# Nebraska Public Health Laboratory Newsletter

A publication of the Nebraska Public Health Laboratory (NPHL) at the University of Nebraska Medical Center
Spring 2001

## The NPHL Takes to the Road

*by Steven Hinrichs, M.D., Director*

A national effort is underway to rebuild the public health communication and reporting infrastructure at the state level. The goal is to improve the detection and prevention of diseases of public health concern through improving communications with community hospital and independent microbiology laboratories. Much of the effort will be directed toward detection of biological agents used in terrorist activities. We recognize that in the past, little information came back to the laboratories or physicians sending samples to the NPHL and that the process for submitting samples was cumbersome and resulted in expense to the submitter.

As a result, the NPHL has initiated a project with the support of the CDC to develop a mechanism for increasing the coordination of laboratory testing for organisms of public health concern. Our goal is to visit every microbiology laboratory in Nebraska to identify needs and concerns regarding the detection and handling of bioterrorism associated organisms or naturally occurring pathogens of public health interest. Standard protocols will be made available based on the interest and requirements of the laboratory. As part of this effort, we will offer educational opportunities such as workshops and teleconferences in different parts of the state. We will also assist in identifying the availability of courier systems to reduce the cost of submitting specimens.

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## NPHL Arbovirus Surveillance Activities

*by Tony Sambol*

Through an agreement with the Nebraska Health and Human Services System (NHHS) and the Centers for Disease Control and Prevention (CDC), the Nebraska Public Health Laboratory (NPHL) has begun to develop diagnostic laboratory services for arbovirus detection. This activity is part of the CDC Cooperative Agreement for West Nile Virus Surveillance and Epidemiological Project that was announced by Nebraska State Medical Entomologist, Dr. Wayne Kramer. During the year 2000 mosquito season, Dr. Kramer directed the statewide collection of surveillance samples for arbovirus screening which included collection of both sera from chicken flocks and mosquitoes from targeted areas. At the NPHL Special Pathogens Laboratory, sera from "sentinel" chickens were tested for antibodies to arboviruses. Additionally, mosquito specimen "pools" (50 mosquitoes per pool) will also be tested for the presence of arbovirus RNA. It is believed that certain areas of Nebraska are at higher risk for the spread of arboviral diseases due to collection of water used in irrigation.

*"Arbovirus Surveillance"*

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## Regulation and Licensure Update for Reporting and Control of Communicable Diseases

*by Peter Iwen*

The latest revision of the state guidelines for the reporting and control of communicable diseases was recently released by the Nebraska Department of Health and Human Services (NHHS). This revision was published in Nebraska Regulations Title 173- "Communicable Diseases" and the effective date was 01-28-01. The current revision reflects the increased awareness by public health officials of the potential for bioterrorism (BT) activities involving infectious disease agents. Within the Title 173 document, Section 1-003.01, Immediate Reports lists the diseases, poisonings and organisms that each laboratory should report immediately to their local health authority. Category B is further defined as: "Clusters, outbreaks or epidemics of any health problem, infectious or other, including food poisoning, influenza or possible bioterroristic attack; increased disease incidence beyond expectations; unexplained deaths possibly due to unidentified infectious causes; any unusual disease or manifestations of illness." (See List 1 on page 2.) A subset of the Category "B" list of agents includes pathogens that are food or water-borne. (See List 2 on page 2).

In addition, Title 173, Section 1-003-01B states that immediate reporting should take place for "Clusters, outbreaks, or unusual events, including possible

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## NPHL Bioterrorism Preparedness Laboratory Update

*by Tony Sambol*

The national effort to become prepared for activities related to bioterrorism should be familiar to everyone working in microbiology laboratories. Many articles, meetings, and seminars have addressed this topic in the past, and will continue to do so in the future. Personnel at the NPHL are working to develop the statewide Laboratory Response Network (LRN) that includes training, planning and preparation for the rapid detection and identification of any biothreat organism that might be encountered. As part of this preparation, a "laboratory self-assessment" was conducted at the Bioterrorism Preparedness Laboratory (BPL) and submitted to the Director of the LRN. Based on this "laboratory self-assessment", the BPL was recently granted a status upgrade to a LRN Level C Laboratory for all biothreat agents except *Clostridium botulinum* toxin testing. This status change was made after new training was obtained and after the addition of equipment and improvement of safety features for the laboratory.

