AccuDiag™
Legionella IgG / IgA / IgM
ELISA Kit

TEST PRINCIPLE

The DAI Legionella IgG/IgA/IgM ELISA test system is designed to detect antibodies to L. pneumophila in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with Legionella antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG/IgA/IgM is added to the wells and the plate is incubated. The Conjugate will react with the antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

SPECIMEN COLLECTION AND PREPARATION

1. It is recommended that the user carry out specimen collection in accordance with NCCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. 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.
3. 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.
4. 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° and 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. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (14).

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 packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v): Controls, Calibrator, and Sample Diluent

1. Plate: 96 wells configured in twelve 1x8-well strips coated with a formalin-inactivated sonicated preparation of L. pneumophila Groups 1-6 antigens. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG/IgA/IgM. Ready to use. One, 15 mL vial with a white cap. Ready to use.
3. Positive Control (Human Serum): One, 0.35 mL vial with a green cap.
4. Calibrator (Human Serum): One, 0.5 mL vial with a blue cap.
5. Negative Control (Human Serum): One, 0.35 mL vial with a green cap.
6. Sample Diluent: One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use.

Note: The Sample Diluent will change color when combined with serum.

7. TMB: One 15 mL amber bottle (amber cap) containing 3,3’,5,5’-tetramethylbenzidine (TMB). Ready to use. Contains DMSO < 15% (w).
8. Stop solution: One 15 mL bottle (red cap) containing 1M H2SO4, 0.7M HCl. Ready to use.
9. Wash buffer concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100 mL clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). Note: 1X solution will have a pH of 7.2 ± 0.2.

NOTES:

1. The antibody response to L. pneumophila may be both specific and nonspecific, since the patient may have antibodies to similar antigens from other Gram-negative bacteria. Optimum times for specimen collection appear to be within the first week of illness, or as soon as possible after the onset (acute specimen), and at least 3 weeks after the onset (convalescent specimen) (3). By the IFA method, a single result of >1:256 is considered presumptive evidence of legionella infection. Diagnostic titers have been reported to be absent in as many as 25% of patients (4), but the use of multiple Legionella species (5,6) as the antigen source and a polyvalent conjugate directed against IgG, IgM, and IgA (7) maximize the accuracy of serological procedures.

SUMMARY AND EXPLANATION

L. pneumophila was identified as the causative agent for Legionellosis (Legionella pneumonia, or Legionnaire's Disease) in 1977 (1). Presently, there are more than 25 species and 33 serogroups in the family Legionellaceae, with at least 18 species associated with pneumonia, accounting for roughly 1-5% of all cases of pneumonia (2). L. pneumophila displays a multitude of morphologies including the bacillus, coccobacillus, and elongated fusiform. Although often difficult to perform, the Gram stain will be Gram-negative.

The antibody response to L. pneumophila may be both specific and nonspecific, since the patient may have antibodies to similar antigens from other Gram-negative bacteria. Optimum times for specimen collection appear to be within the first week of illness, or as soon as possible after the onset (acute specimen), and at least 3 weeks after the onset (convalescent specimen) (3). By the IFA method, a single result of >1:256 is considered presumptive evidence of legionella infection. Diagnostic titers have been reported to be absent in as many as 25% of patients (4), but the use of multiple Legionella species (5,6) as the antigen source and a polyvalent conjugate directed against IgG, IgM, and IgA (7) maximize the accuracy of serological procedures.
1. The following components are not kit lot number dependent and may be used interchangeably with the DAI ELISA Test System: TMB, Stop Solution, and Wash Buffer.

2. The DAI Legionella IgG/IgA/IgM ELISA test system also contains a Component list containing lot specific information inside the kit box, and a Package insert providing instructions for use.

Materials required but not provided

1. ELISA microwell reader capable of reading at a wavelength of 450nm.
2. Pipettes capable of accurately delivering 10 to 200µL.
3. Multichannel pipette capable of accurately delivering (50-200µL).
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

PRECAUTION

1. For In Vitro diagnostic use.
2. Follow all 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 ELISA plate do not contain viable organisms. However, consider the strips potentially biohazardous materials and handle accordingly.
4. The Controls are potentially biohazardous materials. Source materials from which these products were derived are 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, handle these products 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 Microbiological and Biomedical Laboratories”: Current Edition; and OSHA’s Standard for Bloodborne Pathogens (13).
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 refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent, Controls, and Calibrator 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 upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. 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.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate’s enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors. Avoid microbial contamination of reagents. Incorrect results may occur. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/ Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

**EXAMPLE PLATE SET-UP**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Blank</td>
<td>Patient 3</td>
</tr>
<tr>
<td>B</td>
<td>Negative Control</td>
<td>Patient 4</td>
</tr>
<tr>
<td>C</td>
<td>Calibrator</td>
<td>Etc.</td>
</tr>
<tr>
<td>D</td>
<td>Calibrator</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Calibrator</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Positive Control</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Patient 1</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Patient 2</td>
<td></td>
</tr>
</tbody>
</table>

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum. **Note:** The Sample Diluent will undergo a color change confirming that the specimen has been combined with the diluent.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.

