Nebraska Public Health Laboratory Newsletter

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NPHL Updates

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

As the winter approaches, personnel at the NPHL are planning for the "respiratory season" in addition to our other "routine" activities. This newsletter highlights some of these ongoing activities. Patricia Enfield, a TB Program Manager for the NE Department of Health and Human Services provides an interesting update on tuberculosis in Nebraska. Although the number of TB cases continues to be low in our state, our changing population demographics highlights the importance of a reliable laboratory system within our region to provide for early detection and diagnosis of TB. Two activities provide regulatory updates that affect the labs. Karen Stiles, NPHL training coordinator provides an update on the changes that have occurred with the reporting of diseases in Nebraska.. Linda Fell, CLS Program Director, gives an overview of changes that have occurred with credentialing for our profession. She describes the process and gives advice on how the new credentialing will enhance our profession. Finally, to highlight our collaboration with research partners at UNMC, Dr. Marilynn Larson, an Assistant Professor in the Department of Pathology and Microbiology, overviews some research activities concerning tularemia, an endemic disease in Nebraska. This research has resulted in the development of new molecular methods for the detection and identification of *Francisella tularensis*.

Finally, please check out the newly designed NPHL website (www.nphl.org). We are hopeful that you will find this easier to navigate and welcome your comments and suggestions on our new "look". Also, reserve the dates of June 5-8th on your calendar. NPHL will host the national Association of Public Health Laboratories (APHL) Annual Meeting & 5th State Environmental Laboratory Conference in Omaha at the Qwest Center. Hosting this conference in Omaha is a testament to NPHL's active participation in the activities of the APHL. For additional information on this conference, consult the APHL website (http://www.aphl.org/profdev/conferences). We encourage our laboratory partners to participate in this meeting.

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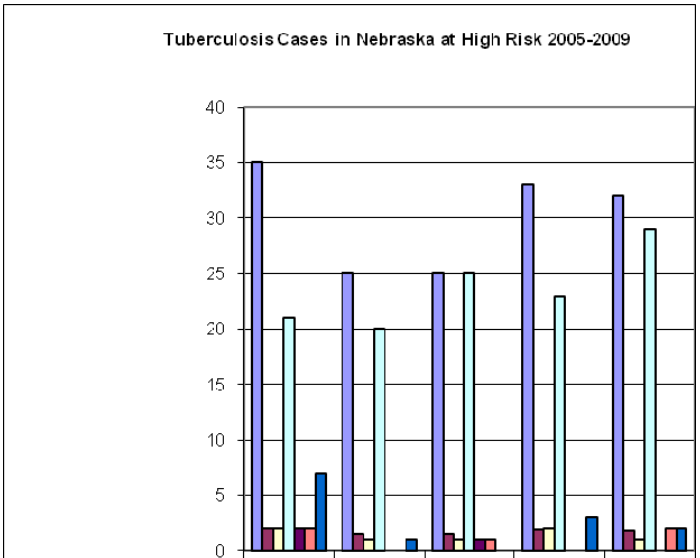
Tuberculosis in Nebraska

By Patricia Enfield, TB Program Manager, DHHS

In the United States, TB has come far from the killer disease that it was at the turn of the 20th century, but it has not yet been eliminated as many people seem to believe. Sixty plus years from when effective chemotherapy was discovered, TB remains one of the leading causes of death in the world today. Each year there are 2 million TB related deaths world wide. Someone in the world is newly infected with TB every second. Because of our global society, it is true that "TB anywhere is TB everywhere".

There were 11,540 TB cases reported in the U.S. in 2009 which is the lowest recorded rate since national TB Surveillance began in 1953. Although the number of cases has gone down, there continues to be a great need for continued research to bring forward new diagnostics and medications if TB elimination is to ever be achieved.

Nebraska reported 32 active TB cases in 2009 which maintains the average of 30 cases per year reported in the last ten years. Of the 2009 cases, 29 were foreign born including three from college campuses and one case of multidrug resistant TB that still remains on treatment. Following is a chart that shows the TB cases in Nebraska for the last five years broken down by high risk categories:



	2005	2006	2007	2008	2009
Reported cases	35	25	25	33	32
Statewide Rate/100,000	2.0	1.5	1.5	1.9	1.8
Homeless	2	1	1	2	1
Foreign Born	21	20	25	23	29
Related to Institutions	2	0	1	0	0
HIV+	2	0	1	0	2
Resistant to => 1 drug	7	1	0	3	2

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In addition to the active cases of TB, there were 173 refugees and immigrants who arrived in Nebraska in 2009 with a Class B1 or B2 status. These classifications mean the individuals had evidence of TB infection and abnormalities on chest x-rays done overseas. They were deemed non-infectious for travel but needed an evaluation soon after arrival in the U.S.

