Herpes Simplex Virus 1,2 IgM (HSV 1,2 IgM)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Qualitative Detection of HSV 1, 2 IgM antibody in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

HSV 1,2 IgM is intended for the Qualitative detection of IgM antibodies herpes simplex virus (HSV) infection, or for evaluating paired sera for the presence of a significant increase in herpes specific IgM. For in Vitro Diagnostic Use Only.

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 newly born of premature infants. Microwell ELISA, HSV 1,2 IgM is an accurate serologic method to detect HSV specific antibody IgM in serum sample.

PRINCIPLE OF THE ASSAY

Purified HSV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the HSV IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic substrate 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 IgM specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS AND COMPONENTS

Materials provided with the test kit:

1. Coated Microwells: Purified HSV 1,2 antigen coated wells.
2. Sample Diluent: Ready to use .
3. Negative Control: Range stated on the label.
4. Positive Control: Range stated on the label.
5. Calibrator.
6. Wash Buffer Concentrate (20X).
7. Enzyme Conjugate: Ready to use.
8. TMB Substrate: Ready to use.
9. Stop Solution: Ready to use.
10. Pack Insert.
11. Plate Sealers.
12. Protocol Sheet.
13. Microwell Holder.

Materials required but not provided

1) Precision pipettes: 10µl, 20-200µl, 100-1000µl
2) Disposable pipette tips
3) Distilled water
4) Disposable Gloves
5) ELISA reader
6) ELISA washer

STORAGE AND STABILITY

1. **HSV 1,2 IgM** kit is stable at 2-8°C up to expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to pink at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable for up to one week when stored at 2-8°C.

SPECIMEN COLLECTION & PREPARATION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

(1) Bring all reagents and specimen to room temperature before use.
(2) Do not pipette any material by mouth.
(3) Do not eat, drink or smoke in the area where testing is done.
(4) Use protective clothing and wear gloves when handling samples.
(5) Use absorbent sheet to cover the working area.
(6) Immediately clean up any spills with sodium hypochlorite.
(7) All specimens and controls should be considered potentially infectious and discarded appropriately.
(8) Neutralize acid containing waste before adding hypochlorite.
(9) Do not use kit after the expiry date.
(10) Do not mix components of one kit with another.
(11) Always use new tip for each specimen and reagent.
(12) Do not allow liquid from one well to mix with other wells.
(13) Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95ml of distilled or deionized water).
2. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding **5µl** of the test samples, Positive Control, Negative Control and Calibrator to **200µl** of sample diluent. Mix well.
3. Dispense **100µl** of diluted serum samples, negative control, positive control and calibrator into the appropriate wells. For the reagent blank, dispense **100µl** of sample diluent in A1 well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for **45 minutes** at **37°C**.
4. Wash each well three times by filling approximately **350µl** diluted wash buffer & blot dry.
5. Dispense **100µl** of enzyme conjugate to each well and incubate for **45 minutes** at **37°C**.
6. Wash each well three times by filling approximately **350µl** diluted wash buffer & blot dry.
7. Dispense **100µl** of TMB Substrate to each well and incubate for **20 minutes** at room temperature, away from direct light.
8. Add **100µl** of Stop Solution to stop the reaction.
9. Read O.D. at 450 - 630 nm with an ELISA reader.

Add **100µl** of sample diluent in A1 Microwell as blank

⬇

Add **100µl diluted NC, PC, Calibrator and samples** into the respective Microwells

⬇

Apply plate sealer and incubate for **45 minutes** at **37°C**

⬇

Wash microwells **3 times** with diluted wash buffer

⬇

Add **100µl** Enzyme conjugate in each well

⬇

Apply plate sealer and incubate for **45 minutes** at **37°C**

⬇

Wash microwells **3 times** with diluted wash buffer

⬇

Add **100µl** substrate in each well

⬇

Apply plate sealer and incubate for **20 minutes** at **18-25°C in dark**

⬇

Add **100 µl** stop solution in each well

⬇

Read results @450nm (Ref.600-700nm) within **15 minutes**

RUN CRITERIA

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 0.150.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The HSV 1,2 IgM Index for Negative and Positive Control should be in the range stated on the labels.

CALCULATION OF RESULTS

1. To obtain Cut off OD value: Multiply the OD of Calibrator by Factor (f) printed on label of Calibrator.
2. Calculate the IgM Index of each determination by dividing the OD values of each sample by cut-off O.D Value.

For example:

If Factor (f) value on label = 0.9
This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator.
Obtained Calibrator O.D. = 0.793
Cut-off O.D. = 0.793 x 0.9 = 0.713
Patient sample O.D. = 1.364
IgM Index = 1.364 / 0.713 = 1.91 (Positive result)
Patient sample O.D. = 0.320
IgM Index = 0.320 / 0.713 = 0.44 (Negative result)

INTERPRETATION OF THE RESULT

HSV 1,2 IgM Index Value	Result
HSV 1,2 IgM Index value <0.90	Negative for IgM antibody to HSV 1,2
HSV1, 2 IgM Index value 0.91-1.19	Equivocal, sample should be retested
HSV 1,2 IgM Index value >1.2	Positive for IgM antibody to HSV 1,2

PERFORMANCE CHARACTERISTICS

The precision of the assay was evaluated by testing three different sera of eight replicates over a period of one week.
The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	8.2 %	7.2 %	6.3%
Inter-assay	9.1 %	8.2 %	6.5%

IMPORTANT NOTE

1. This assay is a temperature sensitive assay. The best temperature condition for this assay is 37°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multichannel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of NC, PC & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. To prevent false negative and false positive IgM test results, caused by the presence of specific IgM and rheumatoid factor (RF) in some specimens, reagents provided in this kit have been formulated to resolve these interferences. However, in specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
3. A negative serological test does not exclude the possibility of past infection. Following primary HSV infection, antibodies may fall to undetectable levels and then be boosted by later clinical infection with the same or heterologous type. Such a phenomenon may lead to incorrect interpretations of seroconversion and primary infection, or negative antibody status. In addition, samples obtained too early during primary infection may not contain detectable antibody. Some persons may fail to develop detectable antibody after Herpes infection.

BIBLIOGRAPHY

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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.
4. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up		Contains sufficient for <n> tests
	Do not reuse		

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