

HSV1,2 IgG ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the Detection of
Herpes simplex virus 1, 2 IgG Antibody
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-HSV1, 2 IgG-96

PISHTAZ TEB DIAGNOSTICS

Introduction

Herpesviridae family includes large number of DNA viruses which known as one of the most common cause of human infection worldwide. *Herpes simplex virus* (HSV) infection caused by two virus types known as HSV1 and HSV2. HSV-1 mostly causes non genital lesions but HSV-2 commonly infects genital organs. Infection caused by Herpes simplex virus type I known as nongenital warts (cold sore) occurs mostly in young children (less than 5 years old in 90% of cases). It affects lips, gingiva, mouth, occasionally cornea and sometimes genital area with very small painful vesicles. In the case of eye infection, painful red eye, feeling of presence of foreign materials in eyes, photosensitivity and uncontrolled tears may be manifested. Direct person to person as well as contact with infected human secretions like saliva, tears, urine and feces are major routes of HSV transmission. Unhealed vesicles are also contagious and contact with their secretion may transmit the virus. HSV2 mostly cause genital lesions in adults and accompanied with sexual activity. HSV infections signs and symptoms appears 4-7 days after contact with infected individual. In most of cases the disease is asymptomatic particularly in pregnant and healthy individuals. Immunocompromised patients or patients under immunosuppressive therapy are affected more frequently. HSV cause very small vesicles in genital area which became extremely painful after rupture. In some affected females, vaginal secretion may appear. HSV neural involvement may provoke fever, headache, vomiting and neck stiffness 3-12 days after genital lesions. In addition to sexual contact, skin contact with respiratory secretion of infected individual can also transmit the infection. Consequently, certain practitioners like dentists, medical staffs and laboratory personnel are at high risk of acquiring infection. Congenital transmission during child birth is also an important route of HSV infection transmission.

Anti HSV1, 2 antibodies increase 4-6 weeks post infection and then declined. Detection of specific antibody against HSV antigens is used for distinguishing acute or chronic infection in asymptomatic patients.

Test Principle

The test principle is based on indirect ELISA technique in which diluted patient serum samples are allowed to react with coated HSV1,2 antigens. After incubation, the HRP -conjugated anti-human IgG is added into the wells. If anti-HSV1,2 IgG was present in samples, they will react with the HRP-conjugated anti-human IgG. After incubation and appropriate washing, a solution of chromogen substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of HSV1, 2 IgG is directly proportional to the color intensity of the test sample.

Materials Provided With Kit

1. Antigen coated plate (1 plate, 96 wells): Microtiter wells coated with HSV1,2 antigens.
2. Sample diluent: 2 vials each contains 50 ml of diluent to dilute samples. The solution contains buffer, protein and stabilizer, ready to use.
3. Conjugate solution: 1 vial, 12 ml volume. The solution contains HRP conjugated anti-human IgG, buffer and preservative. Ready to use.
4. Standard sets: 5 vials, each contains 1.5 ml of standard solution including 0, 10, 50, 100, 200 AU/ml of anti-HSV1,2-IgG standard.
5. Wash solution (20X): 1 vial, 50 ml, Phosphate Buffer Saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative.
6. Chromogen-substrate: 1 vial, 12 ml, contains tetramethyl benzidine (TMB) and hydrogen peroxide, ready to use.
7. Stop solution: 1 vial, 12 ml, 1 molar hydrochloric acid solution.
8. Cardboard sealer.

Materials/Equipment required but not provided with Test Kit

1. ELISA reader with 450 and 630 nm (reference) filters.
2. Precision pipettes (10 and 100 μ l)
3. Distilled water
4. Absorbent paper

General Information

1. Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
2. This kit is just for the detection of Anti-CMV IgG in human serum and plasma.
3. All reagents obtained from human sources are negative for HIV Ag, HIV and HCV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Opened microplates are stable for 4 months.
4. Reagents stability period are marked on their labes.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate the specimen (maximum 2 days at 2-8°C) and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage. Samples

suspected to microbial contamination should not be used.

Reagents & Specimens Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: Warm the vial at 37°C to dissolve possible crystals which formed due to concentration of solution. dilute concentrated wash solution 1:20 with distilled water before use. This solution will be stable for 1 week at 2-8 °C.

Assay Procedure

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag. Test steps should be done sequentially.
2. Dilute samples 1:101 with sample diluent (dilute 10 μ l of sample with 1 ml of sample diluent). Standards set are ready to use and do not need further dilution.
3. Add 100 μ l of each standard as well as diluted test sera into appropriate wells. Duplicate runs are suggested.
4. Seal the plate with cardboard sealer tightly. Mix gently and leave wells for 30 minutes at room temperature (22-28°C).
5. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 μ l of Working wash solution).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 100 μ l of ready to use conjugate solution into all wells.
8. Seal the plate with cardboard sealer tightly. Mix gently and

- incubate wells for 30 minutes at room temperature (22-28°C).
9. Repeat steps of 5 and 6.
 10. Spike 100 µl of chromogen/substrate solution to each well.
 11. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
 12. Add 100 µl of stop solution to the wells to stop reaction.
 13. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available). Reference filter is highly recommended.

