Nebraska Public Health Laboratory Newsletter

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

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Connecting to the Front Lines of Public Health.

by Steven Hinrichs, MD, Director, NPHL

The Nebraska Public Health Laboratory (NPHL) has named Josh Rowland, MBA, MT(ASCP) to assume the position of State Training Coordinator. The purpose of the State Training Coordinator is to provide up-to-date information on public health laboratory testing issues and to serve as a link to laboratory personnel throughout the state, allowing NPHL to connect to what we believe are the front lines of public health. Josh takes over the State Training Coordinator position from Kathy Talmon, MT(ASCP), who has previously served in this position. Kathy will continue to serve as liaison with the CDC and will be responsible for tracking all issues and specimens that are referred to CDC laboratories.

Josh comes to us from Kearney, Nebraska, where he worked for the past six years at the Kearney Clinic as a Medical Technologist. In addition to graduating from UNMC's Medical Technology program, he also has a Bachelor of Science degree in biology from the University of Nebraska at Lincoln and a Master of Business Administration degree from the University of Nebraska at Kearney.

Josh's responsibilities will include expansion of the laboratory training program for biothreat, radiological, and chemical agents. Within the past three months Josh has already visited over 50 laboratories throughout the state and is very pleased with the reception he has been given. Please consider Josh as your advocate and representative and feel free to call or e-mail him for any issues you may have (402-559-6070, jrowland@unmc.edu).

Understanding Vancomycin-intermediate (VISA) and Vancomycin-resistant (VRSA) *Staphylococcus aureus.*

by Paul Fey, PhD, Associate Director, NPHL

Vancomycin has been the "last resort" antibiotic for methicillin-resistant *Staphylococcus aureus* (MRSA) infections for many years. Enterococci acquired vancomycin-resistance (typically mediated by the resistance gene *vanA*) in the 1980's. Since then, scientists and physicians have predicted that staphylococci would also acquire the same resistance mechanism, since it is known that these two organisms exchange genes through conjugative plasmids. Fortunately, staphylococci did not acquire that resistance mechanism until recently.

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Select Agents and Toxins: What is the Role of the Clinical Laboratory?

by Peter Iwen, PhD, Associate Director, NPHL

"For violation of 42CFR Part 73, the Office of Inspector General may impose a penalty of not more than $250,000 in case of an individual, and not more than $500,000 in the case of any other person."

Times certainly have changed for clinical microbiology! In the past, the laboratory focus was on specimen handling, organism identification, and antimicrobial susceptibility testing. Now in addition to these, other considerations have emerged as evident by the new terms now used by the microbiologist such as security risk assessment, bioterrorism, biosafety, select agents and toxins, civil money penalties, EA-101 form, and such. What does all this mean for the average clinical microbiologist? Is my laboratory in compliance with federal mandates? Presented is a brief overview to help answer these questions.

Select agents and toxins are defined as "biological agents or toxins deemed a threat to human, animal or plant health and to animal or plant products." Effective on February 7, 2003, a new federal law was established to regulate the possession, use, and transfer of these agents within the United States. This regulation, referred to as 42 CFR Part 73 (hereafter called Part 73), implements the provisions set forth in the Public Health Security and Bioterrorism Preparedness Act of 2002, which became a federal law on July 12, 2002.

The law affects academic institutions, biomedical centers, commercial manufacturing facilities, research facilities, and yes, clinical and diagnostic laboratories. The major focus of this law is the requirement for registration of a facility that possesses, uses, or transfers a select agent or toxin. Fortunately, for the most part clinical laboratories are exempt from many of the provisions of Part 73, including the need for registration. This exemption applies when the only activities conducted by the laboratory "concern select agents or toxins that are contained in specimens or in isolates from specimens presented for diagnosis, verification, or proficiency testing" (**Table 1**). Although exempt, all clinical laboratories must however, adhere to the reporting and disposal requirements as described in the Part 73 law. These requirements state that, (1) upon identification of a select agent or toxin as the result of diagnosis or verification the result must be reported immediately to the HHS Secretary and to the county and/or state health department, (2) the specimen and isolate containing a select agent or toxin must be transferred to a registered facility or destroyed on-site by a method sufficient to cause inactivation, and (3) a record of the identification and transfer or destruction must be prepared and filed. An abbreviated procedure

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West Nile Virus Laboratory Issues

by Jodi Garrett, MT (ASCP)SM, Amy Armbrust, MT (ASCP), and Steven Hinrichs, MD

West Nile Virus has hit the state of Nebraska with a vengeance and a number of public health efforts are in effect. The NPHL offers the IgM antibody screening test (**Figure 1**) that utilizes West Nile Virus (WNV) reagents provided by Focus Technologies. The role of the NPHL is to assist in determining the severity and extent of disease throughout the state. The NPHL is working with the state program that provides WNV testing at no charge. Once the full extent of WNV disease is determined, the "no charge" program may end. A termination date has not yet been determined.

