DRG® Legionella ELISA (EIA-2814)

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INTENDED USE

The DRG® Legionella ELISA Test is an enzyme-linked immunosorbent assay for the qualitative detection of total antibody (IgG, IgM, IgA) to Legionella pneumophila serogroups 1 – 6 in human sera.

The test is for in vitro diagnostic use only.

SIGNIFICANCE AND BACKGROUND

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.

PRINCIPLE OF THE TEST

The DRG® Legionella ELISA test system is designed to detect IgG/A/M class antibodies to L. pneumophila in human sera. Wells of plastic microwell strips are sensitized by absorption with Legionellla antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibodies 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/A/M is added to the wells and the plate is incubated. The conjugate will react with antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microtiter wells 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 test sample.

MATERIALS PROVIDED

1. **Microplate:**
   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.

3. **Positive Control** (Monkey Serum):
   One 0.35 mL vial with a red cap.
4. **Calibrator** (Monkey 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, pH 7.2 ± 0.2. Ready to use.
   NOTE: Shake well before use.
   NOTE: This sample Diluent will change color in presence of 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 H_2SO_4, 0.7M HCl. Ready to use.
9. **Wash Buffer concentrate 10X:**
   Dilute 1 part concentrate + 9 parts deionized or distilled water.
   One 100 mL bottle (clear cap) containing 10X concentrated phosphate-buffered-saline and Tween 20 solution (blue solution).
   NOTE: 1X solution will have a pH of 7.2 ± 0.2.

Note: Kit also contains:

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

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state and federal laws.
3. The well of the ELISA plate do not contain viable organisms. However, the strips should be considered **potentially biohazardous materials** and handled accordingly.
4. The human serum controls are **potentially biohazardous 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 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", current edition, and OSHA 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, Wash Buffer, and Conjugate 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.
8. The Stop Solution is toxic. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB solution is harmful. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an irritant. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints which can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
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. Wash solutions should be collected in a disposable basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acidic pH should be neutralized before adding to 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 which 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 from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

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MATERIALS REQUIRED BUT NOT SUPPLIED

- Microtiter plate reader capable of reading at a wavelength of 450nm.
- Microliter pipettes capable of accurately delivering 10 and 200 µL.
- Multichannel pipette capable of accurately delivering (50-200µL).
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or plate washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Timer with alarm capable of measuring to an accuracy of ± 1 second.
- Disposal basin and disinfectant, (Example: 0.5% sodium hypochlorite, 10% household bleach).

STORAGE CONDITIONS

1. Store the unopened kit between 2°C and-8° C.
2. Coated microwell strips: Store between 2°C 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 remains blue.
3. Conjugate: Store between 2°C and-8°C. **Do not freeze**.
4. Calibrator, Positive Control and Negative Control: Store between 2°C and-8°C.
5. TMB: Store between 2°C and-8°C.
6. Wash Buffer concentrate (10X): Store between 2°C and-25°C.
   Diluted Wash Bufer (1X) is stable at room temperature (20° to 25°C) for up to 7 days or for 30 days between 2°C and-8°C.
7. Sample Diluent: Store between 2°C and-8°C.
8. Stop Solution: Store between 2°C and-25°C.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out on accordance with NCCLS document M29: Protection of the Laboratory Workers from Infectious Disease.
2. No known test method can offer completer assurance that human blood samples will not transmit infection. Therefore, all blood derivates should be considered potentially infectious.
3. Only freshly drawn and properly stored sera obtained by approved aseptic venipuncture procedures should be used in this assay (8, 9). 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 at 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 which may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

1. Remove the individual kit components 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

	Strip 1	Strip 2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10 mL serum + 200 mL Sample Diluent. NOTE: Shake well before use.) of the and Negative Control, Calibrator, Positive Control and each patient serum. 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 a 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 X.
   Manual Wash Procedure
   a. Vigorously shake out the liquid from the wells.
   b. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps a. and b. for a total of five 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 wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.

