



## LEDIONELLA INDIRECT ANTIBODY FLUORESCENT TEST SYSTEM

### L. pneumophila 1 Ë 6 SCREEN

For *In Vitro* Diagnostic Use

AD LPI180 180 Tests

#### Intended Use

These reagents are intended for use in an indirect immunofluorescent assay for the detection and quantifying of *Legionella pneumophila* antibodies in human serum. The polyvalent reagents utilize antigenic coverage for *Legionella pneumophila* serogroups 1 to 6.

#### Introduction and explanation of the test:

The outbreak in July, 1976 of an acute febrile respiratory illness at a convention of the Pennsylvania American Legion in Philadelphia (1) 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 (2). The disease, which is now commonly termed legionellosis, exhibits a variety of responses from subclinical, asymptomatic infection, and mild influenza-like illnesses to severe multi-systemic disease most commonly recognized as pneumonia (3).

Although several aspects and serologically distinct serogroup have been described thus far (4-8) most of the information has been collected an *Legionella pneumophila*. The *Legionella* indirect fluorescent antibody (IFA) test is an immunofluorescent procedure for detection of antibodies against *L.pneumophila* in human serum (9-14).

Two types of test systems are available, the first contains a polyvalent mixture of *Legionella pneumophila* serogroups 1 to 6 as the antigen substrate. The second test system contains monovalent *L. pneumophila* serogroup 1 as antigen substrate. A fluorescent isothiocyanate (FITC) labelled antihuman globulin conjugate is the indicator reagent. The antihuman globulin conjugate has also been shown to react qualitatively and quantitatively with hyper-immune primate immunoglobulins. (15).

Solid evidence for a diagnosis of legionellosis is obtained when sero-conversion occurs with paired sera run simultaneously (14). There must be a fourfold or greater rise in titer (to at least 1:28) between the acute phase (within the first week) and convalescent phase (3-6 weeks after onset) of illness. 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 (presumptive).

#### Principles of the Test:

The *Legionella* IFA test is performed as a two-step antigen-antibody reaction. The first step occurs when the antigen on the slide is overlaid with dilutions of human serum or primate antiserum positive control. After incubation, the slides are rinsed to remove any excess antibody and dried. In the second step, the antigen (which is complexed with antibody in a positive reaction) is overlaid with fluorescein isothiocyanate (FITC) labelled antihuman globulin. After a second incubation, followed by rinsing and drying, the slides are examined using a fluorescence microscope.

#### Components Available:

- Legionella pneumophila Serogroup 1-6 specific antigen slides.**  
Store at 2-8°C. Refer to the product label for expiration date.
- Lyophilized Legionella pneumophila serogroup 1 Positive Control Primate Serum:**  
Lyophilized control sera (0.5 ml) are standardized to give a 1+ IFA titers within one two-fold dilution above or below the titers stated on the vial. See label for specific titered end-points. The control contains 0.095% sodium azide as preservative. In the lyophilized state, the control sera should be stored at 2-8°C. Refer to the product label for expiration date. Once reconstituted with 0.5 ml distilled water, small aliquots may be stored for 6 months at -20°C or lower or they may be stored at 2-8°C for up to 14 days.
- Lyophilized Legionella pneumophila Negative Control Primate Serum:**  
Lyophilized primate serum has been standardized from non-immunized primate and should be diluted 1:64 for testing purposes. The serum contains 0.095% sodium azide as preservative and is distributed in 0.5 ml amounts. Follow recommended storage procedures as prescribed for positive control sera above.
- Lyophilized FITC-Labelled Goat Antihuman IgG for Legionella IFA:**  
It is a concentrate which must be diluted after reconstitution to the working dilution. The reconstituting volume is 1.5 ml distilled water. See label for recommended dilution and test instructions for dilution procedure. In lyophilized state, the conjugate is stable until expiration date on label, when stored at 2-8°C. Repeated freeze-thaw or prolonged room temperature may deteriorate the conjugate. Sodium azide is added as a preservative at 0.095%.
- Phosphate Buffered Saline pH 7.2 ± 0.1, 0.01M.**  
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, and once reconstituted can be stored at 2-8°C for 1 month. A 10x concentrate may be stored at room temperature for 1 month if desired and a fresh dilution made daily.
- Carbonate-Bicarbonate Buffered Mounting Medium, pH 9.0 :**  
The mounting medium is packaged in 3.0 ml amounts in a dispenser bottle. It contains sodium azide at a 0.095% concentration. It is glycerol-based and will exhibit a slow drop in pH upon exposure to air. It is necessary to tightly cap the bottle when not in use. Storage is a 2-8°C. Refer to label for expiration date.
- Coverslips 22 mm x 70 mm**