Last October, personnel from the NPHL attended a week-long laboratory training session conducted at the Georgia Public Health Laboratory. This "hands-on" laboratory training was conducted by members of the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), and the National Laboratory Training Network (NLTN). Training focused on procedures used at the LRN Level B/C laboratories for identification of *Bacillus anthracis, Yersinia pestis, Francisella tularensis,* and *Brucella* spp.

To expand on the traditional culturing and testing methods used for identification of biothreat agents, personnel at the NPHL have also received training in molecular diagnostic assays developed by the CDC for the LRN Level C Laboratories. Part of the training involved participation in a CDC sponsored multi-site validation study for *Y. pestis* using "real-time" PCR methodology. As they are developed by the CDC, the BPL will participate in additional multi-site validation molecular studies for the identification of other biothreat agents. The significance of the B/C designation means the NPHL will be receiving new assays and will be implementing them into testing protocols when requested. In addition to preparing the NPHL to evaluate agents of bioterrorism, the availability of reagents and protocols to test special pathogens will strengthen the capabilities of the NPHL to aid laboratories throughout the state to handle those pathogens that can cause naturally occurring infections.

One of the most important issues for laboratories throughout the state is to emphasize the collection of either fluids or tissues for diagnostic purposes. Too often physicians collect specimens on swabs even when fluids, such as abcess pus is available. Continued education is needed to make

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*"Regulation and Licensure Update"*

bioterroristic attacks." It is hard for laboratory technologists to be aware of the clinical aspects of this regulation, and the local Laboratory Director should be consulted.

The Nebraska Bioterrorism Preparedness Laboratory would like you to be aware of other conditions that are in the CDC's Category "B" list. (see List 3 below). The occurrence of these agents/ diseases would be highly suspicious as BT associated. We encourage you to also report these immediately to your local health authority.

Please take the time to incorporate these new organisms and diseases into your notification protocol. A draft protocol using NCCLS/CAP guidelines is available from the NPHL by calling Tony Sambol at (402) 559-3032. Rapid response and reporting is critical to the detection and containment of microorganisms causing diseases that could result from a naturally occurring infection or a

**List 1. Category B diseases caused by bioterrorism activity**

Anthrax (*Bacillus anthracis*)
Botulism (*Clostridium botulinum*)
Brucellosis(*Brucella* spp.)
Q fever (*Coxiella burnetii*)
Plague (*Yersinia pestis*)
Smallpox (Variola major)
Tularemia (*Francisella tularensis*)
Glanders (*Burkholderia* {*Pseudomonas*} *mallei*)
Marburg virus Melioidosis (*Burkholderia* {*Pseudomonas*} *pseudomallei*)
Staphylococcal enterotoxin B intoxication
Venezuelan equine encephalitits

**List 2. Subset Category B of food or water-borne pathogens)**

*Salmonella* species
*Shigella dysenteriae*
*Escherichia coli* O 157:H7
*Vibrio cholerae*
*Cryptosporidium parvum*

**List 3. Other Category B organisms and diseases**

Western equine encephalitis
Eastern equine encephalitis
Ricin Toxin Exposure

## Lead Testing and the Public Health Lead Screening Program

*Denise M. Timko, MT(ASCP)SC and Douglas F. Stickle, Ph.D.*

Lead Exposure

Lead is a naturally occurring element that has found use in metal materials throughout history. The atomic symbol for lead, Pb, is derived from the Latin word *plumbum*, for the weight used in a surveyor's plumb line. Lead can be found in concentrated ore deposits in the earth, and its compounds are thus often a detectable component of the natural environment in most regions of the world. As such, there is low-level exposure of humans to lead, as evidenced by the fact that lead can also be detected in blood and tissue in virtually everyone. There it may be regarded as a contaminant, in the sense that lead has no known physiologic or metabolic role. Unfortunately, lead is not chemically inert, and its reactions with organic molecules, both specific and nonspecific, can lead to toxic effects at high and even relatively low levels of exposure.