A. Manual Wash Procedure:
   a. Vigorously shake out the liquid from the wells.
   b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps a. and b. for a total of 5 washes.
   d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20-25°C) for 25 + 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the specimens. Include reagent blank well in the same order as the specimens.

B. Automated Wash Procedure:
If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. Read the plate within 30 minutes after the addition of the Stop Solution.

**ABREVIATED TEST PROCEDURE**

1. Dilute Serum 1:2.
2. Add diluted sample to microwell - 100µL/well.
3. Wash.
4. Add Conjugate 100µL/well.
5. Incubate 25 ± 5 minutes.
6. Wash.
7. Add TMB 100µL/well.
8. Incubate 10 - 15 minutes.
10. Read within 30 minutes.

**RESULTS**

A. Calculations:

1. **Correction Factor**
   A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component Label located in the kit box.

2. **Cutoff OD Value**
   To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.
   \[
   (C.F \times \text{mean OD of Calibrator} = \text{cutoff OD value})
   \]

3. **Index Values or OD Ratios**
   Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

**Example:**

<table>
<thead>
<tr>
<th>Positive Specimens</th>
<th>1.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivocal Specimens</td>
<td>0.91 to 1.09</td>
</tr>
</tbody>
</table>

**B. Interpretations:**

Index Values or OD ratios are interpreted as follows:

B.1. An OD ratio \( \leq 0.90 \) indicates no significant amount of IgG/IgM/IgA antibodies to L. pneumophila detected. A non-reactive result may be equivalent to an IFA titer of less than 1:256. A negative result does not exclude Legionella infection.

B.2. An OD ratio \( \geq 1.10 \) indicates that IgG/IgM/IgA antibodies specific to L. pneumophila were detected and is suggestive of Legionella infection at some time, and may be equivalent to an IFA titer of greater than or equal to 1:256.

B.3. Other laboratory procedures or additional clinical information may be necessary to establish a diagnosis.

Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

**NOTE:** The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present and cannot be correlated of IFA titers.

**PERFORMANCE CHARACTERISTICS**

A. **Comparative Study**
   A comparative study was performed to demonstrate the equivalence of The DAI Legionella IgG/IgA/IgM ELISA test system to another commercially available ELISA test system, and to a IFA test system. The performance of the The DAI Legionella ELISA test system was evaluated in a three-site clinical investigation. One clinical site compared the performance of the DAI product to another commercially available ELISA test system. A second clinical site compared the DAI Legionella ELISA test system to the DAI IFA Test System. The third clinical site compared the DAI Legionella ELISA test system to a commercially available IFA Legionella test system. The study tested a total of 240 specimens. Clinical specimens tested at sites one and two consisted primarily of routine specimens from a reference laboratory in Northeastern United States that tested for normal Legionella serological analysis. Some repository specimens were included which had been previously tested and were found to be positive for antibody to Legionella. Specimens tested at the third clinical site consisted of 22 paired specimens (acute and convalescent) from confirmed cases of Legionella infection. Tables 1, 2, and 3 show a summary of these comparative investigations. Analysis excluded any equivocal specimens.

**Table 1: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site One.**

<table>
<thead>
<tr>
<th>DAI Legionella ELISA Test System</th>
<th>DAI Legionella ELISA Test System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
</tr>
<tr>
<td>Commercial ELISA Test System</td>
<td>12</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2</td>
</tr>
<tr>
<td>Totals</td>
<td>28</td>
</tr>
<tr>
<td>Relative Sensitivity = 12/13 = 92.3% (95% Confidence Interval = 77.8 to 100%)</td>
<td></td>
</tr>
<tr>
<td>Relative Specificity = 67/72 = 93.1% (95% Confidence Interval = 87.2 to 98.9%)</td>
<td></td>
</tr>
<tr>
<td>Relative Agreement = 79/85 = 92.9% (95% Confidence Interval = 85.7 to 98.4%)</td>
<td></td>
</tr>
</tbody>
</table>

* 95% confidence intervals calculated using the exact method.
Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site Two.

