

CALDON BIOTECH INC.
***Chlamydia pneumoniae* IgM**
ELISA

Catalog No. CP095M
 (96 tests)

NAME AND INTENDED USE

The CALDON BIOTECH INC. (CBI), *Chlamydia pneumoniae* IgM ELISA is intended for the use in evaluating of patients with suspected *C. pneumoniae* infection

SUMMARY AND EXPLANATION OF THE TEST

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. pneumoniae* 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

1. Microwell Strips: *C. pneumoniae* coated wells (12 x 8 x 1 wells).
2. Sample Diluent: 1 bottle (22 mL). Ready to use.
3. Calibrator: Yellow Cap. (1.50 mL/vial). Ready to use.
4. Positive Control: Red Cap. (1.50 mL/vial). Ready to use.
5. Negative Control: Blue Cap. (1.50 mL/vial). Ready to use.
6. Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
7. TMB Substrate: 1 bottle (12 mL). Ready to use.
8. Stop Solution: 1N H₂SO₄; 1 bottle (12 mL). Ready to use.
9. Wash Concentrate: 1 bottle (50 mL), 20X concentrate.

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 during storage or usage.

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. 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.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. 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.

well position. Tap the holder to remove air bubbles from the liquid

1. and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with wash buffer.
5. Dispense 100 μ L of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with wash buffer.
7. Dispense 100 μ L of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 μ L of 1N H₂SO₄ to stop reaction.
9. Read O.D. within 30 min at 450 nm using a microwell reader.

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 of serum sample.

PREPARATION FOR ASSAY

1. Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.
2. Prepare washing buffer by adding the contents of the bottle (50 mL, 20X Wash concentrate) to 950 mL of distilled or deionized water in one -liter container. Store at room temperature.

ASSAY PROCEDURE

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 μ L of the sample to 200 μ L of sample diluent. Mix well.
3. Dispense 100 μ L of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 μ L sample diluent in 1A