A number of questions have arisen about the testing procedures and the interpretation of the index value that is reported. To assist you in responding to telephone calls from medical staff, the following explanations have been provided:

What's the difference between a laboratory presumptive positive and a CDC confirmed result? All IgM screening tests, including the Focus Laboratories test used at NPHL incorporate a WNV antigen that in some cases will also detect antibodies to other flaviviruses including St. Louis Encephalitis Virus (SLE). Therefore, to rule out these other possibilities, a number of confirmatory procedures may be used, including determination of specific antibodies to SLE. A monitoring program is in place for SLE in the state and at the present time there is no activity, therefore, SLE infection in humans is unlikely. The State Epidemiologist, Dr. Tom Safranek may request some cases to be referred to the CDC when SLE is a consideration. Currently, positive results from the NPHL should be considered presumptive.

What does an indeterminate result mean? In some situations a cross reaction can be due to heterophile antibodies, in which case the result is reported as indeterminate. Heterophile antibodies are non-specific antibodies that react with antigens in the test kit. If the clinical condition warrants, it may be appropriate to obtain an additional sample for retesting.

How long does it take to get results? Although the laboratory initially intended to perform the test only twice a week, the volume has grown so rapidly that it is now performed daily, Monday through Friday. Results are typically available 48 to 72 hours after specimens are received. The NPHL reports results to the state epidemiologist on specimens submitted through the state program.

How many positive human cases of WNV have been identified? The laboratory has reported over 300 positive cases as of August 22 and approximately 20 to 30 new positives are reported each day. Therefore, the total number of positives is rapidly changing and this frequency may increase until Nebraska has its first hard frost this fall.

Why does NPHL only test for the IgM antibody and not IgG. Dr. Safranek does not recommend screening for West Nile IgG antibody at the present time. All requests for IgG will be forwarded to a reference laboratory for testing and will be billed to the submitter.

Does the NPHL provide other surveillance testing for WNV in Nebraska? To date, the NPHL laboratory has tested 1890 pools of mosquitoes. Each mosquito pool consists of 50 mosquitoes (Genus *Culex*). 683 or 36% of the pools have tested positive. Analysis has shown that 18 of the 21 counties tested have positive WNV mosquitoes in them.

How can I send specimens to the laboratory? If your laboratory is not serviced by a regular courier route to the NPHL, specimen transport arrangements may be made by calling NPHL Client Services at 800-334-0459 or 402-559-2440.

NPHL Bioterrorism Testing Update

by Tony Sambol, MA, Assistant Director, NPHL

The Biosecurity and Special Pathogens Laboratory section of the NPHL has the capacity and capability to test for a variety of agents. Techniques include, culture, DNA detection by polymerase chain reaction (PCR), or detection of whole bacteria or biotoxins by an enzyme-linked immunoassay (ELISA) test known as time-resolved fluorescence (TRF) (**Table 1**). As the CDC develops and releases new assays to state public health laboratories, the NPHL will utilize these procedures and accompanying reagents to perform additional assays.

Table 1. Pathogens and select agents that can be tested at the NPHL.

Agent Type	Species
Bacterial	*Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Brucella* spp., *Burkholderia* spp., *Salmonella* spp., *Shigella* spp., *E. coli* O157:H7, *Vibrio cholerae*, *Coxiella burnetti*, *Chlamydia psittaci*, and *Mycobacterium tuberculosis* (multi-drug resistant)
Viral	SARS-associated Coronavirus, Variola virus (Smallpox virus), Monkeypox virus, Vaccinia virus, Varicella-Zoster virus, and Western and Eastern Equine Encephalitis viruses.
Parasitic	*Cryptosporidium*
Toxin	Ricin, *Staphylococcus* enterotoxin B, and *Clostridium botulinum* toxin

Figure 1.

