# Nebraska Public Health Laboratory Newsletter

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#### **Special Pathogens and the Select Agent Rule**

By Steven Hinrichs M.D., Director, NPHL In this issue, Dr. Peter Iwen discusses the regulations and process for the handling of "select agents", organisms that are of particular concern to the federal government because of their potential use as biological weapons. This situation is a clear indication of how bioterrorism preparedness has affected the everyday work of laboratory technologists, and while no-one enjoys the additional bureaucratic burden of following the complex select agent rules, it's very obvious that Nebraska laboratorians have taken to heart all of the training and communication that has occurred over the past two years. As an illustration of this point, the public health laboratory has received five isolates of Francisella tularensis and two isolates of Brucella spp. for confirmation since January. Prior to the war on terrorism, it was typical to receive only one or two F. tularensis isolates a year. The point is that we don't believe Nebraska is under attack by human terrorists, but rather that when gram negative pleomorphic rods appear on a Gram stain, they are not as easily dismissed as a probable Haemophilus influenzae with unusual biochemicals, but instead, an astute technologist or director will suggest that the specimen be sent to the state public health laboratory for further identification. And it turns out, the suspicion is fully justified as the numbers indicate.

In coordination with the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL), the American Society for Microbiology (ASM) has developed protocols designed to assist clinical microbiology laboratories with techniques for identifying suspicious microorganisms. These Sentinel clinical microbiology laboratory guidelines offer standardized, practical methods to aid microbiologists in ruling out these special pathogens and referring specimens to public health laboratories (LRN Reference Laboratories) for confirmation. These Sentinel laboratory guidelines are the basis for the NPHL's Bioterrorism Preparedness "Train the Trainer" Wet Workshops that we have been hosting throughout the state. The information and training supplied through these workshops are intended to provide the tools needed for the laboratorians to become familiar with identifying special pathogens so that they can "recognize, rule-out, or refer" suspicious organism. Please contact Josh Rowland (jrowland@unmc.edu, 402-559-6070) if you are interested in learning more about these workshops.

In the "Meet the Laboratorian" section, the NPHL newsletter recognizes two individuals, Anita Young and Dr. Don Giger, who for many years have provided outstanding service and expertise to the Nebraska medical and laboratory community. In both cases they have now retired. Anita, while working part-time now for the NPHL, is enjoying having more time to sail with her husband, while Don Giger has decided he is too young to retire completely and is still involved in public health matters. While we are grateful for their years of service, their retirement highlights the fact that over 50% of the laboratory workforce will be joining them in the next five years and we all need to recruit young people into our interesting and rewarding field.

Although we have never published any cartoons in our newsletter, I hope everyone will appreciate the inclusion of a laboratory recipe. The information on page 5 was requested by a hospital technologist for purposes of freezing bacterial isolates.

### **Reporting the Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory**

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

2005

Federal law necessitates that entities who possess, use, or transfer select agents or toxins which are deemed a severe threat to public, animal or plant health or to animal or plant products, be registered either with the US Department of Agriculture (7 DFR 331 and 9 CFR 121) or the US Department of Health and Human Services (42 CFR Part 73). Clinical laboratories however, are exempt from the provisions in this law since the only activity conducted by the laboratory concern select agents or toxins that are contained in specimens or in isolates from the specimen presented for diagnosis, verification, or proficiency testing. The law does require that laboratories, after confirmation of a select agent or toxin, transfer the specimen and isolate to a facility eligible to receive them or destroys the material on-site by some means sufficient to cause inactivation of the agent. As a part of this process, the laboratory is required to prepare a record of the identification and transfer or destruction on a form called, "Guidance Document for Reporting the Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory" (Animal and Plant Health Inspection Service [APHIS]/CDC Form 4). Laboratories that handle serum from a patient who is ultimately confirmed as positive for a select agent or toxin and who may have handled other specimens from this patient that were culture negative for a viable select agent, do not need to file Form 4. However, the laboratory must still immediately report serological confirmation of a select agent-caused disease to the county or State Department of Health. Be advised that multiple scenarios are present in the handling of specimens containing viable select agents and toxins and that ALL laboratories handling a specimen ultimately determined to contain a viable agent or toxin must file the CDC form. Described below (and in Tables 1 and 2 following the article) is the information needed to adequately file the APHIS/CDC Form 4. (The UNMC/NPHL Special Pathogens Laboratory, a unit within the Nebraska Public Health Laboratory [NPHL], is generally listed as the reference laboratory on Form 4 as the laboratory used for confirmation testing to identify a select agent or regulated toxin.)

#### Section 1 (all laboratories complete)

**Legal name of entity**. The legal entity refers to the reporting laboratory's official name.

**Entity registration number**. This number is recorded by the NPHL, while all other laboratories record "Not applicable". **Address.** Self explanatory.

Name of facility director or responsible official, title, telephone, fax, and e-mail. Generally, this is the Laboratory Director but it may also be another responsible person such as the Biosafety Officer. This is the individual who also signs and dates the form after Section 5.

**Select agent or toxin being reported.** Include a scientific name. **Name of facility supervisor.** Usually this is the supervisor of the laboratory handling the specimen.

**Name/strain designation of the select agent/toxin.** Record as "Not applicable"

**Facility ID number.** The specimen accession number is appropriate or leave blank.

**Data regarding characterization.** For laboratories submitting a specimen to a reference laboratory without doing any testing, indicate the following: "Specimen submitted to [list the laboratory] for isolation and/or confirmation". For laboratories submitting an isolate to a reference laboratory for confirmation testing, indicate the following: "Isolate presumptively identified as [give presumptive ID] using the following characteristics [list the methods used] was submitted to [list the laboratory] for confirmation testing indicate the following: "Confirmation identification performed using the following criteria: [list the criteria used]".

**Location where work with specimens was conducted.** For laboratories sending the specimen to a reference laboratory without testing, record "Not applicable". For all other laboratories performing some testing, identify the location of the laboratory. **Biosafety level.** For laboratories sending a specimen to a reference laboratory without testing, record "Not applicable". For laboratories performing tests on the specimen and/or the isolate, list either BSL-2 or BSL-3 depending on the containment of the laboratory identified in the previous inquiry.

#### Section 2 (all laboratories complete)

**Source of select agent isolate.** For all laboratories handling the original specimen, check "Clinical or diagnostic specimen" and complete the species [presumably human] and the type of specimen [Other, may include multiple specimen types]. Check "Environmental sample" if from the environment. Check "Isolate" if the laboratory received an isolate for confirmation testing and indicate the name, address, and telephone number of the laboratory that sent the isolate and the source. Indicate "Other" for situations that do not meet criteria for the other categories.

Name and telephone number of the person familiar with the case. Normally this is the primary physician of the patient from whom the specimen was obtained.

**Description of the disease.** Recorded by the laboratory who originally handled the specimen in consultation with the patient's primary physician. All other laboratories list "Unknown". **Number of isolates.** For laboratories handling multiple specimens containing the select agent or toxin, record the actual number. For a reference laboratory doing confirmation testing, this usually will be 1 isolate.

**Date of onset.** Unless known otherwise, this is the date of specimen collection.

**How diagnosis was made.** Indicate either by "Serological" and/ or by "Positive culture".

**Laboratory that identified the agent.** By and large this would be the "UNML/NPHL Special Pathogens Lab" or the lab may be some other reference facility.

Name, address, and phone of laboratory director. If the reference laboratory was the NPHL, use the following information: Steven Hinrichs, MD, 986495 Nebraska Medical Center, Omaha, NE 68198-6495, 402-559-8301.

#### Sections 3 and 4 (Leave blank)

Section 3 of the form allows for bi-weekly reporting by veterinary diagnostic entities that identify select agents or toxins in areas where the agent is endemic or during outbreaks. Section 4 is used for the reporting of select agent or toxin contained in a specimen presented for proficiency testing. Contact personnel at the NPHL for advice on filing this information when needed.

#### Section 5 (all laboratories complete)

**Date select agent or toxin was identified.** Indicate the isolate confirmation date (whoever did confirmation testing can supply this date).

Amount of agent transferred, destroyed, or retained. The laboratory receiving a specimen and subsequently forwarding the specimen on to a reference laboratory for testing should indicate, "Specimen transferred to (indicate reference laboratory)" A laboratory receiving a specimen for culture and subsequently sending isolate or other material for confirmation testing to the NPHL should indicate, "Isolate transferred to the NPHL and left over specimen (list amount) and other culture material (list amount) destroyed" or "All specimen (list amount) and culture material (list amount) transferred to the NPHL". A laboratory receiving the specimen for culture and subsequently sending an isolate for confirmation testing to a reference laboratory other than the NPHL or doing in-house confirmation testing should indicate, "Specimen [list amount] destroyed and isolate [list amount] transferred (list reference laboratory)" [under this circumstance, additional paperwork will be necessary before a transfer of the isolate from a reference laboratory other than the NPHL where the identification was confirmed or from a local laboratory that does inhouse confirmation testing can take place]. A laboratory retaining an isolate should indicate, "Isolate retained (list amount)". A certificate of registration with the Select Agent Program is necessary in order to retain a select agent (the NPHL has a certificate of registration to possess select agents and toxin.)

