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AESKULISA®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA® Herpes Simplex Virus IgG / IgM

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Content

1 Intended Use 1
2 Diagnostic Relevance 1
3 *AESKULISA*® Test Principle 2
4 Antigen 2
5 *AESKULISA*® Test Components 2
6 Material required, but not provided 3
7 Storage and Stability 3
8 *AESKULISA*® Test Procedure 4
8.1 General Directions for Use 4
8.2 Preparation of Reagents 4
8.2.1 Microtiter Strips (ready-to-use) 4
8.2.2 Calibrators (ready-to-use) 4
8.2.3 Controls (ready-to-use) 5
8.2.4 Sample Buffer (5x conc.) 5
8.2.5 Wash Buffer (50x conc.) 5
8.2.6 Anti-Human IgA, IgG or IgM POD Conjugate (ready-to-use) 5
8.2.7 Substrate (ready-to-use) 5
8.2.8 Stop Solution (ready-to-use) 5
8.3 Preparation of Samples 5
8.3.1 Sample Material 5
8.3.2 Sample Dilution 5
8.3.3 Pre-Absorption of Rheumatoid Factors with *AESKULISA*® IgM 5
8.3.4 Sample Storage 6
8.4 Test Performance 6
8.4.1 Pipetting Scheme 6
8.4.2 Test Procedure 7
8.5 Automated Test Procedure 8
8.6 CSF Diagnostics 8
9 *AESKULISA*® Test Evaluation 9
9.1 Standardization 9
9.2 Quantitative Evaluation 9
9.3 Borderline Range 9
9.4 Measurement Range 9
9.5 Qualitative Evaluation 9
9.6 Criteria of Validity 10
9.7 Interpretation of Test Results 10
10 *AESKULISA*® Performance Characteristics 11
10.1 Analytical Sensitivity and Specificity 11
10.2 Diagnostic Sensitivity and Specificity 12
10.3 Reference Range of Healthy Individuals 12
10.4 Precision 12
11 Safety Measures 14
11.1 Recommendations and Precautions 14
11.2 Disposal 14
12 References 15
13 Regulatory Symbols 16

1 Intended Use

The *AESKULISA*® Herpes Simplex Virus 1/2 IgG und IgM tests are qualitative and quantitative immunoassays for the demonstration of human IgG or IgM antibodies in serum or plasma directed against the Herpes Simplex Viruses 1 and 2. The *AESKULISA*® Herpes Simplex Virus 1/2 IgM test supports the detection of acute infections. The *AESKULISA*® Herpes Simplex Virus 1/2 IgG serves for confirmation of a contact with the pathogen and supports in immune status determination.

The *AESKULISA*® Herpes Simplex Virus 1 (gG1) IgG and *AESKULISA*® Herpes Simplex Virus 2 (gG2) IgG tests are qualitative and quantitative immunoassays for the demonstration of human IgG antibodies in serum or plasma directed against Herpes Simplex Virus 1 (HSV 1) or Herpes Simplex Virus 2 (HSV 2). The *AESKULISA*® Herpes Simplex Virus 1 (gG1) IgG and *AESKULISA*® Herpes Simplex Virus 2 (gG2) IgG tests support in immune status determination as well as in determination of the infecting HSV serotype.

Additionally, the *AESKULISA*® Herpes Simplex Virus 1/2 IgG, *AESKULISA*® Herpes Simplex Virus 1 (gG1) IgG and *AESKULISA*® Herpes Simplex Virus 2 (gG2) IgG tests serve for the determination of intrathecally synthesized antibodies in cerebrospinal fluid.

The interpretation of test results has to be considered in combination with the patient's clinical picture. A diagnosis should not be based on the results of the performed test only, but should be made after all clinical and laboratory findings have been evaluated. For confirmation, further investigations should be carried out. *AESKULISA*® immunoassays are designed exclusively for *in vitro* diagnostic use by qualified personnel only, who are trained, specially advised and familiar with the laboratory methods.

2 Diagnostic Relevance

The Herpes Simplex Virus 1 (HSV 1) and Herpes Simplex Virus 2 (HSV 2), also referred to as Human Herpes Virus 1 (HHV 1) and Human Herpes Virus 2 (HHV 2), are ubiquitous human pathogenic viruses belonging to the family of *Herpesviridae*. Transmission of Herpes Simplex Virus 1 (HSV 1) occurs primarily by saliva transfer and smear contact. Consequently, individuals with *Herpes labialis* present a high risk of infection. Transmission of Herpes Simplex Virus 2 (HSV 2) is strongly associated with mucous membrane contact. Therefore, sexual activity plays a significant role in the spread of the virus.

