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SeroHSV™ IgM

Enzyme -Linked Immunosorbent Assay (ELISA)
for the qualitative detection of specific IgM antibodies to ***Herpes simplex virus Type 1 & 2*** in human serum

Instruction Manual

Test kit for 96 determinations
(Catalog No.152-01)

For ***In Vitro*** Diagnostic Use
For professional use only
Store at 2-8°C. **Do Not Freeze**

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Intended Use

SeroHSV™-IgM kit is an Enzyme Linked Immunosorbent assay (ELISA) for the qualitative detection of specific IgM antibodies to *Herpes simplex 1&2* (HSV) *viruses* in human serum.

The test enables early diagnosis of current infection in a single serum specimen by determination of IgM antibodies

For ***In Vitro*** Diagnostic Use.

Introduction

Herpes Simplex Virus (HSV) is one of the most ubiquitous viruses known to cause acute and recurrent infections in humans. Once acquired, HSV infects the sensory nerve cells innervating the mucosal areas involved in the acute infection, migrates to the regional sensory ganglion and remains latent. When active, the virus may cause the development of vesicles and ulcers. Most of the HSV infected persons may shed the virus periodically even in the absence of clinical manifestations [1, 2, 4, 10].

There are two types of HSV: HSV type 1 (also known as Herpes labialis)- most commonly infects the oral region causing "cold sores". HSV type 2 (commonly known as Herpes genitalis)- most often infects the genital and perianal regions. Both types can cause infections in different sites and can also infect the skin, the eyes and the brain. HSV 2 tendency for recurrent episodes is higher than HSV 1 [1, 5].

HSV type 1 & 2 are transmitted, with or without the presence of sores or other clinical symptoms. The transmission is by saliva, respiratory secretions and by direct contact (sexual contact, skin to skin etc.) [5, 10].

The pathological consequences of herpes infections can be serious, particularly in immune-suppressed patients and in pregnant women [1,4]. Viral transmission to the newborn during labor could result in a life threatening neonatal disease [1]. HSV 2 genital infections are associated with a higher risk of cervical cancer [9].

Primary HSV infections are characterized by a transient IgM response followed by delayed IgG and IgA responses that tend to persist over time. IgM may also be detected in cases of severe reactivation.

Early, accurate diagnosis is important for the initiation of an appropriate treatment and management to reduce the risk of recurrent episodes.

In recent years there has been a tremendous increase in the reported incidences of genital herpes; HSV 2 sero-prevalence in the US is estimated between 20% and 25% by the age of 40 [1, 2]. It is more prevalent among women than men. In STD patients the sero-prevalence may reach 50% [1,9].

Viral isolation, direct fluorescence antigen (DFA) detection and serology tests are currently used for the in vitro diagnosis of HSV infections [6,10]. Positive culture and DFA also enable typing of the virus [10]. Positive culture and/or positive DFA results are regarded as definite results. However, specimen collection, difficulties in sample transportation and length and complexity of the direct detection methods, makes the use of these methods unsuitable for screening purposes. Most available serological assays (using ELISA, IFA and agglutination techniques), although easier to perform, are problematic for the differentiation of the two HSV types due to extensive antigenic homology between HSV 1 and 2. The golden standard assay for differentiating between HSV 1 and 2 antibodies is the Western Blot (WB) technique which is a cumbersome and laborious test and commercially unavailable [1].

Since serum sampling is easy to perform, sero-diagnosis is the most useful method for screening purposes.

Savyon Diagnostics Ltd. has developed ELISA assays enabling the detection of HSV IgG and IgM antibodies (SeroHSV™ 1gG, SeroHSV™ 1gM).

Principle of the Test

1. SeroHSV™ microtiter plates are coated with a mixture of partially purified virus proteins of HSV2 and HSV1
2. The serum to be tested is diluted 1/105 and incubated in the SeroHSV™ plate. In this step HSV specific antibodies are bound to the immobilized antigens.

3. Non-specific antibodies are removed by washing.
4. Anti-human IgM conjugated to horseradish peroxidase (HRP) is added. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex.
5. Unbound conjugate is removed by washing.
6. TMB-substrate is added and is hydrolyzed by the peroxidase, yielding a blue solution of the reduced Substrate.
7. Upon the addition of the stop solution, the blue color turns yellow and the absorbance should be read by an ELISA reader at a wavelength of 450/620nm.
8. The absorbance is proportional to the levels of the specific antibodies that are bound to the coated antigens.

