

Level A Laboratory Procedures for Identification of *Francisella tularensis*

I. General: The procedures described below function to rule out suspected *F. tularensis* using specimens or isolates.

II. Precautions

A. These procedures should be performed in microbiology laboratories that utilize Biological Safety Level 2 (BSL-2) practices; use of biological safety cabinet is recommended. Because of the highly infectious nature of this organism (BSL-3) the state public health laboratory/department should be consulted immediately if tularemia is suspected.

B. Refer to Procedure for Laboratory Safety and Decontamination.

III. Specimen

A. Acceptable specimens

1. Blood culture: Collect appropriate blood volume and number of sets per established laboratory protocol.
2. Biopsied tissue or scraping of an ulcer is preferable; a swab of the ulcer is an acceptable alternative.
3. Aspirate of involved tissue

B. Specimen handling

1. Blood: Transport directly to laboratory at room temperature. Hold at room temperature until placed onto the blood culture instrument or incubator. Do not refrigerate. Follow established laboratory protocol for processing blood cultures.
2. Biopsy: Submit tissue, scraping, or aspirate in a sterile container. For small tissue samples, add several drops of sterile normal saline to keep the tissue moist. Transport at room temperature for immediate processing. If processing of specimen is delayed, keep specimen chilled (2-8°C).
3. Swabs: Obtain a firm sample of the advancing margin of the lesion. If using a swab transport carrier, the swab should be reinserted into the transport package and the swab fabric moistened with the transport medium inside the packet. Transport at 2-8°C; room temperature is acceptable. If processing of specimen is delayed, keep specimen chilled (2-8°C).

C. Rejection criteria

1. Use established laboratory criteria.
2. Dried specimens should be referred to your state public health laboratory.
3. Environmental/nonclinical samples and samples from announced events are not processed by Level A laboratory; submitter should contact the state public health laboratory directly.

IV. Materials

A. Media

1. General nutrient-rich agar: Sheep blood agar (SBA) or equivalent
2. Cysteine-supplemented agar: Chocolate agar (CA), Thayer-Martin (TM) agar, buffered charcoal yeast extract (BCYE), or other similar agar
3. Selective agar: MacConkey agar or eosin methylene blue (EMB)
4. Thioglycolate broth
5. Blood culture, standard blood culture system

B. Reagents

1. Catalase reagent (3% hydrogen peroxide)
2. Gram stain reagents
3. Oxidase reagent
4. XV or *Staphylococcus aureus* ATCC #25923 for satellite test
5. Beta-lactamase test (e.g., Cefinase test reagent)
6. Urease test (e.g., Christensen agar or biochemical kit)

C. Equipment/supplies

1. Microscope slides
2. Heat source for fixing slides: Burner (gas, alcohol), or heat block
3. Staining rack for slides
4. Microscope with high power and oil immersion objectives
5. Bacteriologic loops, sterile
6. Incubator: 35-37°C, ambient atmosphere, CO₂ is acceptable

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources; inclusion does not imply endorsement by the Centers for Disease Control and Prevention, the Department of Health and Human Services, or the Federal Bureau of Investigation.

- V. Quality control:** Perform quality control of media and reagents according to package inserts, NCCLS document M22-A2, and CLIA standards, using positive and negative controls appropriate for each media and reagent. Document all quality control results according to standard laboratory practices.

VI. Procedure

A. Stains and smears: Gram stain

1. Procedure: Perform Gram stain procedure/quality control per standard laboratory protocol.
2. Characteristics: Staining of *F. tularensis* often reveals the presence of tiny, 0.2-0.5- μm X 0.7-1.0 μm , pleomorphic, poorly staining, gram-negative coccobacilli seen mostly as single cells (Fig. A1). The Gram stain interpretation may be difficult because the cells are minute and faintly staining. *F. tularensis* cells are smaller than *Haemophilus influenzae*. Bipolar staining is not a distinctive feature of *F. tularensis* cells.
3. Additional work: Another smear may be prepared for referral to your state public health laboratory.

