Herpes Simplex Virus 1 IgA/IgG/IgM (EIA-2532)

Revised 7 May 2007 **RUO in the USA**

Introduction

The **Herpes simplex virus 1 IgA/IgG/IgM** Enzyme Immunoassay Kit provides materials for the quantitative determination of human IgA/IgG/IgM antibodies against Herpes simplex virus 1 (HSV 1) in serum and plasma. This assay is intended for in-vitro use only. In the United States, this kit is intended for Research Use Only.

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 symtoms 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. The major clinical manifestations associated with HSV 1 infections are gingivostomatitis, keratitis, conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and some letal infections of newborns. HSV 1 causes 85% and HSV 2 15% of oral primary infections. Recurrent infection occurs in form of labial fever blisters. After ulceration and scabbing of these blisters complete recovery occurs within 10 days. The central nervous system may be involved in both primary and recurrent infections. 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 simplex virus 1 IgA/IgG/IgM ELISA is based on the principle of the enzyme immunoassay (EIA). Herpes simplex virus 1 antigen (strain MacIntyre) is bound on the surface of the microtiter strips. Diluted patient serum or ready to use standards and controls are pipetted into the wells of the microtiter plate. A binding between the IgA/IgG/IgM antibodies of the serum and the immobilized 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 IgA/IgG/IgM 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 colour development is terminated by the addition of a stop solution, which changes the colour from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgA/IgG/IgM antibodies is directly proportional to the intensity of the colour.

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Precautions

- The assay calibrators and controls are of human origin and have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All standards, however, should be treated as potential biohazards in use and for disposal.
- The assay reagents contain sodium azide or thimerosal, which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
- The stop solution contains H_2SO_4. If it comes into contact with skin, wash thoroughly with water and seek medical attention. Since the H_2SO_4 used to terminate the colour reaction is corrosive, the instrumentation employed to dispense it should be thoroughly cleaned after use.
- This kit is for in vitro use only. In the United States, this kit is intended for Research Use Only.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents, and wash hands thoroughly afterwards. Microbial contamination of reagents or specimens may give false results.

Storage and Stability

Store all reagents at 2 - 8 °C and use before expiry date. When stored at 2 - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Once the foilbag of the coated microtiter strips has been broken, care should be taken to close it tightly again. The immunoreactivity of the coated microtiter strips is stable for approx. 6 weeks in the broken, but tightly closed bag.
Allow all reagents and required number of strips to reach room temperature prior to use.

Contents of the Test Kit

Microtiter Strips break apart strips coated with Herpes simplex virus 1 antigen.	12 x 8 wells
Negative Control 2 ml, ready to use, human serum diluted by PBS, contains no IgA/IgG/IgM antibodies against HSV 1, addition of 0.01 % potassium tetraiodomercurate, refer to label for exact concentration.	1 vial
Cut-Off Standard 2 ml, ready to use, human serum diluted by PBS, contains a low concentration of IgA/IgG/IgM antibodies against HSV 1, addition of 0.01 % potassium tetraiodomercurate, refer to label for exact concentration.	1 vial

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Weakly Positive Control 2 ml, ready to use, human serum diluted by PBS, contains a medium concentration of IgA/IgG/IgM antibodies against HSV 1, addition of 0.01 % potassium tetraiodomercurate, refer to label for exact concentration.	1 vial
Positive Control 2 ml, ready to use, human serum diluted by PBS, contains a high concentration of IgA/IgG/IgM antibodies against HSV 1, addition of 0.01 % potassium tetraiodomercurate, refer to label for exact concentration.	1 vial
Enzyme Conjugate 11 ml, ready to use, anti human IgA/IgG/IgM conjugated to POD, in protein-containing buffer solution.	1 vial
TMB Substrate Solution 11 ml, ready to use, containing a solution of tetramethylbenzidine (TMB).	1 vial
TMB Stop Solution 11 ml, ready to use, 1 M sulphuric acid (H_2SO_4) Avoid contact with stop-solution it may cause skin irritations and burns.	1 vial
Sample Diluent 60 ml, ready to use, PBS/BSA buffer, addition of 0.01 % potassium tetraiodomercurate.	1 vial
Wash Buffer, concentrate (10x) 60 ml, concentrate, containing PBS buffer with Tween 20, dilute 1 : 10 (1 + 9) with distilled water prior to use (e. g. 10 ml concentrate + 90 ml distilled water).	1 vial

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

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

Material required but not provided

- Automatic pipettes to dispense 5, 50, 100 and 500 µl (a multichannel pipetting device such as Titertek is suitable for adding reagents to the wells)
- Distilled water
- Microtiter plate spectrophotometer (ELISA reader) with 450 nm filter
- Microtiter plate washer

Specimen Collection and Storage

- Serum or plasma (EDTA, heparin) should be used, and the usual precautions for venipuncture should be observed. No special sample pretreatment is necessary. The specimen may be stored at 2 - 8 °C for up to 48 hours, and should be frozen at - 20 °C or lower for longer periods
- Repeated freeze - thawing should be avoided.
- Thawed samples should be inverted several times prior to testing.
- Do not use grossly hemolyzed or grossly lipemic specimens.

Preparation of Samples and Reagents

Samples

Dilute patient sample 1 to 101 with ready to use sample diluent (e. g. 5 µl sample + 500 µl buffer).

For IgM:
In order to avoid interference of rheumatoid factors, patient sera should be treated with RF absorbent (# 590 59). Alternatively, positive results can be confirmed in a second test run. Do not treat the controls!

Wash Buffer
Dilute the wash buffer concentrate with distilled water 1 to 10 (1 + 9)
(e. g. 10 ml concentrate + 90 ml distilled water.). If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 °C for
15 minutes.

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Assay Procedure
General Remarks:

It is recommended to use control samples according to state and federal regulations. The use of control sera or plasma is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.

Once the test has been started, all steps should be completed without interruption.

Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination. For the dispensing of the TMB substrate solution and the TMB stop solution avoid pipettes with metal parts.

Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

Leave sufficient microtiter strips in the strip holder to enable the running of standards, controls, and samples in duplicate, plus one well for the substrate blank. Secure the desired number of microtiter strips in the holder.

Pipet **100 µl of standards, controls and diluted samples** into the appropriate wells of the strips. Leave one well empty for the substrate blank.

Cover plate with the enclosed foil and **incubate for 60 minutes** at room temperature (18 - 24 °C).

Washing: discard the incubation solution, rinse the wells 3 x with 300 µl diluted wash buffer (dilute concentrate 1 to 10 with distilled water) and remove any residual.

Add **100 µl of enzyme conjugate** to each well in sequence. Leave one well empty for the substrate blank.

Cover plate with the enclosed foil and **incubate for 30 minutes** at room temperature (18 - 24 °C).

Washing: discard the incubation solution, rinse the wells 3 x with 300 µl diluted wash buffer (dilute concentrate 1 to 10 with distilled water) and remove any residual.

Promptly pipet **100 µl of the TMB substrate solution** into the rinsed wells. This time also the substrate blank is pipetted.

Cover plate with the enclosed foil and **incubate for 20 minutes** at room temperature (18 - 24 °C) in the dark.

Stop the reaction by adding **100 µl** of **TMB stop solution** to each well.

Shake gently the Microtiter Strips being careful not to let the content come from the wells and **read at 450 nm** within 60 minutes from the stopping.

Calculation of Results
Quantitative 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 %.

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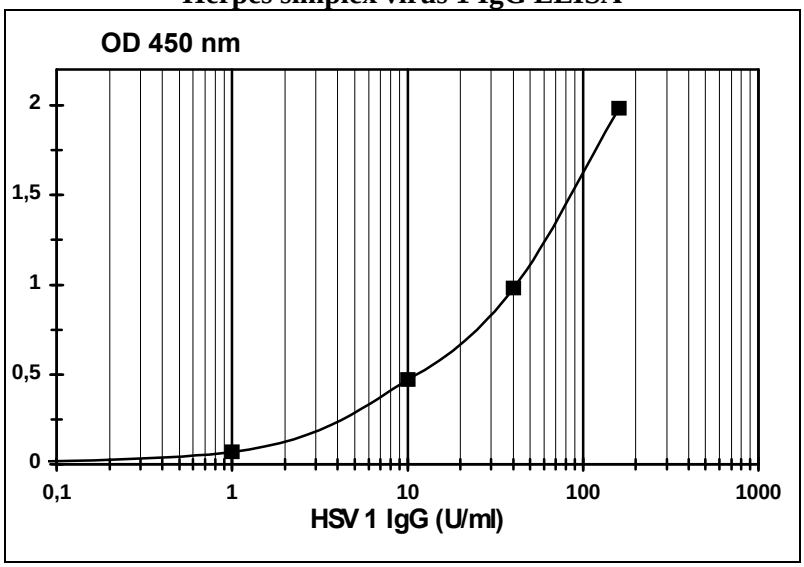
The ready to use standards and controls of the Herpes simplex virus 1 IgA/IgG/IgM ELISA are defined and expressed in Units (U). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards 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, i.e. 4 parameter, spline, logit-log.

Example IgG

Standards	OD Value	corrected OD	Mean OD Value
Substrate Blank	0.022		
Negative Control	0.088/0.093	0.066/0.071	0.069
Cut-Off Standard	0.512/0.530	0.490/0.508	0.499
Weakly Positive Control	1.020/0.985	0.998/0.963	0.981
Positive Control	1.980/2.027	1.958/2.005	1.982

Typical Standard Curve
Herpes simplex virus 1 IgG ELISA



Interpretation

The results of each patient sample can be assessed as follows:

> 12 U/ml	positive
8 - 12 U/ml	borderline
< 8 U/ml	negative

Qualitative Evaluation

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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, should be 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.

Assay Characteristics
Precision
The intra-assay coefficient of variation of the Herpes simplex virus 1 IgA/IgG/IgM ELISA was assessed by a ten-fold determination in a positive serum sample to less than 10 %.

Limitations of use
Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.

Warranty
Any modification of this test as well as exchange or mixture of any components from different lots might influence the results. In such cases there is no claim for a replacement.

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