Validity of the Assay

The assay is to be considered valid if:

1. The mean OD (450 nm) of the zero standard should be lower than 0.1. Higher values indicate inappropriate washing or chromogen/substrate contamination. In such a case, repeat the assay with particular attention into washing steps, the efficiency of the washing device and verify the chromogen color which should be colorless.
2. The mean OD value of standard 10 (the second standard) should be higher than zero standard.
3. The mean OD value of standard 200 should be higher than 1.5. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

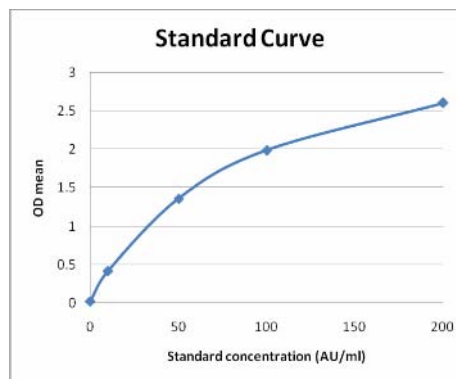
1. Measure absorbance of Standards and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in AU/ml on linear graph paper, with absorbance on the vertical

- (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample, determine the corresponding concentration of Anti-CMV IgG in Au/ml from the standard curve.

In normal population, the cut-off value normally considered as 10 AU/ml. Results lower than 10 AU/ml is considered as negative and those higher than 10 AU/ml should be considered as positive results. Those results between 9-11 AU/ml is considered as suspected results and should be re-evaluated with fresh samples after a while.

Example of Standard curve

| Standards (AU/ml) | Absorbance |
|-------------------|------------|
| 0 | 0.025 |
| 10 | 0.419 |
| 50 | 1.361 |
| 100 | 1.987 |
| 200 | 2.606 |



Note: The standard curve shown in above example is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Performance Characteristics

Sensitivity

To evaluate test sensitivity, 250 positive samples confirmed by chemiluminescence and ELISA were used which all showed positive results. The PT-HSV1,2-IgG-ELISA showed 100% sensitivity which is comparable to other diagnostic kits or methods.

Specificity

To evaluate test specificity, 120 negative sera are tested simultaneously with PT CMV-IgG-ELISA and chemiluminescence method. Results displayed all specimens were negative. According to test results the PT HSV1,2-IgG-ELISA showed 100% specificity.

Test Precision

To verify test repeatability, intra- and inter assay tests were performed on positive, weakly positive and negative sera. Results are shown below:

Intra-assay test results

| | Number of Repeats | Mean Conc. AU/ml | SD | CV % |
|-------------------------|-------------------|------------------|-----|------|
| Negative Sample | 20 | 3.5 | 0.3 | 8.5 |
| Positive Samples | 20 | 137 | 5.2 | 3.7 |
| Weak Positive | 20 | 14 | 0.9 | 6.4 |

Inter-assay test results

| | Number of Repeats | Mean Conc. (AU/ml) | SD | CV% |
|-------------------------|-------------------|--------------------|------|-----|
| Negative Sample | 10 | 4.0 | 0.37 | 9.2 |
| Positive Samples | 10 | 146 | 6.1 | 4.1 |
| Weak Positive | 10 | 15 | 1.0 | 6.6 |



All test were run in duplicate.

References

1. ENGVALI. E. PERLMANN. P. (1997) Enzyme linked immunosorbent assay (ELISA). Quantitative assay for immunoglobulin. J.immunochimistry (8) 871-874.
2. Brooks Georf, Butel Janet, S.Jawetz,melnick & Adelbery's medical microbiology twenty second edition. Mc Grow-Hill 2001.
3. Murray Patrick, R.Rosenthal, Ken. S.Kobayashi, George S. Medical Microbiology fourth Edition...mosby 2002.
4. Roizman B, Knipe DM. Herpes simplex viruses and their replication. In: Knipe DM, Howley PM, editors. Fields Virology. Philadelphia: Lippincott Raven Press; 2001. p. 2399–460.
5. Munday, PE; Vuddamalay, J; Slomka, MJ; Brown, DW. Role of type specific herpes simplex virus serology in the diagnosis and management of genital herpes. *Sex Transm Infect.* 1998 Jun;74(3):175–178.

**Schematic HSV1,2-IgG
ELISA Test Procedure**

Step 1

| Reagent | Standards | Sample |
|----------------|---|---|
| |  |  |
| Standards | 100 µl | None |
| Diluted Sample | None | 100 µl |

Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at room temperature (22-28°C).





Step 2

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.



Step 3

| Ready to use Conjugate Solution | 100 µl | 100 µl |
|---------------------------------|---|---|
| |  |  |

Cover the microplate wells with cardboard sealer and incubate for 30 minutes at room temperature (22-28°C).





Step 4

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.





Step 5

| Chromogen Substrate Solution | 100 µl | 100 µl |
|------------------------------|---|---|
| |  |  |

Incubate wells for 15 minutes at room temperature and dark.

Step 6

| Stop Solution | 100 µl | 100 µl |
|---------------|---|---|
| |  |  |

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).