SERION ELISA classic
Antibody Quantification
Significance of Antibody Quantification

The qualitative activity determination of the individual immunoglobulin classes can provide a first insight into the progression or stage of an infection. However, a simple negative or positive result is often inadequate as a basis for subsequent clinical or therapeutic decisions. In most patient serum samples, IgG antibodies directed against a range of different pathogens can be detected without this being evidence of an acute infection or having other clinical significance. Similarly, IgM and IgA antibodies may persist for evidence of an acute infection or having other clinical significance. For example, IgM and IgA antibodies may persist for evidence of an acute infection or having other clinical significance.

Diagnosis of Infectious Diseases

Serological investigations are important in the diagnosis of infectious diseases. The immune system produces IgM, IgG and IgA antibodies as a vital part of the complex immune response to infection and the presence of pathogens. The detection of these antibodies can be used to great advantage in the diagnostic process.

Fields of Application for Antibody Quantification

The quantitative evaluation of antibody activities is important or even essential...

- for immune status determination e.g. before and during pregnancy, before immunizations in order to prevent complications, before organ transplantsations or blood transfusions of donors and recipients,
- for IgG antibody avidity determination,
- for differentiation of acute from past infections, reinfections and reactivations,
- for demonstration of vaccination success,
- for determination of vaccination requirements,
- for disease staging,
- for therapy monitoring,
- for monitoring of at risk patients with immune deficiencies or immune suppression as well as
- for detection of intrathecally synthesized antibodies in CSF diagnostics.

Challenges in Antibody Quantification

The immune system produces antibodies as a response to the presence of pathogens. The various antigenic epitopes result in the synthesis of a range of antibodies, which differ in immunoglobulin class, concentration, specificity and binding strength, such as affinity and avidity. As a consequence, immunoassays need to detect the activity of a heterogeneous antibody population. Consequently, the classical determination of analytes – analogous to the standards in clinical chemistry – is not applicable to the quantification of antibody activities.

Quantification by using a Standard Curve

Plotting the optical measurement signals (OD) of a serially diluted positive serum sample versus the logarithm of the antibody activity results in a typical sigmoidal curve. This is the basis for quantification of antibody activity in serum samples.

Quantification with the 4 PL Function

The mathematical curve fitting for antibody quantification with SERION ELISA classic immunoassays is, of course, based on the optimal 4-parameter logistic (4 PL) function.

\[
\text{Activity (U/ml)} = e^{c \cdot \ln \left( \frac{D - A}{B} \right)}
\]

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

- Parameter A: Lower asymptote (OD)
- Parameter B: Slope of the curve
- Parameter C: Inflection point
- Parameter D: Upper asymptote (OD)

Institut VirionSerion GmbH establishes a lot-specific 4 PL standard curve for each SERION ELISA immunoassay in multiple test runs under optimal test conditions. The four parameters (I) are indicated on the quality control certificate of each individual SERION ELISA classic test. Consequently, error-prone as well as cost- and time-intensive reconstruction of standard curves by the user of SERION ELISA classic immunoassays is circumvented. The correction factor F is used for adoption of the test level of the user and is described on the following pages.
Economical 1-Point Quantification with SERION ELISA Classic Immunoassays

Despite the processing of SERION ELISA classic immunoassays under standardized conditions, systemic fluctuations lead to interassay variations. However, if the defined incubation times (60 min, 30 min, 30 min) and temperature (37 °C) for SERION ELISA classic immunoassays are adhered to, the fluctuations from test run to test run are very slight and deviations from the standard curve are very small. As a consequence, it is not necessary to generate a new, and furthermore cost-intensive, calibration curve for each test run by using a set of multiple calibrators, as for most ELISA tests from other manufacturers. Instead, the test level of the test run is adjusted to the given 4 PL standard curve. Thereby, interassay deviations are compensated for and antibody activities can be directly derived from the illustrated antibody activity. For the adaption of the test level to the given 4 PL standard curve the correction factor F is calculated by dividing the standard reference OD value with the measured, and consequently test run-specific, standard OD value.

By multiplying all measured OD values obtained from patient samples with the correction factor F, the level of each individual test run is adjusted to the predefined 4 PL standard curve of the SERION ELISA classic immunoassays. The correction factor F is calculated by use of the standard serum (STD) provided with each SERION ELISA classic immunoassay.

Evaluation of Antibody Activity with SERION ELISA Classic Immunoassays

After subtraction of the substrate blank from all measured OD values, calculation of the mean OD value of the standard serum (STD) applied in duplicate and verification of the criteria of validity for the test run indicated in the instructions for use, a range of possibilities are available for the evaluation of antibody activities from optical measurement signals (OD) of patient samples.

By multiplying all measured OD values with a correction factor F, the level of the test run is adjusted to the predefined 4 PL standard curve of the SERION ELISA classic immunoassays. The correction factor F is calculated by use of the standard serum (STD), one single calibrator with defined reference OD and antibody activity.

Using the 4 PL Standard Curve

For evaluation of test results, a quality control certificate containing a figure (not shown) of the lot-specific 4 PL standard curve (1) is provided with each SERION ELISA classic immunoassay. After multiplying the optical measurement signal (OD) of a patient sample with the correction factor F, the corresponding antibody activity can be directly derived from the illustrated standard curve.

Using the Evaluation Table

Using the lot-specific evaluation table of the quality control certificate provided with each SERION ELISA classic immunoassay is another possibility for the interpretation of test results derived from their optical measurement signals (OD). In this case, the appropriate column, e.g. (5), of the table is selected according to the measured OD value of the standard serum. Subsequently, the qualitative interpretation of the measurement signals (OD) of the patient samples can be directly performed. Implementation of the correction factor F is not necessary when using this evaluation procedure.

Borderline Range

The test-specific borderline range (6) of each SERION ELISA classic immunoassay is indicated on the quality control certificate and determines the range for borderline test results. The evaluation of a patient sample below this range indicates a negative test result; test results above the borderline range are interpreted as positive. In consideration of the different seroprevalences, some SERION ELISA classic test kits contain two quality control certificates with alternative borderline ranges for adults and children under four years of age.
**SERION ELISA control - Positive Controls for SERION ELISA classic Immunoassays**

**SERION ELISA control EPSTEIN-BARR VIRUS VCA IgM**

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**Quality Assurance**

The requirements of the guidelines of the German Medical Association foresee the use of positive controls for qualitative and quantitative laboratory tests in order to demonstrate compliance with the mandatory high standards.

Institut Virion/Serion GmbH supports its customers by offering the positive control sera of our product line SERION ELISA control for the qualitative and quantitative determination of antibodies when using SERION ELISA classic immunoassays. The ready-to-use components are, in addition to the controls supplied with the SERION ELISA classic test kits, supplementary external reagents to determine validity of SERION ELISA test runs as well as the precision and reliability of the method. SERION ELISA controls are particularly recommended as an aid to internal quality management in accredited laboratories.

**Target Values and Validity Ranges**

In comprehensive validation studies, target values and validity ranges for each test-specific SERION ELISA control are determined and documented on lot-specific certificates of analysis. These values are recommended as references for the quality management in accredited laboratories when using SERION ELISA controls in combination with SERION ELISA classic test kits.

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**Determinaton of an OD-based Borderline Range**

The test-specific borderline range (6) is indicated on the quality control certificate of a quantitative SERION ELISA classic immunoassay, specified in antibody activities and accordingly expressed in lU/ml (e.g. 20–30 lU/ml). Taking the measured OD value of the standard serum into account, the borderline range may be expressed in OD values for the qualitative interpretation of optical measurement signals derived from patient samples. Therefore, two formulas (7) are indicated on the quality control certificate to be used for calculation of the OD values of the upper and lower limit of the test run-specific borderline range. For instance, if the mean value of the standard serum (STD) is measured at 0.80 OD, the OD range of the threshold is calculated to be 0.324 to 0.403 OD with the formulas mentioned above.

