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SeroHSV™ IgG

Enzyme -Linked Immunosorbent Assay (ELISA)
for the semi-quantitative determination of specific IgG antibodies to
Herpes simplex virus Type 1 & 2
in human serum

Instruction Manual

Test kit for 96 determinations
(Catalog No 151-01)

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

Savyon® Diagnostics Ltd.
3 Habosem St.
Ashdod 77610
ISRAEL
Tel: +972.8.856.2920
Fax: +972.8.852.3176
E-mail: support@savyondiagnostics.com

Intended Use

SeroHSV™-IgG kit is a semi-quantitative Enzyme Linked Immunosorbent assay (ELISA) for the determination of specific IgG antibodies to *Herpes simplex 1&2* (HSV) *viruses* 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 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 HSV1 and HSV2.
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 IgG 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 each calibrator (P10, P50, P100), Negative Control, Positive Control and 1:105 of diluted 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. A151-01M

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

3. **Serum Diluent (blue):** A ready-to-use buffer solution. Contains less than 0.05% Procline as a preservative.
1 Bottle, 30 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. **P10-calibrator:** A ready-to-use *HSV* IgG serum. Contains 10 BU/ml of IgG (arbitrary binding units). Contains less than 0.1% Sodium Azide and less than 0.05% Procline as preservatives.
1 Vial, 2 ml
6. **P50-calibrator:** A ready-to-use *HSV* IgG positive human serum. Contains 50 BU/ml of IgG (arbitrary binding units) .Contains less than 0.1% Sodium Azide and less than 0.05% Procline as preservatives.
1 Vial, 2 ml
7. **P100-calibrator:** A ready-to-use *HSV* IgG positive human serum. Contains 100 BU/ml of IgG (arbitrary binding units) . Contains less than 0.1% Sodium Azide and less than 0.05% Procline as preservatives
1 Vial, 2 ml
8. **Negative Control:** A ready-to-use *HSV* IgG negative human serum. Contains less than 0.05% Procline and less than 0.1% Sodium Azide as preservatives.
1 Vial, 2 ml
9. **Positive Control:** A ready-to-use *HSV* IgG positive human serum. Contains less than 0.05% Procline and less than 0.1% Sodium Azide as preservatives.
1 Vial, 2 ml
10. **Concentrated HRP-Conjugate (300 X):** Horseradish peroxidase (HRP) conjugated anti-human IgG (γ chain specific). 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, 14 ml
12. **Stop Solution :** A ready-to-use solution. Contains 1M H_2SO_4.
1 Bottle, 15 ml
13. **Plate Cover:** **1 units**
14. **Instruction Manual:** **1**

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 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 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 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. 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 (P10, P50, P100), 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: Three wells of calibrators (P10, P50, P100), one well of Negative Control and one well of Positive Control
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 50µl of each of the three calibrators (P10, P50, P100), Negative Control, Positive Control and of 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 IgG should be diluted to working solution shortly before use. Dilute the concentrated HRP-conjugated anti-human IgG 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 IgG is mixed with 3ml of Conjugate Diluent.
12. Dispense 50µl of diluted HRP- 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.

- ***Note:*** *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. P100: OD ≥1.1
2. Negative Control: <10BU/ml (See chapter – Calculation of Test Results)
3. Positive Control: ≥ 30 BU/ml (See chapter – Calculation of Test Results)

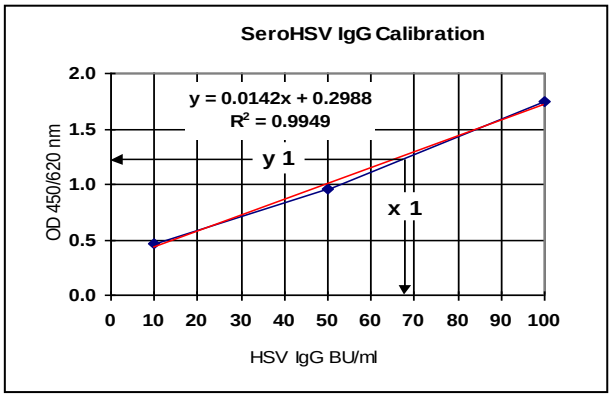
Calculation of Test Results

Manual method, using a squared graph paper:

1. Plot the absorbance values (OD) of the 3 calibrators (P10, P50 and P100) on Y axis versus their concentration (BU/ml) on X axis.
2. Draw the best fitted linear curve through the points.
3. Using the standard curve, interpolate the concentration of the Positive Control and Negative Control in BU/ml.
   Negative Control should be <10BU/ml
   Positive Control should be: ≥ 30BU/ml. If Negative and/or Positive Control are not within specification test should be repeated.
4. Using the standard curve, interpolate the concentration of the tested sample values (in BU/ml) from each absorbance measured (see example 1).

