AccuDiag™
Legionella IgG/IgM
ELISA Kit

INTENDED USE

The Diagnostic Automation, Inc. Legionella IgG/IgM Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the qualitative detection of total antibodies (IgG and IgM) to Legionella pneumophila (Legionella) serogroups 1-6 in serum from patients with clinical suspicion of Legionella Disease. For in vitro diagnostic use. High Complexity test.

SUMMARY AND EXPLANATION

In 1977, a bacterium was isolated and characterized from an outbreak of acute febrile respiratory disease at the Pennsylvania American Legion meeting in Philadelphia in 1976. This bacterium, Legionella pneumophila, was determined to be the etiologic agent in this and other pneumonic diseases in the U.S. and abroad. The symptoms of Legionellosis range from subclinical asymptomatic infections to severe life-threatening pneumonia.1,2,3

Indirect Immunofluorescent Antibody (IFA) assays have been developed and used as supportive evidence of Legionella pneumophila infection. IFA has been well accepted and is in widespread use for the detection and semi-quantitation of antibodies to Legionella pneumophila.4,5 Single titers greater than 256 are considered presumptive laboratory evidence of Legionella infection; and a four-fold (4X) increase in titer (with convalescent greater than 128) is considered laboratory confirmation of infection.6,10,21

Solid phase assays in the form of Enzyme-Linked Immunosorbent Assay (ELISA) and Fluorescent ImmunoAssay (FIA) have been utilized for Legionella pneumophila serology.7,8,9 This test system is an ELISA utilizing a mixture of Legionella pneumophila serogroups 1-6 as the antigen. The antibody response to Legionella pneumophila can be quite varied. Individuals not only attain varied titers, but also give heterological response to different serotypes and species. The heterologous response is variable and is probably characteristic of the individual and does not seem to be mediated by the antigenic nature of the infecting strain.4,10

TEST PRINCIPLE

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials, (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When Legionella antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat antihuman IgG/IgM conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, Tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H2SO4, the contents of the wells turns yellow. The yellow color development correlates with antigenantibody binding in the reaction, and can be read on a suitable spectrophotometer or ELISA microwell plate reader.11,12

SPECIMEN COLLECTION AND PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, nonhemolyzed, non-lipemic, non-icteric). A minimum volume of 50 μL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.10 Early separation from the clot prevents hemolysis of serum.
3. Store samples at room temperature (21° - 25° C) for no longer than 8 hours. Refrigerate the samples between 2° and 8° C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20° C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.

MATERIALS AND COMPONENTS

Materials provided with the test kits
Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.
1. Legionella antigen (serogroups 1, 2, 3, 4, 5, 6 (ATCC # 33152, 33154, 33155, 33156, 33216, 33215), organisms harvested, heat inactivated, solubilized and pooled) coated microassay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. Serum Diluent Type I: Ready for use. Contains ProClin® (0.1%) as a preservative. (96T: one bottle, 30 mL)
3. Cutoff Calibrator (Calibrator): human serum or defibrinated plasma. Sodium azide (0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Cutoff Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature. (96T: one vial, 0.4 mL) *
4. High Positive Control: human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The High Positive Control is utilized to control the high range of the assay. (96T: one vial, 0.4 mL) *
5. Low Positive Control: human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Low Positive Control is utilized to control the assay near the cutoff of the assay. (96T: one vial, 0.4 mL) *
6. Negative Control: human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL) *
Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG/IgM, containing ProClin® (0.1%) as a preservative. (96T: one bottle, 16 mL)

Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. (96T: one bottle, 15 mL)

Wash Buffer Type I (20X concentrate): dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and ProClin® (0.1%) as a preservative. (96T: one bottle, 50 mL)

Stop Solution: Contains a H2SO4 solution, ready to use. (96T: one bottle, 15 mL)

*Note: serum vials may contain excess volume

### Materials required but not provided

- Graduated cylinder (100 mL)
- Flask (1 L)
- Timer – 0 to 60 minutes.
- Micropipettes capable of accurately delivering 10-200 KL volumes (less than 3% CV)
- Deionized or distilled water.
- Paper towels.
- Wash bottle, semi-automated or automated wash equipment.
- Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator’s Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.
- Test tubes for serum dilution.
- Disposal basin and disinfectant (e.g., 0.5% sodium hypochlorite).

### PRECAUTIONS

1. For in vitro diagnostic use.
2. The human serum components used in the preparation of the Controls and Cutoff Calibrators in this kit have been tested for the presence of antibodies to Human Immunodeficiency Virus 1 & 2 (HIV 1&2) and Hepatitis C (HCV) as well as Hepatitis B surface antigen by FDA approved methods and found to be negative. Because no test method can offer complete assurance that HIV, HCV, Hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control and National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution, Stop Solution, and Wash Buffer. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21°-25°C) before running assay. Remove only the volume of reagents that are needed. Do not pour reagents back into vials as reagent contamination may occur.
7. Before opening Control and Cutoff Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. Cross-contamination of reagents and/or samples could cause false results.