#### Additional Materials Required but not Provided:

Test tubes and rack or microtiter system  
Disposable pipettes  
Staining Dish  
Moisture Chamber  
Volumetric Flask (1000 ml)  
Distilled water  
37°C incubator  
Fluorescent Microscope  
Paper Towels

#### Precautions

- Repeated freezing and thawing is detrimental to the conjugate, serum controls and antigen slides.
- Follow the procedural instructions exactly as they appear in this to ensure valid results.
- For *in vitro* diagnostic use.
- Reconstitute reagents carefully and thoroughly with distilled water. Reagents should be free of particulate matter. If reagents become turbid, bacterial contamination should be suspected. If contaminated, the reagent should be discarded.
- All human components have been tested 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.
- Use separate pipette tips for each sample and reagent to avoid cross contamination.
- Reagents should be inspected for evidence of bacterial or fungal contamination.
- Do not reuse substrate slides.

#### Specimen Collection:

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative is strongly recommended. Acute/convalescent sera should be drawn at least 3 weeks apart. Is therefore, strongly recommended that the acute serum should be retained frozen at -20°C to permit simultaneous testing with the convalescent serum in the same titration.

#### Test Instructions:

- Prepared slides:**
  - Allow slides to reach temperature. The slides contain a polyvalent mixture of *Legionella pneumophila* serogroups 1 to 6 in each well. They are designed to permit screening evaluations of three dilutions per patient or control serum. The recommended dilutions for patient screening is 1:64, 1:128 and 1:256. PBS should be used to make dilutions.
  - Place the lowest dilution (1:64) in the bottom row, 1:128 in the middle row and the 1:256 dilution in the top row. This enables testing of three dilutions of six separate serums without cross contamination.
  - After incubation with serum, the slides should be tapped so that a vertical draining pattern will result.
  - For typing and titering purposes, 18 well slides are available. These slides contains all six serogroups on each side, with only one serogroup present in each well. This enables three dilutions of one serum to be tested with each of the six serogroups of *Legionella pneumophila*.
- Conjugate preparation:**
  - Reconstitute the conjugate with 1.5 ml distilled water.
  - Dilute the conjugate to its recommended use dilution using PBS. The recommended dilution of the conjugate is listed on the package label and was established using reference reagents and recommended optical systems.
- Initial Screening Dilutions (1:64, 1:128, 1:256)**  
Initial screening of patient sera should be done once the dilution of the conjugate has been established and the operator has gained sufficient experience with the reagent system.
  - Make a 1:64 dilution of each serum by adding 0.01 ml (10µl) of serum to 0.63 ml of PBS.
  - Make two-fold dilutions of the sera from 1:64 to 1:256 in microtiter plates or test tubes, using equal volumes of sera and PBS.
  - Perform the same dilution sequence for the positive control, but include in the test all dilutions from one two-fold dilution below to one two-fold dilution above the expected end-point titer of the control.
  - Screening slides:** when screening slides are used, place 10 µl of the 1:64 serum dilution in one well of the bottom row, 10 µl of the 1:128 serum dilution in one well of the middle row and 10 µl of the 1:256 serum dilution in one well of the top row. When sera are placed, as stated, on the screening slides, this enables testing of three dilutions of six separate sera without cross contamination.
  - Type and Titer slides:** When Type and Titer slides are used, place 10 µl of lowest serum dilution in the 6 wells of the bottom row, 10 µl of the next highest serum dilution in all 6 wells of the middle row and the 10 µl of highest serum dilution in all 6 wells of the top row. When sera are placed, as stated, on the Type and Titer slides, three dilutions of the same serum can be evaluated against each individual serogroup in each well.
  - Incubate the slides at 19-24°C (room temperature) for 30 minutes in a moisture chamber.
  - After incubation with the serum, the slides should be tapped onto a piece of paper toweling so that the vertical draining pattern will result in the higher dilutions draining over the lower dilutions of the same serum. 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. Place slides in a wash chamber for 10 minutes (2 x 5 minutes) using PBS.
  - Remove the slides from PBS and rinse briefly with distilled water and allow to dry completely.
  - Reconstitute the antihuman globulin conjugate according to directions on the label. Dilute the concentrated conjugate to its working titer as specified on the label. Add 10 µl of conjugate per well. Repeat steps f, g and h.
  - Apply 6-10 drops of mounting medium to each slide and coverslip. Examine slides with a fluorescence microscope.

