ALPHADIA DFA Anti-L Pneumophila

Legionella pneumophila : Groups 1 - 6

IMMUNOFLUORESCENCE ASSAY
FOR THE DETECTION
OF LEGIONELLA PNEUMOPHILA
IN CULTURES AND CLINICAL SPECIMENS

CAT # AD LPD20 20 TESTS

FOR IN VITRO DIAGNOSTIC USE
CONS : 2 - 8°C

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INTENDED USE

These reagents are intended for use in the direct FA for the detection of L. pneumophila serogroups 1 through 6, in cultures and clinical specimens.

INTRODUCTION

The outbreak in July, 1976 of an acute febrile respiratory illness at a convention of the Pennsylvania American Legion in Philadelphia led to an extensive investigation by the Centers for Disease Control (CDC). This resulted in the isolation and identification of the etiologic agent, Legionella Disease Bacterium (LDB), a gram negative rod. The disease, which is now commonly termed Legionnaires'Disease (LD), exhibits a variety of responses from subclinical, asymptomatic infection, and mild influenza-like illnesses to severe multi-systemic disease most commonly recognized as pneumonia.

Although several aspects and serologically distinct serogroups have been described thus far, most of the information has been collected on Legionella pneumophila. At least six distinct serogroups have been described for this species. The direct fluorescent antibody (FA) test may be used for detection of LDB in lung tissues, respiratory tract fluids and cultures. Diagnosis should be confirmed whenever possible by information obtained on histopathological staining, and isolation of the organism; however, the direct FA test provides the most definitive method of identification in the absence of actual isolation, growth and characterization of LDB from the patient.

PRINCIPLES

Direct FA is the simplest and most rapid of the immunofluorescence procedures. Fluorescein isothiocyanate (FITC) labeled globulin conjugate is directed against the antigens prepared. The particular antigen is fixed to a slide and the conjugated globulin is overlaid. The antigen binds to the labeled globulin, forming antigen-antibody complexes which are rendered visible by the excitation of the FITC by the ultraviolet-blue light. When excited by such light, FITC emits longer wavelengths of light in the yellow-green portion of the color spectrum, and the antigens (Legionella cells in this case) are seen as fluorescent yellow-green bacilli.

STORAGE - STABILITY OF COMPONENTS

1. FITC labelled Rabbit anti-Legionella DFA conjugate cat CLPD2 (2.0 ml) with Evans Blue counterstain is to be stored at 2-8°C upon receipt. The conjugate may be aliquoted into small amounts and frozen at -20°C for extended storage.

Note : 2.0 ml vials provide 80 tests.
Upon receipt, store the conjugates at 2-8°C. Check labels for specific expiration dates. Sodium Azide is added as a preservative. The conjugates provided are at their optimum working potency for both sensitivity and specificity.

2.FITC labelled negative rabbit globulin for use in the Legionella Direct FA test. The conjugate is prepared from normal rabbit globulin and is adjusted to the same working dilution as that of the most concentrated immune Legionella conjugates, using 0.25% Evans Blue as the diluent. The product is available in a volume of 2.0 ml (NCLPD2) and contains sodium azide as a preservative. Follow storage instructions for the immune conjugates above. Reconstitute with sterile DI H_2O according to volumes specified on the label. The conjugate may be aliquoted into small amounts and frozen at -20°C for extended storage.

3.Legionella Control/Specimen slide for the Direct IFA test. Antigens are grown on artificial medium and formalin killed antigen suspensions used to prepare slides. The control/specimen slide contains three small pre-applied control wells and two large wells for patient specimen (SLPDT16) . Slides must be stored at 2-8°C or lower. Refer to product label for expiration date.

4.Phosphate Buffered Saline pH 7.2+/- 0.1, 0.01M(PBS1).
The PBS powder is packaged in 10 gr packets which make 1000 ml of buffer when reconstituted with distilled water. Unreconstituted, the buffer is stable at room temperature; refer to product label for expiration date. The PBS contains no preservative, as a 10x concentrate, the reconstituted buffer may be stored at 2-8°C. Working buffer must be used on the day prepared.

5. Mounting medium N° BMM3 - 3ml is stable when stored at 2-8°C. Check label for specific expiration date.

NOTE : All kit components are available separately.

