

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

Catalog No. CT055M
(96 tests)

NAME AND INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

C. trachomatis is an intracellular parasite pathogen that is similar in cell wall structure to gram -negative bacteria. It is the most common sexually transmitted disease (STD) in the US with more than 4 million cases reported annually. The main sites of infection include the GU tract and rectum but conjunctivitis, perihepatitis and reactive arthritis may result. The infection is often asymptomatic, making it difficult to diagnose; as many as 2/3 of infected women are asymptomatic. Women develop mucopurulent cervicitis, and irregular menstrual bleeding or abdominal pain may occur in about 40% of these women. PID is found in about 5% of women. The infection is usually symptomatic in men with dysuria and white/clear discharge occurring. Epididymitis is common. The infection incubates in 7 to 21 days and is commonly found with a second STD pathogen. IgG and IgM antibodies to *C. trachomatis* can be detected with 2 -4 weeks after exposure. IgG remains positive, but the antibody level can drop overtime. ELISA can detect *C. trachomatis* IgM antibody for many months after infection.

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

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

STORAGE AND STABILITY

- Store the kit at 2-8 ° C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- 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.

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 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 100 µL 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. Repeat washing three times with wash buffer.
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. Repeat washing three times with wash buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H₂SO₄ to stop reaction.
9. Read O.D. within 30 min at 450 nm using a microwell reader.

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:

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

INTERPRETATION

The following is intended as a guide to interpretation of CBI *C. trachomatis* 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* IgM by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 Indicative of *Chlamydia* infection.

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 shows 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**

112 patient sera were tested by both CBI *C. trachomatis* IgM ELISA and a reference ELISA method. 15 sera were positive and 94 were negative by both methods (97% agreement). The results are summarized below:

Chlamydia trachomatis seropositivity during pregnancy is associated with prenatal complications Clin Infect Dis 1996; 21(2):424-6.
 5. 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

April 03

	CBI <i>C. pneumoniae</i> IgA ELISA		
	+	-	Total
Reference ELISA	15	2	
+ Kit	17		
+ +	1	94	
-	95		
- -	16	96	
- -	112		
Total			

2. Precision Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	16	1.72	0.063	3.66
2	16	1.42	0.059	4.15
3	16	0.25	0.014	5.65

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10	1.87	0.160	08.55
2	10	1.52	0.117	07.80
3	10	0.28	0.029	10.36

REFERENCES

1. Poussin M; Fuentes V; Corbel C; Prin L; Eb F; Orfila J. Capture-ELISA: a new assay for the detection of immunoglobulin M isotype antibodies using Chlamydia trachomatis antigen. J Immunol Methods, 1997; 204(1):1-12.
2. Dereli D; Coker M; Ertem E; Serter D; Tana, c R; Tez E. Chlamydial infection in infants. J Trop Pediatr 1996; 42(4):233-6.
3. Bas S; Cunningham T; Kvien TK; Glenn as A; Melby K; Vischer TL. The value of isotype determination of serum antibodies against Chlamydia for the diagnosis of Chlamydia reactive arthritis. Br J Rheumatol 1996; 35(6):542-7
4. Gencay M; Koskiniemi M; Saikku P; Puolakkainen M; Raivio K; Koskela P; Vaheri A.