Example 1: Interpolation of results:
On the Y-axis read the absorbency value of the sample (Y1) and draw a horizontal line to the calibration curve.
From the intercept (X1), draw a vertical line to the X-axis. Read the concentration in BU/ml of the sample.

Calibrators	IgG BU/ml	OD 450/620nm
P-10	10	0.470
P-50	50	0.956
P-100	100	1.742
Sample	X1=67	Y=1.256



Interpretation of Results

IgG BU/ml	Result	Diagnostic Interpretation
<10 BU/ml	**Negative** No detectable IgG antibodies	**No indication of *HSV* Infection**
≥10 BU/ml < 50 BU/m	**Positive** Significant level of IgG antibodies	**Indication of current or past *HSV* infection***
≥50 BU/ml	**High Positive** High level of IgG antibodies	**Indication of current or past / chronic *HSV* infection***

* In order to differentiate between past and current infection, it is recommended to take a second sample after 2-4 weeks. If the BU/ml value of the second sample significantly increases current infection is indicated.

In order to evaluate if the difference between the 2 measurements is significant, the ratio between the sera should be calculated as follows:

$$R = \frac{BU2 + 15}{BU1 + 15}$$

BU1 = Concentration in BU/ml of the 1st sample
BU2 = Concentration in BU/ml of the 2nd sample

If **R ≥1.7**, the difference is statistically significant (p=0.005). This equation is not valid when both samples are less than 10 BU/ml.

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	0.998	2.2
Negative	10	0.292	2.4

Inter-assay (between-run) precision:

Sample	No of Replicates	Mean Value	CV%
Positive	10	1.003	5.2
Negative	10	0.285	5.8

Bibliography

1. Ashley R.L., Wold P., clinical Microbiology reviews; Jan 1999, 12(1): 1-8.
2. Di Carlo R.P. Medscape, 1999.Preventing genital HSV infections on a large scale. 13th meeting of the international society for STD research. July 11-14th 1999.
3. Diaz-Mitoma F., Sibbald R., et al; JAMA 1998; 280: 887-92. Oral Famiciclovir for the suppression of recurrent genital Herpes: A randomized controlled trial.
4. The International Herpes Management Forum (IHMF) The 12 management strategies in Herpes, 1997; 1-70.
5. Steben M., Sacks S.L., The Canadian Journal of Human Sexuality. November 1997; 1-14. http://hwcweb.hwc.ca
   Genital Herpes: The epidemiology and control of a common sexually transmitted disease.
6. Screening for genital Herpes Simplex. Guide to clinical preventive services, 2nd ed. Infectious diseases. Columbia-Presbyterian Medical Center. http://cpmcnet.columia.edu
7. Atlanta Maternal-Fetal Medicine, P.G. Clinical Discussions, 1996: 4(4) http://www.atlanta-mfm.com
8. Riott, I., Riott's Essential Immunology, 9th edition. Pp. 112-129
9. Tortora, G.S., Grabowski S.R.; Principles of anatomy and physiology, 8th edition. Pp. 88; 950.
10. Erlich Kims. Safrim Sharon. June 1998: Herpes Simplex Virus. The Aids Knowledge Base. http://www.hivsite.ucsf.edu
11. Brown Z, Selke S, Zeh S, N. Engl. J. Med. 1997; 337:509-15. The acquisition of Herpes Simplex Virus during pregnancy.

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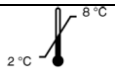
European Authorized Representative: Obelis s.a.
Boulevard Général Wahis 53, B-1030 Brussels, Belgium
Tel: +32.2.732.59.54 Fax: +32.2.732.60.03
E-mail:mail@obelis.net

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