



INTENDED USE

The DRG Mycoplasma IgM ELISA test system 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 pneumonia*e, a prokaryote, is the smallest (10 x 200 nm), 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 laoratory 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 DRG Mycoplasma IgM ELISA test system is designed to detect IgM class antibodies to *M. pneumoniae* in human sera. 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 (u 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 microtiter wells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.

MATERIALS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of test indicated on packaging label.

- 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.
- 2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (μ chain specific). Ready to use. One, 15 mL vial with a white cap. Preservative added.
- 3. Positive Control (Human Serum). One, 0.35 mL vial with a red cap. Preservative added.
- 4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap. Preservative added.
- 5. Negative Control (Human Serum). One, 0.35 mL vial with a green cap. Preservative added.

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- 6. Sample diluent. One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered saline, ((pH 7.2 ± 0.2). Green solution; ready to use. Note: Shake Well Before Use. Preservative added.
- 7. TMB. One 15 mL amber bottle (amber cap) containing 3,3', 5,5'-tetramethylbenzidine (TMB). Ready to use. Contains DMSO ≤ 15% (w).
- 8. Stop solution. One 15 mL bottle (red cap) containing 1M H₂SO₄, 0.7M HCI. Ready to use.
- 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). Contains preservative NOTE: 1X solution will have a pH of 7.2+0.2.

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 wells 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 tese 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 manul "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 human serum controls, Sample Diluent, Conjugate, and Wash Buffer concentrate contain a preservative (sodium azide, 0.1% (w/v) which has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
- 8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- 9. The TMB Solution is HARMFUL; irritating to eyes, respiratory system and skin.
- 10. The Wash Buffer concentrate is an IRRITANT; irritating to eyes, respiratory system and skin.
- 11. Wipe bottom of plate free of residual liquid and/or fingerprints, which can alter optical density (OD) readings.
- 12. Dilution or adulteration of these reagents may generate erroneous results.
- 13. Reagents from other sources or manufacturers should not be used. Follow test procedures carefully.
- 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. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used.
- 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 out and thoroughly rinsed free of all detergents.
- 19. Avoid splashing or generation of aerosols.
- 20. Do not expose reagent 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 acidic pH should be neutralized before adding to bleach solution.
- 24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
- 25. Do not allow the conjugate to come in contact with containers or instruments 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 450 mn.
- Pipettes capable of accurately delivering 10 to 200 uL.
- Multichannel pipette capable of accurately delivering (50-200 uL).
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or plate washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- 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 at 2-8°C.
- 2. Coated microwell strips: Store between 2-8°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 2-8°C. DO NOT FREEZE.
- 4. Calibrator, Positive Control and Negative Control: Store between 2-8°C.
- 5. TMB: Store at $2-8^{\circ}$ C.
- 6. Wash Buffer concentrate (10X). Store between 2 and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25°C) for up to 7 days or for 30 days between 2-8°C.
- 7. Sample Diluent. Store at 2-8°C.
- 8. Stop Solution. Store at 2-25°C.





SPECIMEN COLLECTION

- 1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: <u>Protection of Laboratory Workers from Infectious Disease</u>.
- 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 sued in this assay (10, 11). No antigoagulants 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 múltiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

- 1. Remove the individual kit components from storage and allow them to warm to room temperature (20-25°C).
- Determine the number of microwells hended. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank sould 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 returned to storage between 2° and 8°C.

EXAMPLE PLATE SET-UP					
	1	2			
А	Blank	Patient 3			
В	Neg. Control	Patient 4			
С	Calibrator	Etc.			
D	Calibrator				
E	Calibrator				
F	Pos. Control				
G	Patient 1				
Н	Patient 2				

- 1. Prepare a 1:21 dilution (e.g.10uL of serum + 200uL of Sample Diluent. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.
- 2. To indivudual wells, add 100uL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
- 3. Add 100µL of sample diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank web configuration.
- 4. Incubate the plate at room temperature (20- 25° C) for 25 +/- 5 minutes.
- 5. Wash the microwell strips 5X:

Manual Wash Procedure:

- a) Vigorously shake out the liquid from the wells.
- b) Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
- c) Repeat steps (a) and (b) for a total of 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.

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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 300-350uL/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 conjugate solution to each well at the same rate and in the same order as the specimens were added.
- 9. Incubate the plate at room temperature $(20-25^{\circ}C)$ for 25 + 5 minutes.
- 10. Wash the microwells by following the procedure as described in step 7.
- 11. Add 100μ L 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 (20-25°C) for 10 to 15 minutes.
- 13. Stop the reaction by adding 50μ L 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 sample wells 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 microplate reader wavelength at 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after 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 differ by more than 15% from the mean, discard that value and calculate the mean using the remainint two wells.
- 3. The mean OD value 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: <u>Statistical Quality Control for Quantitative Measurements</u> for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

A. Calculations

1. Correction Factor

A cutoff value for positive samples has been determined by DRG and correlated to the Calibrator. This 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

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results. The correction factor is determined for each lot of kit components and is printed on the data label located in the kit box.