<table>
<thead>
<tr>
<th>DAI Legionella IFA Test System</th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1:128</td>
<td>56</td>
<td>2</td>
<td>7</td>
<td>65</td>
</tr>
<tr>
<td>1:128</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>≥1:256</td>
<td>1</td>
<td>0</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Totals</td>
<td>57</td>
<td>3</td>
<td>27</td>
<td>87</td>
</tr>
</tbody>
</table>

Relative Sensitivity = 16/17 = 94.1% (95% Confidence Interval* = 82.9 to 100%)
Relative Specificity = 56/63 = 88.9% (95% Confidence Interval* = 81.1 to 96.6%)
Relative Agreement = 72/80 = 90.0% (95% Confidence Interval* = 83.4 to 96.6%)

* 95% confidence intervals calculated using the exact method.

Table 3: Calculation of Relative Sensitivity, Specificity and Agreement; Study Site Three. (Individual results for testing acute and convalescent specimens)

<table>
<thead>
<tr>
<th>DAI Legionella IFA Test System</th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1:128</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>1:128</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>≥1:256</td>
<td>3</td>
<td>0</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Totals</td>
<td>22</td>
<td>1</td>
<td>21</td>
<td>44</td>
</tr>
</tbody>
</table>

Relative Sensitivity = 19/22 = 86.4% (95% Confidence Interval* = 72 to 100%)
Relative Specificity = 16/17 = 94.1% (95% Confidence Interval* = 89.2 to 100%)
Relative Agreement = 35/39 = 89.7% (95% Confidence Interval* = 80.2 to 99.2%)

* 95% confidence intervals calculated using the exact method.

With respect to Table 3 above; of the 22 pairs of acute and convalescent specimens, 17/22 were ELISA negative for the acute, and positive for the convalescent. Of the remaining five pairs 3/22 were negative for both acute and convalescent, and 2/22 were positive for both the acute and convalescent. **Note:** Be advised that relative refers to the comparison of this assay’s results to that of a similar assay. There was not an attempt to correlate the assay’s results with disease presence or absence.

B. Reproducibility

To demonstrate inter-laboratory reproducibility of the assay outcome, 6 specimens were evaluated; Two with an IFA titer of <1:128, 2 with an IFA titer of 1:512, and 2 with an IFA titer of ≥1:1024. Five vials of each specimen were prepared for a total of 30 vials. The 30 vials were randomized and simply numbered 1 through 30. The panel was tested in-house and at the two clinical sites. The study demonstrated excellent inter-laboratory reproducibility, with 100% agreement between all three sites.

C. Precision

Precision was evaluated as outlined in CLSI/NCCLS document number EP5-T2: Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition. Both clinical sites performed reproducibility studies using the same eight specimens: two relatively strong positive specimens, two specimens near the cutoff, two that were clearly negative and the kit’s negative control and positive control. On each day of testing, each specimen was assayed in duplicate, at two time points, morning and afternoon, for a total of four replicates per specimen. This reproducibility study ran for a 20-day period, for a total of 80 observations, for each of the eight panel members. A summary appears in Table 4 below:

Table 4: Summary of Precision Testing Conducted at Clinical Sites 1 and 2

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Site</th>
<th>Mean Ratio</th>
<th>Result</th>
<th>SWR*</th>
<th>ST**</th>
<th>Days</th>
<th>Total Observations</th>
<th>Overall % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-1</td>
<td>1</td>
<td>2.264</td>
<td>Positive</td>
<td>0.204</td>
<td>0.438</td>
<td>19</td>
<td>76</td>
<td>10.99</td>
</tr>
<tr>
<td>L-2</td>
<td>2</td>
<td>2.517</td>
<td>Positive</td>
<td>0.138</td>
<td>0.438</td>
<td>19</td>
<td>76</td>
<td>17.42</td>
</tr>
<tr>
<td>L-3</td>
<td>2</td>
<td>2.435</td>
<td>Positive</td>
<td>0.123</td>
<td>0.357</td>
<td>18</td>
<td>80</td>
<td>14.67</td>
</tr>
<tr>
<td>L-4</td>
<td>1</td>
<td>0.271</td>
<td>Negative</td>
<td>0.024</td>
<td>0.040</td>
<td>18</td>
<td>80</td>
<td>8.45</td>
</tr>
<tr>
<td>L-5</td>
<td>2</td>
<td>0.757</td>
<td>Near Cut-off</td>
<td>0.095</td>
<td>0.199</td>
<td>19</td>
<td>76</td>
<td>11.24</td>
</tr>
<tr>
<td>L-6</td>
<td>1</td>
<td>0.606</td>
<td>Near Cut-off</td>
<td>0.079</td>
<td>0.095</td>
<td>19</td>
<td>80</td>
<td>15.72</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>6.414</td>
<td>Positive</td>
<td>0.114</td>
<td>0.297</td>
<td>20</td>
<td>80</td>
<td>4.64</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>0.270</td>
<td>Negative</td>
<td>0.019</td>
<td>0.033</td>
<td>20</td>
<td>80</td>
<td>12.14</td>
</tr>
</tbody>
</table>

