SeroHSV1™

Enzyme -Linked Immunosorbent Assay (ELISA)
for the qualitative detection of specific antibodies to
Herpes Simplex Virus Type 1
in human serum

Instruction Manual

Test kit for 96 determinations
(Catalog No. A240-01)

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

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Intended Use

The SeroHSV1™ kit is a qualitative Enzyme Linked Immunosorbent Competitive Binding Assay (ELISA) for the detection of specific antibodies to Herpes Simplex Virus Type 1 (HSV1) in human serum.

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 the 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 the 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 years. [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 antibodies to HSV (SeroHSV™ IgG, SeroHSV™ IgM) and highly specific self confirmatory assays for the detection of HSV type 1 (SeroHSV1™) and HSV type 2 (SeroHSV2™) antibodies.

Principle of the Test

1. SeroHSV™ microtiter plates are coated with a mixture of partially purified virus proteins of HSV1 and HSV2.
2. Patient serum diluted with Serum Diluent containing HSV1 specific mouse antibodies is incubated in the coated microtiter wells.

3. HSV1 specific antibodies in the patient serum compete with the HSV1 mouse specific antibodies on the HSV1 antigen bound to the well.
4. Unbound antibodies are removed by washing.
5. Horseradish peroxidase conjugated anti mouse antibodies are added and bind to the specific mouse HSV1 antibodies present.
6. Due to the competition, when the level of HSV1 specific antibodies in the patient serum increases, the amount of HSV1 mouse specific antibodies bound to the coated well decreases.
7. Unbound conjugate is removed by washing.
8. TMB Substrate is added and hydrolyzed by the peroxidase enzyme to yield a colored end product.
9. The color intensity is inversely proportional to the amount of HSV1 specific antibodies present in the patient sera.

Assay Procedure

96 well Microtiter Plates coated with HSV1 antigens
↓
Add 50µl of each calibrator: (P35, P80, P100), and of 1:4 diluted Negative Control, Positive Control, and specimens
↓
Cover plate and incubate 1h at 37ºC at 100% humidity
↓
Wash 3 times with Wash Buffer
↓
Add 50µl of 1/100 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. A240-01E

1. ***HSV1* antigen 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 < 0.05% Procline as preservative.
   1 Bottle, 100ml
3. ***HSV1* Serum Diluent (green):** A ready-to-use buffer solution. Contains HSV1 specific mouse antibodies and less than 0.05% Procline as preservative.
   1 Bottle, 30 ml
4. **Conjugate Diluent (purple)**: A ready-to-use buffer solution. Contains less than 0.05% Procline as a preservative**.**
   2 Bottles, each 20 ml
5. **Negative Control:** A ready to use *HSV1* negative human serum. Contains less than 0.1% Sodium Azide as a preservative.
   Negative Control should yield ≥80 "Percent of Signal".
   1 Vial, 2ml
6. **Positive Control:** A ready to use *HSV1* positive human serum. Contains less than 0.1% sodium azide as a preservative.
   Positive Control should yield <70 "Percent of Signal".
   1 Vial, 2ml
7. **P100 - Calibrator:** A ready-to-use calibrator containing pre-diluted mouse HSV1 specific antibodies to be used as the 100 "Percent of Signal". Contains less than 0.1% Sodium Azide and less than 0.05% Procline as preservatives.
   1 Vial, 2 ml
8. **P80- Calibrator:** A ready-to-use Calibrator. Contains pre-diluted mouse HSV1 specific antibodies and HSV1 positive human serum, to be used as the 80 "Percent of Signal" Contains 0.1% Sodium Azide and less than 0.05% Procline as preservatives.
   1Vial, 2 ml
9. **P35 - Calibrator:** A ready-to-use Calibrator. Contains pre-diluted mouse HSV1 specific antibodies and HSV1 positive human serum, to be used as the 35 "Percent of Signal". Contains 0.1% Sodium Azide and less than 0.05% Procline as preservatives.
   1 Vial, 2 ml
10. **Concentrated HRP-Conjugate (100X):** Horseradish peroxidase (HRP) conjugated to anti-mouse antibodies. Contains less than 0.05% Procline as a preservative.
    1 Vial, 0.2 ml
11. **TMB-Substrate:** A ready-to-use solution. Contains 3, 3', 5, 5' - tetramethylbenzidine as a chromogen and peroxide as a substrate.
    1 Bottle, 16 ml
12. **Stop Solution:** A ready-to-use solution. Contains 1M H_2SO_4.
    1 Bottle, 16 ml
13. **Plate Cover: 1 unit**
14. **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 HbsAg, 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. Do not mix components from different lots as 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 reagents 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 30 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. Redissolve 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 calibrators (P100, P80, P35), 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 each Negative and Positive Control and three wells of Calibrators (P100, P80, P35).
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.
4. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. 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, Negative and Positive control 1:4 with the Serum Diluent as follows: Add 20 µl of Negative Control or Positive Control or patient serum to 60µl of Serum Diluent
6. Dispense 50µl of each three calibrators (P100, P80, P35), 1:4 diluted Negative Control, Positive Control, and 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 steps:**
   Manual Wash
   Fill each well with wash buffer up to the end of the well and discard the liquid, repeat this step two more times.
   Automated Wash
   Fill each well with 350µl of Wash Buffer. Repeat this step two more times.
10. Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with conjugate

