**HSV 1 IgG EIA Test Kit**

**INTENDED USE**

The HSV 1 IgG EIA Test Kit is an enzyme immunoassay for the qualitative detection of IgG antibodies to *Herpes Simplex Virus* (HSV) type 1 in human serum or plasma.

For professional in vitro diagnostic use only.

**SUMMARY**

*Herpes Simplex Virus* (HSV) is an enveloped DNA virus belonging to the Herpes virus family which has been characterized by several serotypes. HSV 1 and HSV 2, infection with HSV 1 generally occurs in early childhood causing no symptoms. If symptoms are present, they mostly through sexual contact, with rare occasions occurring before onset of sexual activity. HSV 2 is stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents

Neutralized acids and other liquids should be decontaminated by adding sufficient volume of lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin or contaminated with bacterial or fungal growth.

Serum and plasma specimens may be stored for 2.8°C for up to 7 days prior to assay. For long term storage, specimens should be kept frozen below -20°C.

For the stock plates, a programmable microplate washer capable of aspirating and dispensing 350 μL Positive control plasma can be used if required. The color intensity, which is proportional to the amount of HSV 1 IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

**REQUIREMENTS AND CONDITIONS FOR USE**

- The HSV 1 IgG EIA Test Kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative detection of IgG antibodies to HSV 1 in human serum or plasma. The microwell plate is coated with HSV 1 IgG-Conjugate reagent. The specific antigen is coated against the antigen coated microwell plate and then incubated. If the specimens contain antibodies to HSV 1, it will bind to the antigen coated on the microwell plate to form immobilized antigen-antibody 1 antibody complexes.

- The HSV 1 IgG EIA Test Kit is an immunoassay for the qualitative detection of the presence of IgG antibodies to HSV 1 in serum or plasma specimen. The test utilizes recombinant HSV 1 antibodies to selectively detect antibodies to HSV 1 in serum or plasma.

**SPECIFIC STEPS FOR USE**

**SPECIMEN COLLECTION AND PREPARATION**

1. Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the Concentrated Wash Buffer into a 1000 mL conical flask and add 40 mL of deionized (DI) water, and mix by inversion. Leave A1 as Blank well.

2. Add 100 μL of Negative control plasma or serum to all wells except A1 and W1 (Starting H1: Add 5 μL of Negative control plasma or serum to A1 and W1).

3. Add 5 μL of specimen to assigned wells starting at H1. Then a color change from green to blue will occur if the specimen has been added. A blank well is included to provide an analytical zero.

4. Add 5 μL of specimen to assigned wells starting at H1. Add 5 μL of specimen to assigned wells starting at H1.

5. Add 100 μL of Positive control plasma or serum to all wells except A1 and W1 (Starting H1: Add 5 μL of Positive control plasma or serum to A1 and W1).
1. **Remove the Plate Sealer**
   - Wash each well 5 times with 350 μL of Working Wash Buffer, then remove the liquid.
   - Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.

2. **Add 100 μL of Conjugate to each well except for the Blank well.** (Red Reagent)
   - Cover the microwell plate with the Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.

3. **Wash each well 5 times with 350 μL of Working Wash Buffer.**
   - Mix gently then cover microwell plate with Wash Buffer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.

4. **Cut-Off Value: Well H1 2.336**

5. **Index Value: Specimen/Cut-Off Value 2.336/0.340 = 6.871**

**CALCULATION OF RESULTS AND VALIDITY**

1. Calculate the Mean Absorbance of Negative Control, Cut-Off Calibrator, and Positive Control by referring to the table below.

   **Example of Cut-Off Calibrator Calculation**
   - Mean Absorbance
   - Total Absorbance of Cut-Off Calibrator 0.326
   - Mean Absorbance of Cut-Off Calibrator 0.8562 ± 0.341

2. **Read at 450/630-700 nm within 30 minutes.**
   - Read at 450/630-700 nm within 30 min
   - Mix then cover microwell plate with Wash Buffer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.

**INTERPRETATION OF RESULTS**

**Example of Cut-off calculation below.**

- **Mean Absorbance of Cut-Off Calibrator**
- **Coefficient of Variation of Mean Absorbance**
- **Coefficient of Variation of Mean Absorbance**

**Reproducibility**

- **Intra-Assay: Within-run precision has been determined by using 15 replicates of three specimens:**
  - A low positive, a medium positive, and a high positive.

**Clinical Sensitivity:** 98.5% (92.1-99.9%)*

**Clinical Specificity:** 97.8% (88.2-99.9%)*

**Overall Agreement:** 98.2% (93.8-99.9%)*

**Intra-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens:**

**Clinical Sensitivity:** 97.8% (88.2-99.9%)*

**Clinical Specificity:** 95.9% Confidence Interval

**Check the validation requirements below to determine if the test results are valid.**

1. Calculate the Index Value to obtain qualitative specimen results.

   **Calculation of Index Value**
   - **Cut-Off Calibrator: Well H1 2.336**
   - **Index Value: Specimen/Cut-Off Value 2.336/0.340 = 6.871**

   **Results Qualitative**
   - Negative
   - Positive

   **Interpretation of Results - Qualitative**
   - Multiply the Index Value by 10
   - **Cut-Off Value: Index Value 6.871 x 10**

   **Note:** If the test is invalid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Cut-Off Calibrator. See an example of Cut-off calculation below.

**Performance Characteristics**

**Limitations**

1. The HSV 1 IgG EIA Test Kit is used for the detection of IgG antibodies HSV 1 in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result.

2. Further testing, including confirmatory testing, should be performed before a specimen is confirmed as negative.

3. With all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

**Reagents**

- HSV 1 IgG Substrate A
- HSV 1 IgG Substrate B
- Wash Buffer (25x)
- Positive Control
- Negative Control
- Stop Solution
- Blank Well
- Control Well
- Plate Sealer
- Microwell Plate
- Substrate A
- Substrate B
- Microwell Plate Plate Sealer
- Stop Solution
- Blank Well
- Control Well
- Plate Sealer
- Microwell Plate
- Substrate A
- Substrate B
- Microwell Plate Plate Sealer
- Stop Solution
- Blank Well
- Control Well
- Plate Sealer
- Microwell Plate
- Substrate A
- Substrate B

**Sensitivity and Specificity**

The HSV 1 IgG EIA Test Kit has correctly identified specimens of a mixed titer performance panel and has been compared to a leading commercial HSV 1 EIA test using clinical specimens. The results show that the clinical sensitivity of the HSV 1 IgG EIA Test Kit is 98.5%, and the clinical specificity is 97.8%.

**Interpretation of Results:**

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Cut-Off Calibrator. See an example of Cut-off calculation below.

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, then read the results by referring to the Interpretation of Results table below.

**Performance Characteristics**

**Limitations**

1. The HSV 1 IgG EIA Test Kit is used for the detection of IgG antibodies HSV 1 in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is confirmed as negative.

2. With all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be accurately interpreted to detect an initial test. The results may be affected due to procedural or instrument error.

4. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a Positive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.