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See external label	2°C-8°C	Σ=96 tests	Cat # 1404Z

Herpes Simplex Virus 2 IgM

HSV 2 IgM

Cat # 1404Z

Test	HSV 2 IgM ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect: Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	5ul Serum
Specificity	100%
Sensitivity	99%
Total Time	~ 90 min
Shelf Life	12 -18 Months

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

NAME AND INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

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. DIAGNOSTIC AUTOMATION ELISA HSV 2 IgM is an accurate serologic method to detect HSV 2 specific antibody IgM in serum sample.

PRINCIPLE OF THE TEST

Purified HSV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the HSV 2 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 2 specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell Strips: Purified HSV 2 antigen coated well	(12 X 8 wells)
2. Absorbent Solution: Black Cap	1 vial (22mL)
3. Calibrator: Factor value (f) stated on label. Red Cap	1 vial (150µL)
4. Negative control: Range Stated on label. Natural Cap	1 vial (150µL)
5. Positive control: Range stated on label. Green Cap	1 vial (150µL)
6. Washing Concentrate 10X: White Cap	1 bottle (100mL)
7. Enzyme Conjugate: Red color solution	1 vial (12 mL)
8. TMB Chromogenic substrate: Amber Bottle	1 vial (12 mL)
9. Stop solution	1 vial (12 mL)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
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.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found nonreactive 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 in this kit are intended for use as a integral unit. The components of 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 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. Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 10 x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 µl of the test samples, negative control, positive control, and calibrator to 200 µl of absorbent solution. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl absorbent solution in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells and repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 30 minutes at room temperature.
8. Add 100 µl of 2 **N** HCl to stop reaction.
   Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1. To obtain cut off OD value: Multiply the OD of Calibrator by Factor (1) printed on label of Calibrator.
2. Calculate the HSV2 IgM index of each determination by dividing the OD values of each sample by obtained OD value of Cut off.

For Example:

If Factor (f) value on label = 0.4
Obtained Calibrator O.D. = 1.100

Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition Rubella IgM Index = 1)

Patient sample O.D. = 0.580
Rubella IgM Index = 0.580 / 0.44 = 1.32 (Positive result)

Patient sample O.D. = 0.320
Rubella IgM Index = 0.320 / 0.44 = 0.73 (Negative result)

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 Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The HSV 2 M Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: HSV 2 M Index less than 0.90 are negative for IgM antibody to HSV 2.
Equivocal: HSV 2 M Index between 0.91-0.99 is equivocal. Sample should be retested.
Positive: HSV 2 M Index of 1.00 or greater are positive for IgM antibody to HSV 2.

PERFORMANCE CHARACTERISTICS

Precision:

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	10.2%	8.5%	7.5%
Inter-assay	12.1%	9.7%	8.4%

LIMITATION OF THE PROCEDURE

1. To prevent false negative and false positive IgM test results caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, 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, antibody 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.

REFERENCES

1. Nahmias, A.J., J. Dannenbarger, C. Wickliffe and M. Muther. Clinical aspects of infection with herpes simplex viruses 1 and 2 in the human herpes viruses. An interdisciplinary Perspective (Nahmias, A.J., W.R.Dawdle and R.F. Schinazi eds) New York, Elsevier, pp 3-9, 1981.
2. Vestergaard, B.F., P.C. Grauballe and H. Spanggaard. Titration of herpes simplex virus antibodies in human sera by the enzyme-link immunosorbent assay (ELISA). Acta Pathol. Microbiol. Scand. Sect. B 85:446-448, 1977.
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.

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