Additional materials required but not provided :

Test tubes and rack or microtiter system
Disposable pipettes
Staining dish and slide forceps
Moisture chamber
Distilled water
Fluorescence microscope
Paper towels
37°C incubator

Precautions
These products are for in vitro diagnostic use.
Observe aseptic technique and established precautions against microbiological hazards. Avoid fixation of control wells on slide (SLPDT16).

PREPARATION OF SPECIMEN MATERIAL
During fixation and staining, each slide must be processed separately to ensure that organisms present in one specimen are not transferred to another specimen.

Preparation of culture smears
Note : Process in a safety cabinet.

1. Make suspensions of cultures of known or suspected Legionella bacteria in 1% neutral formalin to give a light turbidity (a McFarland N°1).

2. Prepare smears on double ring or on multi-well slides (SLPDT16 or SLPDTA).

3. Air dry and heat fix.

Preparation of fresh-frozen Tissue (Autopsy or Biopsy)
Note : Process in a safety cabinet.

If tissue is to be culture, do this before making imprints.

1. Using sterile instruments, cut a fresh face of tissue, with the forceps, press and squeeze the tissue against clean slides. If the tissue is so moist that smears may be too thick, first blot on sterile gauze.

Alternatively, grind the tissue with sterile PBS and alundum in a sterile mortar or homogenize the tissue in a Ten Broeck or comparable tissue grinder. Use sufficient PBS to give approximately 10% tissue homogenate. Prepare smears in the same manner as described for formalin-fixed tissue. With larger pieces of tissue, this method produces a more representative sample and reduces sampling error.

2. Air dry and heat fix.

3. Fix smears for 10 minutes by covering with a solution of 10% neutral formalin. Place the slides in a moist chamber to prevent evaporation of the formalin.

4. Drain off the formalin and gently spray rinse with distilled water, or briefly dip each slide into distilled water to remove remainder of the formalin.

5. Air dry.

Preparation of Tissue scrapings for formalin fixed-tissue
1. Select one or more areas of the lung or other tissue for testing. In the lung, areas of dense gray or reddish consolidation are used.

2. Transfer each tissue block to a sterile Petri dish.

3. With a sharp scalpel, cut through these areas to produce new tissue faces for scraping.

4. Grasp the tissue with forceps and holding the scalpel at a right angle to the tissue face, scrape it to produce a fine puree of tissue particles (see limitations of procedure, item 1.).

5. Smear the particles of tissue and tissue fluids onto Alphadia slides (SLPDT16 or SLPDTA) using the scalpel blade to make the smears.

6. Allow the smears to air dry. Gently heat fix.

Preparation of Tissue - Paraffin embedded
Legionella bacteria maintain their serologic integrity through histopathological processing and can be easily demonstrated in tissue sections if reasonable numerous. However, they are not as easily demonstrated in sections as in scrapings of formalin fixed lung or imprints of fresh lung tissue. This is because there are many different levels in the section, and because they are shrunken in size by the histopathological processing.

1. Cut the tissue as thin as possible (4 microns or less) onto Alphadia SLPDT16 or SLPDTA.

2. Fix the sections for approximately 15 minutes at 58-60°C.

3. De paraffin by two passages through xylol, followed by two passages each through absolute ethanol, 95%, and water.

Preparation of Exudates from the Lungs
Note : Process in a safety cabinet.

1. Select a viscous portion of the specimen and prepare smears of moderate thickness on Alphadia slides, (SLPDT16 or SLPDTA) within the 1 to 1.5 cm diameter circles on the microscope slide.

2. Air dry and heat fix.

3. Fix in neutral formalin as described above for fresh tissue.

4. Drain off the formalin and spray rinse or dip the slides briefly into distilled water.

5. Air dry.

Preparation of Pleural Fluids
Note : Process in a safety cabinet.
1. Pleural fluids should be cultured for attempted isolation of the Legionella organisms by contaminants is not a problem.

2. Prepare thin smears, air dry, heat fix and process as described for lung exudates (see limitations of procedures).

TEST INSTRUCTIONS
Note : The polyvalent conjugate (CLPD2) and negative control conjugate (NCLPD2) are used initially to screen specimens. The subgrouping conjugates can be used to examine those specimens which are positive with polyvalent conjugates and negative conjugate.

Apply the conjugates as appropriate to the control/specimen slides (SLPDT16 or SLPDTA).