Since 1999, the percentage of foreign born cases in our state has increased. Nebraska was the first state in the nation to report 100% of our cases in the foreign born in 2005. This population brings with it language and cultural barriers that require a tremendous amount of public health resources to ensure a successful TB treatment outcome. In 2009, the TB Program has been able to meet most of the CDC's National TB Objectives of curing patients within 12 months of initiating therapy, getting drug susceptibilities on all culture confirmed cases; identifying, evaluating and treating contacts to pulmonary cases, getting the Class B1 refugees and immigrants evaluated within 45 days of arrival, and continuing partnerships with tribal health care centers. We do not meet the objective of determining HIV status on every active case of TB but continue to work on this issue.

To control TB, the Nebraska TB Program offers free drugs that are used to prevent and treat the disease. The medications are sent to the person's provider to be dispensed incident to practice. Six hundred fifty four persons utilized this system in 2009. With this system, the program has been able to maintain a data base in the Nebraska Electronic Disease Surveillance System which has allowed the TB Program to track demographic information as well as completion rates of the clients.

Much of the progress in TB control in 2009 was made in applying new laboratory tests for the detection and identification of the *Mycobacterium tuberculosis* complex. The NPHL began offering the *M. tuberculosis* Amplified Direct Detection test in early 2009. This test allows quick turnaround results for persons who are strong TB suspects. In addition, the TB interferon gamma release assay can be used in most circumstances in which the tuberculin skin test is used and TB genotyping is done on all positive TB isolates from throughout the state.

As with many other things, it takes a "village" to control and eventually eliminate TB in our state. The NE TB Program and all its partners continues to diligently work toward this goal. For more detailed statistical and other information on the TB Program, please visit the TB web page at http://www.dhhs.ne.gov/cod/Tuberculosis/tbindex.htm.

Francisella tularensis Research at UNMC

By Marilynn A. Larson, MS, PhD, UNMC Assistant Professor

Francisella tularensis is a pleomorphic gram-negative coccobacillus and the causative agent of tularemia (1). This organism is noted for having a low infective dose with as few as 10 cells able to cause illness and possibly death in numerous mammalian species, including humans. Accordingly, *F. tularensis* is considered a potential biological weapon and classified as a select agent by the Centers for Disease Control and Prevention (CDC). This highly infectious pathogen is predominantly spread by mammalian and arthropod vectors and consists of four subspecies; subsp. *tularensis* (biovar A), subsp. *holarctica* (biovar B), subsp. *novicida*, and subsp. *mediasiatica* (2). Subspecies *novicida* and *mediasiatica* rarely cause tularemia and are considered opportunistic bacteria. Conversely, biovar A and biovar B strains can cause a severe form of tularemia in humans. Biovar A is generally considered more virulent than the biovar B clade and is further subtyped into A.I and A.II strains based on genomic content. Despite the marked differences in virulence and geographical locations of the *F. tularensis* subspecies, they all share >99% genomic content (**Table 1**).

Identification of tularemia in humans is traditionally accomplished by growth characteristics with biochemical analysis and molecular testing or by using serological means, all of which can produce ambiguous results. Although the natural environmental niche for *F. tularensis* is unknown, clinical cases of tularemia occur every year in Nebraska. Therefore, rapid detection of *F. tularensis* and identification of the associated subspecies is important to properly treat the infected host and to assist in the control of tularemia outbreaks by identifying potential reservoirs for this pathogen.

Currently, Dr. Steven Hinrichs' research group at UNMC is focused on developing rapid and reliable molecular detection methods for *F. tularensis*, with a special emphasis on PCR-based assays. To date, several PCR-based protocols have been developed by this group that reliably detect and differentiate the various subspecies and subtypes of *F. tularensis*. In addition, acquisition, assembly, and annotation of the genome from a clinical A.I isolate of *F. tularensis* subsp. *tularensis* has been completed (3), and the assembly of the genome from a clinical A.II strain is near completion. These genome projects have been assisted by bioinformatic specialists at the University of Nebraska-Lincoln and by state-of-the art optical mapping of the bacterial chromosome by OpGen, Inc. Proteomic methodology

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Table 1. Virulence and geographical locations of the *F. tularensis* subspecies.