   Automated Wash Procedure:
   If using an automated wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. Remove microtiter plate from washer, invert plate over paper towel and tap firmly to remove any residual wash solution from the wells.
8. Add 100 µL of the conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate at room temperature (20-25°C) for 25 ± 5 minutes.
10. Wash the plate by following the procedure in step 7.
11. Add 100 µL of the TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50 µL of stop solution to each well, including reagent blank well, at the same rate and the same order as the TMB solution was added. Positive samples will turn from blue to yellow. After adding stop solution, tap plate several times to ensure that the samples are thoroughly mixed.
14. Set the microplate reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the stop solution.

QUALITY CONTROL

1. Each time the assay is run, the Calibrator should 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 determinations. If any of the three Calibrator values differ by more than 15% from the mean, discard that value and calculate the mean of the remaining two values.
3. The mean OD value for the Calibrator and the OD values for the positive and negative controls should fall within the following ranges:

	OD RANGE
Negative Control	≤ 0.250
Calibrator Standard	≥ 0.300
Positive Control	≥ 0.500

   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 value 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

INTERPRETATION OF RESULTS

Calculations

1. Correction Factor

A cutoff OD value for positive samples has been determined by DRG® 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 List 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.

(CF x mean OD of Calibrator = cutoff OD value)

3. Index Value 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:

Mean OD of calibrator	= 0.793
Correction Factor (CF)	= 0.25
Cut off OD	= 0.793 x 0.25 = 0.198
Unknown Specimen OD	= 0.432
Specimen Index Value of OD Ratio	= 0.432 / 0.198 = 2.18

Interpretation

Index values or OD ratios are interpreted as follows:

	Index values or OD Ratio
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 - 1.09
Positive Specimens	≥ 1.10

1. An OD ratio ≤ 0.90 indicates that IgG/A/M antibody to L. pneumophila serogroups 1-6 is not detected. A non-reactive result may be equivalent to an IFA titer of < 1:256. A negative result does not exclude Legionella infection.
2. An OD ratio ≥ 1.10 indicates detection of antibody to Legionella, and is suggestive of Legionella infection at some time, and may be equivalent to an IFA titer of ≥ 1:256. Other laboratory procedures or additional clinical information may be necessary to establish a diagnosis.

3. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested. Specimens which remain equivocal after repeat testing should be tested by an alternate serologic procedure such as the DRG® Legionella IFA test system. An equivocal result does not exclude infection.

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 to IFA titers.

LIMITATIONS OF THE ASSAY

1. A diagnosis should not be made on the basis of 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. The use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens should be avoided. 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 (12).
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, Coxiella burnetii, enteric gram-negative rods, Bacteroides species, Haemophilus species, Citrobacter freundii, and Campylobacter jejuni (10). Therefore, a positive result alone does not indicate infection with Legionella. Additionally, some reports (11) 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 have not been established for matrices other than sera.
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.

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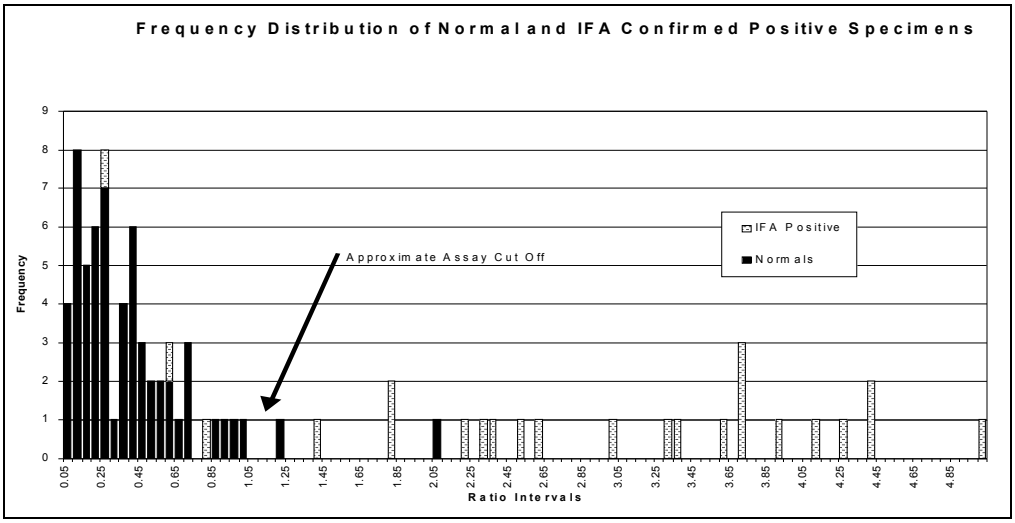
EXPECTED RESULTS