Disposition of select agent after identification. The laboratory that submits a specimen to a reference laboratory for testing should indicate "Other, Specimen submitted to reference laboratory for culture and confirmation testing". The laboratory that receives a specimen for culture and subsequently sends an isolate for confirmation testing to the NPHL should check, "Destroyed on site" and indicate date destroyed and the method of destruction. The laboratory that receives the specimen for culture and subsequently sends all specimen and culture material to a reference laboratory for confirmation testing should indicate, "Other, all materials submitted to reference laboratory for confirmation testing". The laboratory that receives the specimen for culture and subsequently sends an isolate for confirmation testing to a laboratory other than the NPHL (it is assumed that the laboratory has retained a subculture of the isolate submitted) or has done inhouse confirmation testing should check "Transferred" and then call the NPHL personnel to make arrangements for transfer of the identified isolate [an APHIS/CDC Form 2 will need to be proc-

#### (Continued from page 2, Select Agent)

#### essed in consultation with personnel at NPHL]. NOTE: NO **TRANSFER IS TO OCCUR UNTIL FORM 2 HAS BEEN** PROCESSED AND AN AUTHORIZATION NUMBER HAS **BEEN ISSUED BY THE CDC.** A laboratory that receives an isolate for confirmation testing that is subsequently retained after

confirmation [only registered laboratories such as the NPHL can retain these isolates] should check, "Retained".

Is the source expected to provide additional specimens? Usually, check "No" and the anticipated quantity of specimens to be received is then "None" and the anticipated time period to receive specimen is "Not applicable"

Signature. The Laboratory Director [as indicated in Section 1 of the form] must sign and date the form prior to submission to the CDC.

**Conclusion.** As indicated in this review, there are many situations available whereby the APHIS/CDC Form 4 should be processed and submitted to the CDC. The CDC Select Agent Program has indicated [personal communication] that it is not unusual to have multiple copies of the form submitted from a variety of laboratories in reference to one specimen. Complete records are important and CDC personnel actually encourage laboratories to do multiple reporting. Sending samples to the NPHL for confirmation testing actually simplifies the necessary paperwork if a select agent is identified. Although an attempt was made to recognize the common scenarios that laboratories may encounter in the handling of these restricted agents, there will most likely be situations that arise whereby additional information will be needed to fill out and submit Form 4. When these questions occur, please do not hesitate to contact Dr. Iwen at 402-559-7774 for consultation.

 
 Table 1. Who must complete and submit the APHIS/CDC
 Form 4?<sup>a,b</sup>

Laboratories who:

- Handle the original specimen that contained a viable select agent or toxin prior to submission to a reference laboratory for testing
- Conduct the initial plating of the specimen but submit the suspect isolate to reference laboratory for confirmation testing
- Conduct the initial plating of the specimen and perform confirmation testing.<sup>c,c</sup>
- Confirm the identification following isolate submission<sup>c,d,e</sup> (Select agent confirmation is performed at the NPHL in most circumstances)

<sup>a</sup>In many instances, multiple laboratories will handled a specimen containing a select agent or toxin, thus requiring multiple submissions of Form 4.

<sup>b</sup>Laboratories handling serum from a patient who ultimately is confirmed as positive for a select agent by serological testing only do not need to file Form 4, but are still responsible for reporting immediately the result to their county or to the Nebraska Health and Human Services. Go to http://www.hhs.state.ne.us/cod/ codreport.htm for more information about disease reporting.

<sup>°</sup>The laboratory performing confirmation testing is responsible for contacting the CDC for those agents requiring immediate reporting (telephone 404-498-225, facsimile 404-498-2265, or e-mail [Irsat@cdc.gov]).

<sup>d</sup>Agents requiring immediate reporting are listed in the instructions for Form 4.

<sup>e</sup>The UNMC/NPHL Special Pathogens Laboratory has the reagents and protocols necessary to confirm the identification of a select agent or toxin.