***F. tularensis* Subspecies**	**Biovars**	**Geographic Location(s)**	**Virulence**
tularensis	A.I and A.II	North America & Europe	Very High
holarctica	B*	Northern Hemisphere	High
novicida		North America & Australia	Moderate to Low
mediasiatica		Central Asia	Moderate to Low

*** Includes the live vaccine strain (LVS) which has limited use as a protective vaccine in high risk groups**

Changes in Nebraska Reportable Conditions

By Karen Stiles, NPHL State Training Coordinator , MT (ASCP) SM

In 1878, Congress authorized the US Marine Hospital Service (forrunner of the Public Health Service) to collect data regarding cholera, smallpox, plaque, and yellow fever from the US consuls overseas. This information was to be used in instituting quarantine measures to prevent the introduction and spread of these disease into the United States. By 1928, all states, District of Columbia and Puerto Rico where participating in national reporting of 29 specified diseases.

In 1961, The Centers for Control and Prevention (CDC) assumed this responsibility and the National Notifiable Diseases Surveillance System (NNDSS) was established to work with the Council of State and Territorial Epidemiologists (CSTE) to aggregate and compile for publication purposes . Notifiable disease reporting at the local level protects the public health by ensuring the proper identification and follow-up of cases. Public health workers ensure that persons who are ill receive appropriate treatment and trace contacts who need vaccines, treatment, quarantine or education. This data helps public health authorities to monitor the impact, measure disease trends, assess effectiveness of control and preventions measures, identify populations or areas at high risk, allocate resources appropriately, and detect sudden changes in disease occurrence.

The list of notifiable infectious diseases is revised periodically. Public health official at the state health departments and the CDC, continue to collaborate in determining which diseases should be notifiable. Reporting is currently mandated only at the state level (ie. by state legislation or regulation). The list of diseases and other conditions that are considered notifiable, therefore, varies slightly by state. Today, generally all states report the diseases in compliance with the World Health Organization's (WHO), International Health Regulations (IHR). The CDC utilizes a component of the Public Health Information Network called the "National Electronic Diease Surveillance System (NEDSS)" which promotes accuracy, completeness, and timeliness of disease reporting at the local, state, and national levels (1).

Data on selected notifiable infectious diseases are published weekly in the MMWR. In Nebraska , health care providers including physicians, physician assistant (PA), advanced practice registered nurses (APRN) and laboratories are required to submit reports of communicable disease and poisonings as described in our state statue Title 173 NAC 1. These can be found on the Nebraska DHHS website: http://www.hhs.state.ne.us/cod/codreport.htm .

The list and frequency of reporting these conditions in Nebraska is found in the Title 173 NAC 1-004.01 through 1 -004.06. Recent changes to this list had been approved by Gov. Dave Heineman and took effect on May 6, 2010. Changes that have occurred in the Nebraska regulations are summarized in the next column.

Please see complete report for a listing of all immediate, within 7days, and monthly reportable conditions on the NPHL webite at: http://www.nphl.org/documents/reportablediseasesforlabsMay62010.pdf

Newly added reportable diseases requiring immediate notification:

Coccidiomycosis (*Coccidioides immitis spp.*)
Eastern equine encephalitis (EEE virus)
Hantavirus pulmonary syndrome
Influenza due to novel or pandemic strains (includes highly pathogenic avian influenza virus)
Meningitis (*Haemophilus influenzae* or *N. meningitidis*)
Monkeypox virus infection
Rocky Mountain Spotted Fever (*Rickettsia rickettsii*)
Ricin poisoning
Rift Valley fever
Severe Acute Respiratory Syndrome (SARS-associated coronavirus)
Tick-born encephalitis, virus complexes
Viral hemorrhagic fever (including but not limited to Ebola, Marburg, and Lassa fever virus)

Newly added reportable diseases and conditions requiring notification within 7 days:

