TO: Laboratories, Microbiologists, and Public Health

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RE: Laboratory Handling and Diagnostics for Francisella tularensis

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Background

During 2015 year-to-date, 18 cases of tularemia (10 confirmed and 8 probable) have been 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. In particular, type B tularemia has been isolated from the blood from four persons with confirmed infection for whom typing was performed. Please refer to the following information for guidance on handling and processing samples and specimens that might contain Francisella tularensis organisms.

Tularemia

Tularemia is a rare disease caused by a gram negative coccobacillus, Francisella tularensis, which infects humans and animals in the US and Northern Hemisphere. It is a Tier I select agent because it has a low infectious dose (as few as 10 colony forming units), can be aerosolized, and has been developed as a bioweapon in the past.

The bacterium is subdivided into several species with type A (tularensis) and type B (holarctica) accounting for most human illness. Type A is more virulent and most frequently associated with rabbits. Type B typically causes less severe illness and is more often linked to muskrats, beavers, and voles. Both types cause a range of symptoms from localized skin lesions and lymphadenopathy to respiratory impairment or systemic disease marked by fever and generalized malaise.

Tularemia can be transmitted by contact with infected animals or contaminated food, water, or soil; inhalation due to aerosolization; or by insect bites (e.g., ticks). Human-to-human transmission does not occur. Laboratory transmission can occur as the result of accidental inoculation, inhalation (aerosol, droplet), or contact of skin or mucous membranes with potentially infectious samples including blood, spinal fluid, lesion exudates, urine, respiratory secretions, or material from infected animals or vectors.

Tularemia can be diagnosed from blood through antibody testing, DNA isolation, or culture. Samples suspected of containing F. tularensis organisms should be handled at Biosafety Level 2 (BSL2) at a minimum. Biosafety Level 3 (BSL3) practices must be used when handling cultures or when performing necropsies or animal studies. Notably, an open culture plate outside of BSL3 precautions (e.g., on an open bench) DOES represent an exposure risk.
Personal protective equipment (PPE) for BSL2 work includes disposable gloves, lab coat, and face and eye protection for manipulations outside a biosafety cabinet. Please refer to your facility-specific protocols for information regarding PPE for BSL3 work.

Decontamination can be achieved using a 1 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**

Please review the direct Gram Stain report before opening culture plates. Note that *Francisella tularensis* is 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 protocol. If any trigger points are present, only open agar plates and perform testing in a certified BSL2 Safety Cabinet.

Trigger points for hazardous organisms include:
- Slow or poor growth at 48 hours
- No organisms seen in direct specimen Gram stain of sterile body site, but slow growth on culture
- 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
- Suggestive patient history or diagnosis

Trigger points specifically for *Francisella tularensis* include:
- Tiny pleomorphic poorly stained Gram negative coccobacilli
- Interpretation of Gram stain very 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 blood agar (BAP) if cultured from blood, subsequent passage to BAP may fail to grow

If trigger points are present, perform the LRN Rule-out protocol as defined in the *NPHL Bench Guide for Hazardous Pathogens* [http://nphl.org/documents/NPHLBenchGuide_FINAL20131221.pdf](http://nphl.org/documents/NPHLBenchGuide_FINAL20131221.pdf)

If a hazardous organism **CAN** be ruled out by the LRN protocol, it can then 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. Please also contact the Nebraska Public Health Laboratory (NPHL) **AS SOON AS POSSIBLE** by one of the following methods:
- STATPack™ or STATPack Lite™, if available in your laboratory (contact 402.559.3590 if assistance is needed)
- If STATPack not available, dial NPHL pager at 402.888.5588
If NPHL approves, SHIP AS “CATEGORY A”:

- Omaha laboratories should NOT use the routine 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

- Laboratories outside of Omaha (including Lincoln) 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 to package and ship a Category A agent. If you are not set up with NPHL packaging materials, please contact NPHL for assistance.

As a bioterrorism (BT) agent, if *Francisella tularensis* is confirmed at NPHL, the originating laboratory will be asked to complete Centers for Disease Control and Prevention (CDC) Forms WITHIN 24 HOURS. NPHL will assist you with exact method to complete. We recommend that other managerial staff be familiar with this process, as described at: [http://nphl.org/bioTerror.cfm#Select](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 Tularemia Overview: [www.cdc.gov/Tularemia/](http://www.cdc.gov/Tularemia/)


(Guidance for decontamination and handling spills)