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Mycoplasma IgM

REF

Catalog No.E22-316

IVD

For In Vitro Diagnostic Use Only

INTENDED USE

The Immunospec Mycoplasma IgM ELISA test provides a means for the qualitative detection of IgM antibodies to Mycoplasma pneumoniae in human sera. When performed according to these instructions, the results of this test may aid in the diagnosis of M. pneumoniae infections in the adult population.

SIGNIFICANCE AND BACKGROUND

Mycoplasma pneumoniae is the most common cause of pneumonia and febrile upper-respiratory tract infections in the general population (except for influenza A) (1-5). Other nonrespiratory complications may also develop with this disease in virtually any organ system, with insult ranging from mild to life-threatening (6-8).

Mycoplasma pneumoniae, a prokaryote, is the smallest (10 x 200nm), and simplest self-replicating microorganism known, and more closely resembles a bacterium rather than a virus. However, because it lacks a cell-wall, a resistance to cell-wall-active antibiotics is obvious (i.e., penicillin, cephalosporins (1)). This concern for diagnostic, or at least therapeutic accuracy in the early management of community-acquired infections is particularly critical in very young or elderly patients where very little temporal margin of error exists.

Until recently, the routine laboratory diagnosis of this infection has been limited to insensitive and/or non-specific assays (i.e., cold agglutinins, complement-fixation, culture isolation). Species-specific antibodies to surface antigens are now known to exist. They are protective, and are readily detected by ELISA; even in the early stages of the disease. The diagnosis therefore, is best achieved serologically (9).

PRINCIPLE OF THE ELISA ASSAY

The Immunospec Mycoplasma IgM EUSA test is designed to detect IgM class antibodies to M. pneumoniae in human sera. Wells of plastic microwell strips are sensitized by passive absorption with M. pneumoniae antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains anti-human IgG which precipitates and removes IgG and rheumatoid factor from the sample leaving IgM free to react with the immobilized antigen. During sample incubation, any antigen specific IgM 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 IgM (chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound 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.

MATERIALS PROVIDED

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).

PLATE		1. Plate. 96 wells configured in twelve 1x8-well strips coated with a sonicated, inactivated preparation of M. pneumoniae strain FN. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ		2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgM (μ chain specific). Ready to use. One, 15 mL vial with a white cap.
CONTROL	+	3. Positive Control (Human Serum). One, 0.35 mL vial with a red cap.
CAL		4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap.
CONTROL	-	5. Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
DILSPE		6. Sample Diluent. One 30 mL bottle (blue cap) containing Tween-20, bovine serum albumin, phosphate-buffered-saline, and goat anti-human IgG (γ-chain specific), (pH 7.2 ± 0.2). Purple solution, ready to use. Note: Shake Well Before Use. (Product #: 005M).
SOLN	TMB	7. TMB: One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO ≤ 15% (w).
SOLN	STOP	8. Stop solution: One 15 mL bottle (red cap) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF	10X	9. Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) 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.

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 suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The welts 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 Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (12).
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 that 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 solution should be collected in a disposal 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 acid pH should be neutralized before adding to bleach solution.

24. Do not use EUSA 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 kit.

MATERIALS REQUIRED BUT NOT PROVIDED:

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 200µL
- Multichannel pipette capable of accurately delivering (50-200 µL)
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.

Disposable pipette tips.

- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

STORAGE CONDITIONS

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. Calibrator, Positive Control and Negative Control: Store between 2°and 8°C.

5. TMB: Store between 2°and 8°C.

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

7. Sample Diluent: Store between 2°and 8°C.

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

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS 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 (10, 11). 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.

GENERAL PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (2°-25°C).

2. Determine the number of microwetls 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

	1	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.: 10µL of serum + 200 µL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.

4. To individual wells, add 100L of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.

5. Add 100 µL of Sample Diluent to well Al as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.

6. Incubate the plate at room temperature (20°and 25°C) for 25 ±5 minutes.

7. Wash the microwell strips 5X

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 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 (bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated microwell 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 100µL 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 the plate at room temperature (20°and 25°C) for 25 5 minutes

10. Wash the microwells by following the procedure as described in step 7.

11. Add 100 µL of 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°and 25°C) for 10 to 15 minutes.

13. Stop the reaction by adding 5O µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.

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. 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 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:

	OD Range
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 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

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 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. (CFx mean OD of Calibrator = cutoff CD 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:

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 or OD Ratio	=	0.432 / 0.198 = 2.18

B. Interpretations:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	≤ 0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥ 1.10

1. In reporting results, an OD ratio < 0.90 indicates no detectable IgM antibodies to M. pneumoniae. A non-reactive result indicates no current or previous infection with M. pneumoniae as determined with the Immunospec Mycoplasma IgM ELISA test system.