Acinetobacter spp., all isolates (labs doing ELR only)
Arboviral infections (including, but not limited to, West Nile virus, St Louis encephalitis virus, Western equine encephalitis virus and Dengue virus)
Carbon monoxide poisoning
Chancroid (*Haemophilus ducreyi)*
Clostridium difficile
Creutzfeldt-Jakob Disease (14-3-3 protein from CSF or any Laboratory analysis of brain tissue suggestive of CJD)
Cyclosporiasis *(Cyclospora cayetanensis)*
Enterococcus spp., all isolates (labs doing ELR only)
Hansen's Disease (*Mycobacterium leprae)*
Histoplasmosis (*Histoplasma capsulatum)*
Influenza deaths, pediatric (< 18 years of age)
Influenza, all tests (labs doing ELR only)
Influenza, rapid tests summary report only (labs only)
Klebsiella spp., all isolates (labs doing ELR only)
Lymphocytic choriomeningitis virus infection
Lymphogranuloma venereum (*Chlamydia trachomatis)*
Meningitis, including viral bacterial and fungal (reported within 7 days except *Haemophilus influenza* and *Neisseria meningitides* to be reported immediately)
Mycobacterium spp. (including MTB complex, and all "atypical" species, to include culture, nucleic acid tests, or positive histological evidence indicative of tuberculosis infection or disease)
Necrotizing fasciitis
Poisoning or illness due to exposure to radiologic exposure
Respiratory syncytial virus infection, (laboratories only)
Rotavirus infection (labs doing ELR only)
Staphylococcus aureus, all isolates (labs doing ELR only)
Toxoplasmosis, acute (*Toxoplasma gondii)*
Varicella primary infections (chicken pox)
Varicella mortality (all ages)

Reference

1. **National Notifiable Diseases Surveillance System** http://www.cdc.gov/ncphi/disss/nndss/nndsshis.htm

What's in a Name? A Taxonomic Overview of the Genus *Cronobacter sakazakii*

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

The family *Enterobacteriaceae* has undergone numerous taxonomic changes and currently includes 49 different genera (List of Prokaryotic Names with Standing in Nomenclature, http://www.bacterio.cict.fr/e/enterobacteriaceae.html). The *Cronobacter* (Gr.n. *Cronos*, one of the Titans of mythology who swallowed each of his children as soon as they were born; N.L. masc. n. *bacter*, a rod; N.L. masc. n. *Cronobacter*, a rod that can cause infection in neonates) is a recent new genus to accommodate the biogroups of "*Enterobacter sakazakii"* [1].

Over the years, members of the genus *Enterobacter* have confounded commercial systems more than most of the other genera in the *Enterobacteriaceae* because of the heterogeneity within several of the species. In 1980, the identification of "*Enterobacter sakazakii*" was defined as a novel species by Farmer et al., however the existence of divergent biogroups at the time suggested that these organisms may represent multiple species [2]. Thus, in 2008, *Cronobacter* was given as a valid genus name to accommodate the 16 biogroups of the emerging opportunistic pathogen known formerly as "*Enterobacter sakazakii*" [1].

The genus *Cronobacter* now includes 5 valid species: *C. dubliniensis* (formerly biogroups 6, 10, and 12), *C. malonaticus* (biogroups 5, 9, and 14), *C. muytjensii* (biogroup 15), *C. sakazakii* (biogroups 1-4, 7, 8, 11, and 13), and *C turicensis* (biogroup 16). All of these species have been associated with human disease with *C. sakazakii* described as the most common identified species of this genus in the clinical laboratory. One major characteristic of these species is their association with contamination of milk powder and, consequently, powdered infant formula which represents a significant health risk to neonates [3].

The biochemical characteristics that define the genus are oxidase-negative, catalase-positive, lactose fermenting, facultatively anaerobic gram negative rods with a distinctive yellow colony pigment. Commonly used biochemical tests can be utilized to separate the *C. sakazakii* species complex from the common *Enterobacter* species (**Table 1).**

Table 1. Major phenotypic characteristics to differentiate *Cronobacter sakazakii* species complex from the common *Enterobacter* species.

	Enterobacter aerogenes	*Enterobacter cloacae*	*Cronobacter sakazakii* species complex
Characteristics:			
LDC	P	N	N
ADH	N	P	P
ODC	N	P	P
Acid from	P	P	N
Yellow pigment	N	N	P

Abbreviations: N, <10% positive; P, >90% positive; LDC, lysine decarboxylase; ADH, arginine dihydrolase; ODC, ornithine decarboxylase.

Although sequencing of the 16S rRNA gene is a useful method to separate among the *Cronobacter* species, biochemical tests can also be done for this separation**.** It is important to realize that most of the commercial assays for gram negative species identification do not separate the *Cronobacter* species. Unless specific attention is paid to the biochemicals described in **Table 2**, a phenotypic identification of an "*Enterobacter sakazakii*" would be more appropriate to report as "*Cronobacter (Enterobacter) sakazakii* species complex".