11. Concentrated HRP-Conjugate should be diluted to working solution shortly before use.

Dilute the concentrated HRP-Conjugate 1/100 with conjugate diluent. For example: for two strips prepare a minimum of 1ml Conjugate as follows: 10 µl of Concentrated HRP- Conjugate is mixed with 1ml 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 prior to 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. $O.D._{P100} \geq$ **0.8**
2. Positive Control should yield <70 "Percent of Signal".
3. Negative Control should yield >80 "Percent of Signal".

Calculation of Test Results

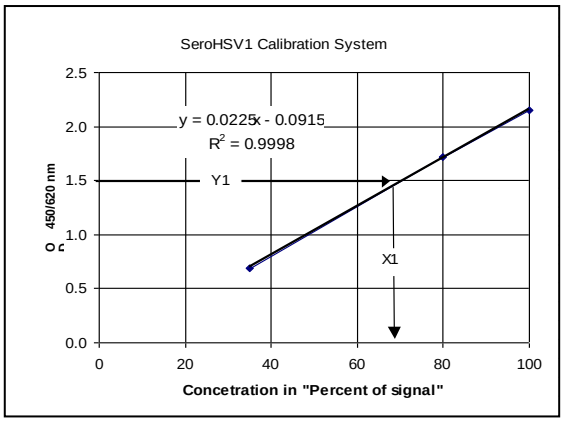
Manual method, using a squared graph paper:

1. Plot the absorbance values (OD) of the 3 Calibrators (P100, P80, P35), on the Y axis versus their respective concentrations ("Percent of Signal") on X axis.
2. Draw the best fitted linear curve through the points.
3. Using the standard curve, interpolate the "Percent of Signal" activity of each of the tested samples from each absorbance measured (see example 1).

Example 1: Interpolation of results:

On the Y-axis read the absorbance value of the sample (y1) and draw a horizontal line to the calibration curve (intercept). From the intercept, draw a vertical line to the X axis (x1). X1 is the "Percent of Signal" obtained with the y1 sample (see following Figure).

	OD 450/620nm	Concentration in "Percent of Signal"
P100	2.152	100
P80	1.72	80
P35	0.693	35
Sample	Y1=1.5	X1=70.7



Percent of Signal"

Concentration of mouse antibodies

Max ← → 0

Concentration of human antibodies

Interpretation of Results

Correlation between the observed concentration of "Percent of Signal" vs. the presence of HSV 1 specific antibodies

Conc. In "Percent of Signal"	Result	Diagnostic Interpretation
X>80	Negative	No detectable levels of HSV1 antibodies
75≤X ≤80	Borderline	Presence or absence of detectable (borderline) levels of HSV1 antibodies cannot be determined. A second serum sample should be obtained after 14-21 days and tested. (When 2nd sample is borderline the results should be considered negative.
X<75	Positive	Detectable levels of HSV1 antibodies

Test Limitations

1. No single serological test should be used for final diagnosis. All clinical and laboratory data should be taken into consideration.

2. Samples obtained too early during primary infection may not contain detectable antibodies. If *HSV1* infection 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 OD Value	CV%
Positive	10	0.49	6.6
Negative	10	1.98	2.1

Inter-assay (between-run) precision:

Sample	No of Replicates	Mean OD Value	CV%
Positive	10	0.57	11.20
Negative	10	2.16	5.09

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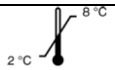	Temperature Limitation
	Consult instructions for use
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
EC REP	Authorized European Representative