Note: Although it is recommended that the slides be examined the same day of testing, they can be examined within 24 hours if they are protected from light and stored at 2-8°C.

**4. Titration of Sera**

- a. Patient's sera, which have not reached their end point when tested at 1:64, 1:128 and 1:256; should be titrated to their 1+ reaction utilizing screening or Type and Titer.
- b. Control sera should be included in each run. The control sera should be tested at one two-fold dilution above and below its recommended end point titer, utilizing its homologous antigen.

**Reading Standard:**

- 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

1. Record the staining intensity at each dilution. The staining intensity of the cells may vary, however, the degree of staining is based on the overall appearance of the antigen preparation.
  2. The ALPHADIA positive control used with the ALPHADIA antigen and conjugate should be read before evaluating an unknown serum.
  3. The serum titration end-point (titer) is the highest dilution of serum giving a 1+ yellow-green fluorescence (e.g. if the last 1+ is seen at a dilution of 1:512, the titer = 512).
- Note: the slides contain polyvalent screening mixture of Legionella pneumophila 1-6 antigens. Particular attention should be made regarding the distribution, morphology and numbers of cells per serogroup when reading the fluorescence of the positive control sera. Each serogroup should result in approximately 75-150 cells/400X magnification. For the slides contain individual serogroups per well, less variation of fluorescence will be seen per well when compared with the polyvalent mixture.

**Quality Control:**

1. The conjugate and control antigens must demonstrate the stated titers of the control sera within one two-fold dilution or the test run must be considered invalid.
2. Light sources, magnification objectives, and eyepieces influence intensity of staining. Reading of end-points with each microscope assembly must be made with reference to the positive control serum used with the antigen(s) and conjugate provide. A conjugate dilution change can be made in the event the positive control titer is > rater than 1+.

**Interpretation of Results:**

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

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

**Negative:** A single titer of less than 256. In paired sera, less than a four-fold increase in titer and less than 128 in the convalescent phase serum.

**Results on Paired Serum**

**Results on Single Serum**

|   | Acute | Convalescent | Report       |   | Test | Report       |
|---|-------|--------------|--------------|---|------|--------------|
| 1 | <64   | 64           | Negative     | 1 | <64  | Negative     |
| 2 | <64   | 128          | Positive     | 2 | 64   | Negative     |
| 3 | <64   | 256          | Positive     | 3 | 128  | Negative     |
| 4 | 64    | 128          | Negative     | 4 | 256  | Inconclusive |
| 5 | 64    | 256          | Positive     | 5 | 512  | Inconclusive |
| 6 | 64    | 512          | Positive     | 6 | 1024 | Inconclusive |
| 7 | 128   | 256          | Inconclusive |   |      |              |
| 8 | 512   | 1024         | Inconclusive |   |      |              |

Titrated end-points should be obtained for each serum.

