HSV-1/2-IgG-ELISA PKS medac

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HSV-1/2-IgG-ELISA PKS medac

Enzyme immunoassay with Pipetting Control System for the quantitative detection of IgG antibodies to Herpes simplex virus 1 and 2 (HSV-1/2) in serum and cerebrospinal fluid (CSF)

Cat. no.: 105-PKS

FOR IN VITRO DIAGNOSTIC USE ONLY

INTRODUCTION

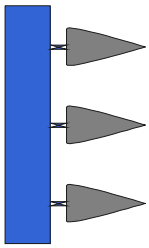
The herpes simplex virus (HSV) belongs to the family of human pathogenic *herpesviridae*. There are two types, HSV-1 and HSV-2, that share 85 % of genetic homology. The envelope glycoprotein G determines type-specificity of the virus. Lifelong latent persistence in the organism after primary infection is typical for HSV. Reactivations occur in about 50 % of latently infected and more frequently in immunocompromised people. Heterologous as well as homologous secondary infections are possible. HSV are common worldwide. In Germany the prevalence of HSV-1 antibodies is more than 90 % in adults whereas nearly 15 % possess antibodies to HSV-2 with increasing tendency.

Virus transfer occurs via mucosa or skin contact. Infections manifest as oro-facial (HSV-1) or genital (mainly HSV-2) herpes. Type-specific localisation of the disease is not obligatory because the two virus types may cause infections in both body regions.

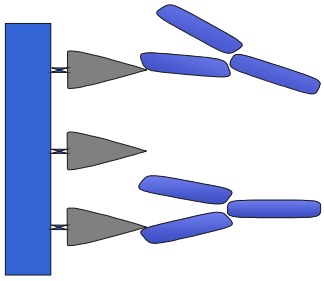
Diagnosis of primary or reactivated HSV infection in the symptomatic/active phase of disease is carried out in most cases by clinical signs or by direct detection of the pathogen. HSV laboratory diagnosis is mainly used in cases of exanthema with unclear genesis, suspected herpes encephalitis, generalised infection of immuno-compromised or newborn patient and genital infection during pregnancy. The determination of antibodies is used preferably for confirmation of immunity as well as for differentiation of early phase of infection from recurrence. Seroconversion of IgG confirms a primary infection. The detection of a HSV-1/2-specific intrathecal antibody synthesis in serum-CSF pairs is a part of differential diagnosis in chronic autoimmune disease of the central nervous system.

The HSV-1/2-IgG-ELISA PKS medac is an assay for the quantitative detection of specific IgG antibodies. The test is also suitable for the detection of a specific antibody index in serum-CSF pairs.

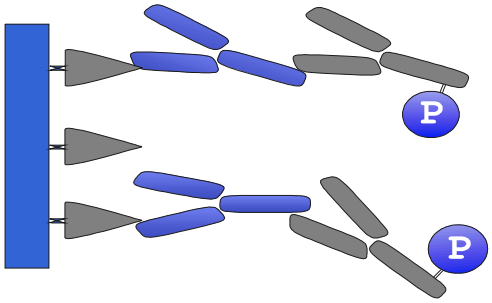
TEST PRINCIPLE



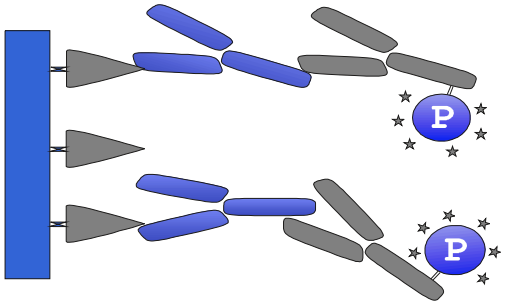
The plate is coated with HSV-1/2-antigen.



The HSV-1/2-specific antibodies from the specimen are selectively bound to the antigen.



Peroxidase-conjugated anti-human IgG antibodies bind to the HSV-1/2-IgG antibodies (P = peroxidase).



Incubation with TMB-substrate (*). The reaction is stopped by the addition of sulfuric acid. The absorption is read photometrically.

Advantages of the test

- ☞ The Pipetting Control System allows to monitor visually each pipetting step through colour changes.
- ☞ The breakable microwell strips permit efficient use of the test.
- ☞ Suitable for automation on open ELISA devices.
- ☞ Single-point quantification, no standard curve needed.
- ☞ No additional calibration curve for diagnostics of CSF needed.

