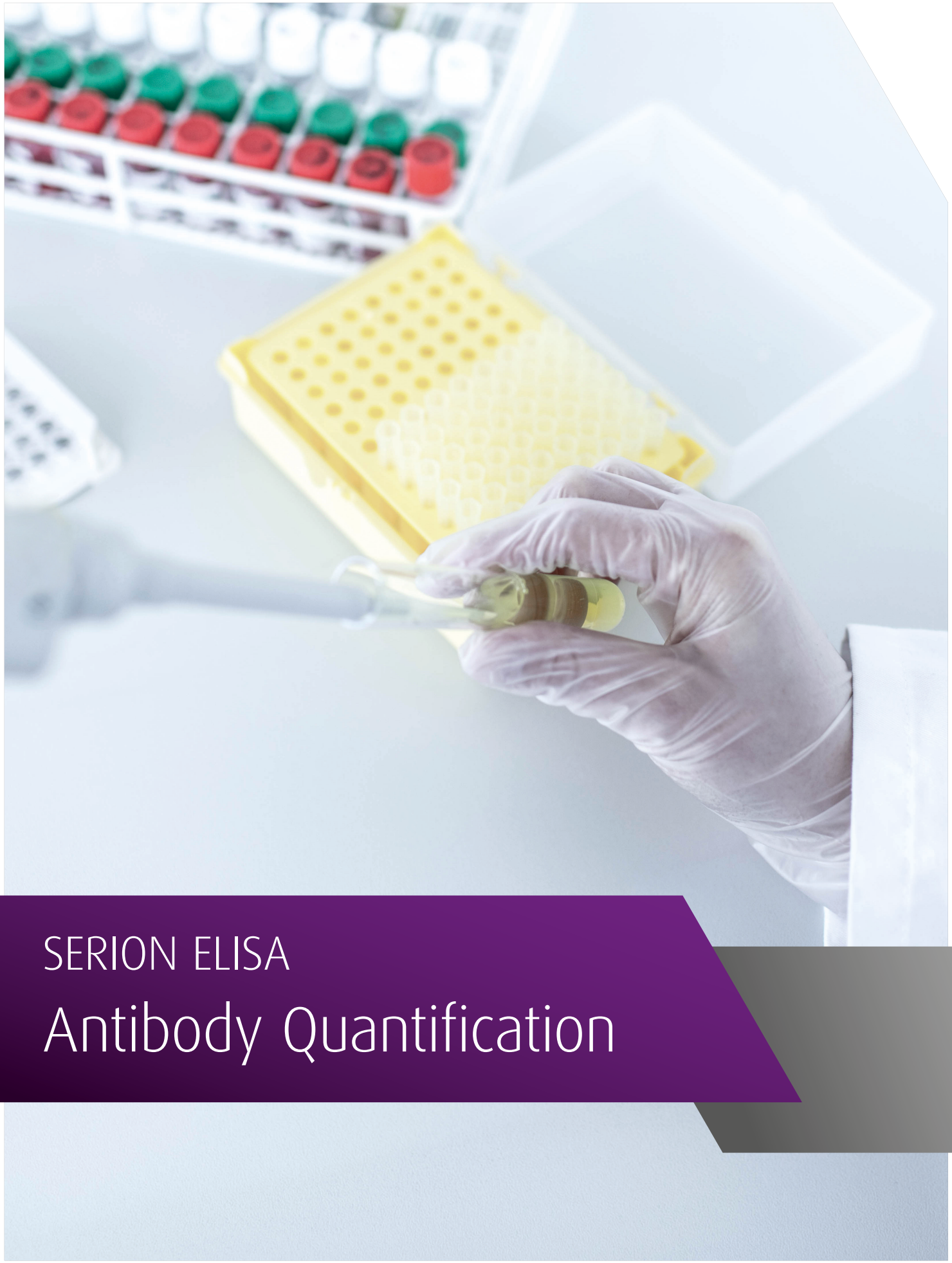
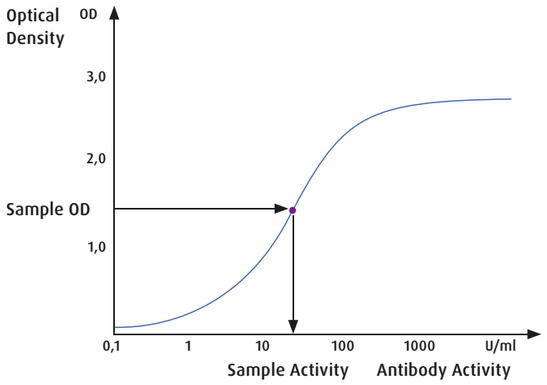


