

Product information

Information about other products is available at: www.demeditec.com



User's Manual

Legionella IgG/IgM/IgA ELISA

Enzyme Immunoassay for the qualitative determination of
IgG/IgM/IgA-class antibodies to *L. pneumophila* in human serum



REF DE2814

 96 wells

***Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.***

Table of Contents / Inhaltsverzeichnis / Tabella die Contenuti / Tabla de Contenidos

1	INTENDED USE	3
2	PRINCIPLE OF THE TEST	3
3	MATERIALS PROVIDED.....	4
4	PRECAUTIONS	5
5	MATERIALS REQUIRED BUT NOT PROVIDED.....	6
6	STORAGE CONDITIONS.....	6
7	SPECIMEN COLLECTION	6
8	ASSAY PROCEDURE	7
9	QUALITY CONTROL.....	8
10	INTERPRETATION OF RESULTS.....	9
11	LIMITATIONS OF THE ASSAY	10
12	EXPECTED RESULTS	11
13	PERFORMANCE CHARACTERISTICS.....	12
14	REFERENCES / LITERATURE.....	14
	SYMBOLS USED WITH DEMEDITEC ASSAYS	16

1 INTENDED USE

The DEMEDITEC 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.

1.1 Significance and Background

Scientists identified *L. pneumophila* 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 one to five percent 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 three weeks after the onset (convalescent specimen) (3). By the IFA method, considering a single result of greater than or equal to 1:256 is 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.

2 PRINCIPLE OF THE TEST

The Legionella 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.

3 MATERIALS PROVIDED

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrators and Sample Diluent

1. **Microplate** **SORB** **MT**: 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** **ENZ** **CONJ**: Conjugated (Horseradish Peroxidase) goat anti-human IgG/IgA/IgM. Ready to use. One 15 mL vial with a white cap.
3. **Positive Control** **CONTROL** **+** (Human Serum): One 0.35 mL vial with a red cap.
4. **Calibrator** **CAL** (Human Serum): One 0.5 mL vial with a blue cap
5. **Negative Control** **CONTROL** **-** (Human Serum): One 0.35 mL vial with a green cap
6. **Sample Diluent** **DIL**: 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: This Sample Diluent will change color when combined with serum.*
7. **TMB** **SUB** **TMB**: One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.
8. **Stop Solution** **STOP** **SOLN**: One 15 mL bottle (red cap) containing 2 N acidic solution, 0.7 M HCl. Ready to use.
9. **Wash Buffer Concentrate 10x** **BUF** **WASH** **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.*

Kit also contains:

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

4 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 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 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, 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, Conjugate and Wash Buffer 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 from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System..

5 MATERIALS REQUIRED BUT NOT PROVIDED

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

6 STORAGE CONDITIONS

1. Store the unopened kit between 2-8 °C.
2. Coated microwell strips: Store between 2-8 °C. Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
3. Conjugate: Store between 2-8 °C. **Do not freeze.**
4. Calibrator, Positive and Negative Control, TMB and Sample Diluent: Store between 2-8 °C.
5. Stop Solution: Store between 2-25 °C.
6. Wash Buffer concentrate (10X): Store between 2-25 °C.
Wash Buffer (1X): 20-25 °C for up to 7 days, 2-8 °C for 30 days.

7 SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out on accordance with CLSI document M29: Protection of the Laboratory Workers from Infectious Disease (Current Edition).
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. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (8, 9). Do not use if there are any added anticoagulants or preservatives. 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 - 8 °C, for no longer than 48 hours. If a 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.

8 ASSAY 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 Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank 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 - 8 °C.

EXAMPLE PLATE SET-UP

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

3. Prepare a 1:21 dilution (e.g.: 10 µL serum + 200 µL 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 100 µL of each diluted control, calibrator and patient specimen. 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 °C - 25 °C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 X.

a) Manual Wash Procedure

1. Vigorously shake out the liquid from the wells.
2. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
3. Repeat steps a. and b. for a total of five washes.
4. 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 disinfectant at the end of the days run.

b) Automated Wash Procedure:

If using an automated wash system, set the dispensing volume to 300 - 350 µL/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.

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 °C - 25 °C) for 25 ± 5 minutes.
10. Wash the microwells 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 °C - 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. 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 450 nm 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.

9 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.
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, Positive Control, and Negative Control should fall within the following ranges:

	<u>OD RANGE</u>
Negative Control	≤ 0.250
Calibrator	≥ 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 but 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 CLSI document C24: Statistical quality Control for Quantitative Measurements for Guidance on appropriate QC practices

10 INTERPRETATION OF RESULTS

10.1 Calculations

1. Correction Factor

The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.

2. Cutoff OD Value

To obtain the Cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.
($CF \times \text{mean OD of Calibrator} = \text{Cutoff OD value}$)

3. Index Value / OD Ratios

Calculate the Index Value / 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	= $0.793 \times 0.25 = 0.198$
Unknown Specimen OD	= 0.432
Specimen Index Value of OD Ratio	= $0.432 / 0.198 = 2.18$

10.2 Interpretation

Index values / OD ratios are interpreted as follows:

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

1. An OD ratio ≤ 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.
2. An OD ratio ≥ 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. 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 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 to IFA titers.

