

Francisella tularensis

(Tularemia)

***Francisella tularensis* is a very tiny, faintly staining Gram-negative pleomorphic rod. The bacterium can be cultivated in a variety of cysteine supplemented nutrient media under aerobic conditions. The colonies form slowly with mature growth seen around 72hrs. Tularemia can infect the human body through the, lungs (pulmonary), or skin (lesions) and causes clinical symptoms based on transmission mode. Cultures should be manipulated under BSL-2 conditions using BSL-3 practices.**

Collection of Clinical specimens

Collect specimens before antibiotic treatment of patient has begun.

Specimens

- **Material from primary lesion (pustule or ulcer)**
 - Cleanse lesion and surrounding area with 70% alcohol; allow to dry.
 - Incise pustule with sterile scalpel.
 - Express several drops of fluid and collect in sterile capillary tube fitted with a rubber bulb.
- **Material from draining lymph node**
 - Collect several drops of **exudate** in a sterile capillary tube fitted with a rubber bulb.
- **Lymph node perfusion**
 - Inject 2 ml sterile saline into an enlarged, intact lymph node. Withdraw fluid.
- **Sputum, gastric aspirates, pharyngeal washings, pleural fluid and bronchial secretions**

Collect these specimens especially in cases of typhoidal or pulmonary tularemia.

 - Sputum, gastric aspirates, pharyngeal washes
 - Obtain in early morning before patient drinks water or washes teeth.
 - For pharyngeal washes, patient should gargle with 15 ml sterile nutrient broth and expectorate into sterile container with cover. If possible, collect several daily samples.
- **Conjunctival scrapings-** Collect in cases of oculoglandular tularemia.

- (Traverse area with sterile swab using gentle pressure.)
- **Biopsy specimens**
 - Process in Ten Broeck grinder with 2 ml sterile saline. (May also grind using sterile mortar and pestle. If grinding tools not available, macerate tissue with sterile wooden stick and use stick to inoculate media.)
- **Blood**

F. tularemia is recovered from blood only infrequently. (If isolation from blood is attempted, the best chance of recovery is very early in disease or in untreated fulminating disease.)

 - Collect at least 3 ml blood, without anticoagulant.
- **Urine and feces** – rarely yield *F. tularensis*
- **Serum** – for serologic diagnosis
 - Collect acute phase specimen as soon as possible after onset of illness.
 - Collect convalescent phase serum 2 to 3 weeks after collection of acute serum.
 - Ship and store frozen. (If freezing is not possible, preserve sera by adding 0.1 ml of a 1 % Merthiolate solution per 1 ml of serum.)

Processing of Clinical Specimens

Divide specimens into 2 portions, one for freezing (for future use), and one for immediate culture.

- **Freezing**
 - Place specimen in small tube containing 0.5 ml sterile nutrient broth at neutral pH.
 - Size of container should be such that, when filled, only a small volume of air will be present.
 - Freeze at – 30 to – 70 C.
 - Rate of freezing not critical, but subsequent thawing should be accomplished quickly at 37 C.
- **Cultures**

General considerations:

 - Whenever possible, prepare smear directly from clinical material for Gram staining.
 - Specimens should be inoculated directly onto culture media as soon as possible, preferably at bedside. If necessary to hold specimen for more than a few hours, keep it moist with sterile broth medium or saline and freeze.

- Plated media should have minimal surface moisture. Moisture may inhibit the growth of *F. tularensis*.
- If it is necessary to send specimen to another facility for testing, package with dry ice.

- Media
 - Cysteine heart agar supplemented with 9% heated (chocolatized) sheep red blood cells (CHAB) – medium of choice
 - Other media supporting growth of *F. tularensis*:
 - Modified Mueller-Hinton agar
 - Chocolate agar supplemented with IsoVitalex
 - Modified charcoal yeast agar
 - Chocolate agar and Thayer-Martin agar may also be used.
 - Sheep blood agar (SBA) will support the growth of wild-type *F. tularensis*. However, on subsequent passages, isolates grow poorly or not at all on this medium.
 - Modified Thayer-Martin medium may be useful if specimen is contaminated.
- Inoculation and incubation
 - Use syringe (needle removed) or pipet fitted with safety suction device to transfer material to medium.
 - Inoculate specimens onto plates of a cysteine-rich agar medium and onto a SBA plate for comparative purposes.
 - In addition to inoculating specimens directly onto plated media, inoculate a portion into brain heart infusion broth supplemented with 1% IsoVitalex to enhance recovery of the organism. Incubate 24-48 h and then inoculate broth culture onto agar plates.

Pustule or ulcer material, conjunctival scrapings, material from draining lymph node, sputum

- These specimens may have high concentrations of *F. tularensis* and/or normal flora
 - Inoculate 0.2 ml per plate.
 - Streak with inoculating loop for isolation.