**Limitations of Procedure:**

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

**US**

|                         |                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------------------|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Component</b>        | PBS Powder Packets             | <b>Precautionary Statement</b><br><b>Prevention:</b><br>• Wash thoroughly after handling.<br>• Wear eye/face protection<br><b>Response:</b><br>• IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.<br>• If eye irritation persists: Get medical advice/attention.<br><b>Storage/Disposal:</b><br>• Dispose of content and/or container in accordance with local, regional, national, and/or international regulations. |
|                         | Mounting Medium                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Pictogram</b>        |                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Signal Word</b>      | <b>WARNING</b>                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Hazard Statement</b> | Causes serious eye irritation. |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

**EU**

|                         |                                    |                                                                                                                                                                                                                                                                                                                                                                        |
|-------------------------|------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Component</b>        | PBS Powder Packets                 | <b>Precautionary Statement</b><br><b>Prevention:</b><br>P264 Wash thoroughly after handling.<br>P280 Wear protective gloves and clothing.<br><b>Response:</b><br>P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if easy to do. Continue rinsing.<br>P337+P313 If irritation persists, get medical advice/attention. |
|                         | Mounting Medium                    |                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Pictogram</b>        |                                    |                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Signal Word</b>      | <b>WARNING</b>                     |                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Hazard Statement</b> | H319 Causes serious eye irritation |                                                                                                                                                                                                                                                                                                                                                                        |

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|-----------|------------|-------------------------------------------------------------------------|
| <b>EC</b> | <b>REP</b> | Emergo Europe<br>Molenstraat 15<br>2513 BH The Hague<br>The Netherlands |
|-----------|------------|-------------------------------------------------------------------------|



|                                                                                     |                                                                                                                                                                              |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|    | Manufactured by<br>Prodotto da<br>Fabricado por<br>Fabriqué par<br>hergestellt von                                                                                           |
| <b>REF</b>                                                                          | Catalog number<br>Numero di catalogo<br>Número de Catálogo<br>Numéro de catalogue<br>Katalognummer                                                                           |
| <b>LOT</b>                                                                          | Lot<br>Lotto<br>Lote<br>Lot<br>Charge                                                                                                                                        |
| <b>EC</b> <b>REP</b>                                                                | EC Authorized Representative<br>Rappresentante Autorizzato CE<br>Representante Autorizado CE<br>CE Représentant autorisé<br>EG autorisierter Bevollmächtigter                |
|    | EC Declaration of Conformity<br>Dichiarazione di Conformità CE<br>Declaración de Conformidad CE<br>CE Déclaration de Conformité<br>EG Konformitätserklärung                  |
|    | Number of tests<br>Numero di test<br>Número de determinaciones<br>Nombre de tests<br>Anzahl der Teste                                                                        |
|    | See instructions for use<br>Vedere le istruzioni per l'uso<br>Consultar la instrucciones de uso<br>Voir instructions<br>Gebrauchsanweisung beachten                          |
|    | Expiration date<br>Data di scadenza<br>Caducidad<br>Date d'Expiration<br>Haltbarkeitsdatum                                                                                   |
|   | Store at 2-8 C / 35-46 F<br>Conservare a 2-8°C/35-46 F<br>Almacenar a 2-8°C / 35-46°F<br>Conservar à 2-8°C<br>Bei 2-8°C / 35-46°F lagern                                     |
|  | Caution<br>Attenzione<br>Precaución<br>Précautions<br>Achtung                                                                                                                |
|  | Potential biological risk<br>Potenziale rischio biologico<br>Riesgo potencial biológico<br>Biohazard<br>Risque Biologique Potentiel<br>Potentielle biologische<br>Gefährdung |
| <b>RFU</b>                                                                          | Ready for use<br>Pronto all'uso<br>Listo para su uso<br>Prêt à l'emploi<br>Gebrauchsfertig                                                                                   |
| <b>IVD</b>                                                                          | For in vitro diagnostic use<br>Per uso diagnostico <i>in vitro</i><br>Para uso solo in vitro<br>Usage in vitro<br>Für in-vitro diagnostische<br>Verwendung                   |
| <b>RUO</b>                                                                          | For research use only<br>Solo per ricerca<br>Para uso solo en investigación<br>Pour recherche<br>Nur für Forschungszwecke                                                    |
| <b>IUO</b>                                                                          | For investigational use only<br>Solo per uso investigativo<br>Para uso solo en investigación<br>Pour investigation<br>Nur für Forschungszwecke                               |
| <b>IFA/DFA</b> <b>PBS</b>                                                           | Phosphate Buffered Saline<br>Tampono salino fosfato<br>Fosfato Salino Tamponado<br>Tampon phosphate salin<br>PBS                                                             |
| <b>SOR</b>                                                                          | Sorbent<br>Assorbent<br>Sorbente<br>Absorbant<br>Sorbens                                                                                                                     |