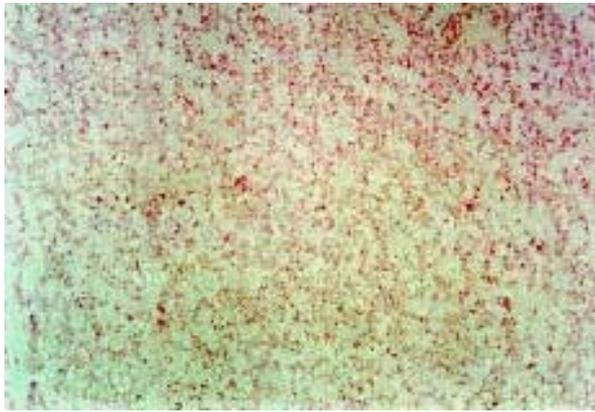


Figure A1. Gram stain of *F. tularensis*, X1000

B. Cultures

1. Use established inoculation and plating procedures. For tissues, use established laboratory procedure to inoculate media (e.g., grind, touch-preparation, or a sterile wood stick). Tape plates shut in 2 places to prevent inadvertent opening (alternative to taping is acceptable).
2. Incubation of cultures
 - a. Temperature: 35-37°C
 - b. Atmosphere: Ambient, use of 5% CO₂ is acceptable.
 - c. Length of incubation: Hold primary plates for 5 days. If it is known that patient has been treated with bacteriostatic antibiotics, then hold plates for up to 7 days to allow bacteria recovery time.
3. Characteristics: *F. tularensis* grows in commercial blood culture media. These organisms require cysteine supplementation; therefore, *F. tularensis* may at first grow on SBA, but upon subsequent passage will fail to grow on standard SBA. On cysteine supplemented agar plates, it is a gray-white, opaque colony, usually too small to be seen at 24 h on most general media such as CA, TM, and BCYE. After incubation for 48 h or more, colonies are about 1-2 mm in diameter, white to gray to bluish-gray, opaque, flat, with an entire edge, smooth, and have a shiny surface (Fig. A2a and A2b). *F. tularensis* will not grow on MacConkey or EMB plates.



Figure A2a. *F. tularensis* SCHU strain on 6% sheep blood agar, 72 h. Note its inability to grow well on SBA.



Figure A2b. *F. tularensis* SCHU strain on chocolate agar, 72 h

C. Biochemical reactions/tests

1. Procedure: Use established laboratory procedures for catalase, oxidase, beta-lactamase, XV (or satellite), and urease tests.
2. Interpretation: According to established laboratory practice
3. Additional notes: Commercial biochemical identification systems are not recommended at this stage.

VII. Interpretation and reporting (Fig. A3)

A. Suspected criteria: Any isolate, from the respiratory tract, blood, or lymph node, containing the major characteristics noted below should be suspected as *F. tularensis*. Warning: Refer to Limitations, VIII. C.

1. Tiny, poorly staining gram-negative coccobacilli seen mostly as single cells (Fig. A1); morphology on Gram stain may be indistinct because the cells are so small; pinpoint colonies on chocolate agar and often on sheep blood agar at 24 h and more visible (1-2 mm) colonies after 48 h (Fig. A2a and A2b).
2. No growth on MacConkey/EMB
3. Oxidase negative
4. Catalase weakly positive
5. Beta-lactamase positive
6. Satellite or XV test negative
7. Urease test negative

B. Reporting/appropriate action

1. Level A laboratories should consult with state public health laboratory director (or designate) prior to or concurrent with testing if *F. tularensis* is suspected by the physician.
2. Immediately notify state public health laboratory director (or designate) and state public health department epidemiologist/health officer if *F. tularensis* cannot be ruled out and a bioterrorist event is suspected. The state public health laboratory/state public health department will notify local FBI agents as appropriate.
3. Immediately notify physician/infection control according to internal policies if *F. tularensis* cannot be ruled out.

4. Preserve original specimens pursuant to a potential criminal investigation and possible transfer to an appropriate LRN laboratory. FBI and state public health laboratory/state public health department will coordinate the transfer of isolates/specimens to a higher-level LRN laboratory as appropriate. Start chain-of-custody documentation if appropriate.
5. Obtain guidance from the state public health laboratory as appropriate (e.g., requests from local law enforcement or other local government officials).
6. If *F. tularensis* is ruled out, proceed with efforts to identify using established procedures.