Toxic Effects of Lead

Specific effects of lead include inhibition of enzymes in the heme synthesis pathways used to produce functional hemoglobin, the oxygen-carrying molecule that is packaged in red blood cells. Thus, one hallmark effect of chronic toxic lead exposure is anemia. Nonspecific toxic effects of lead are also deleterious to health. Like other metals, lead can bind nonspecifically to proteins. It is for this reason that excess lead exposure can lead to buildup of lead in tissues. Lead binding to protein can disrupt intramolecular bonds that are necessary for the proper structure and function of the protein. And, because circulating lead is excreted through the kidney, kidney function is particularly susceptible to the toxic effects of this metal, in either chronic or acute overexposure. The nervous system is also susceptible to deleterious, nonspecific effects of lead that can cause a decrease in nerve conduction velocity. Importantly, chronic exposure even to relatively low levels of lead can have negative effects on various aspects of development in children (such as hearing and growth). Acute, high-level exposure to lead can cause encephalopathy and death.

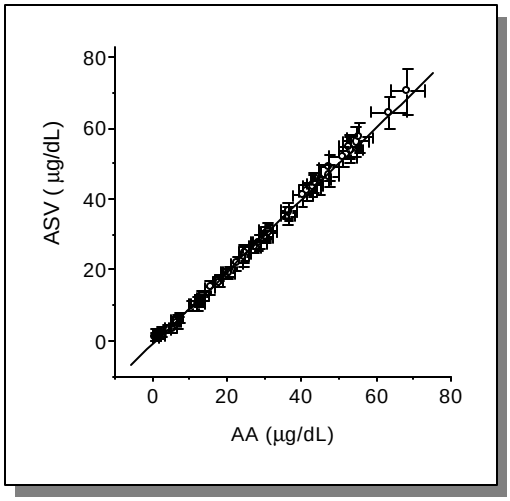


**Figure 1**. Comparison of national survey results for ASV and AA measurements of blood lead.

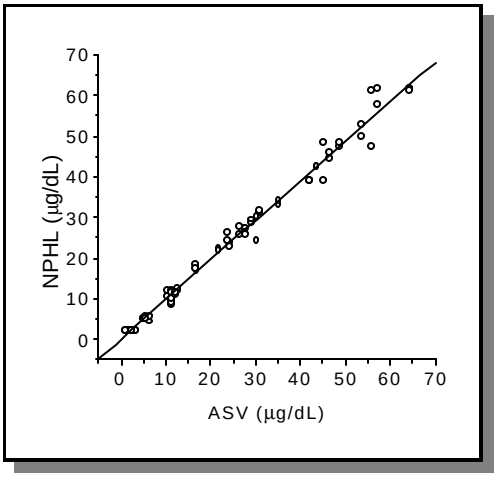


**Figure 2**. Comparison of NPHL survey results using ASV and national survey results using ASV.

Acute toxicity due to lead exposure requires treatment. In addition to medical management of kidney failure, treatment consists of chelation therapy. Chelation is the removal of metal ions by a medication that has a high affinity for the metal. A number of chelating agents are available to treat lead toxicity. Chelation therapy can rapidly remove lead from the blood, and thereby prevent any further deposition into tissues. However, the therapy has less effect on the rate of removal of lead from tissues. This is evidenced by the "rebound" of circulating blood levels to higher values after cessation of chelation therapy. Nonetheless, chelation therapy is an important component of management in cases of acute toxicity. Chelation therapy is recommended for circulating blood levels of > 45 µg/dL (2.2 µM). Therapy is monitored by documenting increased rates of lead excretion in urine.

