AccuDiag™ Legionella Antibody (Group 1-6) IFA Kit

REF 331008-6D

IVD See External Label 2°C 8°C 10 x 8 wells

Legionella Antibody (Group 1-6) IFA	
Principle	Indirect Fluorescent Antibody method
Sample	10 µL serum
Incubation Time	80 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Easy to use with minimal equipment and expertise
- High sensitivity and specificity
- Versatile tool to detect wide range of antigens and antibodies
- Visual Intrepretation of results using Fluorescence microscope

INTENDED USE

The Diagnostic Automation, Inc. Legionella Antibody IFA (Group 1 - 6) 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.

SUMMARY AND EXPLANATION

The DAI IFA Legionella Antibody (Group 1 - 6) Test System is an immunofluorescence procedure for the detection of L. pneumophila antibodies in human serum (1, 5, and 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.

ASSAY PRINCIPLE

The Diagnostic Automation, Inc. Legionella Antibody (Group 1 - 6) Test System is designed to assay the level of Legionella antibodies in human sera. The assay 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 one 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 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 Assay Procedure) It must be noted that the DAI Legionella (Group 1-6) Test system utilize only Groups 1 through 6 Legionella antigens.

SPECIMEN COLLECTION & PREPARATION

1. DAI recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2-8°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 that may cause loss of antibody activity and give erroneous results.

MATERIALS AND COMPONENTS

Materials provided with the test kit

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. Sorbent contains Sodium Azide (<0.1% w/v) as a preservative.

Reactive Reagents

1. **Legionella pneumophila Antigen Substrate Slides:** Ten, 8 -well Slides containing fixed L. pneumophila organisms (Groups 1- 6) standardized to produce optimum reactivity. Also includes a desiccant pouch.
2. **Conjugate:** Goat anti-human globulin (IgG, IgA, and IgM) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, clear-capped, bottle. Ready to use.
3. **Positive Control (Monkey Serum):** Will produce positive apple-green fluorescence of the organisms. One, 0.5mL, red-capped, vial. Ready to use.
   Note: Monkey serum is substituted for positive human serum because it has not been possible to obtain adequate volumes of positive human sera for most of the L. pneumophila serogroups or others species of Legionella. Also, positive monkey serum reacts with the anti-human FA conjugate to approximately the same degree as positive human sera.

4. **Negative Control (Human Serum)** Will produce no detectable staining of the organisms. One, 0.5mL, green-capped, vial. Ready to use.
5. **Diluent:** Three, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. Note: The Diluent will change color when combined with serum.
6. **Phosphate-buffered-saline (PBS):** pH 7.6 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
7. **Mounting media (Buffered Glycerol):** Two, 3.0 mL, white-capped, dripper tipped vials.

Note: Kit also contains:

1. ***Component list containing lot specific information is inside the kit box.***
2. ***Package insert providing instructions for use.***

Materials required but not provided

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish. A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
6. Cover slips, 24x60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e: 10% household bleach – 0.5% Sodium Hypochlorite).
12. Incubator: 35-37°C.

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

TRANSMITTED LIGHT Light Source: Mercury vapor 200W or 50W		
Excitation Filter	**Barrier Filter**	**Red Suppression Filter**
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten – Halogen 100W		
KP490	K510 or K530	BG38

INCIDENT LIGHT Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	**Dichroic Mirror**	**Barrier Filter**	**Red Suppression Filter**
KP500	TK510	K510 OR K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

ASSAY PROCEDURE

1. Remove slides from storage and allow them to warm to room temperature (20-25°C.) Tear open the protective envelope and remove slides. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.**
2. Identify each well with the appropriate patient sera and Controls.
   NOTE: The Controls are intended to be used undiluted.
   Prepare a 1:32 dilution (e.g.:10µL of serum + 310 µl of diluent) of each patient serum. **The Diluent will undergo a color change confirming that the specimen has been combined with the Diluent.** Patients should be screened at 1:128 and 1:256. These dilutions can be prepared by further diluting the 1:32 dilution (1:4 and 1:8 respectively) using PBS as if one were preparing serial, two-fold dilutions.
   a. Users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in the Diluent. When evaluated by DAI, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive Control.
   b. When titrating patient specimens, initial dilutions should be prepared in Diluent and all subsequent dilutions should be prepared in the Diluent or PBS only.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at room temperature (35-37°C) 30 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash Slides for two, 5 minute intervals, changing PBS between washes.
7. Remove Slides from PBS. Rinse Slides briefly with deionized or distilled water and airdry Slides. Do not disturb the organisms in the wells.
8. Add 20µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope. Patient

NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.

RESULTS

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. If paired sera specimens are being assayed to determine acute infection, both specimens must be tested at the same time using identical lots of reagents.

Intensity	Definition of Cell-Wall 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 of the cells, yellow-brown autofluorescence may occur.

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.

LIMITATIONS OF THE 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.

EXPECTED VALUES

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).

PRECAUTIONS

1. For In Vitro Diagnostic Use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety 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 Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens.
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay.** Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
7. The Diluent, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. All components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose off according to local regulations.
22. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

STORAGE CONDITIONS

2°C – 8°C	Unopened Test System.
	Mounting Media, Conjugate, Sample Diluent, Slides, Reactive and Non-Specific Controls.
	Rehydrated PBS (Stable for 30 days)
2°C – 25°C	Phosphate-buffered-saline (PBS) Packets.

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax: 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	2024-03
Brand Name	AccuDiag™
REF 331008-6D	AccuDiag™ - Legionella Antibody (Group 1-6) IFA
EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands www.cepartner4u.eu
Revision Date: 2012-19-27	