VIROTECH HSV Screen IgG/IgM ELISA
(HSV Screen IgG/IgM ELISA)

Order No.: EC108.00

HSV Screen IgG Liquor/CSF Standards

Order No.: EC108L60

HSV Screen IgG Liquor/CSF AI Ctrl-Set

Order No.: EN108L65

Color Coding: red metallic

FOR IN VITRO DIAGNOSIS ONLY

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1. Intended Use

The VIROTECH HSV Screen IgG/IgM ELISA is used for the semi-quantitative and qualitative detection of specific IgG and IgM antibodies to *Herpes simplex Virus* (HSV-1/2) in human serum. The VIROTECH HSV Screen IgG/IgM ELISA uses a combination of purified HSV 1 and HSV 2 antigens. Differentiation between HSV 1 and HSV 2 is only possible if glycoproteins G are used. If there is a positive finding in HSV screening, we therefore recommend subsequent differentiation with a type-specific ELISA or VIROTECH HSV IgG LINE Immunoblot (gG1, gG2).

The serology is suitable for the detection of the immune status and as Herpes exclusion.

The IgM result must not be observed isolated from the IgG result.

The diagnosis of the genital-herpes must be confirmed with the pathogen detection.

The serology is not suitable for the detection of new-born Herpes, as the immune system of a baby is not completely developed at the date of its birth. However it can be used in retrospect to measure the transplacentally transferred anti-HSV IgG-antibodies.

2. Diagnostic Relevance

Herpes simplex viruses are widely spread throughout the population. The transmitted results from direct contact with infected secretions from either a symptomatic or an asymptomatic host. Therefore the contamination starts already in the early child-age. However these primary infections remain asymptomatical in over 90% of the cases, a latent infection is established in the regional ganglia as a rule. For the understanding of the pathogenesis of HSV-infection the fact that latent persistent viruses in the ganglia cells may be reactivated is of important meaning. The further spreading of the virus is favourabled by the asymptomatical virus expression throughout saliva and genital secretion. In the orafacial area the HSV1-infections prevail, whereas in the genital area the infections are mostly caused by HSV 2. Only a small part (5-30%) is generated by HSV 1 (1).

The genital HSV 1-infections recurrent considerably more rarely than HSV 2-infections. A previous infection with genital HSV1 seems to give a certain protection of infections with HSV 2 respectively allays the symptoms or entirely prevent them(1). A previous oral HSV 1 infection does not protect against a genital HSV 2 infection (2). The clinical picture of genital herpes corresponds those of other ulceration of the sexual organs and has therefore to be differentiated against *Haemophilus durcreyi, Treponema pallidum* and *Chlamydia trachomatis*.

Viral isolation, direct fluorescent antibody (DFA) testing, and serology can be used to diagnose HSV infections. Disadvantages of the first two methods are however, length of culture time, specimen collection and transport difficulties, procedural complexity, and other variables that are associated with DFA and culture (3, 4). However, due to the significant cross-reactivity between HSV 1 and HSV 2, the serological assays, that use virus lysates as antigens, are not sufficiently suited to differentiate HSV 1 infections from HSV 2 infections. Due to the high contamination with HSV 1, the serological status for HSV 2 can be detected hardly reliable with such methods (1).

Intrathecal IgG-antibodies occur only 8 – 10 days after the clinical symptoms in a present Herpes encephalitis. IgM antibodies are not regularly developed, but if so, it is in short term appearance and in very low concentration. This means the serology can be used as a confirmatory tool of the clinical diagnosis retroactively.

Herpes simplex CSF diagnosis

In contrast to the serological diagnosis of HSV infections, what is most important in CSF diagnosis is the reliable detection of endogenous synthesis of pathogen-specific antibodies in the CNS, rather than any differentiation between the pathogen species HSV-1 and HSV-2. As a result of the combination of highly purified HSV-1 and HSV-2 lysate antigens in the VT HSV screening test, this test system provides a very suitable screening test for the CSF diagnosis of HSV infections of the CNS, as it is highly sensitive. A broad spectrum of highly purified HSV antigens is used in the VT-HSV screen. This not only leads to the desired high sensitivity, but also to the equally desirable specificity with respect to differentiation from CNS infections with other neutrotropic pathogens of the herpes virus group.

We therefore recommend that the antibody index (AI) should initially be determined in Herpes simplex diagnostic testing with the HSV Screen ELISA.

If there is the additional aim of achieving differentiation between HSV1 and HSV2 after detection of an HSV-CNS infection, this can be achieved with the help of the two species-specific gG1 and gG2 ELISA tests.

Limits: The level of pathogen-specific antibodies against the gG1 or gG2 epitopes in the CNS at the time when the CSF sample is taken may still (or already) be too low to increase the AI. Therefore, if the gG1 or gG2 test is performed alone this may give

a false negative result in some cases or in specific cases, meaning that the antibody index is neither not calculable, or is normal.

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents (IgG and IgM Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgG negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
7. **IgM negative Control, 1300µl,** human serum with protein-stabilizer and preservative, ready to use
8. **IgM cut-off Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
9. **IgM positive Control, 1300µl**, human serum with protein-stabilizer and preservative, ready to use
10. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
11. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
12. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
13. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- and IgM-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off

control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.

2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgM-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$VE\ (\text{positive control}) = \frac{OD\ (\text{positive control})}{OD\ (\text{cut - off control})} \times 10$$

$$VE\ (\text{patient serum}) = \frac{OD\ (\text{patient serum})}{OD\ (\text{cut - off control})} \times 10$$

9.3 Interpretation Scheme IgG and IgM

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
4. In case of a positive IgM result a review of the result by observing the IgG titercourse is recommended.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
1. Therapy with acyclovir can influence antibody formation (5).
2. Cross-reactions with other members of the herpes group cannot be totally excluded.

10. Performance Data

10.1 Sensitivity and specificity

IgG

The detection of IgG in 336 sera was compared with the VIROTECH HSV 1 (gG1) IgG/IgM ELISA and VIROTECH HSV 2 (gG2) IgG/IgM ELISA.

Sera group (n=336)		VIROTECH HSV Screen IgG/IgM ELISA		
		negative	borderline	positive
Overall evaluation of VIROTECH HSV 1 (gG1) and HSV 2 (gG2) ELISA	negative	83	4	10
	borderline	1	0	4
	positive	5	1	228

10 sera with borderline findings were excluded from the analysis.

On the basis of these results, the sensitivity for IgG was calculated as 97.9% and the specificity as 89.2%.

In addition, the detection of IgG in 336 sera was compared with the VIROTECH HSV LINE.

Sera group (n=336)		VIROTECH HSV Screen IgG/IgM ELISA		
		negative	borderline	positive
Overall evaluation of VIROTECH HSV IgG LINE Immunoblot	negative	68	2	2
	borderline	18	3	11
	positive	3	0	229

34 sera with borderline findings were excluded from the analysis.

On the basis of these results, the sensitivity for IgG was calculated as 98.7% and the specificity as 97.1%.

IgM

The detection of IgM in 344 sera was compared with the VIROTECH HSV 1 (gG1) and HSV 2 (gG2) ELISA.

Sera group (n=344)		HSV Screen ELISA		
		negative	borderline	positive
Overall evaluation of VIROTECH HSV 1 (gG1) and HSV 2 (gG2) ELISA	negative	293	10	28
	borderline	2	2	3
	positive	0	0	6

17 sera with borderline findings were excluded from the analysis.

On the basis of these results, the specificity for IgM was calculated as 91.3%.

Because of the low number of positive sera, the sensitivity for IgM cannot be given.

10.2 Cross-reactivity

IgG

40 potentially cross-reactive sera, with positive serological findings for at least one of the following pathogens - EBV, CMV, VZV, parvovirus and measles - were tested with the VIROTECH HSV 1 (gG1) IgG/IgM ELISA, VIROTECH HSV 2 (gG2) IgG/IgM ELISA ELISA and the VIROTECH HSV IgG LINE Immunoblot.

All sera which were negative in both HSV reference systems were also negative in the HSV Screen ELISA. This gives the specificity of 100% for the above panel.

IgM

40 potentially cross-reactive sera, with positive serological findings for at least one of the following pathogens - EBV, CMV, VZV, parvovirus and measles - were also tested for IgM with the VIROTECH HSV 1 (gG1) IgG/IgM ELISA and VIROTECH HSV 2 (gG2) IgG/IgM ELISA.

2 of 40 sera with negative findings in the HSV reference system gave positive findings in the HSV Screen. This gives a specificity of 95% for the above panel.

10.3 Prevalence (expected values)

IgG

The following table gives the results found with the VIROTECH HSV Screen IgG/IgM ELISA for the selected sera groups. They are compared with epidemiological data taken from the literature.

Sera group (n=256)	Borderline/ Positive in the HSV Screen ELISA	Literature Values for HSV-1	Literature Values for HSV-2
Blood donors (n=80)	77.5 %	62% in the USA 79% in Germany (6)	7.6% in Great Britain 15% in Germany (6)
Children sera (n=40)	32.5 %	30%: 1-5 years 45%: 6-11 years 50%: 12-16 years in Germany (6)	< 2%: 1-5 years < 3%: 6-11 years ca. 8%: 12-16 years in Germany (6)
Prostitute sera (n=40)	95 %	91-95% in Germany (7)	64% for STD patients in the USA (5) 78% in Germany (2)
HIV patient sera (n=16)	100 %	91% in Germany (6)	59% in Germany (2)
Pregnant women sera (n=80)	81.3 %	ca. 90% in Spain and Italy (6)	8.9% in Germany (6)

IgM

The following table gives the results found with the VIROTECH HSV Screen ELISA for the selected sera groups.

Sera group (n=224)	Borderline /Positive in the HSV Screen ELISA	Literature Values for HSV-1 and -2
Blood donors (n=80)	5.1 %	
Children sera (n=40)	7.5 %	
Prostitute sera (n=40)	12.5 %	
HIV patient sera (n=24)	16.7 %	
Pregnant women sera (n=40)	2.5 %	**0.5-2%** in Germany (8)

10.4 Intra-assay variation coefficient (repeatability)

In one assay, strips of various plates of one lot were tested with one serum. This gave a variation coefficient of < 9%.

10.5 Inter-assay variation coefficient (reproducibility)

3 sera were tested in 10 independent tests in different laboratories and with different operators. This gave a variation coefficient of under 15%.

11. Literature

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8. Sauerbrei, A., Wutzler, P. (2006) Herpes simplex and varicella-zoster virus infections during pregnancy: current concepts of prevention, diagnosis and therapy. Part 1: Herpes simplex virus infections. In: *Med Microbiol Immunol*, Springer-Verlag.

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ IgG-Samples – Dilution 1:101

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

▼ IgM-Samples - Dilution 1:101 Rheumafactor-absorption with RF-SorboTech

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation	**30 minutes at 37°C**	**100 µl Patient Samples** blank value (Dilution Buffer) and controls
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Conjugate Incubation	**30 minutes at 37°C**	**100 µl Conjugate** IgG, IgM
⇩		
Wash 4times		**400 µl Washing Solution** Remove Residues on a Cellulose Pad
⇩		
Substrate Incubation	**30 minutes at 37°C**	**100 µl Substrate**
⇩		
Stopping		**50 µl Stopping Solution** shake carefully
⇩		
Measure Extinctions		**Photometer at 450/620nm** **(Reference Wavelength 620-690nm)**