Public Health Lead Screening Programs

The goals of public health lead screening programs are to identify individuals who have a higher than expected blood lead level, and to find and remove sources of exposure. Exposure is primarily due to the former use of lead in household paints. In recognition of this issue, and based in part on evidence of undue exposure among children, the Centers for Disease Control (CDC) in 1997 issued specific guidelines for public health lead screening programs (see http:// www. cdc. gov/ nceh/ lead/ guide/ guide97. htm)). This document, and ongoing summaries of the statistics and demographics of lead screening given periodically in the CDC's *Morbidity and Mortality Weekly Report*, provide detailed history of the public health concern and intervention in lead exposure. The guidelines define elevated blood lead concentration as >10 µg/dL (480 nM), and recommend that screening by public health programs be targeted to those children most vulnerable to undue exposure. A CDC publication specifically addresses targeting of Medicaid patients as a high-risk group (see http:// www. cdc. gov/

mmwr/ PDF/ RR/ RR4914. pdf).

Measurement of Lead in Whole Blood

Because lead in blood is concentrated in erythrocytes, screening tests are conducted using whole blood rather than serum or plasma. Blood lead is conventionally analyzed by one of two methods, either atomic absorption (AA) or anodic stripping voltammetry (ASV). AA is analogous to simple absorbance spectrophotometry. The sample is volatilized by a furnace apparatus. Absorption of light of a specific wavelength characteristic of lead is measured and compared to a standard curve from which the sample lead concentration is determined. ASV is based on electrochemistry. As current is passed through a circuit that includes the liquid sample, the instrument's anode tip becomes plated by metals from the sample. Reversing the process strips the anode of specific metals at specific voltages, and the current generated at the characteristic voltage for lead enables determination of the original lead concentration by comparison to a standard curve. AA has the advantages that it can be automated and requires only infrequent calibration if properly maintained. The capital investment and maintenance required for AA is significantly greater than that for ASV, however.

Analytical proficiency surveys provide a national peer review measure of quality control for lead testing, and also provide excellent data for comparison of AA and ASV methods. Both AA and ASV yield accurate and reliable measurements of lead concentrations, as evidenced by data from the College of American Pathologists (CAP) surveys and by the Wisconsin surveys (a program that is administered by the U.S. Public Health Service). As shown in **Figure 1**, lead concentrations determined by AA and ASV are statistically indistinguishable. Data from NPHL are indistinguishable from national data (**Figure 2**). The "coefficient of variation" (CV), a measure of analytical precision, is between 5% and 10% for both ASV and AA at all but low lead concentrations.

An individual proficiency survey result (a singleton measurement of a sample) will meet CDC acceptability criteria if its value is *a*) within ± 10% of the mean, or *b*) within ± 4 µg/dL from the mean, whichever range is greater.

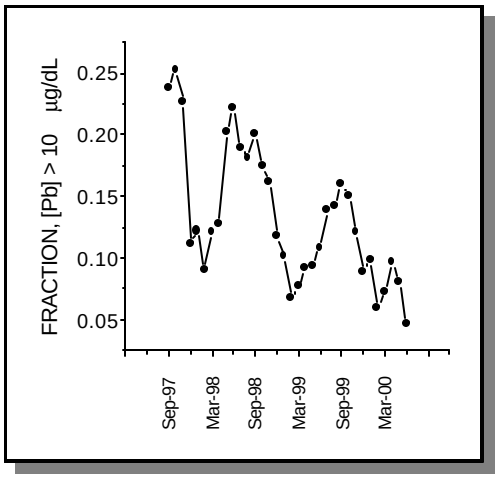


**Figure 3**. Cycle of positive lead screens.

For example, given a mean value of [Pb] = 10 µg/dL (the cutoff value for a positive screening test, and for which the CV is approximately 10%), 95% of the survey results will typically be between 8 and 12 µg/dL – a difference of 50% between low and high values, but well within the acceptable range of 10 ± 4 µg/dL. It is important to note that such differences are unlikely to have any distinct medical significance. Such uncertainty is an inherent aspect of any analytical procedure, and a known component of screening procedures using singleton measurements with a fixed (and usually conservative) cutoff value for positive results.