#### **Table 2.** Checklist for the reporting of a select agent or toxin following diagnosis and verification.

- Report immediately to the CDC by telephone, facsimile, or e-mail when required<sup>a</sup> Note: Only required for laboratories performing confirmation testing.
- Report immediately to the county or to Nebraska Health and Human Services. Go to http://www.hhs.state.ne.us/cod/ codreport.htm for more information about disease reporting.
- Dispose of the select agent or toxin<sup>b,c</sup> Note: Either by transfer to the NPHL or by onsite destruction.
- Obtain a copy of the APHIS/CDC Form 4 from the CDC or NPHL web site<sup>d</sup>
- Complete sections 1, 2, and 5
- Make 3 copies of the completed form
- Send the original Form 4 to the CDC, one copy to the NPHL, and one copy retained by the laboratory for three years<sup>e</sup>

<sup>a</sup>The instructions to the APHIS/CDC Form 4 lists those agents requiring immediate reporting to the CDC (telephone, 404-498-2255; facsimile, 404-498-2265; or e-mail, Irsat@cdc.gov)

<sup>b</sup>Only laboratories registered by the Select Agent Program may retain materials containing a select agent or toxin.

<sup>c</sup>Subcultures of select agents identified by a reference laboratory other than the NPHL should be sent to the NPHL for banking. Transfer of isolates with a confirmed select agent or toxin will require additional paperwork. Personnel at the NPHL will coordinate this transfer.

<sup>d</sup>Refer to the CDC website at http://www.cdc.gov/od/sap/ downloads2.htm or to the NPHL website at http:// www.nphl.org/news.html#Select to obtain Form 4.

<sup>e</sup>Send the completed form to the Centers for Disease Control and Prevention, Select Agent Program, 1600 Clifton Road NE, Mailstop E-79, Atlanta, GA 30333.

## **Bioterrorism/Chemical Terrorism Procedures on** www.nphl.org

By Josh Rowland, State Training Coordinator, NPHL

Bioterrorism and chemical terrorism procedures, along with related information are now available on our website (www.nphl.org). The procedures were developed by the CDC and the ASM. The bioterrorism procedures listed are meant to be used by Sentinel (Nebraska Laboratory Network Level-A and *Level-B*) laboratories. The procedures described function to "recognize and rule-out or refer" bioterrorism agents from clinical specimens. Those isolates that cannot be ruled-out and are hence presumptively identified as bioterrorism agents or special pathogens should be referred to the NPHL for confirmatory testing.

Select Agent information is also available (See Reporting the Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory article on page 1).

The chemical terrorism information on the web site is intended for all clinical laboratories in Nebraska. This information, developed by the CDC, details how clinical specimens (blood and urine) should be collect from patients in a real or suspected chemical terrorism event. In addition to specimen collection information, packaging guidelines and supporting

(See BT/CT Procedures continued on page 5)

# Meet the Laboratorian(s) - Don Giger and Anita Young

#### Compiled by Josh Rowland, State Training Coordinator, NPHL

This issue of the NPHL Newsletter will feature two laboratorians that have 60 years of combined laboratory experience. Dr. Don Giger and Anita Young (see Page 5) have had long careers in Laboratory Medicine at the Omaha Veterans Affairs (VA) Medical Center and The Nebraska Medical Center, respectively. Both have recently retired, although each continues to work in the laboratory on a part time basis.

#### Don Giger, Ph.D.

#### What are you doing now?

I have retired from VA employment, (as the Microbiol-

ogy Supervisor at the Omaha VA Medical Center) but am still a part-time employee of Creighton Medical Laboratories (Pathology Department). I have begun my 26<sup>th</sup> year as a "contributed services" member of the Creighton University Faculty in the Department of Medical Microbiology/ Immunology. My present responsibilities include teaching in the Creighton Medical and Dental schools and participating in the Pathology Resident training program. What got you interested in pursuing a career in science?



While enrolled in the Biological Science Department at Cal-State University, Long Beach, a friend got me a part-time job washing glassware in the laboratory of a community hospital. After getting a B.Sc. degree, I entered the two year Medical Technology Training Program and on completion passed my California State licensure and ASCP Registry exams. I was eligible for the military draft and, on the advice of one of our pathologists, applied for a direct commission as a Laboratory Officer in the Army.

