Herpes simplex virus 2 IgA ELISA

Enzyme immunoassay (microtiter strips) for the qualitative and quantitative determination of IgA antibodies against Herpes simplex virus 2 in human serum and plasma.

REF RE56431

Σ 12x8

2-8°C

EU: IVD U.S.: For research use only. Not for use in diagnostic procedures.

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1. **INTENDED USE**

Enzyme immunoassay (microtiter strips) for the qualitative and quantitative determination of IgA antibodies against Herpes simplex virus 2 in human serum and plasma.

2. **SUMMARY AND EXPLANATION**

The Herpes simplex viruses type 1 and 2 are ubiquitous pathogens of humans that usually cause either asymptomatic infection or mild skin and mucosal diseases. HSV 1 causes 85% and HSV 2 15% of oral primary infections. HSV 1 causes different clinical symptoms in about 10% of the primary infections like gingivostomatitis, keratitis, conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and some lethal infections of newborns. 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.

HSV 2 may cause other symptoms like the Herpes genitalis syndrome occurring principally in adults. The preceding primary infection will be transmitted via sexual contact. HSV 2 is a virus suspected to induce cervix carcinoma in women. In some cases a HSV 2 caused meningitis occurs that is much milder than a HSV 1 caused encephalitis. The most severe complication of genital HSV infection is the neonatal disease. 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 with 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.

3. **TEST PRINCIPLE**

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgA. After the substrate reaction the intensity of the color developed is proportional to the amount of IgA-specific antibodies detected. Results of samples can be determined directly using the standard curve.

4. **WARNINGS AND PRECAUTIONS**

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. Some reagents contain sodium azide (NaN₃) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN₃ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.
5. STORAGE AND STABILITY
The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA, Heparin)
The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

<table>
<thead>
<tr>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-8 °C</td>
<td>&gt;2 days</td>
</tr>
</tbody>
</table>

7. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Symbol</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 12 x 8</td>
<td>MTP</td>
<td>Microtiter Plate Break apart strips. Coated with specific antigen.</td>
</tr>
<tr>
<td>1 x 15 mL</td>
<td>ENZCONJ IgA</td>
<td>Enzyme Conjugate IgA Red colored. Ready to use. Contains: anti-human IgA, conjugated to peroxidase, protein-containing buffer, stabilizers.</td>
</tr>
<tr>
<td>1 x 4 x 2 mL</td>
<td>CAL</td>
<td>Standard A-D 1; 10; 50; 200 U/mL Ready to use. Standard A = Negative Control Standard B = Cut-Off Control Standard C = Weakly Positive Control Standard D = Positive Control Contains: IgA antibodies against HSV II, PBS, stabilizers.</td>
</tr>
<tr>
<td>1 x 60 mL</td>
<td>DILBUF</td>
<td>Diluent Buffer Ready to use. Contains: PBS Buffer, BSA, &lt; 0.1 % NaN3.</td>
</tr>
<tr>
<td>1 x 60 mL</td>
<td>WASHBUF CONC</td>
<td>Wash Buffer, Concentrate (10x) Contains: PBS Buffer, Tween 20.</td>
</tr>
<tr>
<td>1 x 15 mL</td>
<td>TMB SUBS</td>
<td>TMB Substrate Solution Ready to use. Contains: TMB.</td>
</tr>
<tr>
<td>1 x 15 mL</td>
<td>TMB STOP</td>
<td>TMB Stop Solution Ready to use. 0.5 M H2SO4.</td>
</tr>
<tr>
<td>2 x</td>
<td>FOIL</td>
<td>Adhesive Foil For covering of Microtiter Plate during incubation.</td>
</tr>
<tr>
<td>1 x</td>
<td>BAG</td>
<td>Plastic Bag Resealable. For dry storage of non-used strips.</td>
</tr>
</tbody>
</table>

8. MATERIALS REQUIRED BUT NOT SUPPLIED
1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volumes: 5; 100; 500 µL
2. Calibrated measures
3. Tubes (1 mL) for sample dilution
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer
9. **PROCEDURE NOTES**

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.

2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.

3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.

4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.

5. Use a pipetting scheme to verify an appropriate plate layout.

6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.

7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.

8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. **PRE-TEST SETUP INSTRUCTIONS**

10.1. **Preparation of Components**

The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with 4 strips (32 determinations).