2. Place slides in a moisture chamber.

3. Stain for 20-30 minutes at room temperature.

4. Tap off excess conjugate and rinse slides with a gentle stream of PBS (do not aim directly at the specimen). Avoid even momentary intermixing of conjugates of different specificities or reactivities on the smear.

5. Immerse slides in PBS for 10 minutes, rinse briefly in distilled water.

6. Remove slides and air dry.

7. Add 4-5 drops of mounting medium coverslip.
Examine the smears using the 10x objective initially, then switch to the 40x to the 63x immersion objective. Confirm observations with the 100x oil immersion objective.

STABILITY OF THE FINAL REACTION
Stained slides should be examined immediately or kept in the dark and moist at 2-8°C until ready to examine on the day of staining or overnight.

READING STANDARD
4+ = Brillant yellow-green cell wall staining.
3+ = Bright yellow-green cell wall staining.
2+ = Definite but dull yellow-green staining. Cell wall becomes less defined.
1+ = Dim yellow-green staining. Diffuse staining of cell.

QUALITY CONTROL
The conjugates and control antigens must demonstrate the following cell-wall staining reactions, or the test run must be considered invalid.

1. The serogrouping conjugates must stain their homologous control antigen at 3+ to 4+ intensity. Somatic cross-reactions between serogroups will be predominantly less than 2+, however, a few cells per microscopic field may be brighly stained 3-4+.

2. The negative conjugate must not stain any of the 6 serogroup antigens.

INTERPRETATION OF RESULTS
Legionella bacteria are pleomorphic bacilli. Organism in culture are usually longer than those seen in tissues. In older cultures, swollen rods and other bizarre forms may be seen. The bacteria may produce flagella in vivo and in culture. These flagellar antigens are commonly shared among the Legionella serogroups.

1. In clinical specimens, except sputa, the following criteria are used to evaluate and report test results.

a. 25 typical bacteria with bright (3+ to 4+) cell-wall fluorescence per smear : FA-positive.
b. Typical bacteria with bright cell-wall fluuorescence per smear : Numbers only reported.
c. No typical bacteria with bright cell-wall fluorescence per smear : FA-negative.

d. Bacteria atypical morphology which stain brightly, or bacteria with stain 2+ or less : FA-negative.

2. In sputa, organisms are never numerous; therefore, observation of 5 or more typical bacteria with bright cell-wall fluorescence is considered positive.

3. Cultural isolates which are morphologically typical and exhibit bright cell-wall fluorescence are considered positive.

4. Positive results should be reported as Legionella pneumophila serogroup 1,2 etc.

LIMITATIONS OF PROCEDURE

1. Lung tissue from a person with Legionella is usually quite pliable; however, if the tissue is rubbery or spongy, a positive test is unlikely.

2. Plural fluids tend to form a fibrin clot on the slide, and unless handled carefully, the entire film may be dislodged during processing.

3. Stains of the lower respiratory tract are usualy more difficult to interpret.

4. Unwanted fluorescence may occur with white blood cells and bacteria such as staphylococci, diplococci and streptococci due to antibodies in the serum of the immunized rabbit or to non-specific reaction of the IgG molecule with cell-wall components such as protein A. These are morphology and staining characteristics of Legionella. One strain of Ps, fluorescence has been found which is brightly and specifically stained by the working dilution of a Legionella conjugate.

Evans Blue is added to conjugate at a dilution which effectively counteracts background fluorescence such as autofluorescence.

5. When viewing lower respiratory tract smears, at least a five minute search is necessary before calling the smear negative, since relatively few Legionella organisms are seen in these specimens.

6. This test is presumptively diagnostic for Legionella. Whenever possible, diagnosis should be confirmed by isolation of the organism and conventional microbiological techniques.

PRECAUTIONS

1. All human components have been tested by radioimmunoassay for HBsAg and HTLVIII/LAV by an FDA approved method and found to be negative. Not repeatedly reactive. However, this does not assure the absence of HBsAg or HTLVIII/LAV. All human components should be handled with appropriate care.
2. Do not use components beyond their expiration date.
3. Follow the procedural instructions exactly as they appear in this insert to insure valid results.
4. For in vitro diagnostic use.
5. Observe aseptic technique and established precautions against microbiological hazards.
6. Avoid fixation of control wells on slide. (SLPDTC)

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