West Nile Virus Antibody IgM Test Requirements

Method:	Capture IgM ELISA	
Availability:	Monday through Friday STAT Testing is not available	
Specimen:	Blood or CSF CSF must be paired with a serum specimen	
Collect:	6.0 ml SST/ red/black clot tube	
Volume:	Serum: 2.0 ml serum or 0.5 ml minimum CSF: 0.5 ml minimum	
Transport:	Refrigerated. If sample cannot be transported to the laboratory the same day as collected, please centrifuge and separate serum from cells. Arrangements for specimen Transportation can be made by calling NPHL at 800-334-0459 or 402-559-2440. Frozen specimens are acceptable.	
CPT:	Serum:	86317
	CSF/Serum:	86317 x 2
Reference Serum:	Negative:	<0.9 Index
	Equivocal:	≥ 0.9 - ≤1.1 Index
	Positive:	>1.1 - >15.0 Index
Reference CSF:	Negative:	<0.9 Index
	Equivocal:	≥ 0.9 - ≤1.1 Index
	Positive:	>1.1 - >15.0 Index

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Understanding VISA/VRSA

The overwhelming majority of *S. aureus* isolates are vancomycin-susceptible, having an MIC in the range of 0.5-2 μg/ml. Vancomycin-intermediate *S. aureus* (VISA) isolates were first described in Japan in 1997 (Hiramatsu *et al.*, 1997) and subsequently isolated in the United States later that same year. These isolates have a vancomycin MIC between 8-16 μg/ml. The mechanism of resistance in VISA isolates is not completely understood but involves the thickening of the peptidoglycan layer. "True" vancomycin-resistance within *S. aureus* (vancomycin-resistant *S. aureus*-VRSA) was not described until 2002 (Chang *et al.*, 2003). Since that time, two unrelated *S. aureus* strains have been isolated in the United States. These isolates have vancomycin MICs ≥32 μg/ml and the resistance is mediated by *vanA* (encoded on a conjugative plasmid). It is important to note that the vancomycin-intermediate result in VISA isolates is not mediated by the same resistance mechanism as found in the enterococci (*vanA*).

Detection methods

Most, but not all, routine automated susceptibility testing methods will detect VISA. These methods include both conventional Microscan® panels (Dade MicroScan, West Sacramento, CA), Vitek® (bioMerieux, Hazelwood, MO.), Vitek® 2, and E-test (using a 0.5 MacFarland suspension; AB Biodisk, Piscatway, NJ) (Marlowe *et al.*, 2001; Tenover *et al.*, 1998; Walsh *et al.*, 2001). Notably, standard 24 hour disk-diffusion methodology will not detect VISA and is therefore not a recommended (Tenover *et al.*, 1998). Laboratories that rely on disk diffusion are recommended to use the vancomycin agar screen plate (composed of brain-heart infusion agar containing 6 μg/ml of vancomycin) which reliably detects VISA. VISA may appear atypical (small-pinpoint colonies) on standard laboratory media and may take 48 hours to grow. Both VRSA isolates were detected using automated susceptibility testing methods, however, more research is needed to fully determine the best methodology to detect these highly resistant pathogens.

Figure 1 denotes a flow chart for the identification of VISA and VRSA. VISA and VRSA are extremely rare in the United States. There have been fewer than 15 confirmed cases of VISA in the United States and only 2 confirmed cases of VRSA. Consequently, all *S. aureus* isolates that are either intermediate or resistant to vancomycin must be confirmed before reporting. If you have any questions regarding VISA or VRSA, or susceptibility testing in general, please call Dr. Paul Fey at 402-559-2122.

Chang, S., Sievert, D. M., Hageman, J. C., Boulton, M. L., Tenover, F. C., Downes, F. P., Shah, S., Rudrik, J. T., Pupp, G. R., Brown, W. J., Cardo, D. & Fridkin, S. K. (2003). Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. *N Engl J Med* **348**, 1342-1347.

