mycobacterium nebraskense*, a Newly Recognized Slow Growing Opportunistic Pathogen

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

Researchers at the University of Nebraska Medical Center recently described a previously uncharacterized bacterial species isolated from multiple patients with pulmonary disease. The Judicial Commission of the International Committee on Systematic Bacteriology has officially validated the new species now named *Mycobacterium nebraskense* (ne.bras.ken'se, referring to the State of Nebraska). Distinct mycobacterial sequences characteristic of the 16S rRNA gene and the ITS-1 region target along with uniqueness of the mycolic acid profile and other phenotypic characteristics, confirmed that the isolate represented a novel *Mycobacterium* species. Phylogenetic analysis using the 16S rRNA gene sequences showed that *M. nebraskense* is closely related to other slow growing *Mycobacterium* species such as to *M. kansasii, M. scrofulaceum, M. malmoense*, and *M. avium*. The isolation of this new species from the sputum of 5 immunocompromised patients with respiratory symptoms suggests a likely causative association between infection with this pathogen and pulmonary disease. The type strain has been deposited into the American Type Culture Collection (ATCC BAA-837$^T$) and into the German culture collection (DSM 44803$^T$).

Questions about this new pathogen can be directed to Dr. Peter Iwen at 402-559-7774.

**Reference**

**Mohamed, A. M., P. C. Iwen, S. Tarantolo, and S. H. Hinrichs.** 2004. *Mycobacterium nebraskense* sp. nov.: a new slow growing schotochromogenic *Mycobacterium* species. Int. J. Syst. Evol. Microbiol. **54:2057-2060.**

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## New Information System Developed for The Public Health Laboratory: ELIRT™

*By John Glock, Senior Software Developer, NPHL*

A new computer information system for web-based laboratory ordering and reporting has been developed for the Nebraska Public Health Laboratory (NPHL). The new system was created to replace the Public Health Laboratory Information Program (PHLIP) in use at the NPHL since 1999. At the time PHLIP was developed it was the first web-based laboratory test ordering and reporting system for public health laboratories in the country. However, much has changed in the information technology field and the structure of PHLIP limited the ability to increase speed of operation, a problem that became increasingly obvious as the database grew rapidly over the past four years. Planning for a totally new system was begun to incorporate all of the lessons learned by the NPHL through discussions with clients or customers.

The name of Electronic Laboratory Information and Retrieval Technology was selected to reflect that the new system was more than an upgrade as symbolized by the acronym ELIRT. Beta testing of the ELIRT System began in early March and the roll-out will continue through the month. "We are excited by the increase in speed and the new functionality of ELIRT", said Brian Lenz, NPHL's Client Service Coordinator. "The system has a number of features that people will appreciate, but the most important is the quick response time."

The ELIRT System is composed of several components with the primary element being the improved web-based graphical user interface (GUI) for laboratory test order entry and result inquiry. The look and feel of the new system was designed to optimize its usability and overall efficiency.

PHLIP users had asked for enhanced keystroke function and extensive use of drop down menus and pick lists. Most importantly, the ELIRT system allows the user to order additional tests on the same patient without re-entering demographics and clinic information.

The system utilizes a web-based portal technology called *Guardian* that was developed by the Information Management Services Division of the State of Nebraska. This system has been customized for use with the ELIRT system and provides secure, sign-on capabilities that controls access based on previously defined responsibility and authorization. The primary benefit to the user of *Guardian* is the function of single sign-on, and the ability to access authorized components without the need for multiple passwords. These components of the system define the user's profile. Unlike the PHLIP application, ELIRT fully utilizes the user management capabilities of *Guardian* allowing all aspects to be administered in one place.

An additional feature of ELIRT is the ability to generate a variety of customized reports for specific needs of the laboratory or clinic users. Most importantly, the system allows for the export of data without the need for specialized applications. Of special interest to the Epidemiology programs at the state, county and health district level, the ELIRT system provides for automated submission of Electronic Lab Reporting (ELR) of reportable conditions to the Nebraska National Electronic Disease Surveillance System (NEDSS).

Questions about the operation of the ELIRT system can be directed to Brian Lenz at 402-559-7897 or John Glock at 402-559-3592.

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