INSTRUCTIONS FOR USE

EN

Anti-M. pneumoniae IgM

REF 3Z17601M
SM3Z17601M

IVD

CE

Rx Only

Σ 96

INTENDED USE

The Anti-M. pneumoniae IgM 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. This assay is for *In Vitro* diagnostic use only.

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). Research shows that species-specific antibodies to surface antigens 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 ASSAY

The Anti-M. pneumoniae IgM is designed to detect IgM class antibodies to *M. pneumoniae* in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption 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 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.

TEST SYSTEM COMPONENTS

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, Calibrator, and Sample Diluent.

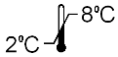
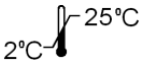
Kit Component	Quantity (Σ 96)	Description
PLATE	1	Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated preparation of M. pneumoniae (strain FH). The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgM (μ chain specific). 15mL, white-capped bottle. Ready to use.
CTRL +	1	Positive Control (Human Serum): 0.35mL, red-capped vial. 21X concentrate.
CAL	1	Calibrator (Human Serum): 0.5mL, blue-capped vial. 21X concentrate.
CTRL -	1	Negative Control (Human Serum): 0.35mL, green-capped vial. 21X concentrate.
DIL SPE	1	Sample Diluent: 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline and goat anti-human IgG (γ-chain specific). Purple solution. Ready to use.
SOLN TMB	1	TMB: 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	Stop Solution: 15mL, red-capped bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASH 10X	1	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTE: The following components are not kit lot number dependent and may be used interchangeably between ELISA kits so long as the component product number is the same: TMB, Stop Solution, and Wash Buffer.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200μL.
3. Multichannel pipette capable of accurately delivering 50 - 200μL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One-liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e., 10% household bleach - 0.5% sodium hypochlorite).

STORAGE CONDITIONS

	Coated Microwell Strips: 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 remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent.
	Stop Solution: 2 – 25 °C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days Wash Buffer (10X): 2 - 25°C

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 (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, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.

25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors 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.

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (10, 11). 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. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (13).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for 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		
	1	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.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL 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 well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 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 day's run.
   b. **Automated Wash Procedure**:
      If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. 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. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. ⟶ *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. ⟶ *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. ⟶ *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

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:

	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, but will not ensure precision at the assay Cutoff.
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 Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *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.
   b. *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)*
   c. *Index Values/OD Ratios*: Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example: Mean OD of Calibrator = 0.793

Correction Factor (CF)	=	0.25
Cutoff OD	=	0.793 x 0.25 = 0.198
Unknown Specimen OD	=	0.432
Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

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

a. An OD ratio ≤0.90 indicates no significant amount of IgM antibodies to *M. pneumoniae* detected. A non-reactive result indicates no current/previous infection.

b. An OD ratio ≥1.10 indicates that IgM antibodies specific to *M. pneumonia* were detected. A reactive test result indicates a past/recent infection.

c. 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.

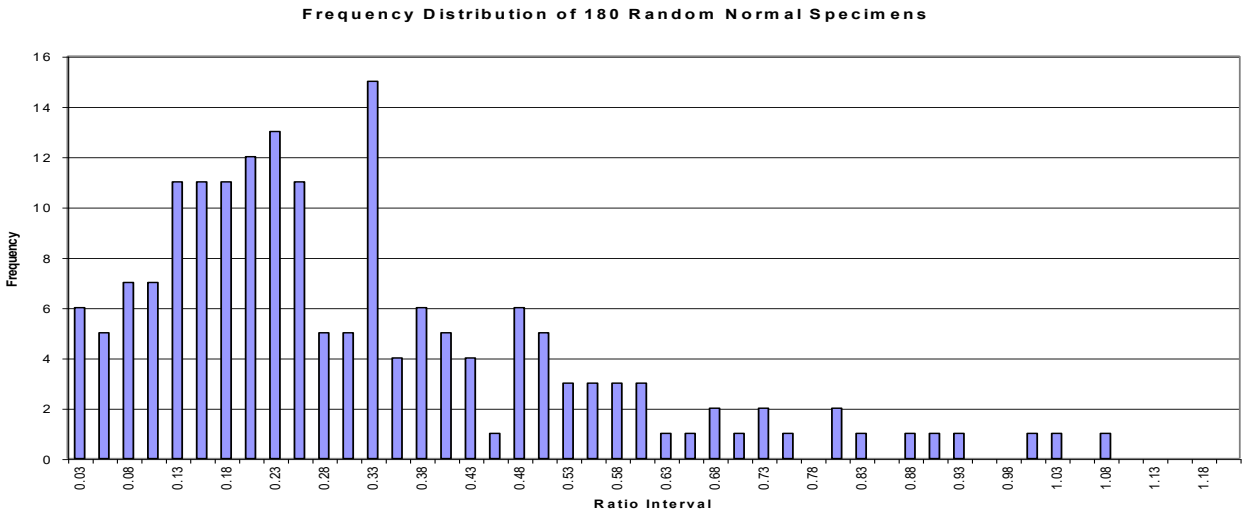
NOTE: The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present and cannot be correlated to IFA titers.

LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis based on Anti-M. pneumoniae IgM results alone. Interpret test results in conjunction with clinical evaluation and results of other diagnostic procedures.
2. If testing a particular specimen occurs early during the primary infection, no detectable IgM may be evident. If there is suspicion of a Mycoplasma infection, take a second sample at least fourteen days later for additional testing.
3. A non-reactive result does not rule out current *M. pneumoniae* infection since the specimen may have been collected before demonstrable antibody was present or after the antibody has decreased below detectable levels. Consequently, demonstration of elevated IgG titers, in conjunction with specific IgM, increases the specificity of serological diagnosis.
4. Avoid the use of hemolytic, lipemic, bacterially contaminated, or heat-inactivated specimens. Erroneous results may occur.
5. ZEUS Scientific has not established assay performance characteristics for matrices other than serum.
6. ZEUS Scientific did not conduct Cross Reactivity Studies on the performance of this assay with certain types of specimens. These specimens include the following: those known to be positive for antibodies to organisms known to be associated with lower respiratory illness (i.e., Influenza A and B, CMV, *C. pneumoniae*, parainfluenza), those closely related Mycoplasma serovars known to cross-react with *M. pneumoniae,* such as *M. genitalium* and *M. hominis*, as well as various Ureaplasma species.
7. Do not use Mycoplasma culture results, or the presence or absence of antibody, to determine the success or failure of therapy.
8. Interpret specimens from immunocompromised patients with caution.
9. Do not perform screening of the general population. Test only when clinical characteristics are present, or exposure is expected.
10. Studies show that the IgG removal system included with this test system will functionally remove the IgG from specimens containing total IgG levels ranging from 300 to 600 mg/mL. Studies were not conducted to establish the effectiveness of this removal system at IgG levels exceeding 600 mg/mL.
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 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. In addition, an in-house study evaluated 180 random normal donor sera. Depiction of results follows in the frequency distribution chart.



PERFORMANCE CHARACTERISTICS

1. **Comparative Studies**

A comparative study was conducted to demonstrate the equivalence of the Anti-M. pneumoniae IgM to the ZEUS IFA Crowntitre® IgM Test System. Technicians evaluated the performance of the Anti-M. pneumoniae IgM on 299 specimens, in a three-site clinical investigation. All clinical sites compared the performance of the ZEUS ELISA to the IFA test system. 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
One	Offsite	Routine specimens which were sent to a reference laboratory in Northeastern U.S. for Mycoplasma serological analysis.	111
One	Offsite	Samples sent to a hospital in the Midwest for Mycoplasma serological analysis.	9
Two	Offsite	Routine specimens which were sent to a reference laboratory in Northeastern U.S. for Mycoplasma serological analysis.	100
Two	Offsite	Repository specimens previously tested for Mycoplasma IgM and were found to be reactive.	2
Three	In-house	Various disease-state paired sera from diagnosed Mycoplasma infections.	62
Three	In-house	Disease-state specimens from confirmed Mycoplasma infections.	15

Table 2: Clinical Site One - Calculation of Relative Sensitivity, Specificity and Agreement

		Anti-M. pneumoniae IgM			
		Negative	**Equivocal**	**Positive**	**Total**
ZEUS IFA	**<1:16**	102	1	0	103
	1:16	8	0	0	8
	≥1:32	2	2	5	9
	Total	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: Clinical Site Two - Calculation of Relative Sensitivity, Specificity, and Agreement

		Anti-M. pneumoniae IgM			
		Negative	**Equivocal**	**Positive**	**Total**
ZEUS IFA	**<1:16**	89	0	7	96
	1:16	0	0	0	0
	≥1:32	0	0	6	6
	Total	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: Clinical Site Three - Calculation of Relative Sensitivity, Specificity, and Agreement

		Anti-M. pneumoniae IgM			
		Negative	**Equivocal**	**Positive**	**Total**
ZEUS IFA	**<1:16**	27	1	10	38
	1:16	0	0	5	5
	≥1:32	3	1	30	34
	Total	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: All Sites Combined - Calculation of Relative Sensitivity, Specificity, and Agreement;

		Anti-M. pneumoniae IgM			
		Negative	**Equivocal**	**Positive**	**Total**
ZEUS IFA	**<1:16**	218	2	17	237
	1:16	8	0	5	13
	≥1:32	5	3	41	49
	Total	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% * 95% confidence intervals calculated using the exact method.

Relative Agreement = 259/281 = 92.2% 95% Confidence Interval* = 88.4 to 95.0%

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 the presence or absence of disease. Make no judgment on the comparison assay's accuracy to predict disease.

2. **Precision and Reproducibility:**

Two clinical sites conducted reproducibility studies using the same eight specimens: two relatively strong positive specimens, two specimens near the cut off, two that were clearly negative and the kit's positive and negative controls. On each day of testing, the technician tested each of the eight specimens in triplicate. The clinical sites conducted this reproducibility study for a three-day period. 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. A summary of this investigation appears in Tables 6 and 7 below.

Table 6: Summary of Intra-Assay Precision Testing Conducted at Clinical Sites One and Two

	Site One Results						**Site Two Results**					
	Day One		**Day Two**		**Day Three**		**Day One**		**Day Two**		**Day Three**	
Sample	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% CV	Mean Ratio	% 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	0.13	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
PC	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 One and Two

	Site One - Three Day Results		Site Two - Three Day Results	
Sample	**Mean Ratio**	**% CV**	**Mean Ratio**	**% 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
PC	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. Evaluate reproducibility at each laboratory, and may vary, depending upon the conditions at the laboratory.

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
Σ n	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication	EC REP	European Commission Authorized Representative
CE	Conformity with Directive 98/79		

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