* Point estimate of within run precision standard deviation.

** Point estimate of total precision standard deviation.

Note: Table 4 depicts the reproducibility results only as an example of those results obtained during the clinical study, using ideal conditions of environment, equipment, and technique. Each laboratory should evaluate reproducibility as it may vary depending upon the conditions at the laboratory.

**QUALITY CONTROL**

1. Each time the assay is performed, the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

<table>
<thead>
<tr>
<th>OD Range</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>&lt;0.250</td>
</tr>
<tr>
<td>Calibrator</td>
<td>≥0.300</td>
</tr>
<tr>
<td>Positive Control</td>
<td>≥0.500</td>
</tr>
</tbody>
</table>

a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.

b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.

c. If the above conditions are not met the test should be considered invalid and should be repeated.

4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.
LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis based on anti-Legionella results alone. Test results for anti-Legionella should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.

2. A positive result suggests infection with one or more of the Group 1-6 species; however, one will not be able to distinguish between species with the results of this ELISA test alone.

3. Avoid the use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens. Erroneous results may occur.

4. Cross-reactivity may occur in sera with infections due to other Legionella species.

5. A negative result does not rule out the possibility of infection with legionella. Serum specimens taken too early during the course of infection may not yet have significant antibody titers. Some culture positive cases of Legionella do not develop antibody to Legionella.

6. Positive results may be due to cross reactivity with antibody generated as a result of non-Legionella infection. Serologic cross-reactions have been reported with P. aeruginosa, several Rickettsia species, Costella burnetti, enteric gram-negative rods, Bacteroides species, Haemophilus species, Citrobacter freundii, and Campylobacter jejuni. Therefore, a positive result alone does not indicate infection with Legionella. Additionally, some reports indicate that a number of apparently healthy individuals may carry antibodies to legionellae; however, a positive result, along with clinical signs and symptoms may indicate possible Legionella infection. Additional serologic testing, such as paired sera analysis by IFA, or other clinical testing such as direct FA and culturing, may be necessary to establish diagnosis.

7. The assay performance characteristics for matrices other than sera have not been established.

8. The affinity and/or avidity of the anti-IgG/IgM/IgA conjugate has not been determined.

9. Although the conjugate is designed to detect human IgG, IgM, and/or IgA, one will not be able to determine which antibody is present with this assay.

10. Early antibiotic therapy may suppress antibody response and some individuals may not develop antibodies above detectable limits.

11. A single positive result only indicates previous immunologic exposure; level of antibody response may not be used to determine active infection.

12. Use of serogroups 1-6 for assessing antibody responses to different Legionella species and serogroups has not been established. Some infected patients may not have detectable levels of antibodies with this assay. Four to eight weeks may be needed to detect an antibody response and antibody levels can fall to undetectable levels within a month of infection.

EXPECTED VALUES

Some researchers have reported background frequencies of elevated antibody levels in a normal population of 1 to 3% for formalin fixed antigen preparations (11). In an evaluation of sixty normal donor sera conducted in-house, one specimen was equivocal (1.7%), two specimens were positive (3.3%), and the remainder (57/60 or 95%) were negative. Below appears a Frequency Distribution of the results of a group of 60 normal donor specimens, and 24 IFA confirmed positive specimens.

STORAGE

1. Store the unopened kit between 2° and 8°C.

2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.

3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.

4. Unopened Calibrator, Positive Control, Negative Control, TMB and Sample Diluent: Store between 2° and 8°C.

1. Wash Buffer concentrate (10X): Store between 2° and 25°C. Wash buffer (1X) is stable at room temperature (20° to 25°C) for up to 7 days or for 30 days between 2° and 8°C.

2. Stop Solution: Store between 2° and 25°C.

REFERENCE