2. In reporting results, an OD ratio≥ 1.10 is reactive for IgM antibodies to M. pneumoniae. A reactive test result indicates an active or recent infection with M. pneumoniae as determined with the Immunospec Mycoplasma IgM ELISA test .

3. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure such as the Immunospec indirect fluorescent antibody (IFA) test procedure.

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.

LIMITATION OF THE ASSAY

1. A diagnosis should not be made on the basis of anti-Mycoplasma results alone. Test results for anti-Mycoplasma should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.

2. If testing a particular specimen occurs early during the primary infection, no detectable IgM may be evident. If a Mycoplasma infection is suspected, a second sample should be taken at least fourteen days later.

3. A non-reactive result does not rule out current M. pneumoniae infection since the specimen may have been collected before demonstrable antibody is present or after the antibody has decreased below detectable levels. Consequently, demonstration of elevated lgG titers, in conjunction with specific IgM, increases the specificity of serological diagnosis.

4. The use of hemolytic, lipemic, bacterially contaminated, or heat-inactivated specimens should be avoided. Erroneous results may occur.

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

6. Performance of this assay has not been tested with specimens known to be positive for antibodies to organisms which are known to be associated with lower respiratory illness (i.e., Influenza A and B, CMV, C. pneumoniae, parainfluenza), and closely related Mycoplasma serovars known to cross-react with M. pneumoniae, such as M. genitalium and M. hominis, as well as various Ureaplasma species. Cross-reactivity studies with such organisms have not been performed with this test system.

7. Mycoplasma culture results or the presence or absence of antibody cannot be used to determine the success or failure of therapy.

8. Specimens from immunocompromised patients should be interpreted with caution.

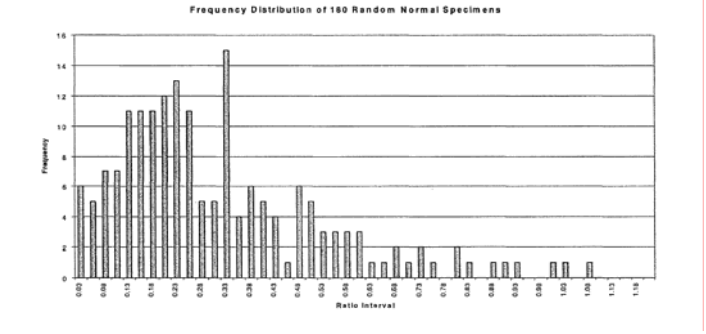
9. Screening of the general population should not be performed. Testing should only be done when clinical characteristics are present or exposure is expected.

10. The lgG removal system included with this test has been shown to functionally remove the lgG from specimens containing total lgG levels ranging from 300 to 600 mg/mL The effectiveness of this removal system at IgG levels exceeding 600 mg/mL has not been established.

11. The prevalence of Mycoplasma IgM antibody is relatively low. Low level prevalence rates of such analytes will affect the assay's predictive value.

EXPECTED RESULTS

The clinical study for this product included 220 random specimens which were sent to a reference laboratory in the Northeastern United States for routine Mycoplasma serological analysis. With respect to this population, 201 /220 (91.4%) were negative, 3/220(1.4%) were equivocal, and 16/220 (7.3%) were reactive. Also, an in-house study was performed to evaluate 180 random normal donor sera. A frequency distribution of the results of that study is shown below.



PERFORMANCE CHARACTERISTICS

Comparative Study

A comparative study was performed to demonstrate the equivalence of the Immunospec Mycoplasma IgM ELISA test system to the Immunospec IgM IFA test system.

The performance of the Immunospec Mycoplasma IgM ELISA test was evaluated in a three-site clinical investigation. All clinical sites compared the performance of the ELISA to the IFA test . A total of 299 specimens were evaluated at the various sites. Table 1 shows a summary of the testing performed at each clinical site. Table 2 shows the results of this comparative testing.