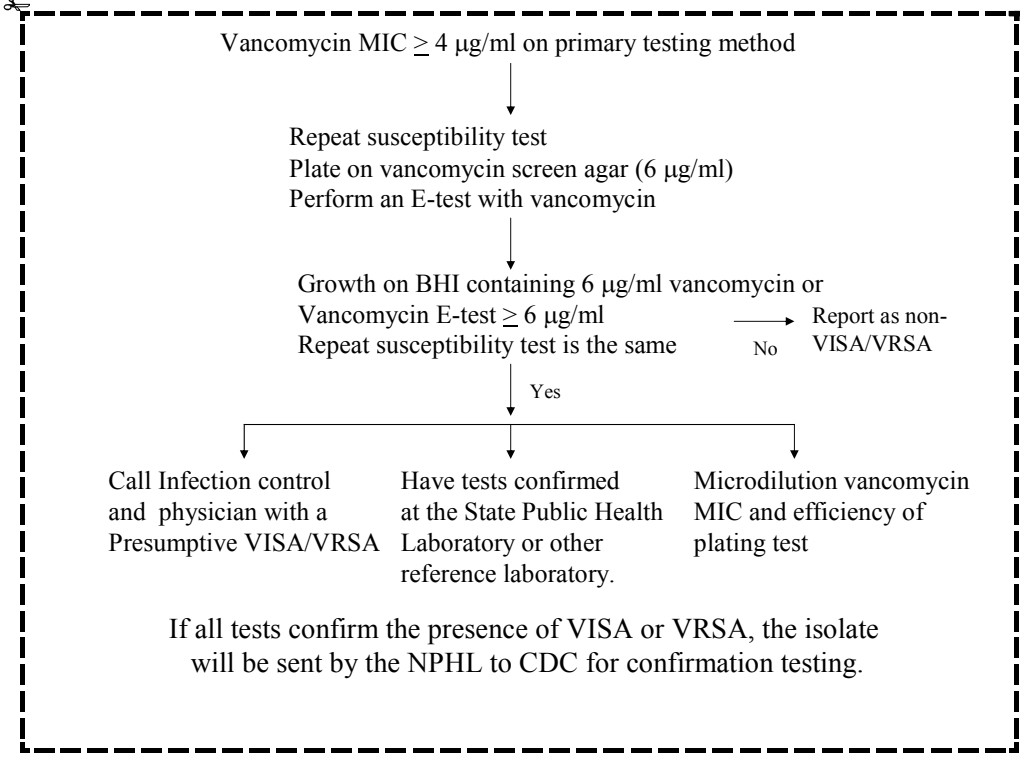
Hiramatsu, K., Aritaka, N., Hanaki, H., Kawasaki, S., Hosoda, Y., Hori, S., Fukuchi, Y. & Kobayashi, I. (1997). Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet* **350**, 1670-1673.

Marlowe, E. M., Cohen, M. D., Hindler, J. F., Ward, K. W. & Bruckner, D. A. (2001). Practical strategies for detecting and confirming vancomycin-intermediate *Staphylococcus aureus*: a tertiary-care hospital laboratory's experience. *J Clin Microbiol* **39**, 2637-2639.

Tenover, F. C., Lancaster, M. V., Hill, B. C., Steward, C. D., Stocker, S. A., Hancock, G. A., O'Hara, C. M., McAllister, S. K., Clark, N. C. & Hiramatsu, K. (1998). Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. *J Clin Microbiol* **36**, 1020-1027.

Walsh, T. R., Bolmstrom, A., Qwarnstrom, A., Ho, P., Wootton, M., Howe, R. A., MacGowan, A. P. & Diekema, D. (2001). Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. *J Clin Microbiol* **39**, 2439-2444.

Figure 1.



(Continued from page 1) ***Select Agents and Toxins***

for the reporting and transfer of a select agent or toxin is described in **Table 2**. To complete a transfer of a select agent or toxin, two CDC forms are required, the CDC form 0.1318 and the CDC form EA-101, copies of these forms are available on the CDC web site (http://www.cdc.gov/od/sap/). The 0.1318 form is used to record that the materials have been transferred or destroyed and the EA-101 form records the transfer to a registered facility. Prior to confirmation of a select agent or toxin such as when a sample (isolate or specimen) is sent to a reference laboratory for diagnosis or verification purposes only, neither an EA-101 form nor 0.1318 form is required. If the reference laboratory identifies a select agent or toxin, the submitting laboratory is immediately notified and it is at this point that the requirements for reporting and transferring the isolate and specimen are implemented.

The NPHL is a select agent and toxin registered facility and will act as the repository for all select agents and specimens on behalf of the state. The Nebraska Health and Human Services System requests that laboratories do not destroy these materials, but they submit these to the NPHL. For additional guidance and/or to request transfer information, contact Dr. Peter Iwen, Responsible Facility Official for the Select Agent Program, at 402-559-7774.

