

Revised 16 July 2008

RUO	in the USA
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Store at 2 to 8°C

**SUMMARY OF ASSAY PROCEDURE**

1. Sample dilution 1:40
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5 µl / 200 µl
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2. Three incubations at 37°C
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Diluted Sample 100 µl 30 min.
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Enzyme Conjugate 100 µl 30 min.
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TMB Reagent (One-Step) 100 µl 15 min.
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3. Stop with 100 µl of acid. Read O.D. at 450 nm
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**INTENDED USE**

The HSV-2 IgM ELISA is intended for the detection of IgM antibodies to herpes simplex virus HSV-2.

**INTRODUCTION**

Herpes Simplex Virus is a common pathogen and its primary infection is usually asymptomatic. There are two immunologically distinct types of HSV: Type 1 and Type 2. HSV-1 is generally associated with oral infection and lesions above the waist, and HSV-2 is associated with genital infections and lesions below the waist. Clinical cases primarily are (1) eczema herpeticum with eczematous skin changes with numerous lesions, (2) gingivo-stomatitis, and (3) herpes sepsis, almost only found in premature newborn infants. The HSV-2 IgM ELISA is an accurate serologic method to detect HSV-2 IgM specific-antibody in serum samples.

**PRINCIPLE OF THE TEST**

Purified HSV-2 antigen is coated on the surface of microwells. Diluted patient serum is added to the wells, and the HSV-2 IgM- specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of HSV-2 IgM- specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

**REAGENTS****Materials provided with the kit:**

- Microtiter Wells: purified HSV-2 antigen coated wells (12 x 8 wells).
- Enzyme Conjugate Reagent (red color): 1 vial (12 ml).
- Sample Diluent (blue color): 1 bottle (22 ml).
- Negative Control: Range stated on label. Natural cap (150 µL/vial).
- Cut-off Calibrator: Yellow cap. HSV-2 IgM Index = 1 (150 µL/vial).
- Positive Control: Range stated on label. Red cap. (150 µL/vial).
- Wash Buffer Concentrate (20x): 1 bottle (50 ml).

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- TMB Reagent (One-Step): 1 vial (11 ml).
- Stop Solution (1N HCl): Natural cap. 1 vial (11 ml).

**STORAGE OF TEST KITS AND INSTRUMENTATION**

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.

**WARNING 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components from different lots should not be mixed.
4. This product contains components 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 PREPARATION**

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

**REAGENT PREPARATION**

1. All reagents should be allowed to reach room temperature (18-25 °C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.
1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control, and calibrator by adding 5 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.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. **Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).**
6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.

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8. Remove Enzyme Conjugate from all wells. *Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).*
  9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
  10. Incubate at 37°C for 15 minutes.
  11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
  12. Mix gently for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*
- Note: Make sure there are no air bubbles in each well before reading.**
13. Read O.D. at 450 nm within 15 minutes with a microwell reader.

**CALCULATION OF RESULTS**

1. Calculate the mean of duplicate calibrator value  $x_c$ .
2. Calculate the mean of duplicate positive control ( $x_p$ ), negative control ( $x_n$ ) and patient samples ( $x_s$ ).
3. Calculate the HSV-2 IgM Index of each determination by dividing the mean values of each sample ( $x$ ) by calibrator mean value,  $x_c$ .

**Example of typical results:** (Note: The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.)

<b>Cut-off Calibrator HSV-2 IgM Index = 1.0</b>
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1. Calibrator O.D. = 1.010, 1.017  $x_c = 1.014$
2. Negative Control O.D. = 0.048, 0.047  $x_n = 0.048$   
 HSV-2 IgM Index =  $x_n / x_c = 0.048 / 1.014 = 0.05$
3. Positive Control O.D. = 1.514, 1.667  $x_p = 1.591$   
 HSV-2 IgM Index =  $x_p / x_c = 1.591 / 1.014 = 1.57$
4. Patient sample O.D. = 1.870, 1.871  $x_s = 1.871$   
 HSV-2 IgM Index =  $x_s / x_c = 1.871 / 1.014 = 1.85$

**QUALITY CONTROL**

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The HSV-2 IgM Index for Negative and Positive Control should be in the range stated on the Certificate of Analysis.

**INTERPRETATION**

Negative: HSV-2 IgM Index less than 0.90 is negative for IgM antibody to HSV-2.

Equivocal: HSV-2 IgM Index between 0.91-0.99 is equivocal. Sample should be retested.

Positive: HSV-2 IgM Index of 1.00 or greater is positive for IgM antibody to HSV-2.

**PERFORMANCE CHARACTERISTICS**

**Specificity and Sensitivity:**

A total of 94 patient samples were used to evaluate specificity and sensitivity of the test. The HSV-2 IgM ELISA test results were compared to a commercial ELISA kit results:

		Reference HSV-2 IgM ELISA			
		N	E	P	Total
HSV-2 IgM ELISA	N	51(D)	0	3(B)	54
	E	0	0	0	0
	P	2(C)	0	38(A)	40
	Total	53	0	41	94

Sensitivity =  $A / (A+B) = 38 / 41 = 92.7\%$

Specificity =  $D / (C+D) = 51 / 53 = 96.2\%$

Accuracy =  $(A+D) / (A+B+C+D) = 89 / 94 = 94.7\%$

**Precision:**

The Precision of the assay was evaluated by testing three different sera of 20 replicates over 4 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low Positive	Positive
Intra-assay	6.9%	6.6%	5.8%
Inter-assay	8.2%	7.1%	6.5%

**LIMITATIONS OF THE PROCEDURE**

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

**REFERENCES**

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3. Coleman, R.M., L. Pereira, P.D. Bailey, D. Dondero, C. Wickliffe, and A.J. Nahmias. Determination of herpes simplex virus type-specific antibodies by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 18 (1983) 287.