Results from the Lead Screening Program in Douglas County

Public health-sponsored lead screening has been conducted by Douglas County for more than three years. Data provided by Douglas County for the fraction of positive screens ([Pb] > 10 µg/dL) from among all screening tests is shown in **Figure 3**. Two aspects of these data are striking. First, there is a yearly cycle in the number of positives, with higher positive rates in late summer and lower positive rates in late winter. This presumably reflects increased exposure of children to lead during the outdoor summer months. Second, the data clearly demonstrate a continuous decrease in the screening test positive rate since the inception of the program. A national trend of decreasing positive rates in lead screening tests has been noted by the CDC (Update: Blood Lead Levels -- United States, 1991-1994. *MMWR* 1997; 46(7):141-6) and was attributed in part to programmatic success in the removal of lead from common environmental sources of exposure. Additionally, the trend in Douglas County may in part reflect an expansion of the program to include a greater fraction of low prevalence populations. The absolute numbers of screenings has increased over this period from approximately 200/month to approximately 500/month, and the demographic characteristics of the expanded population are likely also to have broadened during this time.

Importance of Proper Specimen Collection

Proper specimen collection is an essential component of the success of the lead screening program. We all recognize that collection of blood from children can be a difficult task! Nonetheless, only a small volume is required (200 µL). It is important that the sample be properly anticoagulated after collection by mixing of the contents of the collection with the anticoagulant in the collection tube. Instructions for proper collection techniques are available from NPHL. A detailed discussion regarding collection procedures is also available in the CDC lead screening program guidelines. As with all patient specimens, it is good practice to send the specimen to the laboratory for testing without delay.

The Future of Lead Screening

A successful screening program will eventually result in eliminating sources of childhood lead exposure, whether in the home or playground. Development of a portable blood lead analyzer for use outside of the laboratory could greatly assist screening efforts. Interventions to reduce lead hazards include improving the quality of housing (e.g., improving standards for rental property, maintenance and cleaning), and programs for better education and awareness about lead. Citizens and public health agencies must work together toward these goals and to build upon the successes achieved to date.

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The arbovirus family consists of over 500 viruses that are transmitted by arthropod insects, most commonly the mosquito. Approximately 150 viruses in this group are associated with human illness. Symptoms of arbovirus infection range from a mild febrile illness with headache, to encephalitis. Severe infections can cause lingering neurological sequelae, which can rarely result in death of the infected individual. Arboviruses found in Nebraska include the St. Louis encephalitis (SLE) and Western equine encephalitis (WEE) viruses. According to the CDC, there have been 27 laboratory-confirmed human cases of WEE and 14 cases of SLE in Nebraska since 1964. Dr Kramer using funds provided by the CDC, detected SLE in two mosquito specimen pools out of 1,359 collected in Scottsbluff County in 1994 and 1995. In 1995, 36 out of 2,788 mosquito specimen pools tested positive for WEE. Thirty of the 36 positive mosquito pools were collected in Scottsbluff County, with four from the City of Norfolk and two from the City of Grand Island.

To expand on arbovirus surveillance, West Nile virus (WNV) has now been included in the testing done in Nebraska. This virus, which had been previously restricted to Africa, West Asia and Southern Europe, was detected in the Summer of 1999 for the first time in North America in the New York City area. It has now been found in many areas of the Northeastern United States. Crows, house sparrows and other birds are susceptible to WNV and can act as carriers of the virus once infected. Epidemiologists are concerned that WNV may spread further west in the US via migratory birds within the next few years.

In the future, arbovirus surveillance for detection of SLE, WEE, and WNV activities in Nebraska will include the following: 1. active bird surveillance monitoring of chickens located throughout the state; 2. active surveillance sampling of mosquito populations located in higher risk areas; 3. the evaluation of dead birds; and 4. enhanced passive human surveillance by alerting health care providers to monitor for cases of encephalitis and aseptic meningitis. The combined efforts of these groups will assist various programs within the NHHS network to build upon the existing infrastructure of arbovirus surveillance. It is hoped that enhanced planning and development of a more effective arbovirus surveillance and response program will aid in the prevention and control of arboviral outbreaks within Nebraska.

## *Salmonella* Serotyping Underway at the NPHL

*by Tony Sambol*

The NPHL recently completed the first full year of *Salmonella* serotyping. Prior to 2000, no serotyping had been performed in the State of Nebraska. The CDC estimates that from 1 to 4 million cases of salmonellosis occur each year in the U.S. with approximately 500 deaths. About 40,000 of these infections are confirmed by culture, with most isolates serotyped at state public health laboratories. The CDC tests about 1,000 problem isolates that are referred from the state public health labs each year. Serotyping is an important epidemiological tool to determining the source of disease outbreaks .

