2.   **Precision**
Intra-Assay Study

Serum	No. of Replicates	Mean (OD)	Standard Deviation	Coefficient of Variation %
1	16	1.45	0.091	6.2
2	16	1.21	0.061	5.4
3	16	0.22	0.014	6.4

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.37	1.120	8.7
2	10	0.88	0.087	9.88
3	10	0.27	0.026	9.62

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2008-12-18

CALBIOTECH
A life science company

Mycoplasma Pneumoniae IgM ELISA
Catalog No. MP021M (96 Tests)

INTENDED USE

The Calbiotech, Inc. (CBI), *Mycoplasma pneumoniae* (*M. pneumoniae*) IgM ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgM class antibodies to M. *pneumoniae* in human serum or plasma.

SUMMARY AND EXPLANATION

Mycoplasma pneumoniae is a pathogen with spectrum of clinical presentations ranging from asymptomatic to pronounced pneumonia. Symptoms start from 6 to 32 days after exposure with headache, malaise, cough, sore throat and fever. The illness can last from a few days to a month or more. Detection by ELISA of *M. pneumoniae* IgM antibodies or demonstration of a significant increase of specific IgG antibodies is strong evidence for recent infection in the appropriate clinical setting. Specific IgM antibodies typically increase significantly 1 week after clinical onset and specific IgG levels rise in the second week. *M. pneumoniae* IgM can, however, persist for more than two years after infection, and therefore, detection of specific IgM does not accurately indicate the time of infection. Primary infection and reinfection may be distinguished by the presence of elevated specific IgA and of specific IgM in primary infections and by the presence of elevated specific IgA in the absence of specific IgM in reinfections. In general, the absence of specific IgM in serum collected 10-20 days after onset is strong evidence against primary pneumonia due to *M. pneumoniae*.

PRINCIPLE OF THE TEST

Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated with *M. pneumoniae antigen*	12x8x1
2.	Sample Diluent: 1 bottle (ready to use)	22 ml
3.	Calibrator: 1 Vial (ready to use)	1.5ml
4.	Positive Control: 1 vial (ready to use)	1.5ml
5.	Negative Control: 1 vial (ready to use)	1.5ml
6.	Enzyme conjugate: 1 bottle (ready to use)	12ml
7.	TMB Substrate: 1 bottle (ready to use)	12ml
8.	Stop Solution: 1 bottle (ready to use)	12ml
9.	Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

6. Graph paper

STORAGE AND STABILITY
1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS
1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. This kit is USA FDA exempt product.
3. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING
1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

REAGENT PREPARATION
Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26 °C).

ASSAY PROCEDURE
Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL
The test run may be considered valid provided the following criteria are met:

1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should be greater than 1.2.

INTERPRETATION
The following is intended as a guide to interpretation of *M. pneumoniae* IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation
<0.9   No detectable antibody to *M. pneumoniae* IgM by ELISA.
0.9-1.1   Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1   Detectable antibody to *M. pneumoniae* IgM by ELISA.

LIMITATIONS OF THE TEST
1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and has shown no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
4. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity
127 patient sera were tested by this *M. pneumoniae* IgM ELISA and a reference ELISA method. 38 sera were positive and 82 were negative by both methods (94% agreement). The results are summarized below:

		M. pneumoniae IgM ELISA		
		+	–	Total
Reference ELISA Kit	+	38	3	41
	–	4	82	86
Total		42	85	127