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See external label



2°C-8°C



Σ= tests



cat.#331008-4

Legionella Test System

Catalog No. 331008-4

PLEASE READ THIS MATERIAL BEFORE USING THE KIT

INTENDED USE

The Diagnostic Automation Indirect Legionella test system is an indirect fluorescent antibody (IFA) assay designed for the detection of *L. pneumophila* antibodies in human serum, and is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

The Legionella Indirect Fluorescent Antibody (IFA) test system is an immunofluorescence procedure for the detection of *L. pneumophila* antibodies in human serum (1,5,8). The specificity of this IFA test is enhanced when paired sera from patients with symptoms of Legionellosis are tested. When possible, the test should be used in conjunction with isolation of the organism from either biopsy or autopsy material or demonstration of the organisms in tissue specimens (2).

PRINCIPLE OF THE IFA ASSAY

The Diagnostic Automation Legionella IFA test system is designed to assay the level of Legionella antibodies in human sera. The test system employs heat-killed Legionella bacterium as the substrate antigen and polyvalent anti-human FITC labeled globulin as the antibody indicator. The reaction occurs in two steps:

1. The first involves the interaction of Legionella antibodies in the patient's serum with the Legionella antigen in the test well of the slide.
2. The second step is the reaction between the anti-human conjugate and the Legionella antibody attached to the Legionella antigen. When examined under a fluorescence microscope using near ultra-violet blue light, the FITC emits apple-green staining in a positive assay (see Test Procedure for details). It must be noted that the IFA Legionella Screen Kits utilize only Groups 1 through 4, or Groups 1 through 6 *L. pneumophila* antigens (depending on the test kit ordered). Commercially prepared Group 1 antigen slides are

available separately (Product #:15002-A) and in kit form (Product #:15001-1), and must be treated in the same manner as the polyvalent screening kit described herein.

COMPONENTS

Reactive Reagents:

1. *Legionella pneumophila* substrate slides containing fixed *L. pneumophila* substrate (antigen) standardized to produce optimum reactivity. Ready to use once removed from the freezer and equilibrated to room temperature (20-25°C). Slides should be allowed to reach room temperature before opening the foil pouch. Use thawed slides the same day. Do not refreeze thawed slides. (Product #:15001: Group 1-4, Product #:15001-6: Group 1-6).
2. Goat anti-human globulin (IgG, IgA, and IgM) labeled with FITC: Contains 1.25% bovine albumin (adequate for approximately 40 tests). Rehydrate with distilled water according to label on conjugate. Once rehydrated this reagent is at its routine test dilution. Do not dilute further. See label for expiration date. (Product #: 15003).
3. Legionella Positive Control Sera (Monkey): (Includes individual vials for *L. pneumophila* Groups 1 through 4. See each vial label). These high-titered positive sera are diluted with phosphate-buffered-saline. Rehydrate with distilled water according to label on positive control. See side of control vial for endpoint titer. (Polyvalent Group 1 through 4 positive control is also available (Product #:15004)).

Monkey sera is substituted for positive human sera because it has not been possible to obtain adequate volumes of positive human sera for most of the *L. pneumophila* serogroups or other species of Legionella. Also, positive monkey sera reacts with the anti-human FA conjugate to approximately the same degree as positive human

sera. See label for expiration date. (Product Series #: 15004).

4. Legionella Negative Control Serum (Human): Composed of normal human sera with no detectable Legionella antibody as determined by IFA. Rehydrate with distilled water according to label on negative control. This volume should be adequate for 40 tests. See label for expiration date. (Product #: 15005).

Non-reactive Reagents:

1. Phosphate-buffered-saline (PBS): 10 gms/packet, sufficient to make one liter. See label for expiration date. (Product #: 0008LT).
2. Buffered Glycerol (mounting media): 3mL vial. See label for expiration date. (Product #: 12009).

NOTE: All sera, conjugates, diluents, and buffered glycerol contain a preservative which may be toxic if ingested.

PRECAUTIONS

- For *in vitro* diagnostic use.
- The preservative may be toxic if ingested.
- Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected and the reagent evaluated for the bacteria. If contaminated, the reagent should be discarded.
- The components of each kit are matched for optimum performance. Reagents from different lots should not be interchanged. Follow procedures carefully.
- **BEFORE PROCEEDING, READ ENTIRE TEST PROCEDURE.**
- Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (13).