Lymph node perfusion, pleural fluid, bronchial secretions

- Transfer 0.2 ml to surface of plate at center.
- Spread with sterile, bent glass rod.

Gastric aspirate, pharyngeal washings, blood

- Culture at least 3 ml of specimen, distributing the amount among several plates.
- Transfer inoculum to surface of plate at center.
- Spread evenly with sterile, bent glass rod.

Tissues

- Transfer 0.2 ml to surface of plate at center.
- Spread evenly with sterile, bent glass rod.
- or**
- If specimen was not processed in grinder:
- Punch tissue several times with sterile wood stick.
- Use stick with material on it to inoculate agar plate.
- Incubate at 35-37 C in air for at least 72 h.
- Hold for 10 days if specimen was taken from patient treated with antibiotics.
- Observe daily.

Examination of Cultures and Gram Stain

Colony Morphology

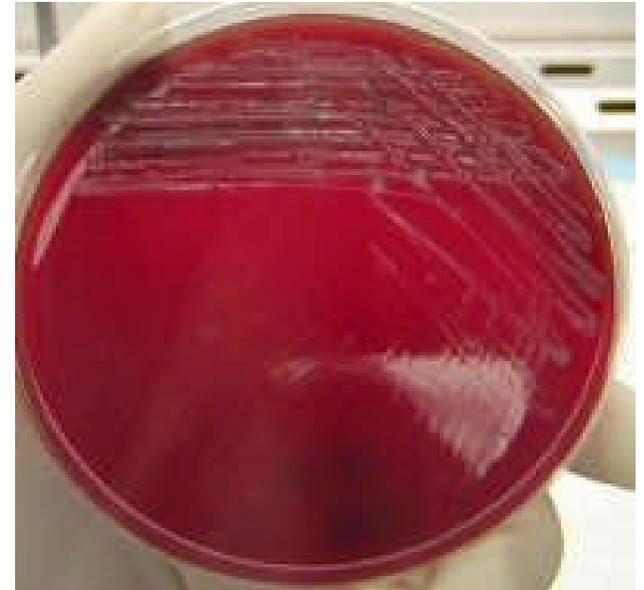
24 h – colonies too small to be seen

48 h on:

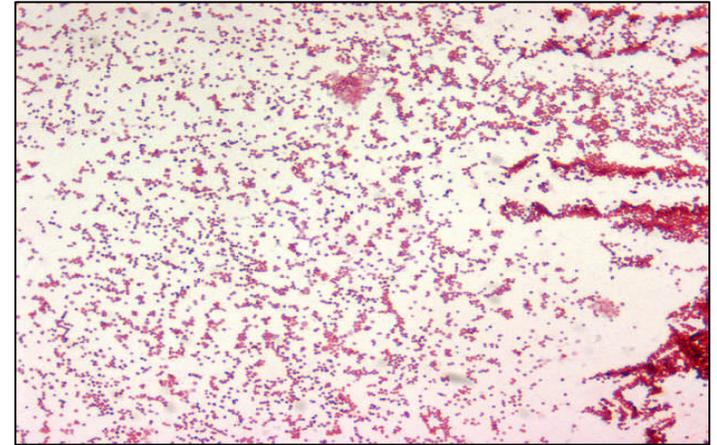
- SBA plate – colonies 1-2 mm in diameter, gray-white, opaque, little or no hemolysis of sheep blood.
- Thayer-Martin and chocolate agar plates – colonies blue-white to gray, flat, entire, smooth, shiny
- CHAB plates – colonies 2-4 mm in diameter, *greenish*-white, butyrous; have *opalescent* sheen (more pronounced on extended incubation); medium around areas of confluent growth or isolated colonies may also appear greenish.
- MAC plate – no growth
- (BCYE) Modified charcoal yeast agar - colonies blue-white to gray, flat, entire, smooth, shiny

Gram stain

- Tiny, Gram-negative rods; 0.2-0.5 um x 0.7-1.0um
- Pleomorphic, poorly-staining



- Cells may appear to be coccoidal. This is because cells exhibit bipolar staining. The darker staining areas (which appear coccoid-like) actually are the ends of bacillary forms whose centers stain even more faintly than their ends.



Identification Flow Chart

Tiny Gram-negative coccobacilli from blood, lymph node aspirate, lesion, or respiratory specimens.

-

Isolates growing slowly on Chocolate agar, poorly or not at all on Blood agar at 72 hours

-

Beta lactamase positive

-

Catalase weakly positive

-

Oxidase negative

-

XV or Satellite negative

-

Urease negative

-

Refer to State Laboratory

610-280-3464