2. <u>Cutoff OD Value</u>

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.

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

3. Index Values or OD Ratios

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

B. Interpreations:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Rati
Negative Specimens	\leq 0.90
Equivocal Specimens	0.91-1.09
Positive Specimens	<u>≥</u> 1.10
1 In non-article an OD ratio < 0.00 india	stee as detectable IoM antibadies to M

- 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*.
- 2. In reporting results, an OD ratio ≥ 1.10 is reactive for IgM antibodies to *M. pneumoniae*. A reactive test result indicates a past or recent infection with *M. pneumoniae*.
- 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 DRG indirect fluorescent antibody (IFA) test procedure.

NOTE: The magnitude of the measured result above the cut-offs 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 IgG 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.





- 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 Micoplasma 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. Micoplasma 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 IgG removal system included with this test system has been shown to functionally remove the IgG from specimens containing total IgG 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 Micoplasma IgM antibody is relatively low. Low level prevalence rates of such analyts will affect the assay's predicitive 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 Micoplasma serological análisis. UIT 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 DRG Microplasma IgM ELISA test system.

The performance of the DRG Mycoplasma IgM ELISA test system was evaluated in a three site clinical investigation. All clinical sites compared the performance of the DRG ELISA to the IFA test system. 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.

Site	Location	Specimen Characteristics	n
1	Off-Site	Routine specimens which were sent to a reference laboratory in Northeastern U.S. for Micoplasma serological analysis	111
1	Off-Site	Samples sent to a hospital in the Midwest for Micoplasma serological analysis	9
2	Off-Site	Routine soecunebs which were sen to a reference laboratory in Northeastern U.S. for Micoplasma serological analysis	100
2	Off-Site	Repository specimens previously tested for Micoplasma IgM and were found to be reactive	2

Table 1. Summary of Clinical Testing.





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

	Clinical Site 1							
		DRG Myvcoplasma IgM ELISA						
		-	+/-	+	Totals			
DRG	<1:16	102	1	0	103			
IFA	1:16	8	0	0	8			
Results	<u>></u> 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 = 102/02 = 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							
	DRG Myvcoplasma IgM ELISA							
		-	+/-	+	Totals			
DRG	<1:16	89	0	7	96			
IFA	1:16	0	0	0	0			
Results	<u>></u> 1:32	0	0	6	6			
	Totals	89	0	13	102			

Relative Sensitivity = 6/6 = 100%Relative Specificity = 89/96 = 92.7%Relative Agreement = 95/102 = 93.1% 95% Confidence Interval* = 54.1 to 100% 95% Confidence Interval* = 96.4 to 97.0% 95% Confidence Interval* = 86.4 to 97.2%

* 95% confidence intervals calculated using the exact method.









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Table 4:	Calculation	of Relative	Sensitivity,	Specificity	and Agreement;
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	DRG Myvcoplasma IgM ELISA						
		-	+/-	+	Totals		
DRG	<1:16	27	1	10	38		
IFA	1:16	0	0	5	5		
Results	<u>></u> 1:32	3	1	30	34		
	Totals	30	2	45	77		

Relative Sensitivity = 30/33 = 90.9%Relative Specificity 27/37 = 73.0%Relative Agreement = 57/70 = 81.4% 95% Confidence Interval* = 75.7 to 98.1%
95% Confidence Interval* = 55.9 to 86.2%
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;

DRG Myvcoplasma IgM ELISA						
		-	+/-	+	Totals	
DRG	<1:16	218	2	17	237	
IFA	1:16	8	0	5	13	
Results	<u>></u> 1:32	5	3	41	49	
	Totals	231	5	63	299	

Relative Sensitivity = 41/46 = 89.1%Relative Specificity = 210/235 = 92.8%Relative Agreement = 250/281 = 92.2% 95% Confidence Interval* = 76.4 to 96.4%

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; <u>Review Criteria For In Vitro Diagnostic</u> <u>Devices for Detection of IgM Antibodies to Viral Antigens</u>. Reproducibility studies were conducted at both clinical sites using the same specimens.

Briefly, six specimens were tested, two relatively strong positive specimens, two specimens near the cut off, and two which were clearly negative. Additionally, the kit's Negative Control and High Positive Control were included as additional panel members, for a total of eight specimens. On each day of testing, each of the eight specimenes 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.





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Table 6 Summary of Intra-Assay Precision Testing Conducted at Clinical Sites 1 and 2												
			Results	s At Site (One				Results	At Site T	wo	
	Day	One	Day	Two	Day 7	Three	Day	One	Day	Two	Day	Three
No.	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%	Mean	%
	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.	Ratio	C.V.
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
HCP	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 InterAssay Precision Testing							
Co	onducted at Clinica	al Sites 1 and 2					
	Three day result	s-Site 1	Three day res	ults-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	0.08	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 tose 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.

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ABBREVIATED TEST PROCEDURE

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

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