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.



PERFORMANCE CHARACTERISTICS

1.1 Comparative Study

A comparative study was performed to demonstrate the equivalence of the DRG® Legionella ELISA test system to another commercially available ELISA test system, and to a Legionella IFA test system.

The performance of the DRG® Legionella ELISA test system was evaluated in a three-site clinical investigation.
One clinical site compared the performance of the DRG® product to another commercially available ELISA test system.
A second clinical site compared the DRG® ELISA to the DRG® Legionella IFA.
The third clinical site compared the ELISA test system to a Legionella IFA test system.

Briefly, there were a total of 240 specimens tested; 109 at site one, 87 at site two, and 44 at site three. Clinical specimens tested at sites one and two consisted primarily of routine specimens which were sent to a reference laboratory in Northeastern United States 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. Equivocal specimens were excluded from any further analysis.

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

		DRG Legionella ELISA Result			
		+	-	±	Totals
Commercial	+	12	1	2	15
ELISA	-	5	67	10	82
	±	11	0	1	12
Test System	Totals	28	68	13	109

Relative Sensitivity = 12/13 = 92.3% (95% Confidence Interval* = 77.8 to 100%)
Relative Specificity = 67/72 = 93.1% (95% Confidence Interval* = 87.2 to 98.9%)
Relative Agreement = 79/85 = 92.9% (95% Confidence Interval* = 85.7 to 98.4%)
** 95% confidence intervals calculated using the exact method.*

Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site Two.

		DRG® Legionella ELISA Result			
		-	±	+	**Totals**
DRG®	**< 1:128**	56	2	7	65
Legionella IFA	**1:128**	0	1	4	5
Test System	**≥ 1:256**	1	0	16	17
	Totals:	57	3	27	87

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)

		DRG® Legionella ELISA Result:			
		-	±	+	**Totals**
	<1:128	16	0	1	17
Legionella IFA	**1:128**	3	1	1	5
Test System	**≥1:256**	3	0	19	22
	Totals:	22	1	21	44

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. No judgment can be made on the comparison assay's accuracy to predict disease.

1.2 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.

1.3 Precision

Precision was evaluated as outlined in document number EP5-T2: Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition, as published by the National Committee for Clinical Laboratory Standards (NCCLS), Vilanova, PA. Reproducibility studies were conducted at both clinical sites using the same specimens.

Briefly, six specimens were tested, two relatively strong positive specimens, two specimens near the cut-off, and two which were clearly negative. Additionally, the kit's negative control and high positive control were included as additional panel members at site one, for a total of eight specimens. On each day of testing, each of the eight specimens were assayed in duplicate. Also, on each day of testing, the assay was performed twice; once in the morning and once in the afternoon, for a total of four replicates for each specimen, daily. The clinical sites conducted this reproducibility study for a twenty day period, for a total of 80 observations for each of the eight panel members. A summary of this investigation appears in Table 4 below:

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

Specimen	Site	Mean Ratio	Result	SWR*	ST**	Days	Total Observations	Overall % CV
L-1	1 2	2.264 2.517	Positive	0.204 0.138	0.249 0.438	19 19	76 76	10.99 17.42
L-2	1 2	2.277 2.435	Positive	0.101 0.123	0.209 0.357	18 20	72 80	9.20 14.67
L-3	1 2	0.479 0.245	Negative	0.024 0.023	0.040 0.049	18 20	72 80	8.45 19.91
L-4	1 2	0.281 0.077	Negative	0.013 0.020	0.032 0.027	19 20	76 80	11.24 35.33
L-5	1 2	1.055 0.757	Near Cut-off	0.081 0.049	0.199 0.091	19 20	76 80	11.32 12.07
L-6	1 2	0.845 0.606	Near Cut-off	0.033 0.060	0.079 0.095	19 20	76 80	9.36 15.72
Positive Control	1	6.414	Positive	0.114	0.297	20	80	4.64
Negative Control	1	0.270	Negative	0.019	0.033	20	80	12.14

* Point estimate of within run precision standard deviation.

** Point estimate of total precision standard deviation

NOTE: The reproducibility results depicted in Table 4 are presented only as an example of those results obtained during the clinical study, using

ideal conditions of environment, equipment, and technique. Reproducibility should be evaluated at each laboratory, and may vary depending upon the conditions at the laboratory.

REFERENCES

1. McDade JE, Shepard CC, Fraser DW, Tsai TR, Redus MA, Dowdle WR, and the Laboratory Investigation Team: Legionnaires' disease. Isolation of a bacterium and demonstration of its role in other respiratory disease. N. Engl. J. Med. 297:1197-1203, 1977.
2. Tilton RC, Balows A, Hohnadel DC, and Reiss RF, Editors: Lower respiratory tract specimens. IN: Clinical Laboratory Medicine, Mosby Year Book, Inc., St. Louis, MO. pp 591-603, 1992.
3. Wilkinson HW: Manual of Clinical Immunology - Second Edition:Immune Response to Legionella pneumophila. Rose NR, Friedman H, editors. pp 500-503 (1980). Published by Am. Society for Microbiology, Washington, DC.
4. Harrison TG, Taylor AG: Timing of seroconversion in legionnaires' Disease. Lancet (2):795, 1988.
5. Wilkonson HW, Reingold AL, Brake BJ, McGiboney DL, Gorman GW, Broome CV: Reactivity of serum from patients with suspected Legionellosis against 29 antigens of legionellaceae and Legionella-like organisms by indirect immunofluorescent assay. J. Infect. Dis. 147:23-31, 1983.
6. McIntyre M, Kurtz JB, Selkon JB: Prevalence of antibodies to 15 antigens of legionellaceae in patients with community-acquired pneumonia. Epidemiol. Infect. 104:39-45, 1990.
7. Wilkinson HW, Farshy CE, Fikes BJ, Cruce DD, Yealy LP: Measure of immunoglobulin G-, M-, and A-specific titers against L. pneumophila and inhibition of titers against non-specific, gram negative bacterial antigens in the indirect immunofluorescent test for legionellosis. J. Clin. Microbiol. 10: 685-689, 1979.
8. Procedures for the collection of diagnostic blood specimens by venipuncture - Second Edition: Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
9. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
10. Edelstein P: Laboratory Diagnosis of Legionnaires Disease; an Update from 1984, pp 7-11. In: Legionella, Current Status and Emerging Perspectives. Barbaree J, et al, editors. Published by American Society for Microbiology, 1993.
11. Paszko-Kolva C, Shahamat M, Keiser J, and Colwell R: Prevalence of Antibodies Against Legionella Species in Healthy and Patient Populations, pp 24-26. In:Legionella, Current Status and Emerging Prespectives. Barbaree J, et al, editors. Published by American Society for Microbiology, 1993.
12. Bangsborg J, et al:The E. coli Immunosorbent as used in serodiagnosis of legionella infections studied by crossed immunoelectrophoresis. A PMIL 96:177-184, 1988.
13. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

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