**Upper (UB) and lower (LB) OD value of the borderline range:**

UB: 0.504 x MV (STD) = 0.504 x 0.80 OD = 0.405 OD  
LB: 0.405 x MV (STD) = 0.405 x 0.80 OD = 0.324 OD

**Using the 4 PL Function**

The mathematical evaluation of the exact quantitative antibody activity derived from OD signals of patient samples is performed by using the 4 PL function specified on the quality control certificate to be used for calculation of the exact quantitative antibody activity derived from OD signals of patient samples.

**Software**

For the automated or software-supported evaluation of antibody activities derived from OD measurement signals of commercially available ELISA readers, the software SERION easyANALYZE as well as the Microsoft® Excel®-based software tool SERION activity are available on request.

**Automation**

SERION ELISA classic immunoassays are suited for automated processing on ELISA automat and are validated for use with Immunomat.

The special formula (8) is particularly applicable for the evaluation of patient samples when using automat or software tools that do not support the quantitative result evaluation based on the 4 PL function with implementation of a correction factor F.

**Limits of Quantification**

The test-specific limits of quantification (9) are specified on the quality control certificate of each SERION ELISA classic immunoassay. Within this measurement range the required linearity of dilution as well as a high intra- and interassay precision have been demonstrated for reliable antibody quantification.

In case a patient sample with an elevated antibody activity yields a test result above the upper limit of quantification, the sample may be analyzed at a higher dilution. The resulting antibody activity must be multiplied by the additional dilution factor in order to obtain the correct antibody activity of the sample.

**Standardization**

The following SERION ELISA classic immunoassays are calibrated with international standard preparations provided by the World Health Organization (WHO) and allow for the evaluation of test results expressed in International Units per milliliter (IU/ml).

- SERION ELISA classic Bordetella pertussis IgA/IgG
- SERION ELISA classic Bordetella pertussis Toxin IgA/IgG
- SERION ELISA classic Diphtheria IgG
- SERION ELISA classic Measles Virus IgG
- SERION ELISA classic Parvovirus B19 IgG
- SERION ELISA classic Rubella Virus IgG
- SERION ELISA classic Tetanus IgG
- SERION ELISA classic Toxoplasma gondii IgG
- SERION ELISA classic Varicella-Zoster Virus IgG

Further SERION ELISA classic immunoassays are calibrated with standard preparations provided by the Paul Ehrlich Institute in Langen (Germany) or the Robert Koch Institute in Berlin (Germany):

- SERION ELISA classic Bordetella pertussis IgG
- SERION ELISA classic Diphtheria IgA/IgG
- SERION ELISA classic Mycobacterium avium intracellulare 1 IgG
- SERION ELISA classic Varicella-Zoster Virus IgG

If neither international nor national standard preparations are available, Institut Virion/Serion GmbH has validated internal standard preparations for calibration of the remainder of the SERION ELISA classic immunoassays. In these cases, the resulting antibody activity is expressed in test-specific units per milliliter (U/ml).

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Highlights of SERION ELISA classic Immunoassays

- Comprehensive SERION ELISA classic product portfolio
- SERION ELISA classic Immunoassays for the analysis of serum, plasma and, when applicable, CSF samples
- Standardized conditions for combination of all SERION ELISA classic tests in one microtiter frame
- High cost efficiency by using break-apart microtiter strips and economical 1-point calibration by use of a single standard serum
- Exact quantification of pathogen-specific IgA, IgG and IgM antibody activities by use of the precise 4-parameter logistic function (4 PL)
- Standardized evaluation of antibody activities calibrated with international standard preparations of the World Health Organization (WHO) with results expressed in IU/ml, if available
- Fast and quantitative data evaluation by use of the software SERION easyANALYZE or the Microsoft® Excel-based software-tool SERION activity
- Excellent diagnostic efficiency with high sensitivity and specificity values by use of carefully selected antigens and optimized coating conditions for microtiter plates
- High precision and linearity within the measurement range
- Detection of intrathecally synthesized antibodies for CSF diagnostics, if applicable
- Compatibility with conventional ELISA Washer and Reader systems
- Application on Immunomat and comparable automates
- CE-approved
- External positive SERION ELISA controls according to modern quality management guidelines