Herpes Simplex Viruses are responsible for a variety of diseases, ranging from *Herpes labialis* and *Herpes genitalis* infections, to Herpes simplex encephalitis and – in neonates – *Herpes neonatorum*. Some 90 % of primary infections with Herpes Simplex Virus 1 (HSV 1) are subclinical whereby the remaining 10 % may present with conjunctival and corneal inflammation as well as the characteristic blisters on the lips (*Herpes labialis*). Such pustular eruptions may spread and become life threatening in eczema sufferers. Further complications may be encephalitis or meningoencephalitis. Around 12 % of primary infections with Herpes Simplex Virus 2 (HSV 2) are clinically apparent with spontaneous abortion, vulvovaginitis, or penile involvement (*Herpes genitalis*). Following infection, the Herpes Simplex Viruses persist life-long in the body. Following a latent phase, declining immunity may allow the viruses to reactivate.

For laboratory confirmation of Herpes Simplex Virus infections, direct and indirect detection methods are available. In routine diagnostic services, indirect demonstration of Herpes Simplex Virus infections is performed by the serological demonstration of HSV specific IgG and IgM antibodies.

3 *AESKULISA*® Test Principle

The *AESKULISA*® (AESKU Enzyme Linked Immunosorbent Assay) is an immunoassay, which is particularly suited to the determination of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the *AESKULISA*® microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in the patient´s sample are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme peroxidase, detects and binds to the immune complex. A colorless substrate is then converted into the colored product. The signal intensity of this reaction product is proportional to the antibody activity in the sample and is measured photometrically.

4 Antigen

Antibody detection with *AESKULISA*® Herpes Simplex Virus 1/2 IgG und IgM immunoassays is based on a mixture of purified and inactivated Herpes Simplex Viruses HSV 1 (strain MacIntyre) and HSV 2 (strain MS).

Antibody detection with *AESKULISA*® Herpes Simplex Virus 1 (gG1) IgG is based on an affinity purified internal fragment of glycoprotein gG1 of HSV 1. Antibody detection with *AESKULISA*® Herpes Simplex Virus 2 (gG2) IgG is based on an affinity purified internal fragment of glycoprotein gG2 of HSV 2.

5 *AESKULISA*® Test Components

Test Component	Color of Solution	Color of Cap	Pieces / Volume
Break apart microtiter test strips MP each with eight antigen coated single wells (altogether 96), 1 frame. The test-specific coating material is inactivated.	-	-	12 pieces
Calibrator A – D CAL (ready-to-use) Human serum or chimeric antibody in protein containing solution (BSA); colored; preservative ProClin. The antibody activities of the calibrators are indicated on their labels and on the quality control certificate of the *AESKULISA*® immunoassay.	yellow*	white	4 x 1.5 ml
Positive Control CON + (ready-to-use) Human serum or chimeric antibody in protein containing solution (BSA); colored; preservative ProClin.	yellow*	red	1 x 1.5 ml
Negative Control CON – (ready-to-use) Human serum or chimeric antibody in protein containing solution (BSA); colored; preservative ProClin.	yellow*	green	1 x 1.5 ml
Sample Buffer SB 5x**, 5x conc.** Protein containing solution (BSA); colored; preservative < 0.1 % sodium azide. The sample buffer of *AESKULISA*® IgM immunoassays contains Rf absorbent.	IgG, IgA: yellow IgM: green	white	1 x 20 ml

Wash Buffer WASHB 50x **, 50x conc.** Solution with tween 20; colored; preservative ProClin.	green	white	1 x 20 ml
Anti-Human IgA, IgG or IgM Conjugate CONJ (ready-to-use) Anti-human IgA, IgG or IgM polyclonal antibody, conjugated to horseradish peroxidase, stabilized with protein containing solution (BSA); colored; preservative ProClin.	IgA: red IgG: blue IgM: green	IgA: red IgG: blue IgM: green	1 x 15 ml
Substrate SUB (ready-to-use) Stabilized TMB/H_2O_2.	colorless	black	1 x 15 ml
Stop Solution STOP (ready-to-use) 1 M Hydrochloric Acid (HCl).	colorless	white	1 x 15 ml
Quality Control Certificate	-	-	1 piece
Instruction for Use	-	-	1 piece

*Color intensity is increasing with antibody activity.

6 Material required, but not provided

- Common laboratory equipment including glassware (cylinder 100 – 1000 ml), test tubes for dilutions, vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100 – 1000 µl).
- Photometer for microtiter plates with filter, wavelength 450 nm, recommended reference wavelength 600 nm – 690 nm (e.g. 620 nm)
- Microplate washing device (300 µl repeating or multichannel pipette or automated wash system)
- Adsorbent paper
- *Aqua dest*.
  AESKULISA® immunoassays are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur. Ph. 4th ed.).

7 Storage and Stability

The microtiter test strips should always be stored with desiccant in the properly sealed aluminum bag. If stored properly in their original containers and at 2 – 8 °C / 35.6 – 46.4 °F, all reagents and the microtiter plate are stable – even after opening – until their expiry date indicated on the label. Diluted solutions are stable at 2 – 8 °C / 35.6 – 46.4 °F for four weeks.

8 *AESKULISA*® Test Procedure

8.1 General Directions for Use

Optimum results can only be achieved if the instructions are strictly followed. Only use *AESKULISA*® reagents when using *AESKULISA*® immunoassays. The test components must not be exchanged for reagents of other manufacturers.

Microtiter plates, calibrators, controls and conjugates of *AESKULISA*® immunoassays are test- and lot-specific and must not be used in other lots. The evaluations of the calibrators and controls are indicated on the quality control certificate provided with the *AESKULISA*® test kit. Washing solution, substrate and stop solution can be used for all *AESKULISA*® immunoassays irrespective of lot and test.

The sample buffer of the *AESKULISA*® IgA and IgG immunoassays can be used for all *AESKULISA*® IgA and IgG immunoassays (REF 6xxx) irrespective of lot and test. The sample buffer of the *AESKULISA*® IgM immunoassays contains Rf absorbent and can be used for all *AESKULISA*® IgM immunoassays for the diagnosis of infectious diseases (REF 6xxx) irrespective of lot and test.

To avoid contamination, aseptic techniques should be used when removing aliquots from the reagent tubes. Conjugate and substrate solution should never be pipetted with tips that are contaminated with other reagents. Reproducibility of test results is dependent on thorough homogenization of the reagents. Therefore, reagent and sample dilutions should be agitated before use. Inappropriate dilution may result in a loss of sensitivity.

Be sure to pipette carefully and comply with the given incubation times and temperatures. Adequate washing avoids test unspecificities.

Avoid the exposure of reagents to strong light during storage and incubation. Never allow the test components to reach temperatures higher than 37 °C / 98.6 °F. Reagents must be tightly closed after use to avoid evaporation and contamination. Take care to not mix-up the caps of the vials. The test components must not reach temperatures above 37 °C / 98.6 °F.

AESKULISA® immunoassay test runs are only valid if the validation criteria are fulfilled.

8.2 Preparation of Reagents

All components and the microtiter plate must be brought to room temperature (20 – 25 °C / 68 – 77 °F) before use. Liquid reagents must be mixed thoroughly. For the dilution of buffer concentrates only clean glass ware must be used.

8.2.1 Microtiter Strips (ready-to-use)

The microtiter test strips are labeled with abbreviations for the coated antigen.

8.2.2 Calibrators (ready-to-use)

The calibrators CAL A – CAL D are ready-to-use and must not be diluted any further. Calibrators must be used for each test run, independent of the number of microtiter test strips to be used.

8.2.3 Controls (ready-to-use)

The positive control CON+ and the negative control CON– are ready-to-use and must not be diluted any further. Controls must be used for each test run, independent of the number of microtiter test strips to be used.

Depending on national guidelines, laboratories can also validate their own controls and use them alternatively.

8.2.4 Sample Buffer (5x conc.)

The concentrated sample buffer is to be diluted 1:5 with distilled water prior to use (e. g. 20 ml + 80 ml). The sample buffer of *AESKULISA*® IgM immunoassays contains Rf absorbent.

8.2.5 Wash Buffer (50x conc.)

The concentrated wash buffer is to be diluted 1:50 with distilled water prior to use (e. g. 20 ml + 980 ml).

8.2.6 Anti-Human IgA, IgG or IgM POD Conjugate (ready-to-use)

The conjugate is ready-to-use.

8.2.7 Substrate (ready-to-use)

The TMB substrate must always be pipetted with brand new tips in order to avoid contamination. Avoid intense exposure of TMB solution to light.

8.2.8 Stop Solution (ready-to-use)

The stop solution is ready-to-use.

8.3 Preparation of Samples

8.3.1 Sample Material

The use of freshly collected serum or EDTA plasma samples is recommended. Icteric, lipemic, hemolytic or bacterially contaminated samples should not be used. Samples with particles should be cleared by centrifugation (< 1000 x g). The supernatant should be taken off and used for further analysis. Samples must not be thermally inactivated.

8.3.2 Sample Dilution

The samples are to be diluted 1:101 (e. g. 10 µl + 1000 µl) with 1x sample buffer and mixed thoroughly.

8.3.3 Pre-Absorption of Rheumatoid Factors with *AESKULISA*® IgM

Rheumatoid factors (Rf) are autoantibodies mainly of the IgM class, which preferably bind to IgG immune complexes. The demonstration of pathogen-specific IgM antibodies might lead to false-positive test results by the presence of such unspecific rheumatoid factors. Furthermore weak-binding pathogen-specific IgM antibodies might be displaced by stronger-binding IgG antibodies leading to false-negative IgM test results. Therefore, the sample buffer of *AESKULISA*® IgM immunoassays contains a specific Rf absorbent. Rf absorption is performed by dilution of the patient´s sample in 1x dilution buffer of the *AESKULISA*® IgM immunoassay and subsequent incubation for a minimum of 15 minutes at room temperature.

8.3.4 Sample Storage

Patient samples should be used within 8 hours, respectively stored tightly closed at 2 – 8 °C / 35.6 – 46.4 °F up to 48 hours. Extended storage is possible at ≤ -20 °C / -4 °F. Avoid repeated freezing and thawing.

8.4 Test Performance

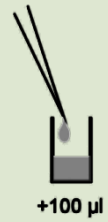
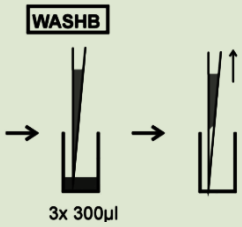
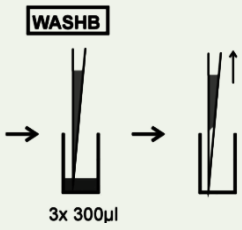
8.4.1 Pipetting Scheme

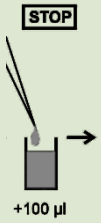
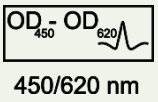
Depending on the intended quantitative or qualitative test evaluation when using *AESKULISA*® immunoassays, the following pipetting scheme is recommended:

	Quantitative Evaluation						Qualitative Evaluation				
	1	2	3	4			1	2	3	4	
A	CAL A	P3				A	CON–	P5			
B	CAL B	P4				B	CAL B	P6			
C	CAL C	P5				C	CAL B	…			
D	CAL D	P6				D	CON+				
E	CON–	…				E	P1				
F	CON+					F	P2				
G	P1					G	P3				
H	P2					H	P4				
	CAL A	Calibrator A					CON–	Negative Control			
	CAL B	Calibrator B					CAL B	cut off Control			
	CAL C	Calibrator C					CON+	Positive Control			
	CAL D	Calibrator D									
	CON–	Negative Control									
	CON+	Positive Control									

8.4.2 Test Procedure

Place the required number of cavities in the frame and prepare a protocol sheet.
For manual use processing at room temperature is recommended.

Step	Symbol	Description
1. Addition of calibrators, controls, and diluted samples		Addition of each 100 µl ready-to-use calibrators, controls and diluted samples into the appropriate wells.
2. Sample incubation		Incubation for 30 +/- 3 minutes at 20 – 32 °C / 68 – 89.6 °F.
3. 3 x Wash		Aspirate the solution, fill each well with 300 µl 1x wash buffer, aspirate the washing solution and repeat the washing procedure another two times; dry by tapping the microtiter plate on a paper towel.
4. Addition of conjugate		Addition of each 100 µl ready-to-use conjugate solution into the appropriate wells.
5. Conjugate incubation		Incubation for 30 +/- 3 minutes at 20 – 32 °C / 68 – 89.6 °F.
6. 3 x Wash		Aspirate the solution, fill each well with 300 µl 1x wash buffer, aspirate the washing solution and repeat the washing procedure another two times; dry by tapping the microtiter plate on a paper towel.
7. Addition of substrate		Addition of each 100 µl ready-to-use substrate solution into the appropriate wells.

8. Substrate incubation		Incubation for 30 +/- 3 minutes at 20 – 32 °C / 68 – 89.6 °F. Avoid exposure to strong light.
9. Addition of stop solution		Addition of each 100 µl ready-to-use stop solution into the appropriate wells using the same order as pipetting the substrate.
10. Incubation		Optional: Incubation for 5 minutes.
11. Agitation		Agitate the microtiter plate carefully for 5 seconds.
12. Analysis		Read optical density (OD) within 30 minutes at 450 nm against a recommended reference wavelength of 620 nm.

8.5 Automated Test Procedure

The automated processing of *AESKULISA*® immunoassays is performed analogous to manual use. The specified test procedure must be adhered to. The *AESKULISA*® immunoassays are evaluated for use with a range of different instruments; the corresponding assayfiles are available on request. For automated processing of *AESKULISA*® immunoassays on other instruments, evaluation of assayfiles by the test kit supplier in collaboration with and instrument provider is recommended. The correct automated processing of *AESKULISA*® immunoassays must finally be validated by the user.

8.6 CSF Diagnostics

The *AESKULISA*® Herpes Simplex Virus 1/2 IgG, *AESKULISA*® Herpes Simplex Virus 1 (gG1) IgG and *AESKULISA*® Herpes Simplex Virus 2 (gG2) IgG tests serve for the determination of intrathecally synthesized antibodies in cerebrospinal fluid. The test procedure is described in a separate instruction for use, which is available on request.

9 *AESKULISA*® Test Evaluation

9.1 Standardization

Calibration of the *AESKULISA*® Herpes Simplex Virus IgG and IgM tests was performed using internal reference serum samples. Quantitative test results are expressed in U/ml.

9.2 Quantitative Evaluation

Generally, the quantitative data evaluation is recommended when using *AESKULISA*® immunoassays. For generation of a standard curve, the optical measurement signals (optical density, OD) of the calibrators are plotted against their antibody activity (in IU/ml or U/ml). The antibody activities of the calibrators are indicated on the lot-specific quality control certificate provided with the *AESKULISA*® test kit. For optimal results, log/lin coordinates and a 4-parameter logistic (4 PL) fit is recommended. Using the generated curve, antibody activities of the samples can be directly evaluated from the optical measurement signals.

9.3 Borderline Range

The borderline range of the *AESKULISA*® immunoassay is specified on the quality control certificates provided with the test kit and indicate the range of borderline test results. Values below this range indicate a negative test result; values above the borderline range are interpreted positive. As a consequence of different seroprevalences and vaccination programs in individual countries, we recommend to verify the borderline range by own analysis and adapt if necessary.

9.4 Measurement Range

The measurement range of the *AESKULISA*® immunoassay is specified on the quality control certificate provided with the test kit. The linearity of dilution as well as a high precision and reproducibility of test results has been demonstrated within this range in comprehensive evaluation studies. Samples with test results above the upper limit of quantification should be reported as >max. Samples with test results below the lower limit of quantification should be reported as <min. In case a patient sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

9.5 Qualitative Evaluation

The qualitative data evaluation when using *AESKULISA*® immunoassays is performed by comparison of the optical measurement signal (optical density, OD) of the patient´s sample with the mean optical measurement signal of the calibrator B (cut off calibrator CAL B) tested in duplicate. If the patient´s sample reaches OD values within the borderline range of +/- 20 % around the mean OD of the cut off calibrator CAL B, the sample is considered as equivocal. Samples with higher OD values are evaluated as positive, samples with lower OD values are evaluated as negative.

9.6 Criteria of Validity

The following criteria of validity have to be fulfilled for a valid test run:

- OD CAL A < 0.3
- OD CAL A < OD CAL B < OD CAL C < OD CAL D
- OD CAL D > 1.3
- The negative control must be evaluated as negative.
- The positive control must not be evaluated as negative.
- By use of quantitative *AESKULISA*® immunoassays, the positive control must present an antibody activity within the validity range indicated on the quality control certificate of the *AESKULISA*®.
- By use of qualitative *AESKULISA*® immunoassays, the variation of the OD values of the cut off calibrator B, tested in duplicate, must not be higher than 20 %.

If these criteria are not met, the test is not valid and must be repeated.

In case of an invalid test run, the expiration dates of the (ready-to-use) reagents, the storage conditions, the incubation times and temperatures, the pipettes, the washer incl. washing cycles, the photometer as well as other devices used should be verified. If no explicable cause for an invalid test run or other aberrant results can be identified, please contact the supplier or manufacturer of the test kit.

9.7 Interpretation of Test Results

A positive test result in *AESKULISA*® immunoassays confirms the presence of specific antibodies. A negative result indicates that no clinically relevant antibody activity against the pathogen are present in the patient´s sample, but does not exclude the possibility of an acute infection. In case of a borderline result, a reliable evaluation is not possible. A definitive diagnosis can only be achieved by testing paired serum samples, taken at one to two week intervals, in parallel.

During the course of a primary HSV infection, the humoral response is characterized by the initial manufacture of IgM, followed – somewhat delayed – by IgG antibodies. Consequently, primary HSV infections can be identified serologically by the demonstration of a seroconversion. IgM antibodies directed against Herpes Simplex Viruses may persist for months and may complicate the interpretation of serological test results. In addition, IgM may also be detectable during HSV reactivations. Furthermore, polyclonal stimulation of antibody production by the presence of other pathogens may result in the synthesis of HSV-specific IgM antibodies. The HSV-specific IgG antibodies generally persist life-long. Cross-reactions of antibodies directed against other herpes viruses (e. g. VZV, CMV, EBV) cannot be excluded.