Table 1. Select agents and toxins that are most likely to be detected in the clinical microbiology laboratory.*

Bacillus anthracis	*Burkholderia pseudomallei*
Brucella abortus	*C. botulinum,* neurotoxin producing
Brucella melitensis	*Francisella tularensis*
Brucella suis	*Coccidioides immitis*
Burkholderia mallei	*Yersinia pestis*

*A complete list of select agents and toxins can be found on the CDC web site (http://www.cdc.gov/od/sap/).

Laboratory Assessment

by Josh Rowland, MT(ASCP)

One objective that I have as the State Training Coordinator for the NPHL is to identify the educational/training needs of laboratorians across Nebraska. To fulfill this objective, a laboratory assessment survey has been created and distributed to those laboratorians that recently attended the "Bioterrorism Preparedness Symposium" held in Grand Island, Norfolk, and Lincoln. The assessment will be distributed to the other laboratorians either in person or through the mail over the next couple of months.

This assessment will help personnel at the NPHL understand what laboratory needs exist in Nebraska and assess the means in which educational and training materials should be distributed. The majority of the training materials already supplied to laboratories have been bioterrorism oriented. Future plans include expanding the educational and training programs to offer materials to laboratorians on other subjects of personal importance. In addition, the assessment will be used to identify which training formats are preferred by laboratorians. These formats could include:

1. On-site training
2. Wet workshops at off-site locations
3. Online training via Internet with interactive learning
4. On-site materials/manuals/posters
5. Correspondence courses
6. Teleconference courses
7. Videotaped lectures for self-study
8. Lectures on CD-ROM for self-study

The NPHL plans to provide a majority of the training materials through Internet or videoconferencing. With the large geographical area of Nebraska, we believe that taking advantage of this technology is the best way to reach all laboratorians. Additionally, the NPHL website (www.nphl.org), will become a repository for both current topics and older training materials.

Table 2. Laboratory procedure for the reporting and transfer of select agents and toxins identified in Nebraska laboratories as a result of diagnostic or verification testing.

1. Report results **(immediately)**

Douglas County Health Dept.	402-444-7214	(if in Douglas County)
Lincoln-Lancaster County Health Dept.	402-441-8053	(if in Lancaster County)
Nebraska Health and Humans Services	402-471-2937	(all other counties)
U.S. Health and Human Services (CDC)	404-498-2255	(all counties)

2. Prepare a record of the identification on CDC Form 0.1318 **(within 7 days after ID)**
-to fill out Section 5 of the form contact the NPHL
-make two copies of the completed form (one for lab records and one for the NPHL)
-submit the original form to:
CDC, Select Agent Program, 1600 Clifton Road NE, Mailstop E-79, Atlanta, GA 30333

3. Prepare a record of the transfer on CDC Form EA-101 **(within 7 days after ID)**
-to fill out Sections 1 and 2 of the form contact the NPHL
-submit the original form to the NPHL

4. Complete the transfer of materials to the NPHL **(as soon as possible)**
-follow protocols for the shipment of infectious substances
-include a copy of the CDC Form 0.1318 and the original EA-101 form with the shipment

5. Filing of the EA-101 form **(after receipt of materials)**
-personnel at the NPHL will send a copy of the completed EA-101 form to both the sender and the CDC

"On the Road Again……Building the Nebraska Laboratory Network"

by Tony Sambol, MA, Assistant Director, NPHL

Much has taken place since the last NPHL Newsletter article was written on building the Nebraska Laboratory Network (Spring 2001). This process started with funding that the NPHL received through NHHSS from the Centers for Disease Control and Prevention (CDC). Nebraska was one of the three State Public Health Laboratories (SPHL's) who received funding to develop and employ novel ideas to enhance public health infrastructure within the state. Although this process had began prior to 2001, the events of Sept 11th, and the following Anthrax Crisis delayed the implementation of the Nebraska Laboratory Network. Reported here are the accomplishments that have been made, with your help, in this area since 9/11/2001.

