IgG Antibody to Herpes Simplex Virus Type 2 (Anti-HSV-2) ELISA

INTENDED USE
Anti-HSV-2 (IgG) ELISA is intended for the qualitative detection of IgG antibodies to herpes simplex virus type 2 in human serum or plasma.

INTRODUCTION
Herpes simplex virus (HSV) is an enveloped, DNA-containing virus morphologically similar to the other members of the Herpetoviridae family. Two naturally occurring variants of HSV, with different biologic and epidemiologic characteristics, are recognized by restriction-endonuclease or antigenic analysis. Both types of virus cause infections in humans which range in severity from cold sores to encephalitis. HSV Type 1 (HSV-1) generally infects the mucous membrane of the eye, mouth and mucocutaneous junctions of the face, and is also one of the most common causes of severe sporadic encephalitis in adults. HSV Type 2 (HSV-2) is usually associated with mucocutaneous genital lesions: genital herpes is now one of the most common sexually transmitted diseases. The association between the site of infection and the HSV type involved is not, however, exclusive. Once infection occurs, HSV persists in a latent state in sensory ganglia from where it may re-emerge to cause periodic recurrence of infection induced by many stimuli, which may or may not result in clinical lesions. Immunocompromized patients are more likely to have frequent HSV recurrences. This suggests that serum antibody and virus-specific cell-mediated immunity contribute to recovery. Pregnant women who develop genital herpes are two to three times more likely to have spontaneous abortions or deliver a premature infant than are pregnant non-infected women. Active virus excretion in genital secretions of pregnant women may result in severe neonatal HSV infection contracted when the infant passes through an inflected genital tract. When HSV lesions are present during delivery, 40% to 60% of the neonates can be affected. Transmission of HSV infection to neonates is associated with high morbidity and mortality rates if untreated. By five years of age, 35% of children have antibody to HSV-1 and 80% of adults by age 25 will have specific antibodies to HSV-1. Since HSV-1 and HSV-2 share common antigenic determinants, antibody directed against one viral type may cross-react with the other viral type. Recurrent infections often occur with both viral types despite the presence of circulating antiviral antibodies. Rapid and accurate diagnosis of HSV infection is necessary to ensure early implementation of selective antiviral chemotherapy and to minimize spread of infection. The first humoral immune response to infection is the synthesis of specific anti-HSV IgM antibody which becomes detectable one week after infection. Normally this is a proof of recent or recurrent infection. Specific IgG antibody generally appears two to three weeks after primary infection, but may fall in titre after a few months. Patients with recurring disease often do not show an increase in titre. Detection of IgG allows assessment of the patient’s immune status and provides serological evidence of prior exposure to HSV. This may aid in the diagnosis of recent (primary or recurrent) HSV infection in paired sera by the presence of seroconversion to HSV-1 or HSV-2 antibody.

PRINCIPLE OF THE TEST
anti-HSV-2 (IgG) ELISA is based on indirect ELISA. Microwells are pre-coated with HSV-2 antigens. Once the sample is added, anti-HSV-2 (IgG), if present, binds to pre-coated HSV-2 antigens. After incubation and wash procedures, enzyme conjugate reagent is added, and the anti-human IgG inside binds to anti-HSV-2 (IgG ) attached to the solid phase in the previous step. After another incubation and wash procedures, add substrate solution and chromogen solution to initiate a chromogenic reaction. Once the color development is completed, add the stop solution, and then read the absorbance of each sample. The color intensity is directly proportional to anti-HSV-2 (IgG) concentration.

MATERIALS
MATERIALS PROVIDED
1. Coated Wells: microplate with HSV-2 antigen coated wells (1 plate, 96 wells).
2. Enzyme Conjugate Reagent: horseradish peroxidase (HRP) labeled anti-human IgG in stabilizing buffer (1 vial, 11.5 ml).
3. Negative Control: human serum/plasma non-reactive for anti-HSV-2 IgG, diluted in buffer with preservatives (1 vial, 1.0 ml)
4. Positive Control: human serum/plasma reactive for anti-HSV-2 IgG, diluted in buffer with preservatives (1 vial, 1.0 ml)
5. Wash Fluid Concentrate: PBS-Tween (1 bottle, 50.0 ml, 20x)
6. Substrate Solution: hydrogen peroxide (1 vial, 7.5 ml)
7. Chromogen Solution: 3, 3’, 5, 5’-tetramethylbenzidine (TMB) (1 vial, 7.5 ml)
8. Stop Solution: 1.0 M H2SO4 (1 vial, 7.5 ml)
9. Sample Diluent: buffer solution with preservatives (1 bottle, 11.5 ml)

MATERIALS REQUIRED BUT NOT PROVIDED
1. Micropipettes and multichannel micropipettes of appropriate volume (the use of accurate pipettes with disposable plastic tips is recommended)
2. Distilled water
3. Vortex mixer
4. Absorbent paper or paper towel
5. Incubator
6. Disposable reagent troughs
7. Instrumentation
   a) Automated microplate strip washer
   b) Microplate reader

STORAGE OF TEST KIT AND INSTRUMENTATION
1. Unopened test kits should be stored at 2 – 8°C upon receipt. The test kit may be used throughout the expiration date of the kit (1 year from the date of manufacture). Refer to the package label for the expiration date.
2. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened components will remain stable for at least 2 months, or until the expiry date, whichever is earlier, provided it is stored as prescribed above.

SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE
1. Plasma specimens may be used with this test but serum is the recommended specimen type for this assay.
2. When plasma specimen is used, it is recommended to use 1.5 g/L EDTA, 10.9 mmol/l sodium citrate or 20 – 30 U/ml heparin as the anticoagulant.
3. Collect all blood samples observing universal precautions for venipuncture.
4. Any turbidity and particulate matters might interfere with the test, hence must be removed by centrifugation before testing.
5. Allow samples to clot before centrifugation.
6. Specimens could be stored at room temperature for up to 8 hours. For specimens which are not to be assayed within 8 hours of collection, they must be stored at 2 – 8°C for no more than 48 hours. Specimens to be transported or stored for a longer period should be stored frozen at -20°C or a lower temperature. Avoid multiple freeze-thaw cycles. After thawing, ensure specimens are thoroughly mixed and brought to room temperature before being assayed.
7. Avoid grossly hemolytic and lipemic specimens.
8. Do not add sodium azide into the specimen as a preservative.
PRECAUTIONS AND WARNINGS
1. For in vitro diagnostic use only.
2. This package insert must be fully understood prior to operation. The operation must be strin- gently in accordance with the instruction for use.
3. Micropipette tips are not interchangeable to eliminate cross contamination.
4. Specimens added must be mixed thoroughly. The presence of bubbles must be eliminated.
5. The microtiter plate must be washed completely. Each well must be fully injected with Wash Fluid. The strength of injection, however, is not supposed to be too intense to avoid overflow. Each wash cycle, liquids in each well must be dried. The microtiter plate should be stroked onto absorbent paper to remove residual water droplets. It is recommended to wash the microtiter plate with an automated microplate strip washer.
6. Wear disposable gloves when dealing with specimens and reagents. Wash hands after operations. All specimens must be regarded as potentially infectious. Waste material must be disposed of safely according to relevant local and national requirements.
7. Avoid any skin contact with all reagents. Stop Solution contains H2SO4, in case of contact, wash thoroughly with water.
8. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION
1. Obtain the assays from the fridges. Place at room temperature (18 – 25°C ) and equilibrate for at least 30 minutes.
2. Mix the reagents by gently inverting or swirling.
3. Calibrate the temperature of the incubator at 37°C. Only use after the temperature is stabilized.

IMPORTANT NOTES
1. Do not use reagents after expiry date.
2. Do not mix or use components from kits with different lot numbers.
3. Do no reuse the plate covers.
4. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
5. Replace caps on reagents immediately. Do not switch caps.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder. Prepare data sheet with sample identification.
2. Leave 1 well for the blank; add 100 µl of Negative Control to the next 3 wells, then 100 µl of Positive Control to the following 2 wells. Add 100 µl of Sample Diluent into each of the rest of the wells, and then add 10 µl of specimen into each of the wells with added Sample Diluent.
3. Mix thoroughly by shaking on a vortex mixer for 10 seconds. Cover the microplate with a lid, incubate at 37°C for 45 minutes.
4. Wash 6 times (an automated microplate strip washer is recommended); strike the microtiter plate onto absorbent paper at the end of the last wash cycle.
5. Add 100 µl of Enzyme Conjugate Reagent into each well except for the blank well.
6. Repeat steps 3 and 4.
7. Add 50 µl of Substrate Solution, then 50 µl of Chromogen Solution into each well including the blank well. Gently mix and incubate at 37°C for 10 minutes without exposure to sunlight.
8. Add 50 µl of Stop Solution to each well. Mix thoroughly on a vortex mixer.
9. Immediately after mixing, read the absorbance of each well at 450 nm using 620 – 630 nm as the reference wavelength. Alternatively, the actual absorbance can be obtained by subtracting the absorbance of each well at 450 nm with the absorbance of the blank well at 450 nm.

INTERPRETATION OF RESULTS
1. Test is valid only if absorbance of Positive Control ≥0.7, and absorbance of Negative Control ≤0.1.
2. Calculation of the cut-off value
   Cut-off value = 0.1 + mean absorbance of Negative Control replicates (in case the mean absorbance of Negative Control replicates < 0.05, use 0.05 instead of the actual mean). The specimen is positive when the absorbance ≥ the cut-off value, otherwise, the specimen is negative.

PERFORMANCE CHARACTERISTICS
1. Sensitivity
   The sensitivity reaches 100% (145/145)
2. Specificity
   The specificity is 93.39% (537/575)
3. Precision
   After 10 replicate tests, the precision was calculated to be ≤15%.

LIMITATIONS
1. This assay is only suited for aiding in the diagnosis of HSV-2 infected patients, not to be used to screen blood sources.
2. This assay is not suited for monitoring the therapeutic treatment for HSV-2 infections.
3. As with other sensitive immunoassays, there is a possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of entire content of wells completely before adding the wash solution.
4. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by a physician is needed for a final diagnosis.
5. The test is for research use, further manufacturing and export only.

REFERENCES

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