PRO-LAB DIAGNOSTICS

LEGIONELLA PNEUMOPHILA SEROGROUP 1 DIRECT FLUORESCENT ANTIBODY KIT

(for *in vitro* diagnostic use)

PRODUCT CODE PL.241

Σ 50

UK CA CE

INTENDED USE

The Direct Fluorescent Antibody Reagent is intended for the presumptive (serological) identification of *Legionella pneumophila* serogroup 1 from culture isolates[1,2].

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.[3]

The most available techniques used for laboratory confirmation of identifying *Legionella* isolates are the serological methods which are based on hyperimmune rabbit antisera containing antibodies directed against the somatic lipopolysaccharide or "O" antigen.[4] However, many *Legionella* species and serogroups have antigens in common[5], cross-reactions are seen when polyclonal antibodies are used for serological identification[5]. 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 specimens[6] and the Direct Fluorescent Antibody (DFA) test used to identify *Legionella* in such cultures. Although the DFA test is sensitive and highly specific, diagnosis should be confirmed by biochemical characterization whenever possible[6.,7,8].

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 will bind specifically to any *Legionella pneumophila* serogroup 1 antigen present in the isolate. If no *Legionella* serogroup 1 antigen is present the antibody reagent will not bind and is removed in the washing step.

The FITC-labelled antibody-antigen complex is detected by exposing the slide to ultraviolet or blue violet light. Excitation by ultraviolet or blue violet light causes the FITC to fluoresce in the longer (visible) wavelengths producing a blue/green or yellow/green color. *Legionella* cells will appear as bright yellow-green bacilli under these conditions.

REAGENTS AND MATERIALS PROVIDED

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[10] and 0.095% sodium azide is included as preservative. The DFA Reagent is packaged 0.5 ml per bottle.
2. PL.312 Positive Control - *Legionella pneumophila* serogroup 1:
   Culture of *L. pneumophila* serogroup 1 is 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 1.0 ml per bottle.
3. PL.311 Negative Control -*Legionella* non-*pneumophila*:
   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 1.0 ml per bottle.
4. PL.315 Mounting Medium:
   The mounting medium is buffered at pH 8.5. It contains glycerol, and an agent to retard photobleaching 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. Some reagents contain a small amount of sodium azide. Sodium azide can reach explosively with copper or lead plumbing if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used if the reagents are flushed down the sink.
4. Universal precautions should be taken in handling, processing and discarding all clinical specimens. All test materials should be considered potentially infectious during and after use and should be handled and disposed of appropriately.
5. Process slides individually and avoid cross contamination with staining reagents.
6. Never allow staining reagent to dry on the slide during staining procedure.
7. Interpretation requires personnel who have experience in fluorescence microscopy and direct fluorescent antibody procedures.
8. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid test results.
9. Product contains material of animal origin and should be handled as a potentail carrier and transmitter of disease.

STORAGE

- FITC-Antibody Conjugate Reagent:
  Store at 2-8°C in the dark. Conjugate is stable to the expiry date shown on the label. Do not freeze.
- Negative Control:
  Store at 2-8°C. Negative Control is stable to the expiry date shown on the label. Do not freeze.
- Positive Control:
  Store at 2-8°C. Positive Control is stable to the expiry date shown on the label. Do not freeze.
- Mounting Medium:
  Store at 2-8°C. Stable to expiry date shown on the label.

SPECIMEN COLLECTION AND PREPARATION

1. Collection and Culture
   Appropriate clinical specimens should be collected using standard medical procedures. Specimens should be cultured as soon as possible following collection, using accepted procedures for *Legionella* (for example see reference [6]). *Legionella* will usually require at least 48 hours before growth is detectable and may take up to 10 days if the isolate is contaminated with other microorganisms or the patient has received antibiotics [6].

2. Preparation of Culture Smears:
   - PROCESS IN BIOLOGICAL SAFETY CABINET
   - Make a light suspension (McFarland Standard 1.0) of colonies of cultures suspected of being *Legionella* in 1% PBS.
   - Prepare smears on double ring or multi-well slide.
   - Air dry and heat gently.
   - Fix smear in 10% neutral formalin for **15 minutes.**
   - Drain and rinse with distilled water, then air dry slides.

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

MATERIALS REQUIRED BUT NOT PROVIDED

1. Biological safety cabinet.
2. Clean microscope slides suitable for fluorescence microscopy.
3. Coverslips.
4. Immersion oil.
5. McFarland Standard 1.0 (Cat. #SD2301)
6. Buffered charcoal yeast extract medium (BCYE).
7. Incubator (35°-37°C).
8. Inoculation loop.
9. Moisture chamber.
10. Sterile distilled water.
11. Sterile petri dishes.
12. Neutral formalin (10%)
13. Fluorescence microscope
14. Phosphate Buffered Saline (available from Pro-Lab as 100 ml of 10X concentrate, Cat. # PL.212.)

TEST PROCEDURE

1. Apply *L. pneumophila* serogroup 1 DFA Reagent (FITC-monoclonal antibody conjugate) to the slide. The entire portion of the slide containing the culture isolate smear should be covered by conjugate reagent.
2. Place the slides in a moist chamber and incubate for 20 to 30 minutes at 37°C.
3. Gently rinse slides individually with PBS to remove the conjugates.
4. Rinse slides with distilled water then air dry. After drying, the slides should be mounted and examined without delay. Slides which cannot be viewed immediately may be stored in the dark for a maximum of 24 hours.
5. Add 4 to 5 drops of mounting medium to slide and apply a coverslip.
6. Using a fluorescence microscope examine slides under a low power (approx. 40x) objective. If fluorescent bacilli are observed, examine under a high power (100x) oil immersion objective to confirm.

QUALITY CONTROL

Both the Positive Control (PL.312) and the Negative Control (PL.311) must be run with each test. All criteria specified in the Interpretation of Results sections 1a, 1b and 1c below must be met for a test to be valid. Do not report test results if any of these criteria are not met.

INTERPRETATION OF RESULTS

1. The following criteria must be met for a test to be valid.
   a. Staining MUST be at least 3+ with typical morphology for a bacillus to be scored as positive.
      4+ = brilliant yellow-green cell wall staining.
      3+ = bright yellow-green cell wall staining.
      2+ = dull yellow green staining. Cell wall not well defined.
      1+ = diffuse, dim yellow green staining of cell.
   b. The DFA reagent conjugate used in the test must produce 3+ to 4+ staining with the Positive Control.
   c. The Negative Control must not react with the DFA reagent.

2. If all of the criteria in section 1 above are met, evaluate test results as follows [9].
   a. Brightly fluorescing bacilli (3+ or stronger): report as FA positive.
   b. No brightly fluorescing bacilli: report as FA negative.

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 assessment of growth requirements and biochemical techniques for *Legionella* bacteria.
2. A negative DFA test does not preclude the presence of species or serogroups of *Legionella* other than *Legionella pneumophila* serogroup 1.
3. 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.
4. The use of these reagents directly with patient specimens or for preparations other than clinical culture isolates has not been established.

REFERENCES

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 = Use by

LOT = Lot number

REF = Catalogue number

 = Manufacturer

EC REP = Authorized Representative in the European Community

 = Contains sufficient for <n> tests

IVD = In vitro diagnostic medical device

 = Temperature limitation

 = Consult instructions for use

PL.315 Canada	**Warning** Causes serious eye irritation. Wash hands thoroughly after handling. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical attention.