The NPHL has been working in many areas to enhance, or in some cases to establish, a relationship with your laboratory. Your patience and willingness to answer phone survey questions regarding laboratory ability to interact with the NPHL and/or the CDC via the Internet has been appreciated. Information gathered from this survey was instrumental in incorporating a laboratory component written into the communication contract that NHHSS sent to all of the hospitals. A key component of this contract was to have a computer placed into all laboratories to allow for Internet access. Prior to May 2003, less than 50% of hospital labs had Internet access; however, this number has now increased to over 80%. Electronic connectivity has been useful to allow for the NPHL to post timely and accurate information on its new webpage (www.nphl.org), or relay information from the NHHSS Health Alert Network (HAN). Recently, laboratory-related information has been sent-out regarding NPHL's ability to perform rule-out testing for the smallpox virus and SARS-associated Coronavirus, and to update information on West Nile Virus and as of late, Monkey pox virus testing. Personnel from NPHL and NHHSS will be collaborating on a survey this summer to further assess your laboratory. This assessment will help NHHSS plan a state-wide laboratory surveillance system for agents of bioterrorism or other emerging diseases.

In addition to telephone and e-mail communication, last fall the NPHL conducted 11 Laboratory Response Network (LRN) Level-A (now called "Sentinel Labs") training sessions in 7 locations that included Scottsbluff, North Platte, Grand Island, Kearney, Lincoln, Norfolk and Omaha. These training sessions addressed the concerns and needs that various facilities had relayed to us through the Bioterrorism (BT) Survey that we put out last summer. We were pleased that 104 laboratorians took time out of their hectic schedules to attend one of these sessions and receive training, and more importantly 74 of the 86 hospitals in our state were represented. It is known that some facilities were unable to send participants because of staffing and workload issues and it is hopeful that all facilities will be able to send participants in the future.

A series of four two-day seminars have recently been presented by the Nebraska Center for Bioterrorism Education. A laboratory break-out session was held as part of these seminars. During these sessions, all laboratorians were invited to attend a "Laboratory Advisory Council" (LAC) meeting to discuss current issues affecting your lab. The input was extremely helpful to the NPHL and the State Epidemiologists to help chart the state's plan of action for testing clinical specimens during this West Nile Virus season. It is anticipated that the LAC sessions will continue in the future.

Finally, as the NPHL continues to enhance the Laboratory Network in Nebraska, it is vital that we receive your input and thoughts as to any training and consultation issues that your laboratory has. This fall, besides updates on biological agent diagnostics, laboratory training will focus on chemical and radiological terrorism events. In addition, Josh Rowland will be gathering information through a Laboratory Assessment Survey this summer, this will allow us to offer training on specific topics of your choice. Personnel at NPHL appreciate the support given and we look forward to a continuing efforts to help build a healthier state.

Shipping Infectious Substances to NPHL

by Josh Rowland, MT(ASCP)

The Nebraska Public Health Laboratory (NPHL) will now provide two types of certified infectious substance (triple-pack) shipping containers. These shipping containers should be used by laboratories for the submission of specimens to the NPHL for banking purposes.

The first type of shipping container has been supplied by the NPHL to Nebraska laboratories since May 2003. These boxes should be used to submit infectious substance specimens to the NPHL on microbiologic agar slant tubes.

A second type of shipping container became available as of August 21, 2003 to submit infectious substance specimens to the NPHL on microbiologic agar plates. The option of the second box type was in response to laboratorians requests that boxes that would support plates were more useful than those that support slants.

Banked organisms include:

Campylobacter spp.
Escherichia coli O157:H7
Listeria monocytogenes
Salmonella spp.
Yersinia pestis
Shigella spp.
VISA/VRSA
Vibrio spp.
Francisella tularensis
Shiga-toxin positive stool culture filtrate
Brucella spp.
Bacillus anthracis
From sterile body sites:
Haemophilus influenzae
Neisseria meningitidis

(other organisms of epidemiological importance)

This change is an effort to help comply with the new Department of Transportation, Hazardous Goods Regulations (DOT 49 CFR) that became effective February 14, 2003. Isolates submitted to the NPHL for banking purposes fall into the "infectious substances category", according to the regulations, and require special packaging by the submitting laboratory. Shipment containers will be available for transportation through the existing NPHL courier system. When these containers are submitted to the NPHL with a specimen for banking purposes, the containers will be decontaminated (if necessary) and re-circulated back to the submitting facility for reuse. Infectious substances, or "highly suspect diagnostic specimens" submitted to the NPHL for purposes other than banking, can be sent using the same infectious substance container.

To request shipping containers, please access the "Supply Order" link at www.nphl.org, or call NPHL Client Services at 1-800-334-0459 or 403-559-2440. For questions, please contact Josh Rowland at 402-559-6070 or by e-mail at jrowland@unmc.edu.

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