Assay Procedure

Wells of microtiter plate coated with Herpes simplex viruses 1&2 antigens
↓
Add 50µl of Cut off serum
Negative control, Positive control,
and of diluted 1/105 specimens
↓
Cover plate and incubate 1h at 37ºC at 100% humidity
↓
Wash 3 times with Wash Buffer
↓
Add 50µl of 1/300 diluted HRP Conjugate
↓
Cover plate and incubate 1 h at 37ºC at 100% humidity
↓
Wash 3 times with Wash buffer
↓
Add 100µl of TMB-Substrate
↓
Cover plate and incubate 15min at room temperature
↓
Add 100µl of Stop Solution
↓
Read absorbance at 450/620nm
↓
Calculate and interpret results

Kit Contents

Test kit for 96 Determinations
Cat. No A152-01M

1. ***HSV coated* microtiter plate:** 96 break apart wells (8x12) coated with *HSV Type 1 & 2* antigens, packed in an aluminum pouch containing a desiccant card.
1 Plate
2. **Concentrated Wash Buffer (20 X):** A PBS - Tween buffer. Contains less than 0.05% Procline as a preservative.
1 bottle, 100ml each
3. **IgM Serum Diluent (red):** A ready-to-use buffer solution. Contains Anti-human IgG antibodies and less than 0.05% Procline as a preservative.
1 Bottle, 50 ml
4. **Conjugate Diluent (green)**: A ready-to-use buffer solution. Contains less than 0.05% Procline as a preservative**.**
1 Bottle, 40 ml
5. **Negative Control:** A ready-to-use *HSV* IgM negative human serum. Contains less than 0.05% Procline and less than 0.1% Sodium Azide as preservatives.
1 Vial, 2 ml
6. **Positive Control:** A ready-to-use *HSV* IgM positive human serum. Contains less than 0.05% Procline and less than 0.1% Sodium Azide as preservatives.
1 Vial, 2 ml
7. **Cut Off Serum:** A ready-to-use *HSV* IgM serum, used for cut off determination. Contains less than 0.1% Sodium Azide and less than 0.05% Procline as preservatives.
1 Vial, 2.5 ml
8. **Concentrated HRP-Conjugate (300 X):** Horseradish peroxidase (HRP) conjugated anti-human IgM (µ chain specific). Contains less than 0.05% Procline as a preservative.
1 Vial, 0.2 ml
9. **TMB-Substrate:** A ready-to-use solution. Contains 3, 3', 5, 5' - tetramethylbenzidine as a chromogen and peroxide as a substrate.
1 Bottle, 14 ml
10. **Stop Solution:** A ready-to-use solution. Contains 1M H_2SO_4.
1 Bottle, 15 ml
11. **Plate Cover:** **1 Unit**
12. **Instruction Manual:** **1 Unit**

Materials Required But Not Supplied

1. Clean test tubes for dilution of patients sera.
2. Disposable plastic vial for dilution of the concentrated HRP- conjugate.
3. Adjustable micropipettes and multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
4. One liter volumetric flask.
5. One 50ml volumetric cylinder.
6. Wash bottle.
7. Absorbent paper.
8. Vortex mixer
9. A 37°C water bath with a lid, or a moisture chamber placed in a 37°C incubator.
10. ELISA-reader with a 450 and 620nm filters.
11. Distilled or double deionized water.

Warning and Precautions

For *In Vitro* Diagnostic Use

1. This kit contains human sera which have been tested by FDA approved techniques, and found to be negative for HBV antigen and for HCV and HIV antibodies. Since no known method can offer complete assurance that products derived from human blood do not transmit infection, all human blood components supplied in this kit must be

handled as potentially infectious serum or blood according to the recommendations published in the CDC/NIH manual "Biosafety in Micro Biological and Biomedical Laboratories, 1988".
2. TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
3. All the components of this kit have been calibrated and tested by lot. It is not recommended to mix components from different lots since it might affect the results.
4. Diluted sulfuric acid (1M H_2SO_4) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.

Storage and Shelf -Life of Reagents

1. All the reagents supplied should be stored at 2-8°C. The unopened reagent vials are stable until the expiration date indicated on the kit pack. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents. **DO NOT FREEZE!**
2. Once the kit is opened, its shelf life is 90 days.
3. Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. Crystals may form in the 20x concentrated Wash Buffer during cold storage, this is perfectly normal. Re-dissolve the crystals by warming the buffer to 37°C before diluting. Once diluted, the solution may be stored at 2-8°C up to twenty one days.