SERION ELISA
Antibody Quantification

serion\diagnostics
Your Partner in Infectious Serology



Plotting the optical measurement signals (OD) of a serially diluted 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.

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.

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 a long time after infection and can complicate any serological interpretation. In such cases, the determination of changes in the antibody activity, detected by successive testing of serum samples, allows for monitoring of the time-dependent course of the disease and provides additional information to help clarify the patient's clinical situation. In fact, the differential quantifiable analysis of the immune response is often decisive for subsequent therapeutic measures. Immunoassays that provide only a simple positive or negative result evaluation, or calculate an antibody index value from sample and cut off ODs, display definite disadvantages when compared to quantitative tests. As a consequence, modern immunoassays provide the quantitative determination of all relevant antibody classes.

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 transplantations 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.

SERION ELISA Immunoassays

SERION ELISA *classic/agile/antigen* tests are quantitative immunoassays for the detection of human antibodies directed against different pathogens responsible for causing infectious diseases.

Quantification by using a Standard Curve

Plotting the optical measurement signals (OD) of a serially diluted positive serum sample versus the logarithm of the calculated antibody activity results in the characteristic sigmoidal shape of a typical curve for antibody-antigen-reactions. The precise correlation of such an experimentally generated standard curve progression by a mathematical function is essential for the exact quantification of antibody activity in patient samples. Imprecision of the curve fitting results in significant discrepancies in antibody quantification. Therefore, modern calibration curves are generally based on nonlinear functions. The recommended mathematical reference method for calibration curves is the 4-parameter logistic (4 PL) function, which optimizes accuracy and precision over the maximal usable calibration range (Findlay and Dillard, 2007). Index determination, non-sigmoidal functions or the test run-specific reconstruction of a standard curve by interpolation between a limited number of calibrators lead to discrepancies in antibody quantification and restricted measurement ranges.

Antibody Quantification with the 4 PL Function

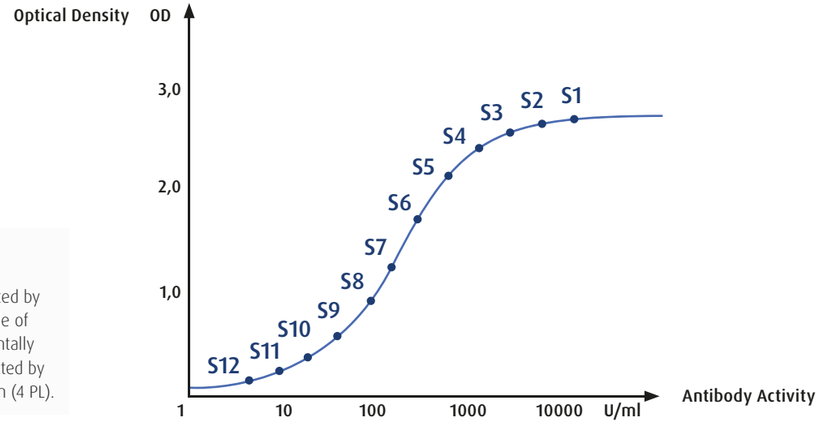
The mathematical curve fitting for antibody quantification with SERION ELISA immunoassays is, of course, based on the optimal 4-parameter logistic (4 PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

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 Virion\Serion 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 (1) are indicated on the quality control certificate of each individual SERION ELISA test. Consequently, error-prone as well as cost- and time-intensive reconstruction of standard curves by the user of SERION ELISA immunoassays is circumvented. The correction factor F is used for adaption of the test level of the user and is described on the following pages.



