NovaLisa®
Herpes simplex Virus 2 (HSV2) IgG ELISA
(HSV2G0540)

Performance Characteristics

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1 Introduction

Herpes simplex is an enveloped DNA virus (150-200 nm in diameter) belonging to the alpha-herpesviridae. Based on antigenic, biochemical and biologically differences it can be divided into two serotypes, HSV-1 and HSV-2. Man is the only known natural host and source of the virus. HSV type 1 typically causes oral herpes, while HSV type 2 typically affects the genital area. Most of the time, HSV-1 and HSV-2 are inactive, or “silent”, and cause no symptoms, but some infected people have “outbreaks” of blisters and ulcers. Once infected with HSV, people remain infected for life. Herpes simplex viruses are amongst the most common infectious agents of man, and either HSV type appears to be capable of infecting similar body sites. A high percentage of the adult populationis seropositive (appr. 90% HSV-1, in dependence on the socio-economic status 10-30% HSV-2). Primary HSV-1 infection usually occurs in early childhood (6 to 18 months of age). HSV-2 usually produces mild symptoms, and most people have no recognized symptoms. Persons at risk are children with inherited T-cell deficiencies and patients who are immunosuppressed because of infection (e.g. HIV), transplantation, or cancer therapy.

HSV-2 can cause potentially fatal infections in infants if the mother is shedding virus at the time of delivery. - HSV-2 may play a major role in the heterosexual spread of HIV: herpes can make people more susceptible to HIV infection, and HIV-infected individuals more infectious.

Species	Disease	Symptoms (e.g.)	Transmission route
HSV-1 HSV-2	Oral herpes (herpes labialis) Genital herpes (herpes genitalis) Neonatal herpes (Herpes neonatorum)	Fever, sore throat, lymphadenopathy, Multiple vesicles in the oral/genital mucous membranes, gingivostomatitis Complication: HSV can cause potentially fatal infections in infants if the mother has a primary infection at the time of delivery	Transmission by droplet infection; Sexually transmitted disease (STD); Perinatal; Congenital

The presence of pathogen or infection may be identified by

- Microscopy
- PCR
- Serology: e.g. by ELISA

2 Intended Use

The Herpes Simplex Virus 2 (HSV 2) IgG ELISA is intended for the qualitative determination of IgG class antibodies against Herpes Simplex Virus 2 (HSV 2) in human serum or plasma (citrate, heparin).

3 Principle of the Assay

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour.
Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Material

NovaLisa® Herpes simplex Virus 2 IgG
Production date: 2015-11
Positive and negative samples

Lot: HSV2G-082-1
Expiry date: 2017-05-31

Test Description

The reproducibility of the NovaLisa® HSV 2 IgG ELISA kit was determined by comparing a minimum of 24 replicates of 3 different samples in one assay (within-run) and by comparing 3 different samples assayed in 12 different runs (between-run).
Acceptance Criterion: CV < 15 %

Results

Table 1: Within-Run Precision

Sample	n	Mean (E)	CV [%]
#1	24	0,648	5,63
#2	24	1,332	3,92
#3	24	1,081	2,62

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
#1	12	21,84	4,25
#2	12	17,43	8,48
#3	12	4,03	8,97

Conclusion

The acceptance criterion was met for all samples.

4.2 Analytical Specificity

4.2.1 Interference from Hemoglobin, Bilirubin and Triglycerides

Introduction

Increased concentrations of possible interference materials such as bilirubin, triglycerides and hemoglobin may interfere with immunoassays creating false-negative or false-positive results.
In order to investigate this topic a literature research in combination with investigation of nearly 100 parameters were performed.

Material and Test Condition

Different members of the NovaLisa® ELISA line were used including assays for the detection of different antibody isotypes (IgA, IgG, IgM, IgG + IgM) to bacteria, viruses, fungi, parasites and worms as well as an antigen ELISA for the detection of TSH.

Defined positive resp. negative or equivocal samples were used.

A certain amount of the potentially interfering substance was added to each sample. The final concentration of each substance was in a pathological range as also described by competitors (10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides). The results obtained with the sample with added “interfering substance” should be 60-140 % of the result of the untreated sample in order to fulfil the specifications.

Conclusion

The internal specifications of 60-140 % were always fulfilled.
Interferences with hemolytic, lipemic or icteric samples were not observed up to a concentration of 10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides.
These results are also in agreement with literature data.