In the fall of 1963 I was assigned to a "Station Hospital" (200-300 beds) in Nürnberg, Germany. During my Army tour of duty I became interested in microbiology (and got to know one of the civilian nurses at the Hospital). Louise and I got married and returned to California in 1966 - about a year later I again became a student, this time in a Master's program in Microbiology. I worked part time as a Medical Technologist while taking classes – my major Professor was Frank Swatek, one of the "oldtime" medical mycologists whose research interests were on the ecology of *Coccidioides immitis*. (Dr. Swatek's mentors had included mycologists CE Smith and J Walter Wilson.) Soil collecting trips to prehistoric American Indian villages, and working

### REMINDER

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1. Click "NEWSLETTERS" on the blue menu bar

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- 2. Newsletters are listed in reverse chronological order
- 3. Click "Get Acrobat Reader" if you do not have the software needed to access the article

in mycology labs as a Teaching Assistant got me hooked on the science of mycology – and seeing the clinical side while working in a hospital lab made it all the more meaningful. I decided to continue my research interests by applying to a recognized Medical Mycology training program at Tulane University. This was the beginning of a four-year stay in New Orleans – a fascinating, historically rich city - and study at Tulane Medical School, an academic medical center with a long tradition of excellence in tropical medicine, parasitology and medical mycology. For a family with small children it combined the usual stresses of low income 'student life' with visits to the lake front, swamp tours, Mardi Gras parades during the weeks before Lent - not to mention some of the most interesting food we have yet to find. For a graduate student, life in this strong microbiology/immunology department having several faculty members with long-standing ties to the medical mycology "community" was a challenge. There were three Ph.D. students there at the time and we learned about pathogenic fungi from the lab perspective, the patient's view (working in the Dermatology clinic at Charity Hospital) and were taught the exacting science of mentors who thrived on detailed study of molds and yeasts.

#### What do you enjoy most about working in a clinical laboratory?

A feeling that what laboratorians are doing has a positive impact on patient welfare, and that many times this significant information can *only* be determined by testing in the laboratory. In microbiology, many results still come from visual impressions – triggered by years of a Technologist's experience.

I enjoyed (and still am enthusiastic about) the problemsolving aspects of laboratory medicine. In the days when we used the Spectronic 20, the Klett colorimeter, an SMA 12 and ... before the days of CLIA or NCCLS or Coulters, there were always challenges in providing correct results. But the biggest changes have taken place in (1) the increase in regulatory oversight of laboratory science, (2) the introduction of instrumentation in microbiology, (3) management of information with computers and (4) molecular/genetic testing in infectious disease diagnosis. (It's hard to believe that "networking" in the VA Medical Center meant driving with exam questions or memos to the Creighton Department of Microbiology/ Immunology. Or that the VA rejected several requests to purchase laboratory instrumentation because "they contained 'a computing device" – this is the same VA system that now relies on a state of the art electronic record in patient care!)

## What advice would you pass on to someone entering the Medical Technology profession?

Keep in mind the science in "Laboratory science" – disciplines in the Laboratory, be it Chemistry, Immuno-Hematology or Microbiology, have a long history of pioneers who were inquisitive about why things work. It is not enough to spend the day generating "results", no matter how accurate. Concentrate on mechanisms, or organisms, or pathways – why a result occurs.

Don't fear being "a specialist" – even though you gain a lot of satisfaction from being a 'jack-of-all-(lab)-trades' you can contribute even more by seeking out a niche where your coworkers will consult with you. And if you enjoy teaching, share that expertise readily.

Keep your idealism and your professionalism active – patients benefit from honesty and integrity and we need to keep their welfare uppermost in our daily activities.

(Meet the Laboratorian(s), continued on page 5)

#### (Continued from page 4, Meet the Laboratorian(s))

#### Anita Young

#### What are you doing now?

I retired from full-time work as a Virologist at The Nebraska Medical Center microbiology laboratory on January 21, 2005. Although I am not working full-time, I have stayed on with the laboratory as "casual labor". I have also started to work at the Nebraska Public Health Laboratory (NPHL) as a surge capacity employee during the West Nile Virus season. At NPHL I prepare mosquitoes for polymerase chain reaction testing as part of the Mosquito Surveillance Program at the Nebraska Health and Human Services System.

When I am not at work I concentrate on catching up on family, house repairs, and traveling that I have been putting off due to working full time.

