

**CALDON BIOTECH INC.**  
**Treponema pallidum**  
**Total Antibody**  
**ELISA**

Catalog No. TP094T  
 (96 tests)

**NAME AND INTENDED USE**

The CALDON BIOTECH INC (CBI), *Treponema pallidum total* (IgG, IgM and IgA) Antibody ELISA is intended for the use in the evaluation of patients with suspected syphilis.

**SUMMARY AND EXPLANATION OF THE TEST**

*Treponema pallidum* is the causative agent of syphilis a contagious and infectious systemic disease characterized by periods of active florid manifestations and by years of symptomless latency. Syphilis is traditionally classified as acquired or congenital, each being further subdivided on the basis of the natural course of the disease. In acquired syphilis, infection is usually transmitted by sexual intercourse. The incubation period of syphilis can vary from 1 to 13 weeks, but usually from 3 - 4 weeks. Untreated patients with primary or secondary syphilis having active lesions are the most infectious, and the risks of contagion are greatest during the first 2 years of infection. Virtually every organ and tissue of the body is affected, including most body fluids. Over 80% of patients have mucocutaneous lesions, 50% have generalized enlargement of the lymph nodes, and about 10% have lesions of the eyes, bones and joints, meninges, liver, and spleen. Mild constitutional symptoms of malaise, headache, anorexia, nausea, aching pains in the bones, and fatigability are often present. Congenital syphilis is the result of passage of *T. pallidum* across the placenta. Clinical manifestations may be present at birth but are more often seen at 3 weeks to 6 months of age. Two types of antibodies are produced by *T. pallidum*: nontreponemal antibodies (reagin) and treponemal antibodies. ELISA for detection of IgG and IgM antibodies is becoming the Gold standard for the diagnosis of syphilis.

**PRINCIPLE OF THE TEST**

Diluted patient serum is added to wells coated with purified antigen. 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 IgG specific antibody in the sample.

**MATERIALS PROVIDED**

- Microwell Strips: *T. pallidum* antigen coated wells (12 x 8 x 1 wells)
- Sample Diluent: 1 bottle (22 mL). Ready to use.
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
- Calibrator: 1 vial (1.50 mL). Ready to use.
- Positive Control: : 1 vial (1.50 mL). Ready to use
- Negative Control: : 1 vial (1.50 mL). Ready to use
- TMB substrate: 1 bottle (12 mL). Ready to use.
- Stop solution: 1N H<sub>2</sub>SO<sub>4</sub> ; 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. **Positive control, negative 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 H<sub>2</sub>SO<sub>4</sub> to stop reaction.

9. Read O.D. within 30 min at 450 nm using 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 *T. pallidum* IgG 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 *T. pallidum*

Antibody by ELISA.

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

>1.1 Indicative of past or current *T. pallidum* infection.

#### LIMITATIONS OF THE TEST

1. 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.

2. Lipemic or hemolyzed samples may cause erroneous results.

#### PERFORMANCE CHARACTERISTICS

##### 1. Sensitivity and Specificity

65 patient sera were tested by both CBI *T. pallidum* antibody ELISA and a reference ELISA method. 16 sera were positive and 45 were negative by both methods (94% agreement).

The results are summarized below:

	CBI <i>T. pallidum</i> antibody ELISA		
	+	-	Total
Reference ELISA	16	2	
+	18		
Kit	2	45	
-	47		
Total	18	47	65

## 2. Precision Intra Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	16	1.35	0.072	5.33
2	16	0.89	0.055	6.18
3	16	0.28	0.021	7.50

## Inter Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10	1.84	0.16	8.69
2	10	1.48	0.14	9.45
3	10	0.29	0.03	10.34

## REFERENCES

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