PRO-LAB DIAGNOSTICS

LEGIONELLA REAGENTS FOR DIRECT FLUORESCENT ANTIBODY TEST

Product Code: , PL.205, PL.206, PL.207, PL.208, PL.209, PL.276, PL.277, PL.278, PL.279, PL.280, PL.281, PL.282, PL.283.

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INTENDED USE

The Direct Fluorescent Antibody Reagents are intended for the presumptive (serological) identification of *Legionella pneumophila* serogroups 2 through 14 from culture isolates[1,2,5].

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 [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[4,6,7].

PRINCIPLE OF THE TEST

The direct fluorescent antibody test is one of the fastest and simplest immunofluorescence procedures. Antibodies directed against *Legionella* 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 antibody reagent. The FITC-labelled antibody will bind specifically to any *Legionella* 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

Legionella pneumophila, single serogroup FITC-conjugated rabbit antisera.

Antisera prepared in rabbits against each of *L. pneumophila* serogroups 2 to 14 are conjugated with FITC. The FITC conjugated antisera 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[11] and 0.1% sodium azide is included as preservative.

The following FITC-Antibody (rabbit) Reagents are available:

Cat.#	
PL.205 *Legionella* DFA Reagent. *L. pneumophila* serogroup 2	0.5 ml
PL.206 *Legionella* DFA Reagent. *L. pneumophila* serogroup 3	0.5 ml
PL.207 *Legionella* DFA Reagent. *L. pneumophila* serogroup 4	0.5 ml
PL.208 *Legionella* DFA Reagent. *L. pneumophila* serogroup 5	0.5 ml
PL.209 *Legionella* DFA Reagent. *L. pneumophila* serogroup 6	0.5 ml
PL.276 *L. pneumophila* DFA Reagent. *L. pneumophila* serogroup 7	0.5 ml
PL.277 *L. pneumophila* DFA Reagent *L.pneumophila* serogroup 8	0.5 ml
PL.278 *L. pneumophila* DFA Reagent *L. pneumophila* serogroup 9	0.5 ml
PL.279 *L. pneumophila* DFA Reagent *L. pneumophila* serogroup 10	0.5 ml
PL.280 *L. pneumophila* DFA Reagent *L. pneumophila* serogroup 11	0.5 ml
PL.281 *L. pneumophila* DFA Reagent *L. pneumophila* serogroup12	0.5 ml
PL.282 *L. pneumophila* DFA Reagent *L. pneumophila* serogroup 13	0.5 ml
PL.283 *L.pneumophila* DFA Reagent *L. pneumophila* serogroup 14	0.5 ml

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 [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

a. Make a lightly turbid suspension (Mcfarland No.1) of colonies of cultures suspected of being *Legionella* in 1% neutral formalin .
b. Prepare smears on double ring or multi-well slide Three sets of slides are required for testing.
c. Air dry and heat gently.
d. Fix smear in 10% neutral formalin for 10 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 Polyvalent Positive Control Antigen (PL.285). Prepare smears as in 2 above.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Positive Control Antigen (available from Pro-Lab in 1 ml size)
   Cat # PL.285 (L. *pneumophila* serogroups 1 to 14)
2. Negative Control Reagent (available from Pro-Lab in 2 ml size)
   Cat # PL.213A Rabbit immunoglobulin conjugated FITC
3. Phosphate Buffer Saline (available from Pro-Lab as 100 ml of 10X concentrate)
   Cat # PL.212
4. Mounting Media (available from Pro-Lab in 10 ml size)
   Cat # PL.213
5. Biological safety cabinet.
6. Bunsen burner.
7. Coplin jars.
8. Clean microscope slides suitable for fluorescence microscopy.
9. Coverslips.
10. Immersion oil.
11. Buffered charcoal yeast extract medium (BCYE).
12. Incubator (35°-37°C).
13. Inoculation loop.
14. Moisture chamber.
15. Sterile distilled water.
16. Sterile petri dishes.
17. Neutral formalin (10%)
18. 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. KP 490 or 2x 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.

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 to remove the conjugates.

4. Immerse slides for 5 minutes in individual coplin jars 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.- 40x) objective. If fluorescent bacilli are observed, examine under a high power (100x) oil immersion objective to confirm.

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C. QUALITY CONTROL

Both the Polyvalent 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.

   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. Monovalent conjugates used in the test must produce 3+ to 4+ staining with the Polyvalent Positive Control antigen.
   c. 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 [8].

   a. Brightly fluorescing bacilli (3+ or stronger): report as FA positive for the appro priate serogroup(s) or species (see 3 and 4 below).
   b. No brightly fluorescing bacilli: report as FA negative.

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 *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 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 *Staphylococci, Streptococci, Flavobacterium, Haemophilus influenzae, Bordetella pertussis, Bacteroides fragilis, Eikkenella corodens, Pseudomonas* including *P. fluoresces, P. maltophila, P. aeruginosa P. putida* 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 [12]. Nonspecific fluorescence can usually be distinguished from the specific reaction with *Legionella* on morphological grounds if one is familiar with the normal morphology and staining and staining characteristics of *Legionella* bacilli [9,10].

5. 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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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

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