### ASSAY PROCEDURE

#### PREPARATION FOR THE ASSAY

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type I to 1 L with distilled and/or deionized H2O. Mix well.

#### METHODS FOR USE

### WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

**H317: May cause an allergic skin reaction.**

**P280:** Wear protective gloves / protective clothing / eye protection / face protection.

**P302 + P352:** IF ON SKIN: Wash with plenty of soap and water.

**P333 + P313:** If skin irritation or rash occurs: Get medical advice/attention.

**P501:** Dispose of contents/container in accordance to local, regional, national and international regulations.

**WARNING**

Serum Diluent and Controls contain < 0.1% sodium azide.

**H302:** Harmful if swallowed

**P264:** Wash thoroughly with plenty of soap and water after handling

**P270:** Do not eat, drink or smoke when using this product

**P301+P312:** IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

**P501:** Dispose of contents/container to in accordance to local, regional, national and international regulations.

### ASSAY PROCEDURE
1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Cutoff Calibrator determinations (one Negative Control, three Cutoff Calibrators, one High Positive Control and one Low Positive Control) per run. A reagent Blank (RB) should be run on each assay. Check software and reader requirements for the correct Calibrator/Control configurations. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

<table>
<thead>
<tr>
<th>Plate Location</th>
<th>Sample Description</th>
<th>Plate Location</th>
<th>Sample Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>RB</td>
<td>2A</td>
<td>Patient #4</td>
</tr>
<tr>
<td>1B</td>
<td>NC</td>
<td>2B</td>
<td>Patient #5</td>
</tr>
<tr>
<td>1C</td>
<td>Cal</td>
<td>2C</td>
<td>Patient #6</td>
</tr>
<tr>
<td>1D</td>
<td>Cal</td>
<td>2D</td>
<td>Patient #7</td>
</tr>
<tr>
<td>1E</td>
<td>Cal</td>
<td>2E</td>
<td>Patient #8</td>
</tr>
<tr>
<td>1F</td>
<td>HPC</td>
<td>2F</td>
<td>Patient #9</td>
</tr>
<tr>
<td>1G</td>
<td>LPC</td>
<td>2G</td>
<td>Patient #10</td>
</tr>
<tr>
<td>1H</td>
<td>Patient #1</td>
<td>2H</td>
<td>Patient #11</td>
</tr>
</tbody>
</table>

RB = Reagent Blank - Well without serum addition run with all reagents. Utilized blank reader.
NC = Negative Control
Cal = Cutoff Calibrator
HPC = High Positive Control
LPC = Low Positive Control

2. Dilute test sera, Cutoff Calibrator and Control sera 1:2 (e.g., 10 μL + 200 μL) in Serum Diluent. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum).
3. To individual wells, add 100 μL of the appropriate diluted Cutoff Calibrator, Controls and patient sera. Add 100 μL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25°C) for 25 minutes +/- 5 minutes.
5. Aspirate or shake out liquid from all wells. Using semi-automated or automated washing equipment add 250-300 μL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three washes) for semi-automated equipment or four times (for a total of five washes) for automated equipment. After the final wash, blot the plate gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading.

**IMPORTANT NOTE:** Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 μL) is recommended. A total of five (5) washes may be necessary with automated equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

6. Add 100 μL Conjugate to each well, including the reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well 25 minutes +/- 5 minutes at room temperature (21° to 25° C).
8. Repeat wash as described in Step 5**.
9. Add 100 μL Chromogen/Substrate solution (TMB) solution to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21° to 25° C) for 10-15 minutes.
11. Stop reaction by addition of 100 μL of Stop Solution (1N H2SO4) following the same order of Chromogen/Substrate addition, including reagent blank well. Tap the plate gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is > 0.150, the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

RESULTS

1. Cutoff Calibrator Value - Calculate the mean value for the Cutoff Calibrator from the three Cutoff Calibrator determinations. If any of the three Cutoff Calibrator values differs by more than 15% from the mean, discard that value and calculate using the average of the two remaining values.
2. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined for each lot of kits. The Correction Factor is printed on the Cutoff Calibrator vial.
3. Cutoff O.D. Value - The Cutoff O.D. Value for each assay is determined by multiplying the Correction Factor by the mean Cutoff Calibrator value determined in Step 1.
4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff O.D. Value determined in Step 3.