Generation of a Standard Curve for SERION ELISA Immunoassays:
A set of at least 10 calibrators is generated by serial dilution of a positive serum sample of defined antibody activity. The experimentally created curve progression is precisely fitted by a sigmoidal 4-parameter logistic function (4 PL).

Quality Control Certificate of SERION ELISA Immunoassays

SERION ELISA *classic* ESR1052M Herpes Simplex Virus 2 IgM EM0218

Qualitätskontrollzertifikat / Quality Control Certificate

Kitcharge / Lot EM0218 **IFU-Version** 105-26 **!New!**
Verw. bis / Exp. 2023-10-31

27.10.2021
Prüfdatum / Date of control

Verwendete Reagenzien / Reagents used	Lot	Standard		Standard Kurve / Standard curve	
Teststreifen / Antigen coated strips	ECM0434	Ref.- Werte / Ref. Values	Gültigkeitsbereich / Validity Range	Parameter (1) A	-0,008
Standardserum / Standard serum	ECM0436	(2) OD 0,88	OD 0,44 - 1,50 (4)	B	0,912
Negativ Kontrolle / Negative control	ECM0435			C	4,027
Konjugat / Conjugate	KJM025+	(3) Units 28,0 U/ml		D	2,553
Quantifizierungsgrenzen / Limits of quantification		(9) U/ml 10 - 500			
Grenzwertbereich / Borderline range		(6) U/ml 20 - 30			

OD Bereich / OD Range 405 nm, Standardserum / Standard serum										
0,44 - 0,49	0,50 - 0,54	0,55 - 0,60	0,61 - 0,65	0,66 - 0,71	0,72 - 0,76	0,77 (5) 0,82	0,83 - 0,87	0,88	U/ml	Interpretation
< 0,37	< 0,42	< 0,46	< 0,51	< 0,55	< 0,60	< 0,64	< 0,68	< 0,71	< 20,0	neg
0,37 - 0,48	0,42 - 0,54	0,46 - 0,60	0,51 - 0,66	0,55 - 0,71	0,60 - 0,77	0,64 - 0,83	0,68 - 0,89	0,71 - 0,92	20,0 - 30,0	gw / borderline
> 0,48	> 0,54	> 0,60	> 0,66	> 0,71	> 0,77	> 0,83	> 0,89	> 0,92	> 30,0	pos

OD Bereich / OD Range 405 nm, Standardserum / Standard serum										
U/ml	0,88	0,89 - 0,96	0,97 - 1,03	1,04 - 1,11	1,12 - 1,19	1,20 - 1,27	1,28 - 1,34	1,35 - 1,42	1,43 - 1,50	Interpretation
< 20,0	< 0,71	< 0,75	< 0,81	< 0,87	< 0,93	< 0,99	< 1,06	< 1,12	< 1,18	neg
20,0 - 30,0	0,71 - 0,92	0,75 - 0,97	0,81 - 1,05	0,87 - 1,13	0,93 - 1,21	0,99 - 1,29	1,06 - 1,37	1,12 - 1,45	1,18 - 1,53	gw / borderline
> 30,0	> 0,92	> 0,97	> 1,05	> 1,13	> 1,21	> 1,29	> 1,37	> 1,45	> 1,53	pos

Formeln für spezielle Auswertesysteme (7) OD = **1,042** x MV(STD) entspricht oberem cut-off/corresponds to upper cut-off 30
Special case formulas OD = **0,808** x MV(STD) entspricht unterem cut-off/corresponds to lower cut-off 20
(8) **Concentration= exp(4,027-ln(2,561/(MV(Sample) x0,88/ MV(STD)+0,008)-1)/0,912)**

