The Legisla Reagents for Direct Fluorescent Antibody Test (for in vitro diagnostic use)

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

**SUMMARY AND EXPLANATION**
During 1976 the Center for Disease Control was involved in an intensive investigation into the cause of an outbreak of acute febrile illness in Philadelphia. The condition, subsequently called Legionnaires Disease, was found to have been caused by a gram-negative rod which was named Legionella Disease Bacterium.

The manifestations of Legionnaires Disease range from asymptomatic infection or mild influenza-like symptoms to severe, sometimes fatal, bronchopneumonia.3

Legionella may be cultured from a variety of clinical specimens - for example an anterior nasopharyngeal smear, urine sediment, sputum, or lung aspirates. In contrast, the isolation rate in culture media is low and the time to growth is variable. A variety of media are used to attempt isolation including the modified buffered charcoal yeast agar medium (BCYE) and buffered charcoal yeast extract medium (BCYE). Legionella pneumophila is aerobic and fastidious and 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.4

The following FITC-Antibody Conjugate Reagents are available:

- L. pneumophila (serogroup 2 to 14)
- L. pneumophila (serogroup 13)
- L. pneumophila (serogroup 14)

**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 Reagents:
Store at 2–8°C in the dark. Conjugate is stable to the expiry date shown on the label. Do not freeze.

**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.6

2. Preparation of Culture Smears:

**PROCESS IN BIOLOGICAL SAFETY CABINET**
- a. Make a lightly turbid suspension (McFarland No. 1) of colonies of cultures suspected of being Legionella in 1% normal saline.
- b. Prepare smears on double ring or multi-well slide. Three sets of slides are required for being evaluated.
- c. Air dry and heat gently.
- d. Immerse slides for 5 minutes in individual coplin jars containing PBS.
- e. Gently rinse slides individually with PBS to remove the conjugates.
- f. Rinse slides with distilled water then air dry. After drying, the slides should be mounted and examined without delay. Slides which can not be viewed immediately may be stored in the dark for a maximum of 24 hours.
- g. Add 4 to 5 drops of mounting medium to slide and apply a coverslip.

3. 2 x KP 490 exciter filter. TK 510 dichronic beam splitting mirror, and K 510 or K 515 barrier filter.

**TEST PROCEDURE**
1. Apply Monovalent conjugate to the first tissue slide, and Negative Control conjugate to the second 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 without removing the conjugates.
4. Immerse slides for 5 minutes in individual conjugate containing PBS.
5. Rinse slides with distilled water then air dry. After drying, the slides should be mounted and examined without delay. Slides which can not be viewed immediately may be stored in the dark for a maximum of 24 hours.
6. Add 4 to 5 drops of mounting medium to slide and apply a coverslip.
7. Using a fluorescence microscope examine slides under a low power (approx. ×40) objective. If fluorescent bacilli are observed, examine under a high power (100x) oil immersion objective to confirm.

**QUALITY CONTROL**
Both the Polyclonal Positive Control Antigen and the Negative Control Conjugate 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**
Legionella bacilli are pleomorphic and antibiotic therapy may lead to delayed appearance of colonies in culture and organisms with uncharacteristic morphology.

1. The following criteria must be met for a test to be valid.

**MATERIALS REQUIRED BUT NOT PROVIDED**

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7. Coplin jars.
8. Clean microscope slides suitable for fluorescence microscopy.
10. Immersion oil.
12. Incubator (35–37°C).
13. Incubation loop.
14. Moisture chamber.
15. Sterile distilled water.
17. Neutal formalin (10%).
18. Fluorescence microscope (transmitted or incident Light).

Monoclonal or binocular fluorescence microscope with 40x and 100x (oil immersion) objectives and the following equipment (or equivalent):

- Cardoid dark field condenser
- 200W ultra-high pressure mercury lamp.
- 150W high pressure xenon lamp.
- Neutral halogen lamp.
- KG 1 or B1/K2 heat absorbing filter.
- KG 38 or KG 23 red suppression filter.
- KP 490 or 2× KP 490 exiter filter.

**PRODUCT CODE PL.205, PL.206, PL.207, PL.208, PL.209, PL.276, PL.277, PL.278, PL.280, PL.281, PL.282, PL.283.**

1+ = diffuse, dim yellow green staining of cell.
2+ = bright yellow-green cell wall staining.
3+ = brilliant yellow-green cell wall staining.
4+ = brilliant yellow-green cell wall staining.
The negative control conjugate must not stain the test samples.

2. If all of the criteria in section 1 above are met, evaluate test results as follows:
   a. Brightly fluorescent bacilli (3+ or stronger): report as FA positive for the appropriate serogroup(s) or species (see 3 and 4 below).
   b. No brightly fluorescing bacilli: report as FA negative.
   c. The negative control conjugate must not stain the test samples.

3. A positive result with a monovalent conjugate indicates that the specific serogroup or species specified by that conjugate is present in the isolate.

LIMITATIONS OF THE PROCEDURE

1. The DFA test is presumptive for the identification of Legionella pneumophila serogroups 2 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 or serogroups of <i>Legionella</i> other than those for which the isolate has been tested.

3. Mixed cultures containing species or serogroups of <i>Legionella</i> other than those for which the isolate has been tested along with small numbers of <i>Legionella pneumophila</i> serogroups 2 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. Nonspecific fluorescence may occur with some strains of <i>Sophylococcus</i>, <i>Streptococcus</i>, <i>Flavobacterium</i>, <i>Haemophilus influenzae</i>, <i>Bordetella pertussis</i>, <i>Bacteroides fragilis</i>, <i>Eikkenella corrodens</i>, <i>Pseudomonas</i> including <i>P. fluorescens</i>, <i>P. maltophilia</i>, <i>P. aeruginosa</i>, <i>P. putida</i> and other gram negative rods, due to natural antibodies in the serum of immunized rabbits or due to nonspecific binding of conjugate to cell wall components.

5. Nonspecific fluorescence can usually be distinguished from the specific reaction with <i>Legionella</i> on morphological grounds if one is familiar with the normal morphology and staining characteristics of Legionella bacilli.

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

REFERENCES


Also available from Pro-Lab:

PL 241 Legionella pneumophila serogroup 1 DFA Kit
[Contains Legionella pneumophila serogroup 1 DFA Reagent (FITC-mouse monoclonal antibodies)] 50 tests

PL 242 Legionella pneumophila serogroup 1 to 14 DFA Kit
[Contains Legionella pneumophila serogroup 1 to 14 DFA Reagent (FITC-mouse monoclonal antibodies)] 50 tests