VIII. Limitations

- A.** The identification of *F. tularensis* should not be attempted with commercial identification systems because of the potential of generating aerosols and the high probability of misidentification.
- B.** Wild-type *F. tularensis* will grow initially on SBA but will grow poorly or not at all upon subsequent passages. Cysteine-enriched media (CA, TM, BYCE) would support growth of subcultures.
- C.** The most common misidentification of *F. tularensis* is *Haemophilus influenzae* (satellite or XV positive), and *Actinobacillus* species (beta-lactamase negative). Identification of isolates by using commercial identification systems is not recommended due to the high probability of misidentification. The Vitek NHI panel may give as high as 99% confidence to the identification of *Actinobacillus actinomycetemcomitans* with strains of *F. tularensis*.

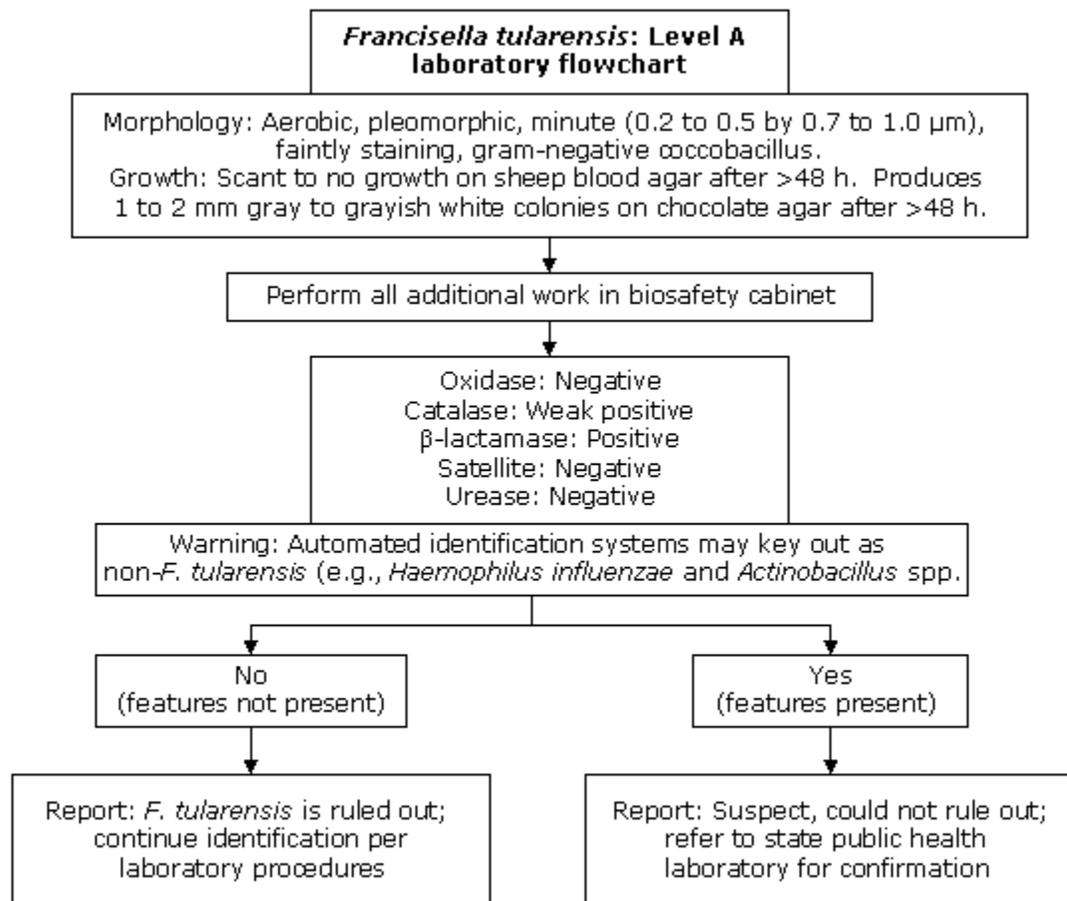


Figure A3: Level A flowchart for *F. tularensis*

XI. References

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