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.

REFERENCES

1. Verkooyen RP; Van Lent NA; Mousavi Joulandan SA; Snijder RJ; van den Bosch JM; Van Helden HP; Verbrugh HA. Diagnosis of Chlamydia pneumoniae infection in patients with chronic obstructive pulmonary disease by micro-immunofluorescence and ELISA. J Med Microbiol 1997; 46(11):959-64
2. Eckman MR, Leinonen M, Syrjala H, et al. Evaluation of serological methods in the diagnosis of Chlamydia pneumoniae during an epidemic in Finland. Eur J Clin Microbiol Infect Dis 1993;12:756-60.
3. Bourke SJ, Lightfoot NF. Chlamydia pneumoniae: defining the clinical spectrum of infection requires precise laboratory diagnosis. Thorax 1995;50(Suppl 1):543-8.
4. Hammerschlag MR, Chirgwin K, Roblin PM, et al. Persistent infection with Chlamydia pneumoniae following acute respiratory illness. Clin Infect Dis 1992;14:178-82.
5. Leinonen, M., Sryjala, H., Kujala, P. and Saikku, P. (1991). Serological diagnosis of Chlamydia pneumoniae (Cpn) in adults. In: Abstracts of 31st ICAAC, Chicago, Illinois, Sept 29 - Oct 2. Washington, D.C. Amer. Soc. Microbiol, 209.
6. Cook PJ, Honeybourne D. Chlamydia pneumoniae. J Antimicrobial Chemother 1994;34:859-73.
7. Nystrom-Rosander C, Thelin S, Hjelm E, et al. High incidence of Chlamydia pneumoniae in sclerotic heart valves of patients undergoing aortic valve replacement. Scan J Infect Dis 1997;29:361-5

2008-12-18

For Research Use Only. Not for use in Diagnostic Procedures.

Cat#: CP095M (96 Tests)
For Order and Inquiries, please contact
Calbiotech Inc.,
10461 Austin Dr, Spring Valley, CA, 91978
Tel (619) 660-6162, Fax (619) 660-6970,
www.calbiotech.com

CALBIOTECH
A life science company

Chlamydia Pneumoniae IgM ELISA

Catalog No.: CP095M (96 Tests)

INTENDED USE

The Calbiotech Chlamydia Pneumoniae IgM ELISA Kit is intended for the detection of IgM antibody to C. Pneumoniae in human serum or plasma.

SUMMARY AND EXPLANATION

Chlamydia pneumoniae, the third recognized of five possible species of Chlamydia (trachomatis, psittaci, pneumoniae, pecorum and an as-yet-unnamed species) was formerly known as Chlamydia spp. Strain TWAR. This respiratory pathogen which causes acute respiratory disease, pneumonia and pharyngitis is often isolated from patients with otitis media with effusion, pneumonia with pleural effusion and in asymptomatic respiratory tract infections. C. *pneuomoniae* causes up to 10% of community-acquired pneumonia cases and it is also a risk factor for coronary heart disease and Guillain-Barré syndrome. Seroprevalence of C.*pneumoniae* among children is low and increases sharply in teenagers, continues to increase until middle age, and remains high (>50%) into old age, suggesting that most people have more than one C.*pneumoniae* infection during their lifetime. Primary chlamydial infection is characterized by a predominant IgM response within 2 to 4 weeks and a delayed IgG and IgA response within 6 to 8 weeks. After acute C.*pneumoniae* infection, IgM antibodies are usually lost within 2 to 6 months IgG antibody titers rise and usually decrease slowly; whereas IgA antibodies tend to disappear rapidly. When primary chlamydia infection is suspected, the detection of IgM is highly diagnostic. In reinfection, IgM level may be rarely detected while IgG and IgA levels rise quickly, often in one to two weeks. IgA antibodies have shown to be a reliable immunological marker of primary, chronic and recurrent infections. These antibodies usually decline rapidly to baseline levels following treatment and eradication of the chlamydia infections.

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 *C.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 designed for research use only.
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 300µl 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 300µl 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 this *C. 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 *Chlamydia pneumoniae* IgM by ELISA.
0.9-1.1	Borderline positive. Follow-up testing is recommend if clinically indicated.
>1.1	Detectable antibody to *Chlamydia pneumoniae* IgM by ELISA