Table 1. Summary of Clinical Testing.

Site	Location	Specimen Characteristics	n
1	Offsite	Routine specimens which were sent to a reference laboratory in Northeastern U.S. for Mycoplasma serological analysis	111
1	Offsite	Samples sent to a hospital in the Midwest for Mycoplasma serological analysis	9
2	Offsite	Routine specimens which were sent to a reference laboratory in Northeastern U.S. for Mycoplasma serological analysis.	100
2	Offsite	Repository specimens previously tested for Mycoplasma IgM and were found to be reactive.	2
3	In-house	Various disease-state paired sera from diagnosed Mycoplasma infections.	62
3	In-house	Disease-state specimens from confirmed Mycoplasma infections.	15

Table 2: Calculation of Relative Sensitivity, Specificity and Agreement; Clinical Site I

		Mycoplasma IgM ELISA			
		-	±	+	Totals
IFA Results	<1:16	102	1	0	103
	1:16	8	0	0	8
	≥1:32	2	2	5	9
	Totals	112	3	5	120

Relative Sensitivity = 5/7 = 71.4% 95% Confidence Interval* = 29.0 to 96.3%
Relative Specificity = 102/102 = 100% 95% Confidence Interval* = 96.4 to 100%
Relative Agreement = 107/109 = 98.2 % 95% Confidence Interval* = 93.5 to 99.8%
* 95% confidence intervals calculated using the exact method.

Table 3:

Calculation of Relative Sensitivity, Specificity, and Agreement. Clinical Site 2

		Mycoplasma IgM ELISA			
		-	±	+	Totals
IFA Results	<1:16	89	0	7	96
	1:16	0	0	0	0
	≥1:32	0	0	6	6
	Totals	89	0	13	102

Relative Sensitivity = 6/6 = 100% 95% Confidence Interval* = 54.1 to 100%
Relative Specificity = 89/96 = 92.7% 95% Confidence Interval* = 85.6 to 97.0%
Relative Agreement = 95/102 = 93.1% 95% Confidence Interval* = 86.4 to 97.2%
* 95% confidence intervals calculated using the exact method.

Table 4:

Calculation of Relative Sensitivity, Specificity, and Agreement. Clinical Site 3

		Mycoplasma IgM ELISA			
		-	±	+	Totals
IFA Results	<1:16	27	1	10	38
	1:16	0	0	5	5
	≥1:32	3	1	30	34
	Totals	30	2	45	77

Relative Sensitivity = 30/33 = 90.9% 95% Confidence Interval* = 75.7 to 98.1%
Relative Specificity = 27/37 = 73.0% 95% Confidence Interval* = 55.9 to 86.2%
Relative Agreement = 57/70 = 81.4% 95% Confidence Interval* = 70.3 to 89.7%
* 95% confidence intervals calculated using the exact method.

Table 5:

Calculation of Relative Sensitivity, Specificity, and Agreement. Combination of Clinical Sites 1, 2, and 3.

		Mycoplasma IgM ELISA Results			
		-	±	+	Totals
IFA Results	<1:16	218	2	17	237
	1:16	8	0	5	13
	≥1:32	5	3	41	49
	Totals	231	5	63	299

Relative Sensitivity = 41/46 = 89.1% 95% Confidence Interval* = 76.4 to 96.4%
Relative Specificity = 218/235 = 92.8% 95% Confidence Interval* = 88.7 to 95.7%
Relative Agreement = 259/281 = 92.2% 95% Confidence Interval* = 88.4 to 95.0%
* 95% confidence intervals calculated using the exact method.

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.

Reproducibility:

Reproducibility was evaluated as outlined in the FDA guidance document; Review Criteria for In Vitro Diagnostic Devices for Detection of IgM Antibodies to Viral Antigens. 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 Positive Control were included as additional panel members, for a total of eight specimens. On each day of testing, each of the eight specimens were assayed in triplicate. The clinical sites conducted this reproducibility study for a three day period. A summary of this investigation appears in Tables 6 and 7 below.

Table 6. Summary of Intra-Assay Precision Testing Conducted at Clinical Sites 1 and 2.