Example:

<table>
<thead>
<tr>
<th>O.D.s obtained for Cutoff Calibrator</th>
<th>Mean O.D. for Cutoff Calibrator</th>
<th>Correction Factor</th>
<th>Cutoff O.D. Value</th>
<th>O.D. obtained for patient sera</th>
<th>Immune Status Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.38, 0.42, 0.40</td>
<td>0.40</td>
<td>0.50</td>
<td>0.50 x 0.40 = 0.20</td>
<td>0.60</td>
<td>0.60/0.20 = 3.00</td>
</tr>
</tbody>
</table>

ANALYSIS

1. The patient’s Index Values are interpreted as follows:

<table>
<thead>
<tr>
<th>ISR ≤ 0.90</th>
<th>Results</th>
<th>Report/ Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
<td>No detectable IgG/IgM antibody by the ELISA test. Should report &quot;Negative for anti-Legionella pneumophila IgG/IgM; may be equivalent to IFA titer &lt;1:256 but does not exclude Legionella infection&quot; (see Limitations).</td>
</tr>
</tbody>
</table>

0.91-1.09  Equivocal Samples that remain equivocal after repeat testing should be restested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken. |

≥ 1.10  Positive Indicates presence of detectable IgG/IgM antibody. Should report "Positive for anti-Legionella pneumophila IgG/IgM, presumptive for Legionella infection and may be equivalent to IFA titer > 1:256. Other laboratory and clinical procedures are necessary to confirm a diagnosis". |

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

1. Cutoff Calibrator and Controls must be run with each test run.
2. Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.
3. Negative Control must be ≤ 0.250 A at 450 nm (when read against reagent blank).
4. Each Calibrator must be ≤ 0.300 A at 450 nm (when read against reagent blank).
5. Positive Control must be $\geq 0.250$ A at 450 nm (when read against reagent blank).
6. The ISR for the Positive and Negative Controls should be in their respective ranges printed on the vial labels. If the Control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.
7. The furnished Controls and Calibrators are manufactured from different lots of material.
8. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
9. Refer to NCCLS C24A for guidance on appropriate Quality Control practices. 17
10. If above criteria are not met on repeat testing, contact Diagnostic Automation/Cortez Diagnostics, Inc.

EXPECTED VALUES
The prevalence rate for the DAI Legionella IgG/IgM ELISA was determined by testing three hundred sixty-seven (367) normal sera with various ages and gender from various regions of the country. Ten were found to be positive, giving a prevalence rate of 2.72%. Common prevalence rates for antibody to Legionella species is 5% to 10% (3). The distribution of ISR Values for the DAI Legionella IgG/IgM ELISA assay, for a normal population, is illustrated in Table 1.

![Distribution of ISR Values in a Normal Population (n=367)](image)

Table 1

<table>
<thead>
<tr>
<th>ISR Values</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>0</td>
</tr>
<tr>
<td>10-20</td>
<td>0</td>
</tr>
<tr>
<td>20-30</td>
<td>0</td>
</tr>
<tr>
<td>30-40</td>
<td>0</td>
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<tr>
<td>40-50</td>
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<tr>
<td>50-60</td>
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<tr>
<td>60-70</td>
<td>0</td>
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<td>70-80</td>
<td>0</td>
</tr>
<tr>
<td>80-90</td>
<td>0</td>
</tr>
<tr>
<td>&gt;90</td>
<td>2</td>
</tr>
</tbody>
</table>

The distribution of ISR Values for the DAI Legionella IgG/IgM ELISA assay for IFA positive population is illustrated in Table 2.

![Distribution of ISR Values in a Normal Population (n=63)](image)

Table 2

<table>
<thead>
<tr>
<th>ISR Values</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>0</td>
</tr>
<tr>
<td>10-20</td>
<td>0</td>
</tr>
<tr>
<td>20-30</td>
<td>0</td>
</tr>
<tr>
<td>30-40</td>
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<td>40-50</td>
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<td>60-70</td>
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<td>70-80</td>
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</tr>
<tr>
<td>80-90</td>
<td>0</td>
</tr>
<tr>
<td>&gt;90</td>
<td>0</td>
</tr>
</tbody>
</table>

PERFORMANCE CHARACTERISTICS

1. % AGREEMENT POSITIVE AND % AGREEMENT NEGATIVE

The DAI Legionella IgG/IgM ELISA was evaluated relative to Legionella IFA at two different sites. The first site was a commercial R&D lab located in Maryland. Thirtythree single IFA positive sera, from an outbreak and samples routinely submitted for Legionella testing, were tested. The results of the study are summarized in Table 3.

![Table 3](image)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA$^+$ $&gt;256$</td>
<td>27</td>
</tr>
<tr>
<td>IFA$^+$ $&lt;256$</td>
<td>3</td>
</tr>
<tr>
<td>IFA$^-$</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
</tr>
</tbody>
</table>

% Agreement positive = 27/30 = 90.00% = 79.0% - 100%

% Agreement negative = 3/33 = 9.09% = 95.6% - 100%

Equivocals were not included in the above calculations. The 95% confidence intervals were calculated using the normal method.

The second site was a clinical laboratory in Pennsylvania. Seventy-two prospective serum for Legionella testing were tested. The results of the study are summarized in Table 4.

![Table 4](image)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA$^+$ $&gt;256$</td>
<td>2</td>
</tr>
<tr>
<td>IFA$^+$ $&lt;256$</td>
<td>70</td>
</tr>
<tr>
<td>IFA$^-$</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
</tr>
</tbody>
</table>

% Agreement negative = 67/68 = 98.53% = 95.6% - 100%

% Agreement = 69/70 = 98.57% = 95.7% - 100%

Equivocals were not included in the above calculations. The 95% confidence intervals were calculated using the normal method.

2. PRECISION

Seven different sera were assayed at two different sites to determine the precision of the assay. An additional three sera were tested at site 1. Each sera was tested ten times each, on three different days at each of the two study sites. The intra- and inter-assay precision for each site is presented in Tables 5 and 6. The inter-site coefficient of variation (CV) for each serum is presented in Table 7.
an increase in ion with Legionella evidence month after ammonia and - established for matrices infection. Testing - o Legionella species tectable levels e pairs had a retest likelihood of conversion by this assay rum analysis, isolation and IFA s cross. In some patients, ement of the e to the e to the t of this assay with a masked serum panel. This does not imply an endorsement of the assay by the CDC.

LIMITATION OF PROCEDURE

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.

2. Icteric, lipemic, hemolyzed, or heat inactivated sera may cause erroneous results and should be avoided.

3. False negatives - A negative result does not rule out infection with Legionella pneumophila or other Legionella species. False negative results may occur when samples are drawn too early after onset. It may take up to 9 weeks post infection for a patient to seroconvert. False negative results may also occur due to the lack of acquisition of antibody. Only 80% of Legionella pneumophila culture proven infections develop diagnostic changes in antibody titer.

4. False positives - A positive antibody response may be due to cross-reacting antibody found in patients with non-Legionella infections. Pneumonia and bacteremias caused by pseudomonas, haemophilus, mycobacteria, Gram negative enteric rods, bordetella, chlamydia, rickettsia, bacteroides, citrobacter and leptospiro have been shown to cause false positive results in Legionellosis serology. Cross-reactivity of this assay with antibodies to the above disease states has not been determined. However, a positive result, along with symptoms and signs of the disease, may indicate the possibility of Legionella pneumophila infection, therefore paired serum analysis, isolation and IFA determinations should be performed to aid diagnosis. Seroconversion by this assay has to be interpreted with caution due to the above listed cross-reactions with other infectious organisms.

5. Cross-reactivity may occur with sera with infections due to Legionella species other than Legionella pneumophila. Cross-reactivity of this assay with antibodies to other Legionella species has not been determined.

6. Testing should not be performed as a screening procedure. The predictive value of a positive or negative serological result depends on the pretest likelihood of Legionella infection. Testing should only be done when clinical evidence suggests Legionella infection.

7. Timing of specimen collection for paired sera may be critical. In some patients, antibody titers may rise to significant levels and fall again to undetectable levels within a month. Other patients may not develop significant antibody levels.

8. This assay detects IgG/IgM antibodies to Legionella pneumophila serogroups 1-6 organisms, whereas other available tests may detect total immunoglobulins (IgG, IgM, IgA).

9. The assay performance characteristics have not been established for matrices other than serum.

10. The affinity and or avidity of anti-Legionella pneumophila IgG and IgM for the Legionella pneumophila antigen have not been determined with this assay. Samples with IgM only may not be detected. Most patients will develop IgG antibodies within the first month after infection; therefore all negative patients should be redrawn and retested.

11. Early antibiotic therapy may suppress antibody response and some individuals may not develop antibodies above detectable limits.
A single positive result only indicates presumptive previous immunologic exposure; level of antibody response may not be used to determine active infection; continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.

Interpretation of results may not be applicable to immunosuppressed individuals.

STORAGE

1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2° and 8° C.
3. Store HRP Conjugate between 2° and 8° C.
4. Store Serum Diluent and 20X Wash Buffer between 2° and 8° C.
5. Store the Cutoff Calibrator, High Positive Control, Low Positive Control and Negative Control between 2° and 8° C.
6. Store the Chromogen/Substrate Solution between 2° and 8° C.
7. Store the Unopened microassay plates at room temperature (21° to 25°C) for up to 5 days, or 1 week between 2° and 8° C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination.

SUMMARY OF ASSAY

1. Dilute serum to 1:21
2. Add 100 μl of Microtubes
3. Add conjugate 100 μl
4. Mix
5. Incubate 25 minutes at room temperature
6. Wash
7. Add TMB solution 100 μl
8. Add stop 100 μl
9. Read at 450 nm

REFERENCES

6. CDC Case Definitions for Public Health Surveillance, Oct. 19, 1990; MMWR R&R.