Dimeski, G. (2008) Interference Testing, Clin Biochem Rev 29, S43 – S48
Tate, J. and Ward, G. (2004) Interferences in Immunoassay, Clin Biochem Rev 25, 105 – 120

4.2.2 Cross-Reactivity

A panel of 18 specimens from patients with confirmed diseases other than Herpes Simplex Virus 2 was tested to establish the analytical specificity of the NovaLisa® Herpes Simplex Virus 2 IgG ELISA.
The specimens were from patients infected with pathogens that may cause similar signs and symptoms to those observed for Herpes Simplex Virus 2 or from individuals with diseases or conditions that have the potential for cross-reactivity.

Material

NovaLisa® Herpes Simplex Virus 2 IgG — Lot: HSV2G-002
Production date: 2001-10 — Expiry date: 2002-11
18 potentially cross-reactive samples

Results

Table 3: Cross-Reactivity

Disease Type	Sample	NTU	Evaluation
Samples positive for:			
CMV / EBV / VZV / RSV / Influenza A	1	6,7	neg
CMV / EBV / VZV / RSV / Influenza A	2	5,8	neg
CMV / EBV / VZV / RSV / Influenza A	3	8,1	neg
CMV / EBV / VZV / RSV / Influenza A	7	3,3	neg
CMV / EBV / VZV / RSV / Influenza A	8	7,5	neg
CMV / EBV / VZV / RSV / Influenza A	9	5,2	neg
CMV / EBV / VZV / RSV / Influenza A	11	4,2	neg
CMV / EBV / VZV / RSV / Influenza A	12	6,1	neg
CMV / EBV / VZV / RSV / Influenza A	13	7,5	neg

Table 4: Cross-Reactivity

Disease Type	Sample	NTU	Evaluation
Samples positive for:			
HSV 1	7280	3,6	neg
HSV 1	6	4,5	neg
HSV 1	7	4,5	neg
HSV 1	10	6,9	neg
HSV 1	80	7,5	neg
HSV 1	21262859	2,3	neg
HSV 1	21260618	1,3	neg
HSV 1	21262670	1,8	neg
HSV 1	21262870	2,5	neg

Summary of the Results

Table 5: Summary

Pathogen/Disease Type	Total Specimens	Positive Result
CMV / EBV / VZV / RSV / Influenza A	9	0/9
HSV 1	9	0/9
Total	18	0/18

Conclusion

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters (antibodies to several infectious agents) did not reveal evidence of false-positive results due to cross-reactions.

4.3 Diagnostic Sensitivity and Specificity

Introduction

The purpose of this study was to determine the efficiency of the assay to discriminate between positive and negative clinical samples.
The evaluation of the diagnostic performance of the NovaLisa® Herpes simples Virus 1 IgG ELISA was carried out internally in comparison to ELISA test systems from DiaSorin, to well defined sera from Labor Limbach and to External Quality Control Schemes.
An external evaluation of the diagnostic performance of the NovaLisa® Herpes simples Virus 1 IgG ELISA was performed at University hospital, Jena (Germany)
(Sauerbrei, A. and Wutzler, P.; Novel recombinant ELISA assays for determination of type-specific IgG antibodies against HSV-1 and HSV-2; J. Virol. Methods (2007) 144, 138-142)
Testing site: University hospital Jena, Germany
Principle investigator: PD Dr. Andreas Sauerbrei

Material

NovaTec ELISA HSV 2 IgG
DiaSorin HSV 2 IgG ELISA
Premier™ HSV-2 IgG type specific ELISA, Gull Laboratories/ Meridian Diagnostics
HerpesSelect™ 1 and 2 IgG Immunoblot from MRL Diagnostics/ Focus Technologies
in-house western blot based of lysates from HSV-2 infected cells
Testing site: University hospital Jena, Germany
Principle investigator: PD Dr. Andreas Sauerbrei

Total number of samples: 413

Results (Summary)

Table 6: Diagnostic sensitivity and specificity

		competitor		
NovaLisa® HSV 2 IgG		positive	negative	**Σ**
	positive	144	6	**150**
	negative	1	262	**263**
	Σ	**145**	**268**	**413**

Diagnostic Sensitivity: 99.31% (95%-width of CI: 96.22% .. 99.98%)
Diagnostic Specificity: 97.76% (95%-width of CI: 95.19% .. 99.17%)
Agreement: 98.3 %

Conclusion:

The acceptance criteria are met.