Table 2. Major phenotypic characteristics to differentiate among the *Cronobacter* species

	Cronobacter species			
	sakazakii	*malonaticus*	*turicensis/ muytjensii*	*dubliniensis*
Indole	N	N	N	P
Carbon utilization:				
Dulcitol	N	N	P	N
Malonate+	N	P	P	P

Abbreviations: N, <10% positive; P, > 90% positive.
+Using malonate phenylalanine broth.

Reference laboratories are available to provide sequence comparison analysis testing to help validate the identification of the *Cronobacter* species or other microbial pathogens when necessary. Although the NPHL does not routinely provide this service, sequencing is available at UNMC to identify microbial pathogens for research purposes. For additional information on the availability of this service, contact Dr. Iwen at 402-559-7774.

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Certification Maintenance Program and Board of Certification

By Linda Fell, MS, MLS(ASCP)^{CM}SH^{CM}

As you know, the ASCP Board of Registry (BOR) and the ASCLS-sponsored National Credentialing Agency (NCA) have merged into the Board of Certification (BOC). This represents great strides for the clinical laboratory science profession!

The merger has resulted in new credentials for medical technologists (MT), clinical laboratory scientists (CLS), medical laboratory scientists (MLS) and medical laboratory technicians (MLT). These credentials are based on the requirement for maintenance of certification through continuing education. The new program is called the Certification Maintenance Program (CMP) and uses the subscript (ASCP)^{CM}.

NCA certification has always required minimum hours of continuing education in order to keep the CLS(NCA) credential current. The BOR adopted similar requirements for MT, MLT and categorical certification earned after January 1, 2004. All current NCA certificants and all BOR certificants holding MT^{CM} or MLT^{CM} credentials are now entitled to use MLS(ASCP)^{CM} or MLT(ASCP)^{CM}. All new graduates who take the BOC examination will be granted these credentials with a time-limited certificate (3 years).

If you were NCA-certified and did not keep current with this certification, you are no longer certified as a MT or CLS. If you were NCA-certified but did not keep your certification current, but were also certified by ASCP before 2004, then your MT(ASCP) credential is valid.

If you earned the MT(ASCP) or MLT(ASCP) credential before 2004, you can continue to use it. However, it does not signify that you have actively kept current with professional updates and issues by participating in continuing education. Thus, it is encouraged that you recertify in order to use the new MLS^{CM}/MLT^{CM} credential.

For complete information, go to http://ascp.org/certification/cmp. Scroll to Step 1 and link to the CMP Information Booklet to see the most current information.

In the second paragraph of the above webpage, there are two links: click on them for information on Voluntary CMP and to learn about the status of credentials.

It will be much better for our profession if all practitioners bear the same credential. Since new graduates will be certified as MLS^{CM}/MLT^{CM}, 'seasoned' practitioners are encouraged to take this opportunity to become MLS^{CM}/MLT^{CM}-credentialed also.

"You're serious about your career and your profession. That's why you want to make sure you can document your competence by participating in the ASCP Board of Certification (BOC) Certification Maintenance Program (CMP). When you add the initials (ASCP)^{CM} after your name, your colleagues will know that you are committed to the highest standards of your profession". - ascp.org

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using mass spectrometry is also being developed by the research group to accurately detect and identify bacterial select agents. All of these methodologies are being developed for deployable clinical and field applications in collaboration with federal partners such as the CDC, Department of Defense, and Association of Public Health Laboratories.

The types of studies described in this report rely on a large collection of diverse strains of *F. tularensis*. Personnel at the NPHL, in collaboration with research groups at UNMC and in consultation with private and public sentinel laboratories in the state, provide the strains necessary for these ongoing research efforts.

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Nebraska Public Health Laboratory

University of Nebraska Medical Center
985900 Nebraska Medical Center
Omaha, Nebraska 68198-5900

Mailing Address

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IN THIS ISSUE

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Editor-in-Chief, Peter Iwen, PhD, D(ABMM) E-mail: piwen@unmc.edu
Editor, Karen Stiles, MT(ASCP)SM E-mail: kstiles@unmc.edu

Please direct suggestions, questions, or comments to: Karen Stiles, Editor, NPHL Newsletter, 985900 Nebraska Medical Center Omaha, NE 68198-5900 or kstiles@unmc.edu.