KIT CONTENTS
Cat. no.: 105-PKS

1. **MTP**
   Microplate: 12 x 8 wells, white-coded (with frame and desiccant vacuum sealed in aluminium bag), breakable, U-form, coated with HSV-1/2-antigen, BSA and pH indicator, ready to use.

2. **CONTROL -**
   Negative control: 1 vial with 1.5 ml, human serum, ready to use, contains BSA, phenol, ProClin™ 300 and gentamycine sulfate.

3. **CONTROL +**
   Positive control: 1 vial with 1.5 ml, human serum, ready to use, contains FCS, BSA, phenol, ProClin™ 300 and gentamycine sulfate.

4. **CAL**
   Calibrator: 1 vial with 1.5 ml, human serum, ready to use, contains FCS, BSA, phenol, ProClin™ 300 and gentamycine sulfate.

5. **WB**
   Wash buffer: 1 bottle with 100 ml, PBS/Tween (10x), pH 7.2 – 7.4, contains ProClin™ 300.

6. **VIR-DIL**
   Sample diluent: 1 bottle with 110 ml, PBS/Tween/BSA, pH 7.2 – 7.4, ready to use, contains ProClin™ 300.

7. **CON**
   Conjugate: 3 vials with 4.5 ml each, goat anti-human IgG, HRP-conjugated, ready to use, stained green, contains BSA, phenol, ProClin™ 300 and gentamycine sulfate.

8. **TMB**
   TMB-substrate: 1 vial with 10 ml, ready to use.

9. **STOP**
   Stop solution: 2 vials with 11 ml each, 0.5 M sulfuric acid (H_2SO_4), ready to use.

1. STORAGE AND STABILITY

Material/Reagent	State	Storage	Stability
Test kit	unopened	2...8 °C	until expiry date
Microplate	opened	2...8 °C in bag with desiccant	6 weeks
Controls/ Calibrator	opened	2...8 °C	6 weeks
Wash buffer	diluted	2...8 °C	6 weeks
Sample diluent	opened	2...8 °C	6 weeks
Conjugate	opened	2...8 °C	6 weeks
TMB-substrate	opened	2...8 °C	6 weeks
Stop solution	opened	2...8 °C	until expiry date

Do not use the reagents after the expiry date.

2. REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

2.1. Water for injection (H_2O redist.). Use of deionised water can disturb the test procedure.

2.2. Adjustable micropipettes.

2.3. Clean glass or plastic containers for dilution of wash buffer and specimen.

2.4. Suitable device for microplate washing (e.g. multistepper or ELISA washer).

2.5. Incubator for 37°C.

2.6. Microplate reader with filters for 450 nm and 620 – 650 nm.

3. PREPARATION OF THE REAGENTS

Before starting the test procedure all kit components must be equilibrated to room temperature.

Calculate the number of wells required.

3.1. Microplate

The aluminium bag has to be tightly resealed together with the desiccant after each removal of wells. Storage and stability of the wells are indicated under point 1.

Note: The microplate wells have a light green colour. Eventually occurring greenish brown stains inside the wells are due to the production process and do not influence the test performance.

3.2. Wash buffer

Mix one volume of wash buffer (10x) with nine volumes of water for injection (e.g. 50 ml wash buffer (10x) with 450 ml water). 10 ml of diluted wash buffer are needed for eight wells.

Crystals in the wash buffer (10x) have to be dissolved by warming (max. 37 °C) and/or stirring at RT.

Do not mix test specific reagents (microplate, controls, conjugate, calibrator) from different kit lots. In contrast to that, sample diluent, wash buffer, TMB-substrate and stop solution are generally exchangeable in all virological ELISA medac.

Reagents from other manufactures must not be used in general.

Valid and reproducible results are only obtained if the test procedure is precisely followed.

4. SPECIMEN

4.1. The test is suitable for serum and CSF samples (for the detection of CSF see 8.).

4.2. Pretreatment of sera, e.g. inactivation, is not necessary. However, they should neither be contaminated with microorganisms nor contain any red blood cells.

4.3. Serum has to be diluted 1:200 with sample diluent. We recommend to prepare an initial dilution of 1:50 (e.g. 10 µl serum + 490 µl sample diluent). For further 1:4 dilution just prepare the volume needed. Samples outside the measuring range can be diluted further.

4.4. **The diagnostic investigation of serum-CSF pairs is described in detail under 8.**

5.A. TEST PROCEDURE

5.1. Cut the aluminium bag above the zip fastener and take out the required number of microplate wells (see 3.1.).

Microplate wells are ready to use and do not have to be pre-washed.

5.2. Leave well A1 empty as blank (see 6.A.). Add 50 µl each of the negative control, positive control as well as the diluted sample in single determination and 50 µl of the calibrator in duplicate to the wells.

After pipetting the samples (pH neutral or basic fluid) the wells turn blue/green. A missing colour change in one well indicates that no sample or control has been added.

If necessary, the microplate wells can be kept up to 30 min at RT before proceeding.

5.3. Incubate the microplate wells for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil.

5.4. After incubation wash the microplate wells three times with 200 µl wash buffer per well. Pay attention that all wells are filled. After washing tap microplate wells on filter paper.

Do not allow the wells to dry out! Proceed immediately!

5.5. Add conjugate (coloured green) to each well (except A1).

50 µl of conjugate have to be pipetted into the wells if the test is done manually.

Please note:
When working with automated devices, 60 µl of conjugate have to be pipetted into each well due to a higher evaporation in the incubation chambers of the devices.

The suitability of the test for automated devices was confirmed during the evaluation of the test. Nevertheless we recommend to verify the compatibility of the test with the devices used in the lab.

5.6. Incubate again for 60 min (± 5 min) at 37 °C (± 1 °C) in a humid chamber or seald with incubation cover foil.

5.7. After incubation wash microplate wells again (see 5.4.).

5.8. Add 50 µl of TMB-substrate to each well (also A1) and incubate for 30 min (± 2 min) at 37 °C (± 1 °C) in a humid chamber or sealed with incubation cover foil in the dark. Positive samples turn blue.

5.9. Stop the reaction by adding 100 µl of stop solution to each well (also A1). Positive samples turn yellow.

Clean microplate wells from underneath before the photometric reading and take care that there are no air bubbles in the wells.

The reading should be done within 15 min after adding the stop solution.

5.B. TABLE FOR THE TEST PROCEDURE

	Blank (A1)	Negative control	Positive control	Calibrator	Sample
Neg. control	–	50 µl	–	–	–
Pos. control	–	–	50 µl	–	–
Calibrator	–	–	–	50 µl	–
Sample	–	–	–	–	50 µl
Incubate for 60 min at 37°C, wash 3x with 200 µl wash buffer					
Conjugate	–	50/60 µl*)	50/60 µl*)	50/60 µl*)	50/60 µl*)
Incubate for 60 min at 37°C, wash 3x with 200 µl wash buffer					
TMB-substrate	50 µl	50 µl	50 µl	50 µl	50 µl
Incubate for 30 min at 37°C in the dark					
Stop solution	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric reading at 450 nm (ref. 620 – 650 nm)					

*) manual/automatic procedure (see 5.5.)

6.A. CALCULATION OF RESULTS (VALIDITY)

* Read OD values at 450 nm (reference wavelength 620 – 650 nm).

* Subtract the OD value of the blank (well A1) from all other OD values.

* Lot-specific data

The lot-specific data sheet provided with the kit contains the following information:

- Lot-specific calibration curve
- Curve parameters a and b
- Nominal OD value of the calibrator
- Lower OD limit of the calibrator
- Nominal concentration range (AU/ml) of the positive control

* Validity criteria

- The OD value of the **negative control** has to be **< 0.150.**
- The unit value of the **positive control** has to be within the nominal range indicated in the lot-specific data sheet.
- The mean OD value of the **calibrator** has to be above the lower OD limit indicated in the lot-specific data sheet.
- Additional validity criteria for the detection of serum-CSF pairs see 8.

Repeat the run if the results do not meet the specification.

* Correction of the results

The measured OD values of the positive control and the samples have to be corrected as follows:

$$OD_{corrected} = \frac{\text{Nominal OD value of the calibrator}}{\text{Measured OD value of the calibrator}} \times OD_{measured}$$

* Quantification of the results

The corresponding concentrations of the corrected OD values in AU/ml can be read from the lot-specific calibration curve (see lot-specific data sheet).

Alternatively, the concentrations can be calculated using the following formula:

$$\text{Concentration [AU/ml]} = b / \left(\frac{a}{OD_{corrected}} - 1 \right)$$

Most of the new ELISA readers allow to program the formula, thus enabling an automated data processing.

The measuring range spans from 11 to 200 AU/ml. Samples above have to be interpreted as > 200 AU/ml. These values must not be extrapolated. The samples have to be retested in higher dilutions.

The cut-off is 12,5 AU/ml.

Grey zone = 11 - 14 AU/ml

Attention! Important Note!

Due to the mathematical algorithm of the quantification negative or not defined mAU values can be obtained in the following cases:

- Highly positive samples with corrected OD values ≥ a are calculated as negative or not defined AU values (not allowed division by 0). These samples have to be retested in higher dilutions or have to be interpreted as > 200 AU/ml.

6.B. INTERPRETATION OF RESULTS/LIMITATIONS OF THE METHOD

* Samples with unit values below the lower limit of the grey zone are reported as **NEGATIVE.**

* Samples with unit values within the grey zone are reported as **EQUIVOCAL.** These samples should be retested together with a fresh specimen taken 14 days later in order to determine a titer change.

* Samples with unit values exceeding the upper limit of the grey zone are reported as **POSITIVE.**

* The results should always be interpreted in connection with clinical data, the HSV-1/2-IgM and HSV-2-IgG results and additional diagnostic parameters.

* Cross reactions, caused by antibodies against other herpes viruses, cannot be excluded in single cases.

* Lipaemic and hemolytic sera do not influence the test results.

7. PERFORMANCE CHARACTERISTICS

We determined the following performance characteristics during the diagnostic evaluation.

7.A. SENSITIVITY AND SPECIFICITY

546 sera (300 from the lab of Prof. Dr. Enders and Colleagues, Stuttgart, 46 from the Reference laboratory for HSV/VZV, Jena, and 200 sera of blood donors from Hanover and Suhl) were measured in comparison to the predefinition during the diagnostic evaluation. The nominal results were defined by means of a HSV-1/2-IgG-ELISA in combination with an immunoblot assay.

The results obtained are shown in the table below:

		Nominal results	
	negative	equivocal	positive
HSV-1/2-IgG-ELISA PKS medac			
negative	158	0	3
equivocal	5	1	0
positive	3*	0	376

*one of these samples has been confirmed to be HSV-IgM positive

Specificity = 95.2 %
Sensitivity = 99.2 %

Concordance: 98.0 %

7.B. PRECISION

Sample	**Intra-assay variation**				**Sample**	**Inter-assay variation**			
	mean AU	**SD**	**CV (%)**	**n**		**mean AU**	**SD**	**CV (%)**	**n**
PC	25.4	1.0	4	22	**PC**	28.8	1.0	3	11
N° 1	4.1	0.3	7	22	**N° 6**	4.4	0.2	5	11
N° 2	18.3	0.5	3	22	**N° 7**	20.3	0.5	2	11
N° 3	103.0	4.2	4	22	**N° 8**	93.9	3.6	4	11
N° 4	114.1	3.8	3	22	**N° 9**	100.7	3.9	4	11
N° 5	157.9	6.2	4	22	**N° 10**	154.9	7.7	5	11

PC = positive control

8. DIAGNOSTIC INVESTIGATIONS OF CEREBROSPINAL FLUID (CSF)

The detection of HSV-specific antibody synthesis in the central nervous system (CNS) during the diagnostic investigation of cerebrospinal fluid is an essential part of the differential diagnostic of chronic autoimmune disease involving the CNS.

The determination of the virus-specific antibody index (AI) is not suitable for acute HSV-associated infection with CNS involvement. In this case virus identification with PCR is the method of choice.

In chronic diseases such as multiple sclerosis, increased AI for measles, rubella and/or Varicella-Zoster as well as HSV are found in about 95 % of all cases.

The identification of HSV-specific intrathecal antibody synthesis is performed by estimation of the AI according to Reiber (Reiber 1987, 1999). In order to calculate the AI the following conditions must be fulfilled:

- estimation of the albumin quotient (Q_{alb}) so as to assess the function of the blood-brain-barrier and to calculate the Limes value in patients with elevated IgG quotients ($Q_{tot} > Q_{lim}$)
- estimation of the total IgG quotient (Q_{tot})

8.1. SPECIMEN

8.1.1. The test is suitable for serum-CSF paired samples.

8.1.2. Pre-treatment of sera and CSF samples, e.g., inactivation, is not necessary. However they should neither be contaminated with microorganisms nor contain any red blood cells.

8.1.3. Serum
In addition to the 1:200 dilution for determination of serostatus, the sera are diluted 1:1,000 with sample diluent. To calculate the AI, a dilution should be chosen so that its AU value will lie inside the assay range of 11 – 200 AU (see 6.A. and 8.4.). If the AU values for both dilutions are within this range, the AU value of the 1:1,000 dilution should be chosen to calculate the AI. If the AU values for both dilutions are above 200 AU, the sample has to be further diluted.

8.1.4. Cerebrospinal fluid
The CSF samples are diluted to a standard dilution 1:5 with sample diluent. If the measured antibody concentration is above the measuring range, the sample has to be further diluted (see 8.4.).

Serum and cerebrospinal fluid have always to be assayed in parallel in the same test run (this also applies to repeat measurements).

8.2. TEST PROCEDURE

The further test setup for HSV-1/2-IgG determination in serum-CSF pairs is performed as described under 5.A.

8.3. DETERMINATION OF TOTAL IgG AND ALBUMIN CONCENTRATIONS

In addition to the HSV-1/2-specific IgG determinations, in each sample pair the total IgG concentration and the albumin concentration in serum and CSF have to be determined.

8.4. CALCULATION OF RESULTS/VALIDITY

* Validity

The validity criteria specified under 6.A. are applicable.

Repeat the run if the results do not meet the specification.

The following points also apply to CSF investigations:

- The assay range for sera extends from 11 – 200 AU.
- Sera with an antibody content < 11 AU in a dilution of 1:200 are considered as sero-negative. In such cases the antibody index cannot be determined.
- In extremely rare cases, sero-negative patients may have HSV-specific antibodies in the CSF indicating an intrathecal antibody production. In these cases further differential diagnostic measures are required.
- The assay range for CSF extends from 4 – 200 AU.
- CSF samples from patients with positive serostatus which at the dilution of 1:5 are below the assay range cannot be calculated. In this case an intrathecal HSV-1/2-IgG antibody synthesis is most unlikely.

* Evaluation

- Calculation of AU values see 6.A.
- Calculation of the pathogen-specific IgG quotient (Q_{spec}).

$$Q_{spec} = \frac{\text{AU CSF x dilution CSF}}{\text{AU serum x dilution serum}}$$

- Calculation of the antibody index

  The pathogen-specific index is calculated from the formula:

  1. $AI = Q_{spec}/Q_{tot}$ (for $Q_{tot} < Q_{lim}$)
  2. $AI = Q_{spec}/Q_{lim}$ (for $Q_{tot} > Q_{lim}$)
  3. $Q_{lim} = 0.93 \times \sqrt{Q_{alb}^{2} + 6 \times 10^{-6}} - 1.7 \times 10^{-3}$

8.5. INTERPRETATION OF RESULTS

* AI values from 0.6 – 1.3 are regarded as the normal range.

* AI values > 1.3 and ≤ 1.5 are regarded as borderline.

* **The pathological range is defined as AI > 1.5.**

* AI values < 0.6 point to analytical errors and cannot be interpreted.

GENERAL HANDLING ADVICES

* To avoid cross contamination do not exchange the vials and their screw caps.

* The reagents have to be sealed immediately after use to avoid evaporation and microbial contamination.

* After use, the reagents have to be stored as indicated to guarantee the shelf life.

* After use, all components of the testkit should be stored in the original package, in order to avoid mixing up the reagents of other test systems or lots (see also 3.).

HEALTH AND SAFETY INFORMATION

* The local occupational safety and health regulations have to be regarded.

* Reagents of human origin have been tested and found to be negative for HBsAg, for antibodies to HIV-1/2 and to HCV. Nevertheless, it is strongly recommended that these materials as well as those of animal origin (see kit contents) should be handled as potentially infectious and used with all necessary precautions.

DISPOSAL CONSIDERATIONS

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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