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

Although I loved English in school, I have always considered myself to be a science nerd despite considering a career in English. As a child I remember going to science fiction movies which always seemed to perk my interest in the sciences. Ultimately I ended up studying science and biology in school and deciding on a career in science.

### Where did you attend school?

Although technically I am not a medical technologist; I graduated with a degree in Biology from Saint Joseph's College in West Hartford, Connecticut in 1964. My first job at the virus laboratory at the Connecticut State Health Laboratory in Hartford, Connecticut lasted until 1968.

The desire to "expand my horizons" brought me to Nebraska later that year after a friend told me about job opportunities at the state's two medical schools; Creighton University Medical School (CUMC) and the University of Nebraska Medical Center

(UNMC). My first job in Nebraska was at CUMC in the biochemistry department working for Dr. Edward Carusi. Dr. Carusi was a researcher who focused on Oncogenic (tumor forming) viruses. My job was to propagate Adenoviruses and extract the DNA for the research. This, of course was before PCR-which made the work very challenging.

I was forced to find a new job after Dr. Carusi's grant ended in 1970. Thankfully, I was offered a job at UNMC by Dr. Roberta White in the Medical Microbiology Virology Laboratory . I worked for Dr. White until 1985 when departmental shifting at UNMC placed the virology laboratory into the Pathology Department at which time virology became part of the clinical laboratory. I continued to work for UNMC in the clinical laboratory until 1997 when UNMC merged with Clarkson Hospital and became The Nebraska Medical Center.

#### What did you enjoy most about working in a clinical laboratory?

Like many who work in clinical laboratories, I love the investigational aspect of the work. I especially enjoy it when a physician appreciates my effort when I contact them with a significant result. When this happens it makes the whole day more enjoyable because I have directly affected patient care.

## What advice would I pass on to someone entering the Medical Technology profession?

Take advantage of every educational opportunity and learn all you can. Don't look at the job as if you are just putting in hours to earn a check. Always remember that what you do and the quality of your work can be very significant in a patient's care and recovery.

#### (Continued from page 3, BT/CT Procedures)

documentation including a chain-of-custody and shipping manifest forms are included. These reference documents are meant to direct laboratorians during a suspected event when collecting human specimens.

Look for NPHL to offer chemical terrorism laboratory preparedness training sessions in the future. Please contact Josh Rowland (402-559-6070, jrowland@unmc.edu) if you have questions.

#### Using nphl.org to order supplies.

- 1. Click "SUPPLY ORDERS" link on the red menu bar
- 2. Enter your NPHL account number and click "Log In"
- 3. Click "Place Orders" and enter volume of supplies
- 4. Click "View Orders" to check status of order
- 5. Order Questions? Call NPHL Client Services at 866-290-1406 (toll free) or 402-559-2440 (local)

#### Make Your Own Freeze Media

By Rhonda Nöel, MT(ASCP), NPHL

To preserve bacterial isolates for future use, prepare a freeze matrix and aliquot to sterile cryovials (with O-ring cap is best). Use LB (Luria-Bretani) broth, BHI (Brain Heart Infusion) broth, or TSB (Tryptic Soy Broth) and add sterile glycerol to create a final concentration of 30% sterile glycerol (v/v) as the matrix. The glycerol is heavy so mix well when working. Aseptically add about 1mL of the mix to the cryovials and store at 4°C. After inoculation with a loop of fresh culture, freeze the same vial at -20°C. The matrix won't freeze completely hard but remains slushy due to the glycerol.

To subculture from frozen stock, stab a hot loop into the vial while keeping the sample as cold as possible. Streak the inoculum to the appropriate plate medium. Immediately refreeze the vial. Freeze thaw cycles decrease the viability of the bacteria. Complete thawing of the sample may require a new preparation from a fresh subculture.

Additional information about cryopreservation of microorganisms can be found at the ATCC website (<u>www.atcc.com</u>).





## Nebraska Public Health Laboratory

University of Nebraska Medical Center 986495 Nebraska Medical Center Omaha, Nebraska 68198-6495

Mailing Address

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Director, Steven H. Hinrichs, M.D. Editor, Josh Rowland, MBA, MT(ASCP) E-mail: shinrich@unmc.edu E-mail: jrowland@unmc.edu

Please direct suggestions, questions, or comments to: Josh Rowland, Editor, NPHL Newsletter, 986495 Nebraska Medical Center Omaha, NE 68198-6495 or jrowland@unmc.edu.