SERION ELISA *classic* Dengue Virus IgG/IgM

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Enzyme-immunoassay for determination of human antibodies

For sale in the U.S. for Research Use Only. Not for diagnostic procedures.

ESR114G SERION ELISA *classic* Dengue Virus IgG

ESR114M SERION ELISA *classic* Dengue Virus IgM

1 INTENDED USE

SERION ELISA *classic* Dengue Virus IgG and IgM tests are quantitative and qualitative immunoassays for the detection of human antibodies in serum or plasma directed against Dengue virus.

2 BACKGROUND

Dengue is currently one of the most important arboviral causes of infectious disease and death in humans. The virus is transferred to humans by the two major mosquito vectors *Aedes aegypti* and *Aedes albopictus*. Approximately 2.5 billion individuals reside in dengue endemic risk areas. According to the World Health Organization, up to 100 million cases of dengue fever occur worldwide each year.

The single-stranded RNA Dengue Virus is a member of the family Flaviviridae. The genome consists of approximately 11kb and encodes three structural and seven non-structural proteins. The Dengue Viruses can be classified into four different serovars namely DEN-1, DEN-2, DEN-3 and DEN-4.

The incubation period for Dengue fever is four to six days. Frequently, Dengue Virus infections are asymptomatic. The classical course of an infection is characterized by fever (DF), headache, myalgias, arthralgia and a typical rash. After an infection, all four serotypes induce a serotype-specific, but not cross-protective, long-term immunity. During secondary infections, additional symptoms such as bleeding (DHF) and shock (DSS) are frequently observed. It has been suggested that DHF may result from follow up infections caused by heterologous Dengue Virus serotypes via antibody dependent enhancement (ADE). Annually, 250,000 to 500,000 cases of dengue hemorrhagic fever (DHF) are recorded with 2-5% taking a fatal course. There is no specific medication for treatment of Dengue virus infection and frequently, adequate management of DHF requires hospitalization.

While the SERION ELISA *classic* Dengue Virus IgM test utilizes inactivated virus lysate, recombinant peptides from the envelope protein are the fundamental component for antibody determination with SERION ELISA *classic* Dengue Virus IgG. In mature virus particles, dimers of the E-proteins on the viral surface initiate the contact to the host cells. The Envelope (E) protein monomer can be divided into three different domains, named DI, DII and DIII. While DI and DIII contain predominately subcomplex and type-specific epitopes, DII presents the major flavivirus group and subgroup cross-reactive epitopes.

3 TEST PRINCIPLE SERION ELISA *classic*

The test strips of the SERION ELISA *classic* microtiter plate are coated with specific antigens of the pathogen of interest. If antibodies in serum samples are present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the immune complex. The colorless substrate p-nitrophenylphosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the analyte in the sample and is measured photometrically.

4 KIT COMPONENTS

Test Components	**Pieces / Volume**
Break apart microtiter test strips each with eight antigen coated single wells (altogether 96 wells) MTP 1 frame. The coating material is inactivated	12 pieces
Standard serum (ready-to-use) STD Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; coloring: Amaranth O.	2 x2ml
Negative control serum (ready-to-use) NEG Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface antigen) and anti-HCV Ab; preservative: < 0.1 % sodium azide; coloring: Lissamin Green V.	2ml
Anti-human IgA, IgG, or IgM conjugate (ready-to-use) APC Anti-human IgA, IgG or IgM polyclonal antibody, conjugated to alkaline phosphatase, stabilized with protein stabilization solution; preservative: 0.01 % methylisothiazolone, 0.01 % bromnitrodioxane.	13ml
Washing solution concentrate (sufficient for 1000 ml) WASH Sodium chloride solution with Tween 20 and 30 mM Tris/HCl, pH 7,4; preservative: < 0.1 % sodium azide.	33.3ml
Dilution buffer DILB Protein containing phosphate buffer with Tween 20; preservative: < 0.1 % sodium azide; colouring: 0.01 g/l Bromphenol blue.	2 x50ml
Stopping solution STOP 1.2 N sodium hydroxide.	15ml
Substrate (ready-to-use) pNPP Para-nitrophenylphosphate in solvent free buffer; preservative: < 0.1 % sodium azide (Substrate in unopened bottle may have a slightly yellow coloring, which does not reduce the quality of the product!)	13ml
Quality control certificate with standard curve and evaluation table INFO (quantification of antibodies in IU/ml or U/ml).	2 pages

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5 MATERIAL REQUIRED BUT NOT SUPPLIED

- Common laboratory equipment
- For IgM detection: SERION Rf-Absorbent, Order Nr.: Z200 (20 ml)
- Photometer for microtiter plates with filter, wavelength 405 nm, recommended reference wavelength 620 nm -690 nm (e.g. 650 nm)
- Incubator 37°C
- Moist chamber
- Distilled water
- Click-Clips (order no. VT120)

6 STORAGE AND STABILITY

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	Unopened After opening, at 2 – 8 °C in closed aluminum bag with desiccant *Strips which are not used must be stored dry in the closed aluminum bag.*	See expiry date Minimum shelf-life four weeks Shelf-life with proper use and storage until expiry date
Control sera / Standard sera	After opening at 2 – 8 °C	See expiry date; 24 months as of production
Conjugate	Ready-to-use solution at 2 – 8 °C *Avoid contamination e.g. by using sterile tips.*	See expiry date; 28 months as of production
Dilution buffer	Unopened After opening at 2 – 8 °C *Discard cloudy solutions.*	See expiry date; 36 months as of production; 24 months
Washing solution	Concentrate after opening at 2 – 8 °C Working dilution at 2 – 8 °C Working dilution at room temperature *Bottles used for the working dilution should be cleaned regularly. Discard cloudy solutions.*	See expiry date 2 weeks 1 week
Substrate	Ready-to-use solution at 2 – 8 °C, stored protected from light *Avoid contamination e.g. by using sterile tips. Discard if solution turns yellow (extinction against distilled water > 0.25 OD).*	See expiry date; 36 months as of production
Stopping solution	After opening at room temperature	See expiry date

7 TEST PROCEDURE SERION ELISA *classic*

7.1 Evidence of Deterioration

Only use SERION ELISA *classic* reagents when using SERION ELISA *classic* immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of SERION ELISA *classic* immunoassays are defined exclusively for the test kit to be used and must not be used in other lots. Dilution buffer, washing solution, substrate and stop solution can be used for all SERION ELISA *classic* immunoassays irrespective of the lot and the test.

There are three different conjugate concentrations for each immunoglobulin class: LOW, MEDIUM, HIGH. The classification is written on each label as follows:

e.g.	IgG +	low concentrated IgG conjugate
	IgG ++	medium concentrated IgG conjugate
	IgG +++	high concentrated IgG conjugate

In rare cases the use of special conjugate is necessary to guarantee consistent quality of our products. Special conjugates are produced in a separate lot and do not carry the “+” sign and are not exchangeable with other conjugates.

Please pay close attention to information on labels!

Unopened, all components of the SERION ELISA *classic* tests may, if stored accordingly, be used up to the expiry dates given on the labels. Reagents may not be used after date of expiry.

Dilution or alteration of the reagents may result in a loss of sensitivity.

Avoid exposure of reagents to strong light during storage and incubation. Reagents must be tightly closed after use to avoid evaporation and contamination.

To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper reclosing. Do not use the strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly reclosed.

Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination. To avoid false positive results ensure not to contact or splash the top-walls of wells while pipetting conjugate. Take care not to mix the caps of the bottles and/or vials.

Reproducibility of test results is dependent on thorough mixing of the reagents. Agitate the flasks containing control sera before use and also all samples after dilution (e.g. by using a vortex mixer).

Be sure to pipette carefully and comply with the given incubation times and temperatures. Significant time differences between pipetting the first and last well of the microtiter plate when dispensing samples and control sera, conjugate, or substrate can result in different pre-incubation times, which may influence the precision and reproducibility of the results.

Optimum results can only be achieved if the instructions are strictly followed.

The SERION ELISA *classic* immunoassay is only valid if the lot-specific validation criteria on the quality control certificate are fulfilled.

Adequate washing avoids test unspecificities. Therefore, the washing procedure should be carried out carefully. All of the flat bottom wells should be filled with equal volumes of washing buffer. At the end of the procedure ensure that the wells are free of all washing buffer in order to avoid uncontrolled dilution effects. Avoid foaming!

Take care not to damage the inscription (pathogen / antibody class) on the microtiter test strips during washing and aspiration to avoid confusion.

