Tularaemia (*Francisella tularensis*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *francisella tularensis*.

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1 PHLN Summary laboratory definition

1.1 Condition:

Tularaemia

1.1.1 Definitive Criteria

- Isolation of *Francisella tularensis* from wound (ulcer) or aspirates or blood.

1.1.2 Suggestive Criteria

- Detection of *F. tularensis* by nucleic acid tests (NAT); or
- Detection of *F. tularensis* by direct fluorescent antibody (DFA) test; or
- Gram negative single poorly staining pleomorphic coccobacilli. Characteristic growth on agar plates (cysteine requirement) from patients with clinical features of tularaemia.

2 Introduction

Tularaemia is a zoonotic disease caused by the gram-negative coccobacillus *Francisella tularensis*, a facultative intracellular pathogen. It was first described in the United States in 1911 and is also known there as “rabbit fever” and “deer fly fever”. Currently four subspecies or biovars are recognized; *F. tularensis* subsp. *tularensis* (type A formerly known as subsp. *nearctica*)), *F. tularensis* subsp. *holartica*, (type B, formerly known as subsp. paleartica) *F. tularensis* subsp. *mediasiatica* and *F. tularensis* subsp. *novicida*. Two subspecies, type A (*F. tularensis* subsp *tularensis/nearctica*) and type B (*F. tularensis* subsp *holarctica/paleartica*) cause disease in man and have been considered as potential biological weapon agents[3]. Type B strains

occur across northern Europe and Japan and generally cause mild illness in humans. Type A strains are restricted to defined geographical foci in North America and cause severe disease[3]. Type B strains have been found naturally in Australia (see below). A strain of *Francisella tularensis* subsp. *novicida*, a low-pathogenic species, was isolated from a wound in Darwin (Whipp et al 2003) and ulceroglandular tularemia caused by *F. tularensis* subsp. *holartica* was reported in a human case bitten by a ringtail possum in Tasmania (Jackson et al, 2012). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3437722/ http://jmm.microbiologyresearch.org/pubmed/content/journal/jmm/10.1099/jmm.0.05245-0. More recently, microbiologists in Sydney demonstrated *Francisella tularensis* subsp. *holartica* variant *japonica* by both PCR and culture in frozen ringtail possum livers dating back to 2003, providing conclusive proof of the existence of this organism in native possums.

Tularaemia is a debilitating illness, rife amongst wild animals and common in the Rocky Mountains, California, Texas, Oklahoma and Martha's Vineyard in the US, as well as parts of Eastern Europe (Kosovo), China, Japan, Scandinavia, and Siberia[1, 3, 5]. *F. tularensis* is a very hardy organism, capable of surviving for weeks and sometimes months in decaying animal corpses[3, 5]. Tularaemia is primarily transmitted to humans by ticks, mosquitoes and wild rabbits, although squirrels, sheep, cats, and dogs have also been identified as carriers[3, 5]. Whilst tularaemia is a highly infectious disease it is rarely spread directly from person to person[3, 5]. Tularaemia is fatal in approximately thirty percent of untreated cases. As a result of its highly infectious nature and its very slow growth patterns, the handling of the organism by laboratory staff represents a considerable risk (particularly in its unidentified state) and in endemic areas *F. tularensis* is a significant cause of laboratory-associated infections[2, 3].

Tularaemia characteristically presents as an acute febrile illness. The route of infection plus the host's response will determine the clinical manifestation of the illness. These may range from an ulcer at the site of cutaneous or mucous membrane inoculation, pharyngitis, ocular lesions, regional lymphadenopathy and pneumonia. Tularaemia is characterised by sudden onset of chills, fever, headache, generalized body aches, coryza, pharyngitis, cough, and chest pain or tightness. Typically the patient will have a temperature of 38–40oC. The incubation period is 2–10 days. Without treatment, nonspecific symptoms usually persist for several weeks, and sweats, chills, progressive weakness, and weight loss characterise the illness[3].

Antibiotic resistance in wild strains is rare but all of them are intrinsically resistant to beta-lactams. Doxycycline and quinolones are the drugs of choice and gentamicin can be used to augment bactericidal effect. Some patients have been successfully treated with ciprofloxacin, but penicillins and cephalosporins are not effective[3, 5].

The usual mode of infection is through puncture of the skin whilst handling infected material or through the bite of an insect (*Dermacentor* and *Ixodes* ticks are active in summer months) or infected mammal[3]. An ulcer will form as the bacteria multiply. From the penetration site the bacteria are transported by the lymphatic system to regional lymph nodes and from there to other sites if the infection is not contained[3]. Tularaemia presents in humans primarily as ulceroglandular disease (45–80% of reported cases), as primarily glandular infection (10–25%) and less frequently, as oculoglandular, septic, oropharyngeal and pneumonic forms[3]. Despite the existence of a pulmonary form of the disease, human-to-human transmission is unusual[2]. Any of the principal forms of

tularaemia may be complicated by bacteraemic spread, leading variously to tularaemic pneumonia (common), sepsis (uncommon), and meningitis (rare)[3]. Other sources of infection include:

a. handling infectious animal tissues or fluids,

b. direct contact or ingestion of contaminated water, food or soil,

c. inhalation of infective aerosols e.g. handling damp hay.