An important field of application for HSV serology – also during latency – is the detection of serotype-specific HSV IgG antibodies in order to identify potential HSV carriers who could infect susceptible individuals. In particular, the identification of HSV 2 serotype-specific antibodies in pregnant women, who present a high risk of transferring the virus to a newborn child, is recommended. The serological typing of an HSV infection is usually performed by identifying IgG antibodies directed against the HSV-subtype specific surface glycoproteins G (gG) of HSV 1 (gG1) and HSV 2 (gG2). Due to the fact that the immune response to the glycoproteins gG1 and gG2 may be delayed during HSV infection, a positive test result in *AESKULISA*® Herpes Simplex Virus 1/2 IgG does not exclude the possibility of negative test results in *AESKULISA*® Herpes Simplex Virus 1 (gG1) IgG and *AESKULISA*® Herpes Simplex Virus 2 (gG2) IgG.

Basic Interpretation Scheme of Serological Test Results

HSV 1/2 IgM Activity	HSV 1/2 IgG Activity	Evaluation
negative	negative	No specific antibodies detectable. In case of reasonable suspicion, another test in one or two weeks is recommended.
positive	negative / positive	Indication of acute HSV infection. For confirmation, further investigations are recommended.
negative	positive	Indication of a past HSV infection / latency.

HSV 1 (gG1) IgG Activity	HSV 2 (gG2) IgG Activity	Evaluation
positive	negative	Indication of a past HSV 1 infection / latency.
negative	positive	Indication of a past HSV 2 infection / latency.
positive	positive	Indication of a past HSV 1 and HSV 2 infection / latency.

The interpretation of test results has to be considered in combination with the patient's clinical picture. A diagnosis should not be based on the results of the performed test only, but should be made after all clinical and laboratory findings have been evaluated. For confirmation, further investigations should be carried out.

10 *AESKULISA*® Performance Characteristics

10.1 Analytical Sensitivity and Specificity

The Limit of Blank (LoB) was assessed by multiple analysis of wells containing sample buffer only. The Limit of Detection (LoD) was assessed by multiple analysis of negative samples.

	Limit of Blank (LoB)	Limit of Detection (LoD)
AESKULISA® Herpes Simplex Virus 1/2 IgG	0.26 U/ml	1.75 U/ml
AESKULISA® Herpes Simplex Virus 1/2 IgM	0.54 U/ml	2.15 U/ml
AESKULISA® Herpes Simplex Virus 1 (gG1) IgG	0.37 U/ml	1.98 U/ml
AESKULISA® Herpes Simplex Virus 2 (gG2) IgG	0.40 U/ml	1.94 U/ml

The analytical specificity of the *AESKULISA*® immunoassays was assessed by addition of potentially interfering substances to samples and determination of their influence on the measurement. A significant influence of hemoglobin (up to 800 mg/dl), bilirubin (up to 20 mg/dl), bilirubin conjugate (up to 20 mg/dl) and triglycerides (up to 3000 mg/dl) on test results was not observed.

10.2 Diagnostic Sensitivity and Specificity

Sensitivity and specificity of the *AESKULISA*® Herpes Simplex Virus IgG and IgM immunoassays were assessed by the analysis of more than 160 serum samples from healthy blood donors and individuals with suspected Herpes Simplex Virus infection using the Herpes Simplex Virus IgG and IgM ELISA immunoassays of leading European manufacturers as reference tests.

	Sensitivity	Specificity
AESKULISA® Herpes Simplex Virus 1/2 IgG	96.8 %	> 99 %
AESKULISA® Herpes Simplex Virus 1/2 IgM	> 99 %	> 99 %
AESKULISA® Herpes Simplex Virus 1 (gG1) IgG	98.1 %	> 99 %
AESKULISA® Herpes Simplex Virus 2 (gG2) IgG	> 99 %	97.4 %

Sera classified as borderline were not included in the calculation of sensitivity and specificity.

10.3 Reference Range of Healthy Individuals

Testing of serum samples from unselected blood donors with *AESKULISA*® Herpes Simplex Virus IgG and IgM immunoassays resulted in the following distribution:

AESKULISA®	Nr. of Samples	Negative	Borderline	Positive
HSV 1/2 IgG	100	14 (14.0 %)	1 (1.0 %)	85 (85.0 %)
HSV 1/2 IgM	100	98 (98.0 %)	2 (2.0 %)	0 (0.0 %)
HSV 1 (gG1) IgG	100	29 (29.0 %)	6 (6.0 %)	65 (65.0 %)
HSV 2 (gG2) IgG	100	86 (86.0 %)	4 (4.0 %)	10 (10.0 %)

10.4 Precision

Precision and reproducibility of test results obtained with *AESKULISA*® Herpes Simplex Virus IgG and IgM were assessed by the determination of the intra- and interassay precision as well as the lot-to-lot variance by the analysis of multiple samples of different antibody activities.

AESKULISA® Herpes Simplex Virus 1/2 IgG

Sample	Extinction (OD)	IgG Activity	Intraassay CV (U/ml)	Interassay CV (U/ml)	Lot-to-Lot CV (U/ml)
Serum 1	0.196	2.7 U/ml	5.2 %	6.9 %	8.6 %
Serum 2	0.994	22.4 U/ml	4.8 %	5.5 %	5.4 %
Serum 3	1.286	34.5 U/ml	5.5 %	7.4 %	7.0 %
Serum 4	1.576	50.8 U/ml	5.9 %	8.1 %	7.2 %
Serum 5	2.132	101.5 U/ml	6.2 %	6.7 %	7.7 %

AESKULISA® Herpes Simplex Virus 1/2 IgM

Sample	Extinction (OD)	IgM Activity	Intraassay CV (U/ml)	Interassay CV (U/ml)	Lot-to-Lot CV (U/ml)
Serum 1	0.239	5.4 U/ml	4.5 %	8.2 %	13.8 %
Serum 2	0.339	9.2 U/ml	4.0 %	8.0 %	14.0 %
Serum 3	0.441	11.7 U/ml	5.4 %	8.5 %	14.5 %
Serum 4	0.537	16.7 U/ml	4.0 %	5.5 %	15.0 %
Serum 5	0.674	22.3 U/ml	3.8 %	5.1 %	13.0 %

AESKULISA® Herpes Simplex Virus 1 (gG1) IgG

Sample	Extinction (OD)	IgG Activity	Intraassay CV (U/ml)	Interassay CV (U/ml)	Lot-to-Lot CV (U/ml)
Serum 1	0.368	6.8 U/ml	5.9 %	9.2 %	7.8 %
Serum 2	0.870	23.2 U/ml	4.4 %	8.1 %	5.8 %
Serum 3	1.093	32.3 U/ml	2.9 %	6.7 %	7.5 %
Serum 4	1.579	59.0 U/ml	4.5 %	6.2 %	7.0 %
Serum 5	1.897	79.1 U/ml	3.2 %	4.0 %	4.7 %

AESKULISA® Herpes Simplex Virus 2 (gG2) IgG

Sample	Extinction (OD)	IgG Activity	Intraassay CV (U/ml)	Interassay CV (U/ml)	Lot-to-Lot CV (U/ml)
Serum 1	0.490	12.6 U/ml	4.0 %	11.7 %	5.3 %
Serum 2	0.990	31.6 U/ml	4.2 %	9.6 %	10.7 %
Serum 3	1.058	34.6 U/ml	2.9 %	7.3 %	7.8 %
Serum 4	1.215	41.0 U/ml	4.1 %	7.0 %	9.2 %
Serum 5	2.026	89.9 U/ml	3.1 %	6.6 %	7.3 %

More comprehensive study reports on additional performance characteristics such as analytical sensitivity, analytical specificity, trueness, precision, accuracy, recovery, linearity, limits of detection and measurement range are available on request.

11 Safety Measures

11.1 Recommendations and Precautions

AESKULISA® immunoassays are designed exclusively for *in vitro* diagnostic use by qualified personnel only, who are trained, specially advised and familiar with the laboratory methods. All kit reagents and human specimens should be handled carefully, using established good laboratory practice. If the product is damaged or product information - including the labelling - is wrong or incorrect, please contact the manufacturer or supplier of the test kit.

Do not pipette by mouth. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Avoid direct contact by wearing disposable gloves, laboratory coat and safety glasses while handling kit reagents or specimens. Wash hands thoroughly afterwards.

This product contains dilutions of human serum samples. Although all serum samples have been tested and found negative for anti-HIV 1 and 2-ab, HBs-Ag (Hepatitis B-Virus-surface Antigen) and anti-HCV-ab, they should be considered potentially infectious. This product contains dilutions of animal origin. Please observe the relevant statutory requirements.

This kit contains potentially hazardous components, which might be irritant to eyes and skin.

Individual test components contain sodium azide (NaN_3) as a preservative. Sodium azide may be toxic if ingested or adsorbed by skin or eyes. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Calibrators and controls as well as patient samples should be considered potentially infectious and handled according to national laws. Patient samples and other potentially infectious material should be decontaminated after the test run.

Reagents should be stored safely and be inaccessible to children.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.

A summary of Safety and Performance is available via Eudamed as well as upon request.

11.2 Disposal

For decontamination and disposal please follow the recommendations of the CDC as well as the relevant local and national statutory requirements.

