TO: Laboratorians, Public Health

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RE: Laboratory Handling and Diagnostics for *Francisella tularensis*

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**Background**

During 2015, 24 cases of tularemia (10 confirmed and 14 probable) were reported to the Nebraska Department of Health and Human Services. This number represents a 25-year high and a three-fold increase compared with 2014. As we approach the outdoor season, laboratory workers need to be aware of laboratory-related issues posed by specimens containing *Francisella tularensis*.

**Tularemia**

Tularemia is an uncommon disease caused by the bacterium *Francisella tularensis*. It infects humans and animals in the US and Northern Hemisphere. Due to its potential as an intentional biological threat, this organism is classified by the federal government as a Tier I select agent. This agent has been used as a biological weapon in the past due in part to the ability to be easily aerosolized and to the low infectious dose (as few as 10 colony forming units).

Tularemia can be transmitted by contact with infected animals (major sources in Nebraska are cats and rabbits) or contaminated food, water, or soil; inhalation due to aerosolization; or by insect bites (e.g., ticks and flies). Human-to-human transmission does not occur. Laboratory acquired infections can occur following exposure to viable cultures of *F. tularensis* as the result of accidental inoculation, inhalation (aerosol, droplet), or contact of skin or mucous membranes.

Tularemia can be diagnosed by testing serum for specific antibodies or by evaluating any clinical material for the presence of *F. tularensis* by DNA analysis or by culture. All suspect cultures can be processed and preliminarily tested following the LRN protocol within the BSL-2 containment laboratory but should be manipulated within a biosafety cabinet (BSC) using the appropriate personal protective equipment (PPE). Identification should not be attempted using an automated system such as a Vitek or MicroScan system, or on a MALDI-TOF. Notably, an open culture plate outside of the BSC (e.g., on an open bench) or within the BSC without the appropriate PPE DOES represent an exposure risk.

Personal protective equipment for BSL2 work includes disposable gloves, lab coat, and face and eye protection.
Decontamination can be achieved using a 5 to 10% sodium hypochlorite solution for 10 minutes followed by a 70% alcohol solution. All laboratory materials should be decontaminated, autoclaved, or placed in biosafety waste as appropriate.

**Laboratory Procedures**

Review the Gram Stain report before opening culture plates (this organism stains as a gram-negative coccobacillus). Major phenotypic characteristics for *Francisella tularensis* include oxidase negative, tube catalase weak positive, and beta-lactamase positive.

Watch for trigger points that indicate the need for the Laboratory Response Network’s (LRN) Rule-Out or Refer protocol. If any trigger points are present, only open the agar plates and perform testing in a certified BSC.

Trigger points for hazardous organisms include:
- Slow or poor growth at 48 hours
- No organisms in direct specimen Gram stain of sterile body site, but slow culture growth
- Gram negative diplococci or coccobacilli in direct specimen Gram stain of sterile site
- No growth, or poor growth, of a gram negative rod on MacConkey agar
- Better growth on Chocolate agar than on sheep blood agar (SBA)
- Suggestive patient history or diagnosis

Trigger points specifically for *Francisella tularensis* include:
- Tiny pleomorphic, often poorly stained gram negative coccobacilli
- Gram stain interpretation difficult due to minute size, often reported as not otherwise specified (NOS)
- Slow growth at ≤48 hours, gray-white, opaque, shiny, or wet colonies
- Better growth on Chocolate agar
- May initially grow on SBA if cultured from blood, subsequent passage to SBA may fail to grow

If trigger points are present, perform the LRN Rule-Out or Refer protocol in the *NPHL Bench Guide for Hazardous Pathogens*


If a hazardous organism **CAN** be ruled out by the LRN protocol, the isolate can safely be handled on an open bench or tested with automated kit systems for identification.

If a hazardous organism **CANNOT** be ruled out by the LRN protocol, please immediately notify the laboratory manager, infection control, and local/state health department. Also contact the Nebraska Public Health Laboratory (NPHL) AS SOON AS POSSIBLE by one of the following methods:
- STATPack™ or STATPack Lite™ (contact 402.559.3590 if assistance is needed)
- If STATPack not available, contact NPHL 24/7 at pager at 402.888.5588

Complete a NPHL Special Microbiology Test Request form and ship with isolate:
- Carbon-copy preprinted with facility account number – document all testing performed.
- Click “Generate Referral Form” on STATPack (full version only), complete patient demographics.
NPHL-approved isolates are shipped as “CATEGORY A”:

- Omaha laboratories should NOT use the routine NPHL courier to ship Category A agents. NPHL will make arrangements for an exclusive courier.
  Make 3 copies of the Shippers Declaration:
  - Keep one copy in laboratory as documentation for 2 years
  - Place one in pouch on lid of box
  - Staple last copy to the Emergency Response Information (ERI) sheet (Guide 158 or Safety Data Sheet) and hand directly to courier
- Most laboratories outside of Omaha - should ship all Category A suspicious agents by FedEx, following the procedures on the NPHL website (www.nphl.org). Shippers are required to be trained and certified by DOT Division 6.2 to package and ship a Category A agent. If you are not set up with NPHL packaging materials, please contact NPHL for assistance.

Since it is a potential bioterrorism (BT) agent, if *Francisella tularensis* is confirmed at NPHL, the originating laboratory needs to complete Centers for Disease Control and Prevention (CDC) Forms WITHIN 24 HOURS. NPHL will assist you with form completion. We recommend that other laboratory managerial staff be familiar with this process, as described at: http://nphl.org/bioTerror.cfm#Select.

**Interpretation Guidelines**

Please refer to manufacturers’ literature for use of specific diagnostic kits and protocols.

The CDC Laboratory Criteria for Diagnosis are as follows:

**Presumptive Diagnosis:**

1. Elevated serum antibody titer(s) to *F. tularensis* antigen (without documented fourfold or greater change) in a patient with no history of tularemia vaccination
2. Detection of *F. tularensis* in a clinical specimen by direct fluorescent assay (DFA)

**Confirmatory Diagnosis:**

1. Isolation of *F. tularensis* from a clinical specimen
2. Fourfold or greater change in serum antibody titer to *F. tularensis* antigen

**Additional Resources**

CDC Biosafety in Microbiological and Biomedical Laboratories Manual: http://www.cdc.gov/biosafety/publications/bmbl5/

CDC Tularemia Overview: www.cdc.gov/Tularemia/

American Society for Microbiology’s Sentinel Lab protocols: http://www.asm.org/index.php/guidelines/sentinel-guidelines

US National Response Team’s Quick Reference on Tularemia: http://nrt.org/ (Guidance for decontamination and handling spills)