The salmonellae are first classified or serogrouped based on their somatic (O) antigens, and then serotyped based on the flagellar (H) antigens. To date, there are 2,435 serovars described by the CDC, with 20 to 35 new serotypes described each year by the World Health Organization (WHO). Currently, the CDC recognizes two species of *Salmonella*, *S. enterica* and *S. bongori*. *S. bongori* contains 18 serovars, and *S. enterica* contains over 2300 serovars, divided among six subspecies.

Most *Salmonella* serotypes isolated from humans belong to *Salmonella enterica* subspecies with the most common serotypes in the U.S. being *Salmonella* serotype Enteritidis and *Salmonella* serotype Typhimurium. To serotype the salmonellae requires the availability of more than 250 O and H typing antisera. The CDC maintains an inventory of the antisera needed to serotype the 100 most common isolates found in the U.S., and these reagents are available for the state public health laboratories to use.

Serotyping in 2000 was performed on isolates sent to the NPHL from 14 different hospital and private laboratories in the state. The NPHL received 184 isolates from human sources. Of these, 179 have been identified and fall into 8 different serogroups and 42 serotypes, some which have rarely been reported in the Midwest. The top ten *Salmonella* serotypes seen in Nebraska for 2000 are shown in Table 1. Clinical laboratories are encouraged to continue submitting all isolates of *Salmonella* to the NPHL for serotyping. Nebraska has had excellent cooperation from the various microbiology laboratories in the state for this activity. The test is performed without charge and the only requirement for processing of the isolate is completion of the "Special Microbiology Requisition Form". In the future, the NPHL Newsletter plans to report *Salmonella* serotypes by county, so listing the county of suspected origin of the *Salmonella* isolate on the requisition will be helpful to generate this data.

**Top ten *Salmonella* serotypes for Nebraska in year 2000**

| *Salmonella* serotype | Serogroup | Number |
|---|---|---|
| 1/ Typhimurium var. Copenhagen | B | 35 |
| 2/ Typhimurium | B | 32 |
| 3/ Enteriditis | D1 | 30 |
| 4/ Heidelberg | B | 12 |
| 5/ Newport | C2 | 11 |
| 6/ Montevideo | C1 | 7 |
| 6/ Paratyphi B var. Java | B | 7 |
| 7/ Derby | B | 4 |
| 8/Agona | B | 3 |
| 8 /St. Paul | B | 3 |

# Nebraska Public Health Laboratory

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*"NPHL Takes the Road"*

The process to build a national network of laboratories is referred to as the Laboratory Response Network (LRN). In Nebraska, current plans call for participation by at least one laboratory in each of the six health planning regions of the state. These laboratories will be designated as Level A. A Laboratory Advisory Council (LAC) will be established composed of the Laboratory Director, or designee, from each of the LRN Level A laboratories. Our long term goal is to develop a statewide electronic reporting system that is tied into the office of the State Epidemiologist. Implementation of a statewide laboratory communication system will begin with an auto-fax messaging system from the NPHL to the designated LRN Level A labs. When fully mature, this communication system will involve networking by the telephone, e-mail and finally laboratory-based electronic reporting involving a "real-time" electronic connection. Our system will be evaluated by several criteria specified by the CDC to demonstrate that it has met the standards for a state-wide surveillance system. We anticipate that the system developed in Nebraska will become a "model system" for other states to follow. We look forward to working with the laboratories through out the state and thank you all in advance for your

***EDUCATIONAL MEETING NOTICE***

**"Resistance in the 21st Century: An Update on Antibiotic Susceptibility"**

Harvey Homes, Ph.D.
Chief of Diagnostic
Microbilogy Section at CDC.

**June 5, 2001 9:00 to 12:00**
**At UNMC**

Registration fee $30.00

**Registration Required**
Call Kathy Talmon
402.552.2106

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