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 +/- 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

92 human sera were analyzed by this HSV 2 IgM Elisa and a commercial Elisa (Test A) as reference method. Out of 92 samples, 7 were positive for the presence of IgM antibodies to Herpes simplex virus 2 by RD-RatioDiagnostics (RD-labs) Elisa, and commercial Elisa also showed 7 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
RD-Labs	7	85	0	0
Test A	7	85	0	0

2. Precision

2. Inter-assay Study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.537	2.3	0.012
SD	0.022	0.19	0.001
CV%	4.1	8.2	10.6

3. Intra-assay study			
No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.423	1.91	0.020
SD	0.022	0.020	0.001
CV%	5.41	1.077	9.10

3. Interference study

Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

REFERENCE

1. S. Land et al.: Rapid diagnosis of herpes simplex virus infections by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 19: 865 (1984).
2. B. Gonik et al.: Comparison of two enzyme-linked immunosorbent assays for detection of herpes simplex virus antigen. J. Clin. Microbiol. 29: 436 (1991).
3. 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).
4. M. Morgan and T. Smith: Evaluation of an enzyme-linked immunosorbent assay for the detection of herpes simplex virus antigen. J. Clin. Micronbiol. 19: 730 (1984).

RD Ratio Diagnostics

Herpes simplex virus 2 IgM Elisa

Catalog No. E-HHM-K34

IVD CE 2°c 8°c

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INTENDED USE

The RD-RatioDiagnostics E-HHM-K34 Herpes simplex virus HSV 2 IgM ELISA is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgM-class antibodies to HSV 2 virus in human serum or plasma. 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 cheratitis 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.Infection with Herpes simplex virus exhibits a strong antibody reaction of IgG class to HSV which can be measured in serum by ELISA as a valuable tool to determine the immune status of the patients.

PRINCIPLE OF THE TEST

The E-HHM-K34 HSV 2 IgM kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with HSV 2 - recombinant derived GD2-protein /antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgM antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added, and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-HSV 2 IgM antibodies present.

REAGENTS

The RD-RatioDiagnostics Herpes simplex virus 2 *IgM* ELISA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED	QUANTITY	CATALOG NO.
HSV 2 – Antigen-Coated Microtitration Strip	One Plate	E-HHM-10
Wash Concentrate	One Bottle	E-WSL-30
Sample Diluent	One Bottle	E-DLB-40
TMB-Substrate	One Bottle	E-TMB-08
Negative control	One Vial	E-HHM-01
Cut off control	One Vial	E-HHM-02
Positive control	One Vial	E-HHM-03
2nd Antibody Conjugate	One Bottle	E-HHM-20
Stopping Solution	One Bottle	E-STP-09
Sorbent M	One Bottle	E-SOR-21

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

MATERIAL RPOVIDED

HSV 2 -Antigen-Coated Microtitration Strips:

One stripholder containing 12x8 (96) microtitration wells coated with Herpes simplex virus recombinant derived GD2 protein / antigen. 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% Brij 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 BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

HSV 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.

2nd Antibody Conjugate:

One bottle, 12 mL, containing anti-human IgM monoclonal antibodies 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 H_2SO_4 in solution. Store at 2-8°C until expiration date.

Sorbent M : One Bottle, 4 ml, containing anti-human IgG , in a phosphate buffer solution with 0.02% proclin. Store at 2°- 8° C.

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 affected 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 by inversion. 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. Add 30 μL Sorbent M only in to the wells of diluted samples.
4. Incubate for 45 minutes at 37°C.
5. 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 uL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
6. Add 100 μL of Enzyme-Labeled 2nd Antibody-Conjugate into each well.
7. Incubate for 45 minutes at 37°C.
8. 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.
9. Add 100 μL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 μL of Stopping Solution to each well using a dispenser.
12. 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.