7.2 Sample Preparation and Storage

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

7.2.1 Dilution of Samples

Before running the test, samples (V1) must be diluted in dilution buffer (V2) as follows:

SERION ELISA *classic* Dengue Virus IgG

V1 + V2 = 1 + 100	add	10 µl sample
	each to	1000 µl dilution buffer

After dilution and before pipetting into the microtiter plate, the samples must be mixed thoroughly to prepare a homogenous solution.

Interference with rheumatoid factors

Rheumatoid factors are autoantibodies mainly of the IgM class which preferably bind to IgG immune complexes. The presence of non-specific IgM antibodies (rheumatoid factors) can lead to false-positive results in the IgM assay. Furthermore, the possibility exists, that weak-binding pathogen-specific IgM antibodies may be displaced by stronger-binding IgG antibodies leading to a false negative IgM result. Therefore it is necessary to pretreat samples with rheumatoid factor-absorbent prior to IgM detection (SERION Rf-Absorbent, Order Nr.: Z200 (20 ml/100 tests)). Rf-absorption is performed by incubation of the sample in Rf-dilution buffer for 15 minutes at room temperature or overnight at 4 °C. The test procedure is described in a separate instruction manual.

Before running the test, rheumatoid factor-absorbent (V1) must be diluted 1+4 in dilution buffer (V2).

V1 + V2 = V3 (1 + 4)	add 200 µl	Rf-absorbent
	each to	800 µl dilution buffer

Samples (V4) must be diluted in this Rf-dilution buffer (V3):

V4 + V3 = 1 + 100	add	10 µl	sample
	each to	1000 µl	Rf-dilution buffer

After dilution and before pipetting into the microtiter plate the samples must be mixed thoroughly to prepare a homogenous solution.

7.2.2 Sample Storage

Samples should not be stored for more than 7 days at 2 – 8 °C. Extended storage is possible at ≤ -20 °C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2 – 8 °C for one week.

7.3 Preparation of Kit Reagents

Bring all reagents to room temperature before testing.

7.3.1 Microtiter Test Strips

The microtiter test strips in frames are packed with a desiccant in an aluminum bag. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions.

7.3.2 Control Sera / Standard Sera

Control and standard sera are ready-to-use and must not be diluted any further. For each test run -independent of the number of microtiter test strips to be used - control and standard sera must be included. The standard sera should be set up in duplicate.

Do not treat control sera with Rf-absorbent.

7.3.3 Anti-human IgA, IgG or IgM AP-Conjugate (ready-to-use)

Conjugates with the same concentration and of the same immunoglobulin class are interchangeable. Avoid contamination of ready-to-use conjugates by using sterile tips.

7.3.4 Washing Solution

Dilute washing buffer concentrate (V1) 1:30 with distilled water to a final volume of V2.

Example:

Buffer concentrate (V_1)	Final volume (V_2)
33.3ml	1000ml
1.0ml	30ml

7.3.5 Dilution Buffer for Samples (ready-to-use)

7.3.6 Substrate (ready-to-use)

Avoid contamination of the ready-to-use substrate solution e. g. by using sterile tips.

7.3.7 Stopping Solution (ready-to-use)

7.4 Overview -Test Procedure

SERION ELISA *classic*
Dengue Virus IgG / IgM
quantitative

In case of IgM detection absorption of rheumatoid factor, see No. 7.2.1; Incubation 15 minutes at room temperature or overnight at 4°C

sample dilution[1]
1+100

Pipette diluted samples and ready-to-use control / standard sera into the microtest wells (100 µl)

▼

INCUBATION 60 Min./ 37 °C
moist chamber

▼

WASH (4 x 300 µl DIL WASH)[2]

▼

Pipette conjugate solution APC (100 µl)

▼

INCUBATION 30 Min./ 37 °C
moist chamber

▼

WASH (4 x 300 µl DIL WASH)[2]

▼

Pipette substrate solution pNPP (100 µl)

▼

INCUBATION 30 Min./ 37 °C
moist chamber

▼

Pipette stopping solution STOP (100 µl)

▼

READ EXTINCTION at 405 nm

[1]Special dilution buffers for the following SERION ELISA *classic* tests:
Borrelia burgdorferi IgG, IgM, EBV EA IgG, Parvovirus B19 IgM and Hantavirus Puumala IgG, IgM

[2]For manual use:
Tap plate at the end of the wash procedure on paper towel.