<table>
<thead>
<tr>
<th>Dilute / Dissolve</th>
<th>Component</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mL</td>
<td>WASHBUF CONC</td>
<td>200 mL bidist. water</td>
<td>1:11</td>
<td>Warm up at 37 °C to dissolve crystals, if necessary. Mix vigorously.</td>
<td>2-8 °C</td>
<td>8 w</td>
</tr>
</tbody>
</table>

10.2. **Dilution of Samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>to be diluted</th>
<th>with</th>
<th>Relation</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma</td>
<td>generally</td>
<td>DILBUF</td>
<td>1:101</td>
<td>e.g. 5 µL + 500 µL DILBUF</td>
</tr>
</tbody>
</table>

Samples containing concentrations higher than the highest standard have to be diluted further.
11. TEST PROCEDURE

1. Pipette 100 µL of each Standard and diluted sample into the respective wells of the Microtiter Plate. In the qualitative test only Standard B is used.

2. Cover plate with adhesive foil. Incubate 60 min at 18-25 °C.

3. Remove adhesive foil. Discard incubation solution. Wash plate 3 x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.

4. Pipette 100 µL of Enzyme Conjugate into each well.

5. Cover plate with new adhesive foil. Incubate 30 min at 18-25 °C.

6. Remove adhesive foil. Discard incubation solution. Wash plate 3 x with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.

7. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.

8. Pipette 100 µL of TMB Substrate Solution into each well.


10. Stop the substrate reaction by adding 100 µL of TMB Stop Solution into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.

11. Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 60 min after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The evaluation of the test can be performed either quantitatively or qualitatively.

13.1. Qualitative Evaluation

The Cut-off value is given by the optical density (OD) of the Standard B (Cut-off standard). The Cut-off index (COI) is calculated from the mean optical densities of the sample and Cut-off value. If the optical density of the sample is within a range of 20 % around the Cut-off value (grey zone), the sample has to be considered as borderline. Samples with higher ODs are positive, samples with lower ODs are negative.

For a quantification, the Cut-off index (COI) of the samples can be formed as follows:

\[
\text{COI} = \frac{\text{OD Sample}}{\text{OD Standard B}}
\]

13.2. Quantitative Evaluation

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline or point-to-point curve, because these methods give the highest accuracy in the data calculation.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.
14. INTERPRETATION OF RESULTS

<table>
<thead>
<tr>
<th>Method</th>
<th>Range</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative</td>
<td>&lt; 8 U/mL</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>8 – 12 U/mL</td>
<td>equivocal</td>
</tr>
<tr>
<td></td>
<td>&gt; 12 U/mL</td>
<td>positive</td>
</tr>
<tr>
<td>Qualitative</td>
<td>&lt; 0.8</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td>0.8 – 1.2</td>
<td>equivocal</td>
</tr>
<tr>
<td></td>
<td>&gt; 1.2</td>
<td>positive</td>
</tr>
</tbody>
</table>

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

15. EXPECTED VALUES

In an in-house study, apparently healthy subjects showed the following results:

<table>
<thead>
<tr>
<th>Ig Isotype</th>
<th>n</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>equivocal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>negative</td>
</tr>
</tbody>
</table>

IgA 88 6.8 % 4.6 % 88.6 %

16. LIMITATIONS OF THE PROCEDURE
Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

Azide and thimerosal at concentrations > 0.1 % interfere in this assay and may lead to false results.

The following blood components do not have a significant effect (+/- 20 % of expected) on the test results up to the below stated concentrations:

<table>
<thead>
<tr>
<th>Blood Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>8.0 mg/mL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>0.3 mg/mL</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>5.0 mg/mL</td>
</tr>
</tbody>
</table>

17. PERFORMANCE

<table>
<thead>
<tr>
<th>Analytical Specificity (Cross Reactivity)</th>
<th>No cross-reactivities were found to: Measles, Mumps, VZV, EBV(VCA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>Mean (U/mL) CV (%)</td>
</tr>
<tr>
<td>Intra-Assay</td>
<td>52 10.0</td>
</tr>
<tr>
<td>Inter-Assay</td>
<td>53 9.3</td>
</tr>
<tr>
<td>Linearity</td>
<td>Range (U/mL) Serial dilution up to Range (%)</td>
</tr>
<tr>
<td></td>
<td>2.7 - 102 1.8 71 - 127</td>
</tr>
<tr>
<td>Recovery</td>
<td>76 – 126 % % Recovery after spiking (n = 3)</td>
</tr>
<tr>
<td>Method Comparison versus ELISA</td>
<td>Rel. Sensitivity &gt; 95 %</td>
</tr>
<tr>
<td></td>
<td>Rel. Specificity &gt; 95 %</td>
</tr>
</tbody>
</table>
18. PRODUCT LITERATURE REFERENCES


LIABILITY: Complaints will be accepted in each mode -written or vocal. Preferred is that the complaint is accompanied with the test performance and results. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2012-01-20