	Results at Site One						Results at Site Two					
	Day One		Day Two		Day Three		Day One		Day Two		Day Three	
Sample	Mean Ratio	Percent CV	Mean Ratio	Percent CV	Mean Ratio	Percent CV	Mean Ratio	Percent CV	Mean Ratio	Percent CV	Mean Ratio	Percent CV
1	2.53	9.7	2.70	8.2	2.95	8.0	2.05	38.0	2.73	15.7	2.46	11.2
2	1.14	13.8	1.09	9.1	1.35	18.5	1.13	7.1	1.25	6.3	1.29	12.9
3	2.42	11.9	2.37	1.3	2.29	6.0	2.49	7.5	3.07	19.5	2.52	4.1
4	1.10	10.6	1.09	5.9	1.04	6.1	0.97	7.5	1.36	21.0	1.13	9.2
5	0.18	23.6	0.18	9.3	0.12	15.7	0.17	15.6	.013	50.0	0.19	8.2
6	0.20	23.1	0.24	5.2	0.17	8.5	0.16	16.1	0.18	14.7	0.23	13.5
NC	0.07	28.5	0.09	15.4	0.09	55.9	0.11	32.8	0.09	22.3	0.11	18.2
HPC	3.25	3.9	3.05	4.4	3.23	5.9	2.98	3.5	3.49	6.2	3.68	7.3

Table 7. Summary of Inter-Assay Precision Testing Conducted at Clinical Sites 1 and 2.

Table 7. Summary of Inter-Assay Precision Testing Conducted at Clinical Sites 1 and 2.

	Three day results – Site 1		Three day results – Site 2	
Sample	Mean Ratio	Percent CV	Mean Ratio	Percent CV
1	2.73	10.0	2.41	22.9
2	1.19	16.6	1.22	10.3
3	2.36	7.2	2.69	15.8
4	1.08	7.3	1.15	20.0
5	0.16	25.2	0.16	27.7
6	0.20	18.9	0.19	20.8
NC	0.09	36.2	0.10	23.5
HPC	3.18	5.1	3.38	10.6

NOTE: The reproducibility results depicted above 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. Tauzon CU, and Murray HW: 'Atypical pneumonias". In: Respiratory Infections: diagnosis and Management. Pennington JE, ed. Raven Press, New York, NY, pp. 251, 1983.
2. Chanock RM, Fox HH, James WD, Gutekunst RR, White RT, Seterfit LB: Epidemiology of M.P. infection in military recruits. Ann. NY Acad. Sci. 143:484-496, 1967.
3. Und K, Bentzon MW: Epidemics of M. pneumoniae infection in Denmarkirom 1958-1974. Tnt. J. Epidemiol. 5:267-277, 1976.
4. Noah ND: M. pneumoniae infection in the united Kingdom. British Med. J. 2:544-546, 1 974.\
5. Foy HM, Kenny GE, Cooney MK, Alan ID: Long-term epidemiology of infections with M. pneumoniae. J. Infect. Dis. 139:681-687, 1979.
6. Murray HW, Masur H, Seterflt LB. and Roberts LB: The protean manifestation of M. pneumcrliae infections in adults. An,. J. Med. 58:229-242, 1975.
7. Cassell GH, and Cole BC: Mycoplasmas as agents of human disease. N. Engl. J. Med. 304:80, 1981.
8. Noriegs ER, Simberkoff MS, Gilroy SJ, et at: Life threatening M. pneumoniae. JAMA 29:1471-1472, 1974.
9. Carter JB, and Carter SC: Acute-phase, Indirect Fluorescent antibody Procedure for diagnosis of Mycoplasma pneumoniae infection. Ann. Clin. Lab. Sci. 13, No. 2, 150-155, 1983.
10. Procedures for the collection of diagnostic blood specimens by venipuncture - Second Edition: Approved Standard (1984). Published by National Committee for Clinical laboratory Standards.
11. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
12. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-641 82, 1991.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100 µL/well
3. ⟶ *Incubate 20 to 30 minutes*
4. Wash
5. Add Conjugate – 100 µL/well
6. ⟶ *Incubate 20 to 30 minutes*
7. Wash
8. Add TMB 100 µL/well
9. ⟶ *Incubate 10 to 15 minutes*
10. Add Stop Solution 50 µL/well - Mix
11. READ

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