7.5 Manual Test Procedure

1 Place the required number of **wells in the frame** and prepare a protocol sheet.

2 Add each **100 µl of diluted sample or ready-to-use controls** into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

IgG/IgM quantitative well no.	
well A1	Substrate blank Negative
well B1	Negative Control
well C1	Standard serum
well D1	Standard serum
well E1	Sample 1....

3 **Sample incubation** for 60 minutes (+/-5 min) at 37 °C (+/-1°C) in moist chamber

4 After incubation **wash** all wells with washing solution (by automated washer or manually):
-aspirate or shake out the incubation solution
-fill each well with 300 µl washing solution
-aspirate or shake out the washing buffer
-repeat the washing procedure 3 times (altogether 4 times!)
-dry by tapping the microtiter plate on a paper towel

5 **Addition of conjugate** Add 100 µl of the ready-to-use IgG/IgM conjugate to the appropriate wells (except substrate blank)

6 **Conjugate incubation** for 30 minutes (+/-1 min)* at 37 °C (+/-1 °C) in moist chamber.

7 After incubation **wash** all wells with washing solution (see above)

8 **Addition of substrate** Add 100 µl of ready-to-use substrate solution to each well (including well for substrate blank!)

9 **Substrate incubation** for 30 minutes (+/-1 min)* at 37 °C (+/-1 °C) in moist chamber.

10 **Stopping of the reaction** Add 100 µl stopping solution to each well, shake microtiter plate gently to mix.

11 **Read extinction** Read optical density (OD) within 60 minutes at 405 nm against substrate blank, reference wave length between 620 nm and 690 nm (e.g. 650 nm).

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* Please note that under special working-conditions internal laboratory adaptations of the incubation times may be necessary.

7.6 Automated Test Procedure

SERION ELISA are suited for processing on automats and evaluated for use with Immunomat™ as well as with DYNEX DSX® and DS2®. The automated processing is performed analogous to manual use. Please note, that under special working-conditions internal laboratory adaptations of the incubation times may be necessary.

7.7 Positive Control / Accuracy Control

For the periodic verification of the test method, in order to fulfill the requirements of laboratory internal quality management systems, we recommend using SERION ELISA *controls* to determine precision and accuracy of SERION ELISA *classic* test runs. The use of SERION ELISA *controls* is described in specific instruction manuals.

8 TEST EVALUATION

SERION ELISA *classic* Dengue Virus IgG/IgM

8.1 Single-Point Quantification with the 4PL Method

Optimized assignment of extinction signals to quantitative values is guaranteed by using non-linear functions, which adjust a sigmoide curve without any further transformation to OD-values. Determination of antibody concentrations with the SERION ELISA *classic* is carried out by the 4 parameter logistic-log-model (4 PL) which is ideal for exact curve-fitting. It is based on the formula:

$$OD = A + \frac{D - A}{1 + e^{B(C - \ln Conc.)}}$$

The parameters A, B, C, and D are representative for the exact shape of the curve:

1	lower asymptote	• parameter A
2	slope of the curve	• parameter B
3	turning point	• parameter C
4	upper asymptote	• parameter D

For each lot the standard curve is evaluated by Institut Virion\Serion GmbH (Würzburg, Germany) in repeated test runs under optimal conditions. Time consuming and cost intensive construction of the standard curve by the user is not necessary.

For evaluation of antibody concentrations a lot specific standard curve as well as a lot specific evaluation table is included with each SERION ELISA *classic* test kit. The evaluation software SERION *evaluate* as well as the Microsoft® Excel-based software tool SERION *activity* are available on request.

To compensate for normal test variations and also for test run control a standard serum is used in each individual test run. For this control serum a reference value with a validity range is determined by the quality control of the producer. Within this range a correct quantification of antibody concentration is ensured.

8.2 Criteria of Validity

- The substrate blank must be < 0.25 OD.
- The negative control must produce a negative test result.
- By use of quantitative SERION ELISA *classic* tests the mean OD-value (after subtraction of the substrate blank!) of the standard serum must be within the validity range, which is given on the lot specific quality control certificate.
- The variation of OD-values of the standard serum may not be higher than 20 %.

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

8.3 Calculation SERION ELISA *classic* Dengue Virus IgG/IgM

8.3.1 Non-automated Evaluation

For the SERION ELISA *classic* test evaluation a lot-specific quality control certificate with standard curve and an evaluation table is included in the test kit so that the obtained OD values may be assigned to the corresponding antibody activities. The substrate blank must be subtracted from all OD values prior to evaluation.

Method 1: Qualitative Evaluation

To fix the cut-off ranges multiply the mean value of the measured standard OD with the numerical data of the quality control certificate (see special case formulas), e.g.:

OD = 0.502 x MW (STD) with upper cut-off
OD = 0.352 x MW (STD) with lower cut-off

If the measured mean absorbance value of the standard serum is 0.64 OD, the range of the cut-off is in between 0.225-0.321 OD.

Method 2:

Continuous Determination of Antibody Activities using the Standard Curve

So called interassay variations (day to day deviations and laboratory to laboratory deviations) are compensated by multiplication of the current measured value obtained with a sample with the correction factor F. This factor is calculated as follows:

$$F = \frac{\text{OD-reference value (of standard serum)}}{\text{OD-current value (of standard serum)}}$$

The procedure is necessary to adjust the current test level of the user with the lot-specific standard curve. First, daily deviations have to be corrected by calculating the correction factor F.

1 The mean of the two OD-values of the standard serum has to be calculated and checked that it is within the given validity range.

2 Calculation of the factor F: the given reference value is divided by the mean of the extinction of the standard serum:

F = reference value extinction STD serum / mean value extinction STD serum.

3 All measured values of samples are multiplied by F.

4 Antibody activities in IU/ml or U/ml can be determined from the standard curve with the corrected values.

8.3.2 Automatic Test Evaluation with Software SERION *evaluate*

After input of the four parameters and the reference value of the standard serum, antibody activities are calculated online from processed and measured SERION ELISA *classic* test runs by the evaluation software SERION *evaluate*.

If the optical density of the standard is out of the validity range, the following message will appear.
„Standard values out of ranges in following groups: Group 1-24." or
„Standard values differ more than 20 % in following groups: Group 1-24."

In these cases the test run is invalid and should be repeated.

Parameters and reference value need to be changed only if there is a change of lot (evaluation table shows parameters and reference values). Correct input of the lot specific data can be checked on the basis of the standard serum activity (in IU/ml or U/ml) assigned to the standard serum. The calculated mean value of the units has to correspond to the unit value indicated on the lot specific certificate. There is an automatic correction of the measured values. In the standard version the printout displays the following:

Sample code
OD-value
IU/ml or U/ml
Evaluation

8.4 Limits of Quantification

The limits of quantification are specified on the quality control certificate of the SERION ELISA *classic* test. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. 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 thereby determined antibody activity must be multiplied by the additional dilution factor.

8.5 Borderline Ranges

The borderline ranges of the SERION ELISA *classic* Dengue Virus IgG/IgM tests are specified on the quality control certificates and indicate the range for borderline test results. Values obtained, when testing a sample, which fall below this range indicate a negative test result; values above the borderline range are interpreted positive. In cases where the results are within the borderline range a definitive interpretation of the result is not possible. In such cases, the test should be repeated in parallel with a follow-up sample taken one to two weeks later (serum pair).

9 SAFETY MEASURES

9.1 Statements of Warning

The SERION ELISA *classic* is designed for use by qualified personnel who are familiar with good laboratory practice.

All kit reagents and human specimens should be handled carefully, using established good laboratory practice.

-This kit contains human blood components. Although all control-and cut-off sera have been tested and found negative for anti-HIV-Ab, HBs-Ag (*Hepatitis B-Virus surface Antigen*) and anti-HCV-Ab, they should be considered potentially infectious.

-Do not pipette by mouth.

-Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

-Wear disposable gloves, laboratory coat and safety glasses while handling kit

reagents or samples. Wash hands thoroughly afterwards.

-Samples and other potentially infectious material should be decontaminated after the test run.

-Reagents should be stored safely and be unaccessible to unauthorized access.

-Stopping solution:  corrosive (C); causes acid burn (R34). Use safety glasses, gloves and laboratory coat while handling!

9.2 Disposal

Please observe the relevant statutory requirements!

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