LEGIONELLA PNEUMOPHILA SEROGROUPS 1 to 14 DIRECT FLUORESCENT ANTIBODY KIT

(for *in vitro* diagnostic use)

PRODUCT CODE PL.242 (50 Tests)

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INTENDED USE

The Direct Fluorescent Antibody Reagents are intended for the presumptive (serological) identification of *Legionella pneumophila* serogroups 1 to 14 from culture isolates[1,2].

SUMMARY AND EXPLANATION

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.[3] However, many *legionella* species and serogroups have antigens in common[4], cross-reactions are seen when polyclonal antibodies are used for serological identification[4]. The *Legionella pneumophila* serogroups 1 to 14 DFA kit utilizes FITC labelled monoclonal antibodies which offer highly sensitive and specific identification of *Legionella pneumophila* serogroups 1 to 14.

Legionella may be cultured from a variety of clinical specimens [5] 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[5,6,7].

PRINCIPLE OF THE TEST

The direct fluorescent antibody test is one of the fastest and simplest immunofluorescence procedures. Monoclonal antibodies directed against *Legionella pneumophila* serogroups 1 to 14 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* serogroups 1 to 14 antigen present in the isolate If no *Legionella* 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 AVAILABLE

1. PL.313 *Legionella pneumophila* serogroups 1 to 14 DFA Reagent (FITC-mouse monoclonal antibodies).
   Monoclonal antibodies prepared in mice against *L. pneumophila* serogroups 1 to 14 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 rabbit serum is present in the reagent as a counterstain[9] and 0.1% sodium azide is included as preservative. The DFA Reagent is packaged 0.5 ml per bottle.
2. PL.314 Positive Control - *Legionella pneumophila*
   Culture of *L. pneumophila* is grown on defined medium, harvested and boiled to produce a positive antigen control. The positive control is packaged 1.0 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. The negative control is packaged 1.0 ml per bottle.
4. PL.316-.Mounting Medium
   Mounting medium consists of glycerol in carbonate buffer pH 8.4-8.6. 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. Conjugate and antigen reagents contain 0.1% 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 when flushing used reagent down the sink.
4. Patient specimens and culture isolates should be considered potentially infectious and precautions appropriate to microbiological hazards must be observed.
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.

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. Control antigen 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 [5]). *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 [5].

2. Preparation of Culture Smears:

PROCESS IN BIOLOGICAL SAFETY CABINET

a. Make a very heavy suspension (Mcfarland No.9) of colonies of cultures suspected of being *Legionella* in 1% PBS. .
b. Prepare smears on double ring or multi-well slide.
c. Air dry and heat gently.
d. Fix smear in 10% neutral formalin for 15 minutes.
e. 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.314) 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.
4. Coverslips.
5. Immersion oil.
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 (transmitted or incident Light).
    Monocular or binocular fluorescence microscope with 40x and 100x (oil immersion) objectives and the following equipment (or equivalent):
    Transmitted illumination
    - cardioid dark field condenser
    - 200W ultra-high pressure mercury lamp, 105W high pressure xenon lamp or 100W tungsten halogen lamp.
    - KG 1 or B1/K2 heat absorbing filter. BG 38 or BG 23 red suppression filter. K 4 90 or 2 x KP 490 exciter filter. K 510 or K 515 barrier filter.

    Incident illumination
    - 50W, 100W or 200Wultra-high pressure mercury lamp, 75W or 150W high pressure xenon lamp, or 50W or 100W tungsten halogen lamp.
    - KG 1 or B1/K2 heat absorbing filter. BG 38 or BG 23 red suppression filter. KP 490 or 2 x KP 490 exciter filter. TK 510 dichronic beam splitting mirror, and K 510 or K 515 barrier filter.

    Tungsten halogen lamps may not always be successfully used with binocular microscopes for either transmitted or incident illumination.
14. Phosphate Buffered Saline (0.01M). It may be purchased from Pro-Lab as PL.212 Phosphate Buffered Saline (10X concentrate). It is supplied as 100 ml of 10X con centrate. Dilute 1 volume of concentrate with 9 volumes of distilled water to produce phosphate buffered saline pH 7.5-7.7.

TEST PROCEDURE

1. Apply *L. pneumophila* serogroups 1 to 14 DFA Reagent (FITC-monoclonal antibody conjugate) to the tissue 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 Antigen and the Negative Control antigen 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 antigen.
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 [8].

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* serogroups 1 to 14. 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 of *Legionella* other than those for which the isolate has been tested.

3. Mixed cultures containing species or serogroups of *Legionella* other than those for which the isolate has been tested along with small numbers of *Legionella pneumophila* serogroups 1 to 14 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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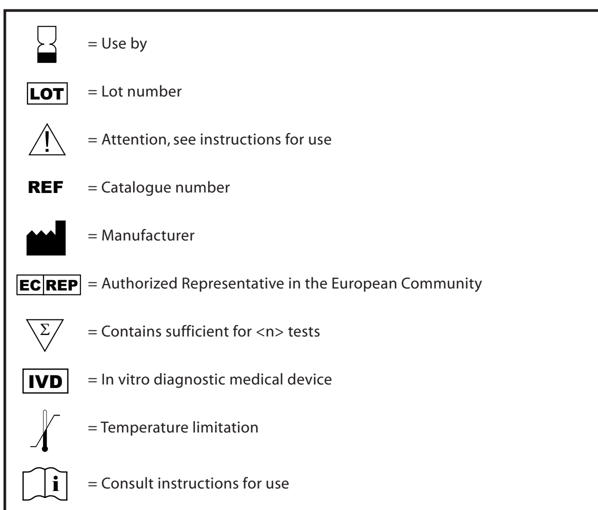

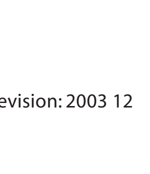

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