F. tularensis has been developed in the past as a biological weapon[1]. There is evidence that the USSR released a concentrated amount of *Francisella tularensis* over German lines near Stalingrad in 1942, resulting in numerous cases on both sides, many of them displaying the pneumonic form of the disease[1]. After the war, the former USSR successfully weaponised *F. tularensis* and developed multi-drug resistant strains, whilst maintaining or enhancing virulence[1]. A biological attack could take the form of aerosol distribution of *F. tularensis*[2]. Large numbers of pneumonia cases in a short period of time with a massive casualty rate might be the first indication of such an attack. The infectious dose by aerosol is approximately 100 – 500 organisms[1].

3 Laboratory diagnosis/tests

3.1 Culture

Francisella tularensis is a fastidious bacterium requiring cysteine for growth. It will not grow on MacConkey agar. Whilst it may at first grow on standard sheep blood agar or chocolate agar, it will fail to grow on subsequent passage unless the medium has been supplemented with cysteine[5]. Laboratories should be aware that some MALDI-TOF libraries will not contain spectra for select agents such as *F. tularensis*, resulting in samples returning results such as “no reliable identification”, causing additional characterisation of isolates, increasing potential for laboratory exposure (Clark et al 2013). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3719498/.

3.1.1 Media

Francisella tularensis will grow on 5% sheep blood agar, chocolate agar, CHAB, Thayer Martin agar or BCYE[3, 5]. However, as noted above, best growth on blood agar will be obtained on cysteine-supplemented media. Growth will also occur in standard blood culture media. Plates should be firmly taped shut and clearly label to prevent inadvertent opening[3, 5].

3.1.2 Suitable specimens

A. Samples from acutely ill patients

- Blood culture
- Tissue biopsy or material scraped from an ulcer (swabs are acceptable)
- Aspirates of tissue at site of lesion

NB. Samples should be labelled as “High Risk” and forwarded to a PHLN laboratory for culture. Samples that are transported immediately may be stored at room temperature, but if a delay is anticipated they should be cooled to 2–8 oC.

B. Samples from post-mortem

- Blood from a vein (if possible)
- Aspirate from lesion
- Scraping from ulcer site

NB. Extensive post-mortem examination is discouraged in cases of suspected tularaemia because of the risk of aerosolising *F. tularensis* present in body fluids, drips, etc.[3].

3.1.3 Test sensitivity

No quantitative data available.

On agar plates supplemented with cysteine (Chocolate agar, CHAB, Thayer-Martin, BCYE or supplemented SBA) at 35-37ºC, growth will be too small to be seen after 24 hours. At 48 hours, colonies will still be very small (1–2 mm), white to grey to bluish-grey, opaque, flat, with an entire edge, smooth with a shiny surface. Plates may be incubated aerobically or in 5% CO2[4, 5]. However, the growth of this organism is not enhanced by CO2[2].

3.1.4 Test specificity

The slow growth of *F. tularensis* and the requirement for cysteine provide clues to the identification of this organism. A stained smear demonstrating poorly staining pleomorphic gram negative coccobacilli that stain as single cells should increase suspicion when suitable symptomology is evident[4].

3.1.5 Predictive values

A negative culture does not exclude *F. tularensis*, especially if there is some doubt over the age and storage in transit of the specimen. A positive culture in the absence of symptoms should be treated with utmost suspicion and should not lead to an automatic diagnosis of tularaemia.

3.1.6 Suitable acceptance criteria

On blood agar, growth is invisible after 24 hours at 37oC. After 48 hours or longer incubation, colonies range in size from 1–2 mm in diameter, are white to grey to bluish-grey in appearance, and are opaque, flat, with an entire edge and smooth with a shiny surface; colonies are not haemolytic. There is no growth on MacConkey or EMB agar. Subcultures on standard sheep blood agar will fail to grow because of the cysteine requirement. There is little or no haemolysis on blood agar. Cultures should be kept for at least 10 days before discarding[3, 4, 5]. The organism grows poorly at 28oC.

3.1.7 Suitable internal controls

Blood agar is a fairly consistent product, but all batches must be validated for suitable performance using a documented internal quality control system. The consistent growth of fastidious and non-fastidious Gram-negative bacilli should be indicative of suitable performance.

3.1.8 Suitable test validation criteria

Biochemical identification should not be attempted with commercial test systems, firstly because they are unreliable, and secondly because of the risk of transmission to staff[4, 5]. Only select PHLN laboratories can identify *Francisella tularensis* definitively.

3.1.9 Suitable external QC program

The RCPA Biosecurity QAP provides an external quality control program that includes virtual modules for *Francisella tularensis* as well as specimen modules using *Francisella novicida.*

3.2 Identification of *Francisella tularensis*

There are two main steps likely to occur in the aetiological diagnosis of tularaemia:

- *Diagnostic laboratories* – In the event of a patient returning from a tularaemia-endemic area or a deliberate biohazard release, clinical samples should go to a routine diagnostic microbiology laboratory for analysis. As soon as there is any suspicion of the organism being *Francisella tularensis*, all culture work should cease. The cultures should be forwarded immediately to a PHLN laboratory by arrangement with senior PHLN lab staff. Likewise, a request to culture for *Francisella tularensis* should result in automatic referral of specimens to a PHLN laboratory.
- *Reference (PHLN) laboratories* – Samples as described above or, where a presumptive diagnosis has already been made and clinical samples are being collected based on this diagnosis, these samples should be referred to a PHLN laboratory for culture and confirmation.

3.2.1 Presumptive identification

- Staining – *F. tularensis* is a small (0.2–0.5 µm by 0.7–1 µm) Gram-negative pleomorphic, encapsulated and weakly staining coccobacillus seen mostly as single cells. It does not exhibit the characteristic bipolar staining of *Y. pestis*. A suspect diagnosis of tularaemia may be made if the direct stain (Gram) made from blood, tracheal or lung aspirates or an ulcer from a patient with compatible clinical symptoms shows small weakly staining Gram negative coccobacilli[3, 4, 5].
- Identification test strips – biochemical test strips and the standard Maldi-TOF profiles are not regarded as being able to provide a definitive identification.
- PCR protocols targeting the *tul4* and *fopA* genes for *F. tularensis* are under development, but have yet to be validated for diagnostic use. The GeneXpert available in some PHLN laboratories has a bioterrorism module that includes rapid molecular confirmation of *Francisella tularensis.*
- Some PHLN laboratories have access to the GeneXpert or Biofire Film Array system which has a bioterrorism panel that covers PCR identification of *Francisella tularensis*.

3.2.2 Definitive identification

Confirmation of the identity of suspect *F. tularensis* requires specialised reagents with limited availability in Australia. These include specific direct fluorescent antibody (DFA) stain and Nucleic Acid Tests (NAT). A slide agglutination test is commercially available in Australia. Suggestive identification

can be achieved with Catalase (weak +ve), Oxidase (-ve), Beta-lactamase (+ve), Urease (-ve) and XV-requirement (-ve)[4]. The most common misidentifications with cultures of *Francisella tularensis* are *Haemophilus influenzae* (XV-requirement) and *Actinobacillus* sp. (beta-lactamase –ve), especially when commercial identification systems are used[3].

3.2.3 Predictive values

A negative result in a biochemical test strip or by MALDI-TOF does not rule out *F. tularensis*. Isolates must be confirmed by the methods outlined above.

3.2.4 Suitable test criteria

An isolate that exhibits characteristic weak staining, has characteristic growth on agar, biochemically confirms as *F. tularensis* and gives a positive specific agglutination test or stains with specific fluorescent antibody stain, with confirmation by NAT (PCR for the *tul4* gene and *fopA* genes and 16S rRNA gene sequencing).

3.2.5 Suitable internal controls

Each batch of reagents tested with positive and negative controls. Results of all QC testing should be recorded and the records maintained.

3.2.6 Suitable validation criteria

Correct reactions exhibited by a recognised control strain of *F. tularensis*.

3.2.7 External QC Program

RCPA Biosecurity QAP.

3.3 Nucleic Acid Detection

F. tularensis is an ideal target for nucleic acid detection because of its fastidious nature and its high infectivity. In house PCR assays have been used successfully to detect *F. tularensis* during outbreaks in humans and animals in endemic areas overseas using conventional PCR formats[6]. Real time PCR has been shown to be significantly more sensitive than conventional assays for detecting *F. tularensis* from animal samples, however there is no data published on its efficacy for human diagnosis. Most assays target the *tul4* gene encoding a 17 kDa lipoprotein which is conserved and cannot be used to differentiate among the subspecies.

3.4 Antigen Detection

A rapid hand held detection assay and antigen capture enzyme-linked immunosorbent assays (cELISA) have been developed to detect the 17 kDa lipopolysaccharide antigen in specimens and environmental samples. The hand held device has an estimated detection limit of 106 bacteria/ml, the cELISA 103 and PCR 102[7]. The antigen detection assay is available in a limited number of laboratories.

3.5 Serological Diagnosis

Serological diagnosis is highly specific and can be useful in culture-negative cases[4]. Acute and convalescent sera can be forwarded to the Centers for Disease Control and Prevention, Atlanta, GA, US. Tests available include microagglutination and tube agglutination[4]. There are also several EIA and immunochromatography-based tests (e.g., the VIRapid® Tularemia immunochromatographic test) which have been evaluated in endemic countries (Chaignat et al, 2014).

4 References

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