

RESULTS

Calculate the mean absorbance for each control and unknown.

Qualitative results:

If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific IgM. Calculate the ratio between the average OD value of the sample and that of the Cut-Off. The sample is considered:

Positive: if the ratio is > 1.1 .

Doubtful: if $\pm 10\%$ of the Cut-Off.

Negative: if the ratio is < 0.9 .

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the late phase of infection, when only IgG antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.
- Heat inactivated sera should be avoided.

QUALITY CONTROL

Subtract the value of the blank from all the other readings. The OD values of Cut off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut off control.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

104 human sera were analyzed by this HSV 1&2 IgM Elisa and a commercial Elisa (Test A) as reference method. Out of 104 samples, 9 were positive for the presence of IgM antibodies to Herpes simplex virus by DIAsource Elisa and commercial Elisa showed 9 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
DIA	9	95	0	0
Test A	9	95		

2. Precision

2. Inter-assay Study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean (OD's)	0.315	1.92	0.013
SD	0.004	0.074	0.001
CV%	1.3	3.8	8.15

3. Intra-assay Study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean (OD's)	0.261	0.55	0.036
SD	0.009	0.015	0.019
CV%	3.6	2.78	7.8

REFERENCE

- S. Land et al.: Rapid diagnosis of herpes simplex virus infections by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 19: 865 (1984).
- B. Gonik et al.: Comparison of two enzyme-linked immunosorbent assays for detection of herpes simplex virus antigen. J. Clin. Microbiol. 29: 436 (1991).
- C. Gleaves et al.: Evaluation of an enzyme immunoassay for the detection of herpes simplex virus (HSV) antigen from clinical specimens in viral transport media. J. Virological Meth. 28: 133 (1990).
- M. Morgan and T. Smith: Evaluation of an enzyme-linked immunosorbent assay for the detection of herpes simplex virus antigen. J. Clin. Microbiol. 19: 730 (1984).

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Herpes simplex virus 1 & 2 IgM Elisa Catalog No. KAPRHGM25



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INTENDED USE

The DIAsource KAPRHGM25 Herpes simplex virus (HSV) 1&2 IgM ELISA is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgM-class antibodies to HSV 1&2 virus in human serum. This assay is intended for *in vitro* use only.

SUMMARY AND EXPLANATION

The Herpes simplex virus (HSV) is a member of the Herpesviridae family, of which two types are known: type 1 (HSV-1) and type 2 (HSV-2), which present slight antigenic differences. HSV-1 causes chiefly oral-facial lesions/cold sores, while HSV-2 is mainly responsible for genital lesions, but this distinction is not definitive, both types occasionally causing infection in either anatomical site. HSV may also cause a form of ocular cherratitis, and lesions of the central nervous system.

HSV can affect practically the whole population. The primary infection is often in a subclinical form and is rarely diagnosed. After a latency period of variable duration, reactivation may occur and viral replication may or may not give rise to clinical lesions. Infection contracted during birth is of particular interest, this being an important cause of morbidity and mortality. It is therefore important to determine the immunitary state of women during pregnancy in order to detect serum conversion. The assay of specific IgM is important for the diagnosis of neonatal infection and encephalitis caused by HSV. The assay of specific IgM is important for the diagnosis of neonatal infection and encephalitis caused by HSV. Moreover, the presence of specific IgM indicates viral activity in progress, although it is not possible to distinguish between primary infection and reactivation.

PRINCIPLE OF THE TEST

The KAPRHGM25 test for the assay of Herpes simplex 1&2 IgM ELISA is based on the principle of the capture of these immunoglobulins and subsequent identification of those, which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The capture is performed using monoclonal antibodies bound to the solid phase (microtitration strips). The antigen is composed of purified protein of Herpes simplex virus 1&2.

REAGENTS

The DIAsource Herpes simplex virus 1&2 IgM ELISA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:

Anti-human IgM Antibody-Coated
Microtitration Strip



Quantity : 1 plate

Wash Concentrate

WASH	SOLN	CONC
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Quantity : 1 bottle

Sample Diluent

DIL	SPE
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Quantity : 1 bottle

TMB-Substrate

CHROM	TMB
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Quantity : 1 bottle

Negative control

CONTROL	-
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Quantity : 1 vial

Cut off control

CONTROL	CO
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Quantity : 1 vial

Positive control

CONTROL	+
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Quantity : 1 vial

HSV 1&2-HRP Conjugate

Ag	HRP
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Quantity : 1 bottle

Stopping Solution

STOP	SOLN
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Quantity : 1 bottle

MATERIAL NOT PROVIDED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Deionized/Distilled water
- Precision pipette to deliver 10 µL, 100 µL, and 1 mL
- Semi-automatic pipette to deliver 100 µL
- Automatic microtitration plate washer
- Absorbent materials for blotting the strips
- Incubator capable of maintaining temperature at 37 +/- 2°C

Antibody -Coated Microtitration Strips

One stripholder containing 12x8 (96) microtitration wells coated with Ani-human IgM antibodies monoclonal. Store at 2-8°C until expiration date. Remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure. Once opened, the product is stable for 4 weeks at 2-8°C.

Wash Concentrate

One bottle, 100 mL, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% per weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

Sample Diluent

One bottle, 100 ml, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

HSV 1&2 IgM Controls

Three vials, negative, cut off and positive , each 2 mL of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

HSV 1&2- HRP Conjugate

One bottle, 12 mL, containing HSV 1&2 antigen labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

TMB-Substrate

One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.

Stopping Solution

One bottle, 15 mL, containing 0.3 M H₂SO₄ in solution. Store at 2-8°C until expiration date.

PRECAUTIONS

For *in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and materials in compliance with applicable regulations.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, April 1999.

WARNING AND PRECAUTION

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at -20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and TMB can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

PREPARATION OF REAGENTS

Wash Solution

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 mL of the Wash Concentrate into a clean container and dilute by adding 900 mL of deionized/distilled water. Mix thoroughly. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.









Microtitration Strips

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Assay Procedure

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step.
4. Incubate for 45 minutes at 37°C.
Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 300 µL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
5. Add 100 µL of HSV -1&2-HRP-Conjugate into each well.
6. Incubate for 45 minutes at 37°C.
Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
7. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
8. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
9. Add 100 µL of Stopping Solution to each well using a dispenser.
10. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

	Consult instructions for use		Manufacturer
	Storage temperature		Contains sufficient for n tests
	Use by		In vitro diagnostic medical device
	Batch code		Catalogue number

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