Serum Collection

Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbid or contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.

Storage

Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If longer storage period is anticipated, aliquot and store the specimens below -20°C. Avoid repeated thawing and freezing.

Test Procedure - Manual

Automation protocol available upon request

A. Preparation of Reagents

1. Bring all components and the clinical specimens to be tested to room temperature. Mix well the cut off serum, Negative Control, Positive Control and the clinical specimens before use.
2. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: One well of Negative Control, Positive Control and two wells Cut Off serum.
3. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
   Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of wash buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

B. Incubation of sera samples and controls

5. Dilute each patient serum 1:105 with the supplied Serum Diluent as follows: Add 10 µl of patient serum to 200µl of Serum Diluent (1/21), and then dilute further by adding 25µl of 1/21 dilution to 100µl of Serum Diluent.
6. Dispense 2x50µl of the Cut Off serum and 50ul of the negative, positive controls and of the 1:105 diluted serum samples into separate wells of the test strip.
7. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
8. Discard the liquid content of the wells.
9. Washing step:
   Manual Wash:
   Fill each well with wash buffer up to the end of the well and discard the liquid, repeat this step three times.
   Automated Wash:
   Fill each well with 350ul of wash buffer and discard the liquid, repeat this step three times.
10. Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with conjugate

11. Concentrated HRP-conjugated anti-human IgM should be diluted to working solution shortly before use. Dilute the concentrated HRP-conjugated anti-human IgM 1/300 with conjugate diluent. For example: for two strips prepare a minimum of 3 ml conjugate as follows: 10 µl of Concentrated HRP-conjugated anti-human IgM is mixed with 3ml of Conjugate Diluent.
12. Dispense 50µl of diluted conjugate into each well.
13. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
14. Discard the liquid content and wash as described in steps 9-10.

D. Incubation with TMB - Substrate

15. Dispense 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature for **15 minutes.**
16. Stop the reaction by adding 100µl of stop solution (1M H_2SO_4) to each well.

E. Determination of Results

17. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

- *N**ote:** Any air bubbles should be removed before reading. The bottom of the ELISA plate should be carefully wiped.*

Test Validation

The following criteria must be met for the test to be valid. If these criteria are not met, the test should be considered invalid and should be repeated.

1. O.D. $_{PC}$ $\geq$ 0.8
2. Ratio: O.D. $_{PC}$ / O.D. $_{Cut\ Off}$ >2
3. O.D. $_{NC}$ < 0.25

Calculation of Test Results

1. The average absorbance value of the Cut off serum run in duplicate should be calculated.
2. In order to normalize the results obtained in different tests, the cut off index (COI) is calculated according to the following formula:
3. **COI =** $\frac{\text{Absorbance of the serum sample}}{\text{Average value of the Cut Off Control}}$

Interpretation of Results

COI	Result	Diagnostic Interpretation
≤ 1.0	**Negative** No detectable IgM antibodies	**No indication of current *HSV* Infection**
1-1.1	**Borderline** Low level of IgM antibodies	**Indication of possible exposure to HSV.** Second sample testing required after 2-4 weeks. [1]
≥1.1	**Positive** Significant levels of IgM antibodies	**Indication of current *HSV* Infection**

[1] In case of borderline results a second serum sample should be taken 2-4 weeks later and tested together with the first sample.
If borderline result is repeated, specimen must be considered negative.

Test Limitations

1. No single serological test should be used for final diagnosis. All clinical and laboratory data should be taken into account.
2. Samples obtained too early during primary infection may not contain detectable antibodies. If HSV is suspected, a second sample should be obtained 2-4 weeks later and tested in parallel with the original sample.

Performance Characteristics

Precision
Intra-assay (within-run) precision:

Sample	No of Replicates	Mean Value	CV%
Positive	10	0.975	2.8
Negative	10	0.212	5.6

Inter-assay (between-run) precision:

Sample	No of Replicates	Mean Value	CV%
Positive	10	0.912	8
Negative	10	0.196	11

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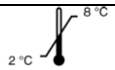	Temperature Limitation
	Consult instructions for use
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
EC REP	Authorized European Representative