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Staining dishes or Coplin jars.

- Distilled water
- Small serological, Pasteur, capillary, or automatic pipettes capable of delivering 0.01mL.
- Coverslips: 24 x 60mm, thickness No. 1.
- Moist incubation chamber.
- Incubator: 35-37°C.
- Acetone
- Small test tubes: 13 x 100mm or comparable.
- Test tube racks.
- Properly equipped fluorescence microscope assembly.

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light fluorescence assemblies:

SPECIMEN COLLECTION

Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (11,12). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10° C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

STORAGE CONDITIONS

- Substrate slides containing *L. pneumophila*: Store at -20°C or lower.
- Goat anti-human immunoglobulin labeled with FITC (lyophilized): Store at 2-8°C. Once rehydrated, stable for 90 days at 2-8°C. Frozen aliquots are stable for 6 months at -20°C or lower. **DO NOT FREEZE AND THAW CONJUGATE MORE THAN ONCE. REPEATED FREEZING AND THAWING DESTROYS ANTIBODY ACTIVITY.**
- Positive Controls (lyophilized): Store at 2-8°C. Stable for 90 days after reconstitution at 2-8°C or 1 year if frozen aliquots are maintained at -20°C or lower.
- Negative Control (lyophilized): Store at 2-8°C. Stable for 6 months after reconstitution at 2-8°C, or 1 year if frozen aliquots are maintained at -20°C or lower.
- Phosphate-buffered-saline: Store at 2-25°C. Rehydrated PBS is stable for 30 days when stored at 2-8°C.
- Buffered Glycerol: Store at 2-8°C.

NOTE:

1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed.
2. Do not freeze and thaw reagents more than once.

Repeated freezing and thawing destroys antibody activity. Do not store in self-defrosting freezer.

PROCEDURES

Preparation of Reagents:

1. Use of commercially prepared slides:
 - a) Remove slides from freezer. Allow slide to reach ambient temperature. Tear open the protective envelope and remove slide containing antigen. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.**
 - b) Antigens on these slides have already been fixed and are ready to use.
2. Rehydrate positive control(s), negative control, and FITC conjugate by adding the appropriate amount of distilled water.
3. Prepare a 1:128 and 1:256 screening dilution of serum in PBS.

Test Procedure:

1. SCREENING OF PATIENT'S SERA

- a) Add the 1:128 and 1:256 dilutions of each patient's serum (acute and convalescent or single serum) to antigen wells.
- b) Add the positive control serum as reconstituted to antigen wells. Add negative control serum to an antigen well. Add PBS to one antigen well to serve as a conjugate control.
- c) Incubate slide(s) in a moist chamber at 35-37°C for 30 minutes.
- d) Rinse the slide(s) briefly in a light stream of PBS. **DO NOT AIM THE STREAM OF PBS DIRECTLY ONTO THE WELLS.** Place slide(s) in wash chamber containing PBS for 10 minutes.
- e) Remove slide(s) from PBS. Rinse the slide(s) briefly with distilled water and allow to air dry.
- f) Add one drop of anti-human conjugate to each well.
- g) Repeat steps c), d), and e).
- h) Apply 2-3 drops of mounting media to each slide and coverslip. Examine slide(s) immediately with an appropriate fluorescence microscope assembly using 40X to 54X objectives.

NOTE:

If delay in examining slides is anticipated, coverslip, seal with nail polish, and store in refrigerator. It is recommended that slides be examined on the same day as testing, or at least within 24 hours from time of

preparation.

2. TITRATION OF PATIENT'S SERA:

- a) Positive sera for which endpoint titers are to be obtained are processed as above using two-fold serial dilutions of each serum beyond 1:128 as previously directed. The positive control endpoint can be found on the label of the control vial.
- b) If paired serum specimens are being assayed to determine acute infection, both specimens must be tested at the same time using identical lots of reagents.

QUALITY CONTROL

To assure optimum results, adhere precisely to the procedure and reagents as described herein. Reading of endpoints with each microscope assembly must be made with reference to the positive and negative control sera used with the antigens and conjugate provided. It is imperative that both positive and negative controls be used with each IFA assay. By achieving acceptable results, the use of the controls validates the procedure performed. Whenever the expected Q.C. results are not obtained, the patient values must not be used.