|                        |                                                                                                                                                                          |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>SLIDE</b>           | Tissue Substrate Slide<br>Vetrini con substrate di tessuto<br>Porto objetos de Sustrato de Tejido<br>Lame portant le substrat tissulaire<br>Gewebe substrat-Objektträger |
| <b>MM</b>              | Mounting Medium<br>Mezzo di montaggio<br>Medio de Montaje<br>Liquide de montage<br>Eindeckmedium                                                                         |
| <b>10x</b>             | Concentration<br>Concentrazione<br>Concentración<br>Concentration<br>Konzentration                                                                                       |
| <b>ENS</b>             | Enhancement solution<br>Soluzione di rinforzo<br>Solución de realce<br>Solution d'amplification<br>Verstärkungslösung                                                    |
| <b>WASHB</b>           | Wash Buffer<br>Tampone di lavaggio<br>Tampón de lavado<br>Tampon de lavage<br>Waschpuffer                                                                                |
| <b>MPS</b> <b>12x8</b> | Microplate Strips<br>Strip per Micropiastria<br>Tiras de micro placa<br>Microplaque<br>Mikrotiterplattenstreifen                                                         |
| <b>CONJ</b>            | Conjugate<br>Coniugato<br>Conjugado<br>Conjugué<br>Konjugat                                                                                                              |
| <b>SUB</b>             | Substrate<br>Substrato<br>Sustrato<br>Substrat<br>Substrat                                                                                                               |
| <b>STOP</b>            | Stop Solution<br>Soluzione bloccante<br>Solución de Parada<br>Solution d'arrêt<br>Stopplösung                                                                            |
| <b>CAL X</b>           | Calibrator(s)<br>Calibratore (i)<br>Calibrador (s)<br>Calibrateur(s)<br>Kalibrator(en)                                                                                   |
| <b>CONTROL -</b>       | Negative Control<br>Controllo Negativo<br>Control Negativo<br>Contrôle Négatif<br>Negative Kontrolle                                                                     |
| <b>CONTROL +</b>       | Positive Control<br>Controllo Positivo<br>Control Positivo<br>Contrôle Positif<br>Positive Kontrolle                                                                     |
| <b>CONJ</b> <b>ONS</b> | Counterstain<br>Colorante di contrasto<br>Contraste<br>Contre colorant<br>Gegenfärbung                                                                                   |
| <b>CS</b>              | Coverslip<br>Coprioggetto<br>Cubre portaobjetos<br>Lamelles couvre-objet<br>Deckgläschen                                                                                 |
| <b>CONJ</b> <b>+</b>   | Positive Conjugate<br>Coniugato Positivo<br>Conjugado Positivo<br>Conjugué Positif<br>Positivkonjugat                                                                    |
| <b>CONJ</b> <b>-</b>   | Negative Conjugate<br>Coniugato Negativo<br>Conjugado Negativo<br>Conjugué Négatif<br>Negativkonjugat                                                                    |
| <b>DIL</b>             | Sample Diluent<br>Diluente del campione<br>Diluyente de muestra<br>Tampon de dilution<br>Probenverdünnungslösung                                                         |