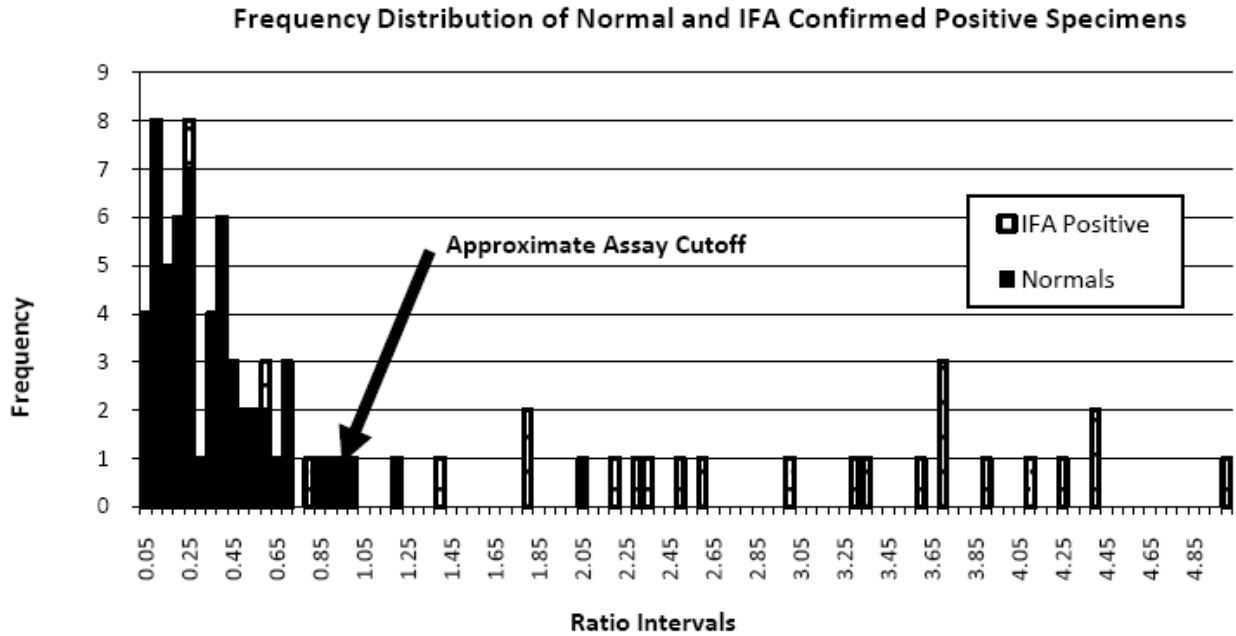
11 LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis based on anti-Legionella results alone. Physicians should interpret test results for anti-Legionella in conjunction with clinical evaluation and 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 (12).
6. Positive results may be due to cross reactivity with antibody generated because 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 antigen detection and culturing, may be necessary to establish diagnosis.
7. 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. Do not use the level of antibody response 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.

12 EXPECTED RESULTS

Some researchers have reported background frequencies of elevated antibody levels in a normal population of 1 - 3% for formalin fixed antigen preparations (11).

In an evaluation of 60 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.



13 PERFORMANCE CHARACTERISTICS

13.1 Comparative Study

Performance of a comparative study demonstrated the substantial equivalence of the ELISA L. pneumophila IgG/IgM/IgA Test System to another commercially available ELISA test system and to an IFA Legionella Test System. A three-site clinical investigation evaluated the performance of the ELISA Legionella IgG/IgM/IgA Test System. One clinical site compared the performance of the ELISA Legionella IgG/IgM/IgA Test System to another commercially available ELISA test system. A second clinical site compared the ELISA L. pneumophila IgG/IgM/IgA Test System to the IFA Legionella Test System. The third clinical site compared the ELISA L. pneumophila IgG/IgM/IgA 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.

	DEMEDIATEC Legionella ELISA Result			
	Positive	Negative	Equivocal	Totals
Commercial ELISA	12	1	2	15
Test System	5	67	10	82
	11	0	1	12
	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.

	DEMEDIATEC Legionella ELISA Result			
	Negative	Equivocal	Positive	Totals
Legionella IFA	56	2	7	65
Test System	0	1	4	5
	1	0	16	17
	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)

	DEMEDIATEC Legionella ELISA Result:			
	Negative	Equivocal	Positive	Totals
Legionella IFA	16	0	1	17
Test System	3	1	1	5
	3	0	19	22
	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 either assays' results with disease presence or absence.

13.2 Precision and Reproducibility

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

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

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

14 REFERENCES / LITERATURE

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 Perspectives*. Barbaree J, et al, editors. Published by American Society for Microbiology, 1993.
12. Bangsberg 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.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità