VIROTECH HSV IgG LINE Immunoblot

(HSV IgG LINE-16)

Order No.: WE130G16

(HSV IgG LINE-32)

Order No.: WE130G32

FOR IN VITRO DIAGNOSIS ONLY

Virotech Diagnostics GmbH
Waldstrasse 23 A2
63128 Dietzenbach, Germany

Tel.: +49(0)6074-23698-0
Fax.: +49(0)6074-23698-900
www.goldstandarddiagnostics.com

CE

Freigabedatum22.04.2022 10:07
REV 21 VIROTECH HSV IgG LINE Immunoblot EN

Contents

1. Intended Use

LINE Immunoblot Testkit for the qualitative detection of specific IgG-antibodies against *Herpes simplex Virus* HSV-1 and HSV-2 in human serum. Based on the usage of subtype-specific glycoproteins G1 (gG1) and G2 (gG2) the diagnostical meaningful differentiation between HSV1 and HSV2 is possible.

2. Diagnostic Meaning

Herpes-simplex-virusses (HSV) are widely spread throughout the population. The transmittance 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 virusses 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 HSV2. Only a small part (5-30%) is generated by HSV1 (11, 14).

One of the most serious consequences of genital herpes is neonatal herpes (2). Without therapy, mortality for untreated infants who develop disseminated infection exceeds 70% with half of the survivors developing neurological impairment (14). Almost all neonate HSV2 infections are acquired by passage through an infected birth canal (7). Most mothers (60-80%) who transmit HSV to their children are asymptomatic at delivery (14). Transmission rates are much higher when the mother is experiencing a primary or initial genital infection (50%) (14) versus a recurrent infection (<5%) (4, 5, 9). CDC recommends that *„...prevention of neonatal herpes should emphasize the prevention of acquisition of genital HSV infection during late pregnancy. Susceptible women whose partners have oral or genital HSV infection, or those whose sex partners infection status is unknown, should be counseled to avoid unprotected genital and oral sexual contact during late pregnancy" (7).*

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 (1, 7). However, due to the significant cross-reactivity between HSV1 and HSV2, the serological assays, that use virus lysates as antigens, are not sufficiently suited to differentiate HSV1 infections from HSV2 infections.

Due to the high contamination with HSV1, the serological status for HSV2 can be detected hardly reliable with such methods.

The genital HSV1-infections recurrent considerably more rarely than HSV2-infections. A previous infection with genital HSV1 seems to give a certain protection of infections with HSV2 respectively allays the symptoms or entirely prevent them(10). A previous oral HSV1 infection does not protect against a genital HSV2 infection (14). 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 (7)*. The differentiation between HSV 1- from HSV 2-infection is therefore very important for the determination of the contamination with HSV1 respectively HSV2. This does also mean it is very important for the identification of potential transmittors and especially for the risk assessment and prevention of the Herpes neonatorum.

3. Principle of Test

Proteins of the pathogen-antigen are transferred to the nitrocellulosis membrane by a micro-dispensing method. The nitrocellulosis membrane is then cut into single strips.

Incubation of the antigen-coated nitrocellulose strips with samples of human serum or plasma permits the detection of specific antibodies. These antibodies develop immuncomplexes with the antigen fixed on the test strip. After removing the unbound antibodies by washing steps, the single nitrocellulose-strips are incubated with alcalic phosphatasis conjugated anti-human IgG-antibodies. After unbound conjugated antibodies have been removed by a further washing step, a visualisation of the antigen/antibody-complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue-violet precipitates at each site („antigen bands") where the conjugated anti-human antibodies have bound. The enzyme/substrate-reaction is stopped through washing the nitrocellulose-strips with aqua dest./deionised. Depending on the observed band pattern one can interpret the presence of specific IgG-antibodies.

4. Package Contents

4.1 Kit for 16 determinations

1.	**IgG Nitrocellulose test strips** with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**1x**	16 strips
2.	**IgG Cut off Control,** human serum, prediluted	**1x**	1,0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**1x**	50 ml
4.	**IgG- Conjugate** (100x conc.) **Anti-human**-(goat)-Alcalic Phosphatasis, with preservative	**1x**	0,7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**1x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**1x**	1 pcs.

4.2 Kit for 32 determinations

1.	**IgG Nitrocellulose test strips** with sprayed antigen, (solid strips stabilised on a plastic foil), sorted in a booklet, ready to use	**2x**	16 strips
2.	**IgG Cut off Control,** human serum, prediluted	**1x**	1,0ml
3.	**Dilution-/ washbuffer**, pH 7.3 (10x conc.), with Tris and preservative	**2x**	50 ml
4.	**IgG- Conjugate** (100x conc.) **Anti-human**-(goat)-Alcalic Phosphatasis, with preservative	**1x**	0,7 ml
5.	**Substrate** (BCIP/NBT), ready to use	**1x**	57 ml
6.	**Evaluation Record sheet** for the notation and deposit of the results	**1x**	1 pcs.

Also available on request:

IgG- Positive control, human serum, prediluted, 0.5 ml.
The positive bands > For the cut--off band, refer to the certificate supplied with the kit.
(Order No.: IgG: WE130P60)

IgG- Negative control, human serum, prediluted, 0.5 ml.
The negative control shows no bands or no bands relevant to the evaluation. > Cut-off band.
(Order No.: IgG: WE130N60)

5. Storage and Stability

Store test kit at 2-8°C. The shelf life of the single components is mentioned on the relevant label; for shelf life of the Kit please refer to the Quality Control Certificate.

1. Do not expose the single kit components to high temperature nor freeze them.
2. Do not use the kit reagents after their expiring date.
3. Do not expose reagents to strong light during storage or incubation.
4. The BCIP/NBT-substrate solution is sensitive to light and has to be stored in dark.
5. **Nitrocellulose test strips** : Use strips immediately after taken out of the bag. Close bag with the not required strips again savely and store at 2-8°C. When putting the results into archives please take care that the nitrocellulose test strips and templates are protected against direct sunlight, to avoid fading of the bands.

Material	Status	Storage	Shelflife
Test Samples	Undiluted	+2 to +8°C	1 week
Test Strips	After Opening	+2 to +8°C (stored in supplied bag)	3 months
Controls	After Opening	+2 to +8°C	3 months
Conjugate	After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	ca. 6h
Substrate	After Opening	+2 to +8°C (protect from light)	3 months
Washing Solution	After Opening	+2 to +8°C (protect from light)	3 months
	Final Dilution (ready-to-use)	+2 to +8°C	4 weeks
	Final Dilution (ready-to-use)	*or* room temperature	2 weeks

6. Precautions and Warnings

1. Only sera, that have been tested and found to be negative for HIV1-ab, HIV2-ab, HCV-ab and Hepatitis-B-surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls and conjugate as well as the antigen strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Use plastic forceps and wear protective gloves when handling the Immunoblot.
3. Please follow the local valid waste disposal regulations.
4. The incubation baths are designed by the manufacturer for a single use. The reuse of the incubation baths is at the risk of the user. If they are to be reused we recommend that after use the incubation baths be disinfected for several hours in 1% sodium hypochlorite solution and then rinsed thoroughly with tap water followed by distilled or deionized water.

7. Additionally required material (not supplied)

1. Incubation tray (if required available with order no.: WE300.08)
2. Rocking platform (vertical not centrifugal)
3. A wash bottle for stopping

4. Pipette or handwasher
5. Micro-pipettes 5 µl - 1500 µl
6. Pipette filler
7. Test tubes, 2-20 ml volume
8. Plastic foreceps
9. Aqua dest. or deionised water
10. Filter paper

8. Examination Material

Either serum and plasma may be used as test materials, even when the package leaflet only mentions serum. Plasma samples may contain any anticoagulant.

9. Test Procedure

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

9.1 Preparation of Samples

1. 15 µl serum or plasma are needed for each patient sample.
2. Blood samples should be taken separately by venous puncture. Serum is separated after complete coagulation (not applicable to plasma). If they are to be stored longer sera have to be frozen at -20°C.
3. Repeated freezing and thawing should be avoided.
4. Sera that are heat-inactivated, lipaemic, haemolytic or microbiologically contaminated, may lead to faulty results and shall therefore not be used.
5. Do not use turbid samples (especially after thawing), centrifuge if necessary (5 minutes at 1000sg), pipette clear supernatant and use in testing.

9.2 Preparation of Reagents

1. To facilitate routine laboratory work, all LINEs and EcoBlots can be processed in a single test run with the same incubation times and the same component - when these are independent of the parameters and batches. The cut off controls now have parameter and batch specific values.
2. Bring the corresponding concentrate to room temperature (20-25°C) before preparing the dilution. Use only high quality Aqua dest./deionised and bring up to room temperature (20-25°C) before usage.
3. Mix dilutions well before starting the test.
4. **Dilution-/Washbuffer:**
   The dilution-/washbuffer is provided as a 10-fold concentrate. Dilute the dilution-/washbuffer concentrate 1:10 with distilled or deionised water (10ml/50ml/100ml concentrate + 90ml/450ml/900ml A distilled or deionised water), mix well. Both the concentrated and the diluted dilution/washing buffer may exhibit a yellow colouration. This colouration does not influence the stability of the dilution/washing buffer or the function or the reliability of the diagnostic test.
5. **IgG conjugate**
   Dilute the conjugate 1 + 100 with finally diluted dilution/washing buffer and mix thoroughly. 1.5 ml conjugate working solution is required for each serum sample. See conjugated dilution table (item: "Test Procedure").
6. **Substrate Solution**
   The substrate solution is delivered ready-to-use.

9.3 Immunoblot Test Procedure

Attention: For the correct performance and evaluation of the HSV LINEs, each test run should include the appropriate parameter and batch-specific cut off controls.

1. Test has to be proceeded at room temperature.
2. For each sample put 1 strip into the channel of a clean incubation tray. Hold strip only at the marked upper end.
3. Pipette 1,5ml ready to use **dilution-/ washbuffer** each and put onto the rocking platform. Take care that the antigen strips are consistently covered with liquid, the strips must not dry out during the whole test procedure.
4. The solid antigen strips are being moistured completely within one minute and can be incubated in supine, lateral position or face-down position.
5. **15 µl patient serum or plasma** or **100 µl of the cut-off or positive / negative control** added by pipetting, if at all possible at the upper marked end of the strip. Incubate patient serum and control for **30 minutes** on the rocking platform. Take care that during pipetting and following pour away no cross-contamination of the single patient samples occur.
6. Aspirate or carefully pour away the liquid out of the channels completely. During the pour away of the liquid, the antigen strips remain at the bottom of the channel. Drain the remaining liquid onto a cellulosis paper.

7. **Washing** of strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate washing buffer always completely. Before ending of the last washing step, prepare the needed amount of fresh conjugate dilution (refer to table).
8. Aspirate or pour away the liquid completely out of the channels (please refer to point 6).
9. Pipette 1,5 ml of the prepared **conjugate dilution** each into the corresponding incubation channel and incubate for **30 minutes** on the rocking platform.
10. Pour away or aspirate liquid completely out of the channels.
11. **Washing** of the strips: Incubate with 1,5 ml ready to use dilution-/washbuffer each for **3 x 5 minutes** on the rocking platform. Pour away or aspirate the washbuffer always completely. Afterwards rinse **1 x 1 minute** with **Aqua dest./deionised.**
12. Pour away or aspirate the liquid completely out of the channels (refer to point 6).
13. Pipette 1,5 ml ready to use **substrate solution** each into the channels and allow to develop **10 ± 3 minutes** on the rocking platform.
14. **Stop** the color reaction by pouring away the substrate solution. Afterwards wash the strips without incubation in between for **3 x** with 1,5 ml **Aqua dest./deionised** each.
15. Pour away the aqua dest./deionised and let the strip dry on a clean cellulosis paper. The background-coloring, that may be observed on the moistured antigen strips disappears completely when the strips are completely dry. Solid antigen strips need a little longer than the conventional antigen strips until they are completely dry.
16. Use the included protocol sheet for the interpretation. The inscription of the high-specific bands on the protocol sheet facilitate the interpretation of the patient samples for you.

For test procedure scheme pls. refer to last page

9.4 Use of Immunoblot-processors

The following instruments have been validated for the automatic processing of the Blots and LINEs: Apollo and Profiblot. All commercially available Blot machines are suitable in principle.

10. Interpretation of Results

For a secure interpretation each LINE is fitted out with two controls:

1. **Serum control:**
   Only after the incubation with patient serum the serum incubation band appears below the markline.
2. **Conjugate control:**
   The LINE strip is fitted out with a conjugate control band which appears after incubation with the conjugate.

The test procedure is valid, if the serum control as well as the internal conjugate control appears clearly visible on the developed antigen strip. Please refer to the protocol sheet for the information of the exact position of the serum- and the conjugate control.

10.1 Interpretation of the patient samples

Please refer to the protocol sheet for position and denotation of reactive bands.
IgG bands: HSV, gG1, gG2

10.2 Usage of the Cut-Off Control

Cut-off band to evaluate the HSV total antigen, gG1 and gG2 bands: gG2 band of the cut-off control

Assessment of the band intensities:

1.	gG1 and gG2 band:	bands < gG2-band of cut-off contol:	negative (-)
		bands = gG2-band of cut-off contol:	borderline
		bands > gG2-band of cut-off contol:	positive (+)
2.	HSV-total antigen:	Bands < gG2 band of the cut-off control:	negative (-)
		Bands ≥ gG2 band of the cut-off control:	positive (+)

10.3 Meaning of the Antigens

Antigen / Description	Meaning of the antigen	Specificity of the Antibodies with the LINE
HSV-total antigen	Native HSV1- and HSV2-total antigen.	Specific
gG1	Recombinant, species specific glycoprotein of Baculovirus-system.	High specifc for HSV1

gG2	Affinity chromatographically purified species specific glycoprotein.	High specific for HSV2

10.4 Interpretation criteria

The interpretation of the serological result shall always include the clinical picture, epidemiological data and further diagnostical parameter.

Recommended interpretation

If only the entire HSV antigen band, only the gG1 band, or only the gG2 band is detected, the result is to be evaluated as implausible and should be verified using a different methodology.

Assessment of bands			**Overall interpretation**	
HSV total antigen (*)	**gG1**	**gG2**	**HSV-1**	**HSV-2**
-	-	-	negative	negative
only one isolated band			implausible	implausible
+	borderline	-	borderline	negative
+	-	borderline	negative	borderline
+	borderline	borderline	borderline	borderline
+	**+**	-	positive	negative
+	-	**+**	negative	positive
+	borderline	**+**	borderline	positive
+	+	borderline	positive	borderline
+	+	+	positive	positive

10.5 Limits of the Test

1. A negative Blot result does not completely exclude the possibility of an infection with HSV1 and/or HSV2. The sample may be taken before the occurance of antibodies, or the antibody titre exists below the detection limit of the test. We recommend IgM-serology in these cases.
2. The therapy with Acyclovir may influence the antibody development (15).
3. The genetic variability of the gG2 protein may lead to gG2 negative HSV2 strains.
4. In rare cases patients may show „inverse"-bands (dark background, white bands), these are not to be considered, means the Immunoblot can not be assessed in such cases. The serum should be checked using other serological methods.

11. Performance Data

11.1 Sensitivity and Specificity compared to Western Blot

Following sera collectives were tested to determine the sensitivity and specificity:
prostitutes (n=30), children (n=10), autoimmune sera (n=3), sera from patients with suspected HSV-infection (n=82).
A Western Blot was used as reference method (finding):

Sera collective (n = 125) 5 unclear sera		**VIROTECH HSV IgG LINE Immunoblot**	
		HSV1 negative	**HSV1 positive**
Finding (Western Blot)	**HSV1 negative**	41	3
	HSV1 positive	4	72

Sera collective (n = 125) 3 unclear sera		**VIROTECH HSV IgG LINE Immunoblot**	
		HSV2 negative	**HSV2 positive**
Finding (Western Blot)	**HSV2 negative**	37	2
	HSV2 positive	7	76

The thus calculated sensitivity/specificity is:

	HSV1	**HSV2**

Sensitivity:	94,7 %	91,6 %
Specificity.	93,2 %	94,9 %

11.2 Sensitivity and Specificity compared to ELISA

Following sera collectives were tested to determine the sensitivity and specificity:
prostitutes (n=30), pregnant women (n=74/75) children (n=58), blood donors (n=69), autoimmune sera (n=14), sera from proficiency testings (n=20), HIV-sera (n=76), potentially cross-reactive sera (n=18), sera from patients with suspected HSV-infection (n=82).
An ELISA was used as reference method (finding):

Sera collective (n = 441) 24 unclear sera		**VIROTECH HSV IgG LINE Immunoblot**	
		HSV1 negative	**HSV1 positive**
Finding (ELISA)	**HSV1 negative**	120	6
	HSV1 positive	8	283

Sera collective (n = 442) 13 unclear sera		**VIROTECH HSV IgG LINE Immunoblot**	
		HSV2 negative	**HSV2 positive**
Finding (ELISA)	**HSV2 negative**	282	6
	HSV2 positive	6	135

The thus calculated sensitivity/specificity is:

	HSV1	**HSV2**
Sensitivity:	97,3 %	95,7 %
Specificity.	95,2 %	97,9 %

11.3 Diagnostical Sensitivity and Specificity

52 respectively 53 clinically defined HSV-sera were tested to determine the diagnostical sensitivity and specificity.

Sera collective (n = 53) 3 unclear sera		**VIROTECH HSV IgG LINE Immunoblot**	
		HSV1 negative	**HSV1 positive**
Diagnostical Finding	**HSV1 negative**	16	0
	HSV1 positive	2	32

Sera collective (n = 52) 1 unclear serum		VIROTECH HSV IgG LINE Immunoblot	
		HSV2 negative	**HSV2 positive**
Diagnostical Finding	**HSV2 negative**	10	0
	HSV2 positive	3	38

The thus calculated diagnostical sensitivity/specificity is:

	HSV1	**HSV2**
Diagnostical Sensitivity:	94,1 %	92,7 %
Diagnostical Specificity.	100,0 %	100,0 %

The in comparison to the sensitivity little lower diagnostical sensitivity can be explained by the serum panel. As these sera also include sera with primary infections, it might be that the IgG antibody development has not yet taken place in each case.

11.4 Cross-Reactivity

The purified native gG2- and the purified recombinant gG1-protein used are high specific.

11.5 Prevalence (expected values)

The following table shows the results of certain sera collectives obtained by VIROTECH Diagnostics. The results show a good correlation with the epidemiologic data described in literature.

Sera collective (HSV1/HSV2)	**HSV LINE HSV1 positive**	**Referemce HSV1**	**HSV LINE HSV2 positive**	**Reference HSV2**
Blood donors (n= 69)	78,3 %	80% in Germany (15) 75% in Germany (10) 80% in Switzerland (6)	8,7 %	15% in Germany (15) 14-18% in Germany (10) 19% in Switzerland (6)
Pregnant women (n=79/78)	79,7 %	70% in Netherlands (13)	6,4 %	-
Children's sera (n=85/82)	28,0 %	30% : 1- 5 years-old 50% : 12-16 years-old (12)	0%	1-5 years <2% 6-11 years: <3% 11-16 years approx. 8% (12)
Prostitutes (n=35/35)	85,7 %	-	71,4 %	78% in Germany (10) 49% in Switzerland (8)
HIV sera (n= 76/76)	88,2 %	91% in Germany (15)	71,1 %	60% (10)

The data correspond well with the references, this is a notice to a good specificity and sensitivity of the test.

11.6 Intra-Assay-Precision (Repeatability)

For the determination of the repeatability, 32 blot strips of a non-cut Nitrocellulose-membrane have been incubated in a first examination testrun with the cut-off control and in a second examination testrun with the positive control. The bands show an uniform intensity on the whole nitrocellulose-sheet.

11.7 Inter-Assay-Precision (Reproducibility)

The determination of the test precision has been effected in 10 independent test runs, both, manually and using automates and all proceeded by different persons.

For HSV-1 a negative serum, a low positive serum and a positive serum have been tested in IgG and for HSV-2 a negative serum, a negative-borderline serum and a positive serum have been tested in IgG.

	HSV1
negative	10 neg.
low positive	10 pos.
positive	10 pos.

	HSV2
negative	10
negative-borderline	5 neg. / 5 borderline
positive	10 pos.

12. References

1. Anzivino, E, D Fioriti, M Mischitelli, A Bellizzi, V Barucca, F Chiarini, V Pietropaolo. 2009. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. Virol J. 6,40
2. Arvin, A, C Prober. 1995. Herpes Simplex Viruses. 876-883. In Murray, P, E Baron, M Pfaller, F Tenover, and R Yolkenet (eds.). Manual of Clinical Microbiology. 6th Ed. ASM, Washington, D.C.
3. Bernstein, DI, LR Stanberry, CJ Harrison, JC Kappes, MG Myers. 1986. Antibody response, recurrence patterns and subsequent herpes simplex virus type 2 (HSV-2) re-infection following initial HSV-2 infection of guinea-pigs: effects of acyclovir. J Gen Virol. 67, 1601-1612
4. Brown ZA, Benedetti J, Ashley R, Burchett S, Selke S, Berry S, Vontver LA, Corey L.. 1991.Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. N Engl J Med. 324, 1247-1252
5. Brown, Z , S Sleke, J Zeh, J Kopelmann, A Maslow, R Ashley, D Watts, S Berry, M Herd, L Correy. 1997. The acquisition of herpes simplex virus during pregnancy. N Engl J Med. 337, 509-515
6. Bünzli, D, Wietlisbach V, Barazzoni F, Sahli R, Meylan PR. 2004. Seroepidemiology of Herpes Simplex virus type 1 and 2 in Western and Southern Switzerland in adults aged 25–74 in 1992–93 : a population-based study. BMC Infect Dis. 4. http://www.biomedcentral.com/1471-2334/4/10
7. CDC. 1998. Guidelines for Treatment of Sexually Transmitted Diseases. MMWR. 47, 1-118
8. Eing, BR Lippelt L, Lorentzen EU, Hafezi W, Schlumberger W, Steinhagen K, Kühn JE. 2002. Evaluation of confirmatory strategies for detection of type-specific antibodies against herpes simplex virus type 2. J Clin Microbiol. 40, 407-413.
9. Prober, C, W Sullender, L Yasukawa, D Au, A Yaeger, A Arvin. 1987. Low risk if herpes simplex virus infections in neonates exposed to the virus at the time of vaginal delivery to mothers with recurrent herpes simplex virus infections. N Engl J Med. 316, 240-244
10. Rabenau HF, Buxbaum S, Preiser W, Weber B, Doerr HW. 2002. Seroprevalence of herpes simplex virus types 1 and type 2 in the Frankfurt am Main area, Germany. Med Microbiol Immunol. 190, 153-160
11. Roizman, B, DM Knipe. 2001. Herpes Simplex Viruses and Their Replication. . In Fields, B, D Knipe, P Howley, et al. (eds.). Fields Virology 4th Ed. Lippincott-Raven, Philadelphia
12. Smith J, N Robinson. 2002, Age-specific prevalence of infection with herpes simplex : a global review. J Inf Dis. 186, 3-28
13. Tunbäck, P, Bergström T, Claesson BA, Carlsson RM, Löwhagen GB. 2007. Early acquisition of herpes simplex virus type 1 antibodies in children-A longitudinal serological study. J Clin Vir. 40, 26-30
14. Whitley, R. 2001. Herpes Simplex Viruses. In Fields, B, D Knipe, P Howley, et al. (eds.). Fields Virology 4th Ed. Lippincott-Raven, Philadelphia
15. Wutzler, P, Doerr HW, Färber I, Eichhorn U, Helbig B, Sauerbrei A, Brandstädt A, Rabenau HF. 2000. Seroprevalence of herpes simplex virus type 1 and type 2 in selected German populations - relevance for the incidence of genital herpes. J Med Virol. 61, 201-207

13. Test Procedure Scheme

Test Procedure in short version

Samples Incubation	**30 minutes**	15 µl Patient serum/ plasma/ 100 µl control in 1,5 ml dilution-/washbuffer each
Washing	**3 x 5 minutes**	with 1,5 ml dilution-/washbuffer each
Conjugate incubation	**30 minutes**	with 1,5 ml working dilution (1 + 100)
Washing	**3 x 5 minutes** **1 x 1 minute**	with 1,5 ml dilution-/washbuffer each with Aqua dest./deionised
Substrate incubation	**10 ± 3 minutes**	with 1,5 ml ready to use substrate solution each
Stopping	**3 x without incubation in between**	with 1,5 ml Aqua dest./deionised each

Conjugate Dilution table (rounded)

Number of strips	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Dilution-/washbuffer	1,5ml	3,0ml	4,5ml	6,0ml	7,5ml	9,0ml	11,0ml	12,0ml	14,0ml	15,0ml
Conjugate-concentrate	15µl	30µl	45µl	60µl	75µl	90µl	110µl	120µl	140µl	150µl
Final volume	1,515ml	3,03ml	4,545ml	6,06ml	7,575ml	9,09ml	11,11ml	12,12ml	14,14ml	15,15ml

Number of strips	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Dilution-/washbuffer	17,0ml	18,0ml	20,0ml	21,0ml	23,0ml	24,0ml	26,0ml	27,0ml	29,0ml	30,0ml
Conjugate-concentrate	170µl	180µl	200µl	210µl	230µl	240µl	260µl	270µl	290µl	300µl
Final volume	17,17ml	18,18ml	20,2ml	21,21ml	23,23ml	24,24ml	26,26ml	27,27ml	29,29ml	30,3ml

Number of strips	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Dilution-/washbuffer	32,0ml	33,0ml	35,0ml	36,0ml	38,0ml	39,0ml	41,0ml	42,0ml	44,0ml	45,0ml
Conjugate-concentrate	320µl	330µl	350µl	360µl	380µl	390µl	410µl	420µl	440µl	450µl
Final volume	32,32ml	33,33ml	35,35ml	36,36ml	38,38ml	39,39ml	41,41ml	42,42ml	44,44ml	45,45ml

Number of strips	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
Dilution-/washbuffer	47,0ml	48,0ml	50,0ml	51,0ml	53,0ml	54,0ml	56,0ml	57,0ml	59,0ml	60,0ml
Conjugate-concentrate	470µl	480µl	500µl	510µl	530µl	540µl	560µl	570µl	590µl	600µl
Final volume	47,47ml	48,48ml	50,5ml	51,51ml	53,53ml	54,54ml	56,56ml	57,57ml	59,59ml	60,6ml