**INTENDED USE**
The Direct Fluorescent Antibody Reagent is intended for the presumptive (serological) identification of *Legionella pneumophila* serogroup 1 from culture isolates.

**SUMMARY AND EXPLANATION**
*Legionella pneumophila* serogroup 1 is the most common etiological agent of Legionnaires’ disease and one of the most frequently identified *Legionella* isolates in environmental samples.1

The most available techniques used for laboratory confirmation of identifying *Legionella* isolates are the serological methods which are based on hyperimmune rabbit antiserum containing antibodies directed against the somatic lipopolysaccharide or “O” antigen.2 However, many *Legionella* species and serogroups have antigens in common,3 reactive with anti-ovalbumin antibodies.4 Due to the availability of monoclonal antibodies for serological identification, the *Legionella pneumophila* serogroup 1 DFA kit utilizes FITC labelled monoclonal antibodies which offer highly sensitive and specific identification of *Legionella pneumophila* serogroup 1.

*Legionella* may be cultured from a variety of clinical specimens5 and the Direct Fluorescent Antibody (DFA) test used for *Legionella* in such cultures. Although the DFA test is sensitive and highly specific, diagnosis should be confirmed by biochemical characterization whenever possible.6,7

**PRINCIPLE OF THE TEST**
Monoclonal antibodies directed against *Legionella pneumophila* serogroup 1 antigens are conjugated to the fluorochrome, fluorescein isothiocyanate (FITC). To form an FITC-labelled antibody reagent.

Isolates to be tested are fixed to a microscope slide and overlaid with the monoclonal antibody reagent. The FITC-labelled antibody-antigen complex is detected by exposing the slide to ultraviolet light which fluoresces at a wavelength different from FITC conjugated to normal antibodies is present in the reagent as a counterstain.

**REAGENTS AND MATERIALS AVAILABLE**

1. PL.310 Legionella pneumophila serogroup 1 DFA Reagent (FITC-mouse monoclonal antibodies):
   - Monoclonal antibodies prepared in mice against *L. pneumophila* serogroup 1 are conjugated with FITC. The FITC conjugated monoclonal antibodies are supplied ready to use. Rhodamine isothiocyanate (a fluorochrome fluorescing at a wavelength different from FITC) conjugated to normal antibodies is present in the reagent as a counterstain.

2. PL.312 Positive Control - *Legionella pneumophila* serogroup 1:
   - Culture of *L. pneumophila* serogroup 1 grown on defined medium, harvested and boiled to produce a positive antigen control. 0.095% sodium azide is included as a preservative. The positive control is packaged 1 ml per bottle.

3. PL.311 Negative Control - *Legionella* nonpneumophila:
   - Culture is grown on defined medium, harvested and boiled to produce a negative antigen control. 0.095% sodium azide is included as a preservative. The negative control is packaged 1 ml per bottle.

4. PL.315 Mounting Medium:
   - The mounting medium is buffered at pH 8.5. It contains glycerol, and an agent to prevent phase beading caused by ultraviolet light. Supplied ready to use. It is packaged 5.0 ml per bottle.

**PRECAUTIONS**

1. Reagents are for IN VITRO DIAGNOSTIC USE ONLY.
2. Do not use reagents after expiry date shown on product label.
3. Contagious or carcinogenic agents contain 0.095% sodium azide. Sodium azide can react explosively with lead or copper if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used to prevent contamination.
4. Patient specimens and culture isolates should be considered potentially infectious and precautions appropriate to microbiological hazards must be observed.

**STORAGE**
- FITC Antibody Conjugate Reagent:
  - Store at 2°C-8°C in the dark. Conjugate is stable to the expiry date shown on the label.
- Negative Control:
  - Store at 2°C-8°C. Negative control is stable to the expiry date shown on the label.
- Positive Control:
  - Store at 2°C-8°C. Control antigen is stable to the expiry date shown on the label.
- Mounting Medium:
  - Store at 2°C-8°C. Stable to expiry date shown on the label.

**SPECIMEN COLLECTION AND PREPARATION**

1. Collection and Culture
   - Appropriate clinical specimens should be collected by using standard medical procedures. Specimens should be cultured as soon as possible following collection, using accepted procedures for *Legionella* (for example see reference). The laboratory should ensure that the specimen is collected within 48 hours of being received. If fluorescent bacilli are observed, examine under a high power objective to confirm.

2. Preparation of Culture Smears:
   - PROCEDURE IN BIOLOGICAL SAFETY CABINET
     - Make a thin suspension (McFarland No.1) of colonies of suspected species of *Legionella*. Apply a small drop of suspension to the slide.
     - Prepare smears on double ring or multi-well slide.
     - Air dry and heat gently.
     - Fix smear in 10% formalin for 15 minutes.
     - Drain and rinse with distilled water, then air dry slides.

3. Preparation of Control Antigen Smears:
   - Each set of culture isolates tested should include smears of the Positive (PL.312) and Negative Control Antigen (PL.311). Prepare smears as in 2 above.

**MATERIAL PROVIDED**

Reagents as described in Reagents and Material Available.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Biological safety cabinet.
2. Bunsen burner.
3. Clean microscope slides suitable for fluorescence microscopy.
5. Immersion oil.
7. Incubator (35°C-37°C).
8. Inoculation loop.
9. Moisture chamber.
10. Sterile distilled water.
11. Sterile petri dishes.
12. Neutral formalin (10%)
13. Fluorescence microscope (transmitted or incident Light).
14. Monoclonal or binocular fluorescence microscope with 40x and 100x objectives and the following equipment (or equivalent):
   - Transmitted Illumination: 300W ultra-high pressure mercury lamp, 105W high pressure xenon lamp, or 100W tungsten halogen lamp.
   - 4x or 10x/bk 23 red suppression filter. Fit 390 or 2 x KP 490 exciter filter. K 510 or K 515 barrier filter.

**LIMITATIONS OF THE PROCEDURE**

1. The DFA test is presumptive for the identification of *Legionella pneumophila* serogroup 1. A positive result should be confirmed by demonstration of growth  in a suitable medium and/or serological techniques for detection of *Legionella* other than *Legionella pneumophila* serogroup 1.

2. Mixed cultures containing species or serogroups of *Legionella* with small numbers of *Legionella pneumophila* serogroup 1 may also give negative results if the quantity of the latter is very low. Use of isolates derived from single colonies can reduce the likelihood of this occurrence.

3. The use of these reagents directly with patient specimens or for preparations other than clinical culture isolates has not been established.

**REFERENCES**


