INTENDED USE

The Herpes 1 IgG Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Herpes 1 in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service. This assay is intended for in-vitro diagnostic use only. Laboratory results cannot be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

GENERAL DESCRIPTION

The Herpes simplex virus type 1 is an ubiquitous pathogen of humans that usually causes either asymptomatic infection or mild skin and mucosal diseases. Antibodies to HSV 1 occur in about 90% of adults. Normally HSV 1 is transmitted by oral secretions or open wounds prior to the age of five. Recently in adults primary infections were observed, too. After the primary infection some viruses establish a latent state in their host cells (mostly ganglial cells). The virus DNA is integrated into the genome of the host cell, where it remains until the infected person dies. After stimulation of the host cell, recurrent infection occurs, which is called an exacerbation, when clinical symptoms appear. The recurrence may be caused by different kinds of traumas, as fever or physiological changes and diseases. Immunosuppressed persons may show a severe clinical course. HSV 1 causes different clinical symptoms in about 10% of the primary infections. HSV 1 causes 85% and HSV 2 15% of oral primary infections. The major clinical manifestations associated with HSV 1 infections are gingivostomatitis, keratitis, conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and some lethal infections of newborns. In some cases HSV 1 infection leads to a meningitis with different neurological symptoms. Persons at an increased risk for serious or prolonged HSV infections are those with eczema, severe burns or a defect in their cell-mediated immunity. The drug Acyclovir is the treatment of choice for most serious HSV infections. The common manifestations of HSV infections are so typical that the infection may be easily diagnosed on clinical recognition alone. The "gold standard" for diagnosis of HSV infection remains isolation of the virus in tissue culture. For typing HSV 1/HSV 2 Western blots or indirect immunofluorescence may be performed. Diagnosis of the primary infection by HSV 1/HSV 2 can be confirmed by a significant rise of the IgG titer within 6 to 10 days. A finished infection can be monitored by the IgG ELISA. In case of a suspicion of HSV encephalopathy it is recommended to perform a parallel determination of both HSV-specific antibodies (IgG and IgM) in serum and liquor.

PRINCIPLE OF THE TEST

The Herpes 1 IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Herpes 1 antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Herpes 1 antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

SPECIMEN COLLECTION AND HANDLING

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20°C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results. For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 μL serum + 500 μL sample diluent).

MATERIALS REQUIRED BUT NOT PROVIDED

1. 5 μL-, 100 μL- and 500 μL micro- and multichannel pipets
2. Microtiter Plate Reader (450 nm)
3. Microtiter Plate Washer
4. Reagent tubes for the serum dilution
5. Bidistilled water
REAGENTS PROVIDED

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Microtiter Strips: 12 strips with 8 breakable wells each, coated with a Herpes 1 antigen (Herpes simplex 1, strain MacIntyre, host cell: African Green Monkey Kidney). Ready-to-use.

Calibrator A (Negative Control): 2 mL, protein solution diluted with PBS, contains no IgG antibodies against Herpes 1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

Calibrator B (Cut-Off Standard): 2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Herpes 1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

Calibrator C (Weak Positive Control): 2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Herpes 1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

Calibrator D (Positive Control): 2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Herpes 1. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

Enzyme Conjugate: 5 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

Substrate: 15 mL, TMB (tetramethylbenzidine). Ready-to-use.

Stop Solution: 15 mL, 0.5 M sulfuric acid. Ready-to-use.

Sample Diluent: 60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

Washing Buffer: 60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

Plastic Foils: 2 pieces to cover the microtiter strips during the incubation.

Plastic Bag: Resealable, for the dry storage of non-used strips.

ASSAY PROCEDURE

1. Reagent And Sample Preparation

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

1) Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.

2) All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.

3) Calibrators and samples should be assayed in duplicates.

4) A calibration curve should be established with each assay.

5) Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

2. Assay Steps

1) Prepare a sufficient amount of microtiter wells for the calibrators, controls and samples in duplicate as well as for a substrate blank.

2) Pipet 100 μL each of the diluted (1:101) samples and the ready-to-use calibrators and controls respectively into the wells. Leave one well empty for the substrate blank.

3) Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.

4) Empty the wells of the plate (dump or aspirate) and add 300 μL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

5) Pipet 100 μL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.

6) Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.

7) Empty the wells of the plate (dump or aspirate) and add 300 μL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.

8) Pipet 100 μL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.

9) Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).

10) To terminate the substrate reaction, pipet 100 μL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.

11) After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.
**EVALUATION**

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

1. **Qualitative Evaluation**
   The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20% around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

2. **Quantitative Evaluation**
   The ready-to-use standards and controls of the Herpes 1 antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials. For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

**ASSAY CHARACTERISTICS**

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes 1 ELISA</td>
<td>8.3%</td>
<td>8.0%</td>
<td>8.6%</td>
</tr>
<tr>
<td>Intra-Assay Precision</td>
<td>9.4%</td>
<td>11.5%</td>
<td>9.4%</td>
</tr>
<tr>
<td>Inter-Assay Precision</td>
<td>5.2-11.8%</td>
<td>6.8-14.8%</td>
<td>5.6-11.0%</td>
</tr>
<tr>
<td>Inter-Lot Precision</td>
<td>1.10 U/mL</td>
<td>1.03 U/mL</td>
<td>1.06 U/mL</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>Recovery 90-95%</td>
<td>92-118%</td>
<td>90-97%</td>
</tr>
<tr>
<td>Linearity</td>
<td>73-117%</td>
<td>91-128%</td>
<td>68-121%</td>
</tr>
<tr>
<td>Clinical Specificity</td>
<td>96%</td>
<td>98%</td>
<td>100%</td>
</tr>
<tr>
<td>Clinical Sensitivity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Cross-Reactivity:</td>
<td>No cross-reactivity to Measles, Mumps and Varicella.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferences:</td>
<td>No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**REFERENCES**