

## CALDON BIOTECH INC. HSV-2 IgM ELISA

Catalog No. H2032M  
(96 tests)

### NAME AND INTENDED USE

The CALDON BIOTECH INC. (CBI), HSV-2 IgM ELISA is intended for use in the evaluation of patients with suspected HSV-2 infection.

### SUMMARY AND EXPLANATION OF THE TEST

HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they're teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure. A significant increase in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV 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: HSV-2 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.
- 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
- 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.
- 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, as 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 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 HSV-2 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 HSV-2 IgM by ELISA.

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

>1.1 Indicative of HSV 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**

137 patient sera were tested by both CBI HSV-2 IgM ELISA and a reference ELISA method. 12 sera were positive and 124 were negative by both methods (99% agreement).

The results are summarized below:

	CBI HSV-2 IgM ELISA		
	+	-	Total
Reference ELISA	12	1	
+ Kit	13	0	124
-	124		
Total	12	125	137

**2. Precision  
Intra-Assay Study**

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	16	1.26	0.047	5.92
2	16	1.03	0.038	3.69
3	16	0.19	0.011	5.79

**Inter-Assay Study**

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10		0.120	08.16
2	10	1.47	0.098	10.54
3	10	0.93	0.020	13.33
		0.15		

**REFERENCES**

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April 03