RESULTS

A serum titration endpoint is the highest serum dilution producing a 1+ apple-green fluorescence. The serum titer is the reciprocal of that endpoint dilution. (e.g., endpoint = 1:512, titer = 512).

LIMITATION OF PROCEDURE

1. Considerable experience in reading endpoints against the polyvalent antigen may be required to obtain the same titers as those obtained with monovalent antigens. Therefore, polyvalent antigen titers should not be used unless user proficiency can first be demonstrated.
2. A serological test should not be used as the only criterion for diagnosis. The patient's clinical data and other laboratory tests should be carefully reviewed by a medical authority before a diagnosis is made.

INTERPRETATION

A four-fold rise in titer ≥ 128 from the acute to the convalescent phase provides evidence of a recent infection with Legionella. A standing or single titer ≥ 256 provides presumptive evidence of infection at an undetermined time. Single titers of less than 256 are not considered evidence of infection.

REFERENCES

1. McDade JE, Shepard CC, *et al*: Legionnaires' Disease. Isolation of a bacterium and demonstration of its role in other respiratory diseases. *New Engl. J. Med.* 297:1197-1203, 1977.
 2. Chandler FW, Hicklin MD, and Blackmon JA: Demonstration of the agent of Legionnaires' Disease in tissue. *New Engl. J. Med.* 297:1218-1220, 1977.
 3. McKinney RM, Thomason BM, Harris PP, Thacker L, Lewallen KR, Wilkinson HW, Herbert GA, and Moss CW: Recognition of a new serogroup of Legionnaires' disease bacterium. *J. Clin. Microbiol.* 9:103-107, 1979.
 4. Goldman M: Fluorescent Antibody methods. Academic Press, New York, pp. 148-149, 1968.
 5. Wilkinson HW, Fikes BJ, and Cruce DD: Indirect Immunofluorescence Test for Serodiagnosis of Legionnaires' Disease: Evidence for Serogroup Diversity of Legionnaires' Disease Bacterial Antigens and for Multiple Specificity of Human Antibodies. *J. Clin. Microbiol.* 9:379-383, 1979.
 6. Brenner DJ, Steigerwalt AG, Gorman GW, Weaver RE, Feeley JC, Cordes LG, Wilkinson HW, Patton C, Thomason BM, and Sasseville KRL: *Legionella bozemanii* species nova and *Legionella dumoffii* species nova: Classification of two additional species of Legionella associated with human pneumonia. *Curr. Microbiol.* 4:111-116, 1980.
 7. Hebert GA, Steigerwalt AG, and Brenner DJ: *Legionella micdadei* species nova: Classification of a third species of Legionella associated with human pneumonia. *Curr. Microbiol.* 3(5):255-257, 1980.
 8. Wilkinson HW, Farshy CE, Fikes BJ, Cruce DD, and Yealy LP: Measure of immunoglobulin G-, M-, and A-specific titers against *Legionella pneumophila* and inhibition of titers against non-specific Gram-negative bacterial antigens in the indirect immunofluorescence test for legionellosis. *J. Clin. Microbiol.* 10:685-689, 1979.
 9. McKinney RM, Thacker L, Harris PP, Lewallen KR, Herbert GA, Edelstein PH, and Thomason BM: Four serogroups of Legionnaires' Disease bacteria defined by direct immunofluorescence. *Ann. Intern. Med.* 90:621-624, 1978.
 10. Morris GK, Steigerwalt A, Feeley JC, Wong ES, Martin WT, Patton CM, and Brenner DJ: *Legionella gormanii* species nova: a new species of Legionella. *J. Clin. Microbiol.* 1980.
 11. Procedures for the collection of diagnostic blood specimens by venipuncture - Second Edition; Approved Standard (1984). Published by national Committee for Clinical Laboratory Standards.
 12. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
- U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. *Fed. Register* 56:64175-64182, 1991.

INTENSITY

DEFINITION OF CELLWALL STAINING

- 4+ = brilliant yellow-green staining of bacteria
- 3+ = bright yellow-green staining
- 2+ = definite but dim staining
- 1+ = barely visible staining
- Neg = absence of yellow-green staining of the cells, yellow-brown autofluorescence may occur



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