12 References

Marchi, S., Trombetta, C.M., Gasparini, R., Temperton, N., Montomoli, E. (2017) Epidemiology of herpes simplex virus type 1 and 2 in Italy: a seroprevalence study from 2000 to 2014. J. Prev. Med. Hyg. *58*, E27 – E33.

Sauerbrei, A. (2014) Diagnostik und antivirale Therapie von Herpes-simplex-Virus-Infektionen. Mikrobiologe *24*, 150 – 8.

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13 Regulatory Symbols

IVD	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγνωστικό μέσο
	- Para uso Diagnóstico in vitro	
REF	- Numero d'ordine	- Cataloge number
	- Référence Catalogue	- Numéro de catálogo
	- Bestellnummer	- Αριθμός παραγγελίας
	- Número de catálogo	
LOT	- Descrizione lotto	- Lot
	- Lot	- Lote
	- Chargen Bezeichnung	- Χαρακτηρισμός παρτίδας
	- Lote	
UDI	- Identificatore univoco del dispositivo	- Unique Device Identifier
	- Identifiant unique de l'appareil	- Identificador único del dispositivo
	- eindeutige Produktidentifizierung	- Μοναδικό αναγνωριστικό συσκευής
	- Identificador único do dispositivo	
CE	- Conformità europea	- EC Declaration of Conformity
	- Déclaration CE de Conformité	- Declaración CE de Conformidad
	- Europäische Konformität	- Ευρωπαϊκή συμφωνία
	- Déclaracão CE de Conformidade	
Σ 96	- 96 determinazioni	- 96 tests
	- 96 tests	- 96 pruebas
	- 96 Bestimmungen	- 96 προσδιορισμοί
	- 96 Testes	
	- Rispettare le istruzioni per l'uso	- See instructions for use
	- Voir les instructions d'utilisation	- Ver las instrucciones de uso
	- Gebrauchsanweisung beachten	- Λάβετε υπόψη τις οδηγίες χρήσης
	- Ver as instrucões de uso	
	- Da utilizzarsi entro	- Use by
	- Utilise avant le	- Utilizar antes de
	- Verwendbar bis	- Χρήση μέχρι
	- Utilizar antes de	
+2°C +8°C	- Conservare a 2-8°C (35.6-46.4°F)	- Store at 2-8°C (35.6-46.4°F)
	- Conserver à 2-8°C (35.6-46.4°F)	- Conservar a 2-8°C (35.6-46.4°F)
	- Lagerung bei 2-8°C (35.6-46.4°F)	- Φυλάσσεται στους 2-8°C (35.6-46.4°F)
	- Conservar entre 2-8°C (35.6-46.4°F)	
	- Prodotto da	- Manufactured by
	- Fabriqué par	- Fabricado por
	- Hergestellt von	- Κατασκευάζεται από
	- Fabricado por	
CON +	- Controllo positivo	- Positive Control
	- Contrôle Positif	- Control Positivo
	- Positiv Kontrolle	- Θετικός ορός ελέγχου
	- Controlo positivo	
CON −	- Controllo negativo	- Negative Control
	- Contrôle Négatif	- Control Negativo
	- Negativ Kontrolle	- Αρνητικός ορός ελέγχου
	- Controlo negativo	
CAL	- Calibratore	- Calibrator
	- Etalon	- Calibrador
	- Kalibrator	- Αντιδραστήριο βαθμονόμησης
	- Calibrador	
CONJ	- Coniugato	- Conjugate
	- Conjugé	- Conjugado
	- Konjugat	- Σύζευγμα
	- Conjugado	
MP	- Micropiastra rivestita	- Coated microtiter plate
	- Microplaque sensibilisée	- Microplaca sensibilizada
	- Beschichtete Mikrotiterplatte	- Επικαλυμμένη μικροπλάκα
	- Microplaca revestida	
WASHB 50x	- Tampone di lavaggio	- Wash buffer
	- Tampon de Lavage	- Solución de lavado
	- Waschpuffer	- Ρυθμιστικό διάλυμα πλύσης
	- Solucão de lavagem	
SUB	- Tampone substrato	- Substrate buffer
	- Substrat	- Tampón sustrato
	- Substratpuffer	- Ρυθμιστικό διάλυμα υποστρώματος
	- Substrato	
STOP	- Reagente bloccante	- Stop solution
	- Solution d'Arrêt	- Solución de parada
	- Stopreagenz	- Αντιδραστήριο διακοπής αντίδρασης
	- Solucão de paragem	
SB 5x	- Tampone campione	- Sample buffer
	- Tampon Echantillons	- Tampón Muestras
	- Probenpuffer	- Ρυθμιστικό διάλυμα δειγμάτων
	- Diluente de amostra	