

## I. Introduction

Culture remains the most sensitive method for the diagnosis of Legionnaires' disease. The reported sensitivity and specificity for culture are 80 to 90% and 100%, respectively. In addition to respiratory tract specimens, *Legionella* has been isolated from blood, pericardial, and peritoneal fluids, as well as prosthetic valves and sternal wounds.

## II. Specimen Collection

- A. Appropriate specimens:
  - 1. Pleural fluid
  - 2. Transtracheal aspirate
  - 3. Bronchial washings/Bronchial Alveolar Lavage (BAL)
  - 4. Lung tissue
  - 5. Sputum
  - 6. Tracheal aspirate
- B. The specimen should be collected in a sterile container.
- C. Order the test as CLEG "*Legionella* Culture".

## III. Processing Procedure

- A. The following media are inoculated:
  - 1. Buffered Charcoal Yeast Extract Agar (BCYE)
  - 2. Selective Charcoal Yeast Extract Agar (BCYES)
- B. The media are incubated at:
  - 1. 5-10% CO<sub>2</sub> at 35°C.
  - 2. Tape the plates closed.

## IV. Interpretation and Reporting

- A. Examine the plates on day 4 and day 7.
  - 1. No growth cultures:
    - a. On Day 4 - Preliminary report: **No *Legionella* isolated to date.**
    - b. On Day 7 - Final report: **No *Legionella* isolated.**
  - 2. Cultures with growth:
    - a. Evaluate colony morphology.
      - i. After approximately three days, *Legionella* colonies are flat, rough and grayish 2 mm colonies on BCYE and/or BCYES agar.
      - ii. Very young colonies of *L. pneumophila* may appear as characteristic convex, ground-glass colonies with speckled green, blue, or pinkish purple iridescent edges. This iridescence can be readily seen through the dissecting scope, if a light source is directed towards the plate at an angle.
      - iii. Colonies of non-*L. pneumophila* species can be mucoid with irregular edges or raised grayish white colonies.
      - iv. If no suspicious colonies are present, report: **Negative for *Legionella*.**
    - b. Gram stain colonies exhibiting suspicious morphology.

- i. Colonies showing the presence of gram-negative rods should be tested further to determine if L-cysteine is essential for growth.
- c. Subculture gram-negative rods to a BAP and BCYE.
  - i. If the organism grows on the BAP, do not pursue *Legionella*. Report: **Negative for Legionella.**
  - ii. If there is no growth on the BAP and growth is present on the BCYE plate, perform the *Legionella* DFA stain on the isolate.
    - 1.) If the *Legionella* DFA stain is positive, report: **Legionella pneumophila.**
    - 2.) If the *Legionella* DFA stain is negative, bring the culture up on Rounds.
- d. Examine for fluorescence under a Wood's Lamp. Certain species of *Legionella*, other than *L. pneumophila*, autofluoresce blue-white, red, or yellow-green under long-wavelength UV light.
- e. Bring all suspicious or confirmed *Legionella* cultures up on Rounds.
- f. Although the *Legionellaceae* share a number of phenotypic characteristics, these characteristics are of limited value in species identification. The usual basis for identification includes the culture requirement for L-cysteine and serotyping. Due to our limited DFA reagents, isolates that fail to grow on the BAP and have been ruled out as *Haemophilus* spp. will be forwarded to the State Health Department for confirmatory testing.

## V. References

- A. Manual of Clinical Microbiology, 8<sup>th</sup> edition, 2003, Chapter 52, pg. 809-823.

Document Control

Effective 03/01/2006

Medical Director Approval: Reviewed by Dr. Schappert 3/10/2010.

Microbiology Director Approval: Dr. Ann Robinson 03/13/2006

Microbiology Supervisor Reviews: Jerry Claridge 03/06/2006, 01/2007, 09/2007, 09/2008, 09/2009, 03/2011, 03/2013, Jason Ammons 05/2015

Revisions & Updates: