SUMMARY AND PRINCIPLES
The outbreak in July, 1976 of an acute febrile respiratory illness at a conference of the Pennsylvania American Legion in Philadelphia (1) led to an extensive investigation by the Centers for Disease Control. This resulted in the isolation and identification of the etiologic agent, then referred to as Legionnaires Disease Bacterium (LD) a gram negative rod (2). Although several species of serologically distinct serogroups have been described (4-9), thus far most of the information has been collected on Legionella pneumophila (10,11).

The disease, which is now commonly termed legionellosis, exhibits a variety of responses from subclinical, asymptomatic infection, and mild influenza-like illnesses, to severe multisystemic disease most commonly recognized as pneumonia (3).

Solid evidence for a diagnosis of legionellosis is obtained when seroconversion occurs with paired sera run simultaneously. There must be a four-fold or greater rise in titer to at least 128 between the acute and convalescent phase sera. A single or standing titer of greater than or equal to 256 may indicate past infection or exposure to Legionella. The diagnostic relevance of such single or sustained titers cannot be determined with currently available epidemiologic data and is reported as inconclusive.

The indirect fluorescent antibody test is used for the detection of human IgG antibody to the antigens of; L. pneumophila, serogroups 1-6 (L.pn 1-6); L. pneumophila, serogroups 7-14 (L.pn 7-14); L. bozemanii 1 and 2; L. dumoffi, L. gormanii, L. micdadei, L. longbeachae 1 and 2; and L. jordanis (L.sp b-); by the indirect fluorescent antibody (IFA) procedure. The MarDx Legionella IFA Test System is not to be used for diagnostic purposes and is intended to be used only when the actual diagnosis is based on an established method or procedure including clinical findings. For Professional Use. For Export Only

PRECAUTIONS
1. Follow the procedure instructions exactly as they appear in this insert to ensure valid results.
2. Always wear suitable protective clothing, gloves and eye/face protection when working with this product.
3. Thimerosal (Mercapto) used as a preservative in some of the reagents, may be toxic if ingested, inhaled, or absorbed through skin and is a reproductive hazard.
4. Some components contain less than 0.1% sodium azide which is toxic if ingested and forms potentially explosive copper and lead azide compounds in waste plumbing lines. Should the reagents come in contact with copper or lead waste plumbing, flush the waste line with large quantities of water to prevent the formation of potentially explosive compounds.
5. WARNING - POTENTIAL BIOHAZARDOUS MATERIAL. Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of antibody to HIV, as well as HBsAg, and found to be negative (were not repeatedly reactive). Because no test method can offer complete assurance that human immunodeficiency virus (HIV), hepatitis B virus, or other infectious agents are absent, these human control reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biohazards in Medical Microbiology and Biomedical Laboratories", 1999 (12).
6. The phosphate buffered saline and mounting medium found in this kit are irritating to the eyes, respiratory system and skin.

STORAGE AND STABILITY
1. Legionella Omni Slides (Prod #30-7109): Store at +2 to +8°C upon receipt. All antigens are stable until the expiration date printed on the product label, when stored at the recommended temperature.
2. Legionella pneumophila, Serogroup 1, Strain Specific, Positive Control Chimeric Mouse (Prod #30-7051): Store at +2 to +8°C. Refer to product label for expiration date.
3. Legionella Negative IFA Control (Prod #30-7500): Store at +2 to +8°C. Refer to product label for expiration date.
4. FITC labeled Anti-human IgG (gamma chain specific) for Legionella IFA (Prod #30-1524): The conjugate should be stored at +2 to +8°C. Refer to the product label for expiration date.
5. Phosphate Buffered Saline, pH 7.5 (Prod #90-1607): Store at +2 to +8°C. PBS is stable at room temperature in its bicarbonate buffered mounting medium.
7. Distilled water.

ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED
1. Test tubes, test tube rack, pipettes, or a microtiter system for preparing titrations.
2. Volumetric flask (1 liter) for PBS.
3. Moist incubation chamber.
4. Slide washing chamber.
5. Fluorescence microscope equipped with FITC filters.
7. Distilled water.

7. Some components in this kit contain 0.1% Proclin 300. At full strength Proclin 300 is corrosive and will cause burns and possibly sensitisation by skin contact.

Xi – Irritant
R43: May cause sensitization by skin contact.
S28-37: After contact with skin, wash immediately with plenty of water and soap. Wear suitable gloves.

MATERIALS PROVIDED:

<table>
<thead>
<tr>
<th>Prod#</th>
<th>Description</th>
<th>Qty</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-7109</td>
<td>Legionella Omni Slides (L.pn 1-6, L.pn 7-14, L.sp b-))</td>
<td>10 ea</td>
</tr>
<tr>
<td>30-7501</td>
<td>L. pneumophila Serogroup 1 Positive Control, IFA</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>30-7500</td>
<td>L. pneumophila Negative Control, IFA</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>30-1524</td>
<td>Legionella FITC IgG Conjugate</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>90-1611</td>
<td>Legionella Mounting Medium (pH 9.0)</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>90-1607</td>
<td>Phosphate Buffered Saline (pH 7.5)</td>
<td>2x10 g</td>
</tr>
<tr>
<td>90-1700</td>
<td>Coverslips, 70x22 mm</td>
<td>12 ea</td>
</tr>
<tr>
<td>90-1718</td>
<td>Blotters, 18 Well</td>
<td>10 ea</td>
</tr>
</tbody>
</table>

REAGENT PREPARATION
1. Allow all reagents to come to room temperature before use.
2. Legionella omni slides (Prod #30-7109), should be brought to room temperature prior to breaking the package seal. Peel back the top portion of the package and remove the slide without touching the antigen wells. The slide is now ready to use. Antigen placement on the slide is as follows:

<table>
<thead>
<tr>
<th>Row</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
</tr>
<tr>
<td>Col2</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
</tr>
<tr>
<td>Col3</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
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<td>1-6</td>
</tr>
<tr>
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<td>1-6</td>
<td>1-6</td>
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</tr>
<tr>
<td>Col5</td>
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<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
</tr>
<tr>
<td>Col6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
<td>1-6</td>
</tr>
</tbody>
</table>

3. Reconstitute each 10 gram vial of PBS (Prod #90-1607) with 1.0 L distilled water.
4. The conjugate (Prod #30-1524) is supplied as a ready to use liquid. No reconstitution is necessary. Mix by vortex or inversion prior to use. Dilute sufficient conjugate for a day’s testing to the recommended working dilution stated on the vial using PBS (Prod #90-1607). The recommended working dilution of the conjugate was established using reference reagents and recommended optical systems. Refer to the product label for the established working dilution.
5. The conjugate may require refrigeration. See Preparation of Controls.
6. The Legionella mounting medium (Prod #90-1611) is used at the concentration provided.
SPECIMEN COLLECTION
Serum should be collected using aseptic technique. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at +2 to +8°C if it is to be analyzed within a few days, and may be held for months at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided (13).

Acute phase sera should be collected within the first week after onset of illness, and convalescent phase sera, 3-8 weeks after onset. In addition, acute and convalescent sera should be tested simultaneously (within the same assay run) so that a more precise comparison of antibody activity is established. For these reasons, acute phase sera should be retained frozen (-20°C or lower) until convalescent sera is ready for testing.

PREPARATION OF CONTROLS
Include the positive and negative controls in each run.

The L. pneumophila Serogroup 1 Positive and the Legionella Negative Control are supplied as a ready to use liquid. No reconstitution is necessary. Mix the control by vortex or inversion prior to use. Prepare dilutions using PBS.

1. The positive control is standardized to give end-point activity within one two-fold dilution above or below the dilution stated on the vial. Include in the test procedure all dilutions from one two-fold dilution below to one two-fold dilution above the expected end-point dilution. Refer to the vial label for the specified dilution end-point of each lot.
2. The negative control is standardized to demonstrate a negative reaction at the dilution stated on the vial. Include in the test the negative control at the stated dilution.

Note: Variations in absolute fluorescence between microscopes can be expected due to the variation in the optical sensitivity of the microscope components including light source, objective lenses, ocular lenses, total magnification, etc. If the controls consistently yield results higher or lower than expected, the conjugate may require retitration. This is accomplished by retesting the controls at appropriate two-fold dilutions of the conjugate using PBS as a conjugate diluent. If retitration of conjugate is required, please call the MarDx technical support department (1-800-331-2291) for assistance.

PREPARATION OF SPECIMENS
Make dilutions of patient serum samples in PBS. The recommended dilutions for patient screening are 1:64, 1:128, and 1:256.

Prepare a 1:64 dilution of each patient serum, e.g., add 0.10 mL of serum to 6.30 mL of PBS. Mix equal volumes of diluted serum and PBS for subsequent two-fold dilutions. Sera producing positive reactions at 1:256 should be titrated to end-point.

TEST PROCEDURE
Note: Initial screening of patients sera should be done once the dilution of the conjugate has been established and the operator has gained sufficient experience with the system.

1. Remove the number of slides needed from the sealed pouches and mark them with a marking pen as necessary.
2. Test sera should be placed on slides according to the scheme given below:

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:64</td>
<td>1:64</td>
</tr>
<tr>
<td>1:128</td>
<td>1:128</td>
</tr>
<tr>
<td>1:256</td>
<td>1:256</td>
</tr>
</tbody>
</table>

Rows:

<table>
<thead>
<tr>
<th>Row 1</th>
<th>1-6</th>
<th>1-6</th>
<th>1-6</th>
<th>1-6</th>
<th>1-6</th>
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</thead>
<tbody>
<tr>
<td>Row 2</td>
<td>7-14</td>
<td>7-14</td>
<td>7-14</td>
<td>7-14</td>
<td>7-14</td>
<td>7-14</td>
</tr>
<tr>
<td>Row 3</td>
<td>b-j</td>
<td>b-j</td>
<td>b-j</td>
<td>b-j</td>
<td>b-j</td>
<td>b-j</td>
</tr>
</tbody>
</table>

Using this format two patient sera can be tested per slide. Control sera should be evaluated on a separate slide. Add just enough diluted serum (10 µL) to cover each reaction site.

3. Incubate slides in a moist chamber at room temperature for 30 minutes.

4. After incubation with sera the slides should be taped onto a piece of paper towels so that a vertical draining pattern will result. This will prevent cross contact of sera from adjacent wells. Direct a gentle stream of PBS in the same vertical direction over the slide using a wash bottle. Do not aim the stream of PBS directly onto the wells.

5. Place the slides in a wash chamber filled with PBS for 5 minutes. Replace wash chamber with fresh PBS and wash slides for another 5 minutes.

6. Remove the slides from the PBS and place, antigen side up, on a dry paper towel. Carefully place the 18 well blotter over the slide so that the blotter is carefully indexed so as not to come in contact with the reaction wells. Hold one edge of the blotter with one hand to keep the blotter in place and apply sufficient gentle pressure with the microscope slide roller to remove the moisture surrounding antigen wells. DO NOT ALLOW THE ANTIGEN WELLS TO DRY.

7. Add just enough titered conjugate (10 µL) to cover each reaction site.

8. Incubate slides as described above (#3).

9. Rinse, wash and blot slides as described above (#4, #5, #6). DO NOT ALLOW THE ANTIGEN WELLS TO DRY.

10. Place 2 to 3 drops of mounting medium on slide and cover with a coverslip avoiding air bubbles.

11. Read slides with a fluorescence microscope.

READING SLIDES

1. Do not attempt to read the slides before the microscope has been switched on for at least 5 minutes.
2. Read slides within one hour. Slides may be read within 24 hours if stored refrigerated in a moist chamber. Allow refrigerated slides to warm to room temperature before reading.
3. The slides should be examined at a total magnification of 40X.
4. Drying may distort the most peripherally situated organisms in the well, therefore disregard the reactions seen with these organisms.
5. The staining intensity of individual cells may vary, however, the degree of staining is based on the overall appearance of the antigen smear.
6. Record reaction intensity at each dilution using the following criteria:

   - 4+ = Brilliant yellow-green cell wall staining.
   - 3+ = Bright yellow-green cell wall staining.
   - 2+ = Definite but dull yellow-green staining.
   - 1+ = Dim yellow-green staining, diffuse staining of cell. Negative = Absence of yellow green specific fluorescence.

7. Read the controls before proceeding to the test sera.

QUALITY CONTROL
1. The positive control serum must demonstrate positive fluorescence within one dilution of the end-point dilution stated on the vial or the test is invalid.
2. The negative control serum must demonstrate the absence of yellow-green specific fluorescence at the stated dilution on the vial or the test is invalid.
3. Reading of test serum end-points with each microscope assembly must be made with reference to the reactivities of the control sera with the antigen slides and conjugate provided.

INTERPRETATION OF RESULTS
The serum titer is the reciprocal of the highest dilution of serum giving a positive (1+ to 4+) reaction, e.g., if the last positive reaction is 1+ at a dilution of 1:512, the titer is 512. Patient sera which have not reached their end-point when tested at 1:64, 1:128, and 1:256 should be retested to determine the reaction end-point.

NEGATIVE: A single titer of less than 256. In paired sera less than a four-fold increase in titer or <128 in the convalescent phase serum.

INCONCLUSIVE: Single or sustained titers of greater than or equal to 256 may indicate past infection or exposure to Legionella species, however, the diagnostic relevance of such titers cannot be determined with currently available epidemiologic data.

POSITIVE: A four-fold or greater rise in titer to greater than or equal to 128 between properly drawn acute to convalescent phase sera provides serological evidence of a recent Legionella infection.

RESULTS ON PAIRED SERA

<table>
<thead>
<tr>
<th>Acute</th>
<th>Convalescent</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1:64</td>
<td>1:64</td>
<td>Negative</td>
</tr>
<tr>
<td>&lt;1:64</td>
<td>1:128</td>
<td>Positive</td>
</tr>
<tr>
<td>&lt;1:64</td>
<td>1:256</td>
<td>Positive</td>
</tr>
<tr>
<td>1:64</td>
<td>1:128</td>
<td>Negative</td>
</tr>
<tr>
<td>1:64</td>
<td>1:256</td>
<td>Positive</td>
</tr>
<tr>
<td>1:64</td>
<td>1:512</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>1:512</td>
<td>1:1024</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

RESULTS ON SINGLE SERA

<table>
<thead>
<tr>
<th>Titer</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1:64</td>
<td>Negative</td>
</tr>
<tr>
<td>1:64</td>
<td>Negative</td>
</tr>
<tr>
<td>1:128</td>
<td>Negative</td>
</tr>
<tr>
<td>1:256</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>1:512</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>1:1024</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>
LIMITATIONS OF PROCEDURE

1. Light sources, total magnification, objective lenses, and ocular lenses influence intensity of staining.
2. Variations in intensities may be observed when different microscope assemblies are used. Testing of sera should not be attempted unless the positive control serum gives the expected titer within one two-fold dilution and the negative control yields negative results.
3. The accuracy in the test often depends on the competency of the operator.
4. This test is presumptively diagnostic for legionellosis. Further work by conventional cultural and biochemical methods is recommended when possible.
5. Clinical specimens of higher titer than the control sera provided may demonstrate brighter fluorescence at the screening test dilution when compared to the control.
6. The patients clinical data and other laboratory tests should be carefully reviewed by a medical authority before a diagnosis is made.
7. The serogroup or species of the infecting strain may not be determined from serologic results because patient sera often have IFA titers against multiple cross-reactive Legionella antigens.

REFERENCES


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