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D-97076 Würzburg

Economical 1-Point Quantification with SERION ELISA Immunoassays

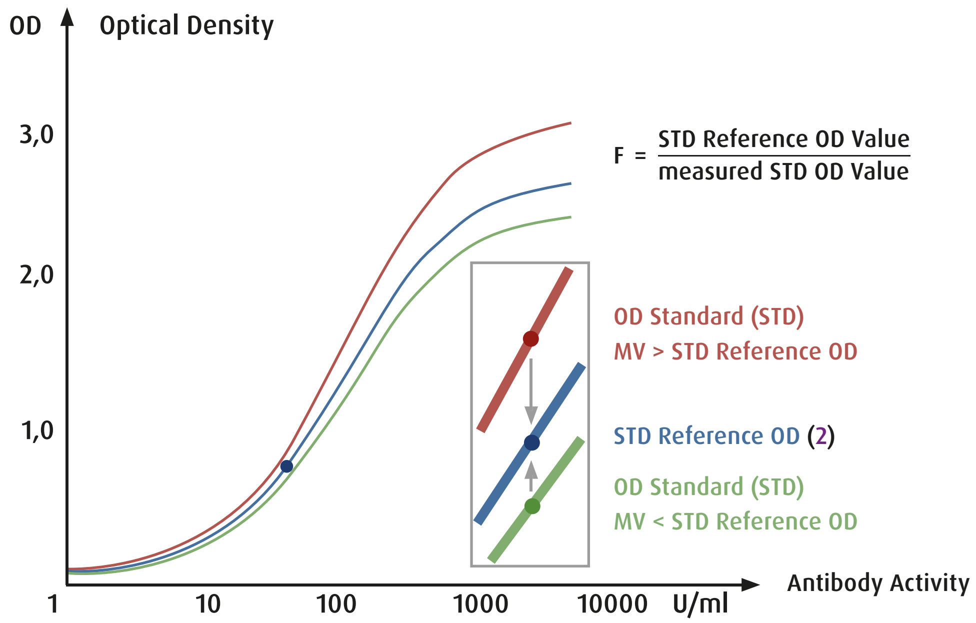
Despite the processing of SERION ELISA immunoassays under standardized conditions, systemic fluctuations lead to interassay variations. However, if the defined incubation times (60 min, 30 min, 30 min) and temperature (37 °C) for SERION ELISA immunoassays are adhered to, the fluctuations from test run 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 user can be adjusted mathematically to the predetermined 4 PL standard curve by implementing a correction factor. The use of a single test-specific calibrator is sufficient in order to compensate for interassay variations (1-Point Calibration). The calibrator for SERION ELISA immunoassays is the so-called standard serum (STD) provided with each individual SERION ELISA test. Its reference OD value (2) and the corresponding antibody activity (3) are indicated on the quality control certificate.

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.

$$F = \frac{\text{STD Reference OD Value}}{\text{measured STD OD Value}}$$

By multiplying the OD values obtained from patient samples with the correction factor F, the level of each individual test run is adjusted to the given 4 PL standard curve. Thereby, interassay deviations are compensated for and antibody activities can be directly evaluated from the 4 PL standard curve. A mathematical adjustment of the test level to the standard curve is ensured if the OD value of the standard serum corresponds with the values within the validity range (4) indicated on the quality control certificate. Thus, the 1-point quantification with SERION ELISA immunoassays guarantees a precise and economical antibody quantification with only one single calibrator.

Principle of 1-Point Quantification by SERION ELISA Immunoassays



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

Evaluation of Antibody Activity with SERION ELISA Immunoassays

After substraction 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.

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. 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 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 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 test kits contain two quality control certificates with alternative borderline ranges for adults and children under four years of age.

Determination of an OD-based Borderline Range
The test-specific borderline range (6) is indicated on the quality control certificate of a quantitative SERION ELISA immunoassay, specified in antibody activities and accordingly expressed in U/ml (e.g. 20 - 30 U/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 = 0.403 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 formula specified on the quality control certificate of the SERION ELISA test with consideration of the correction factor F.

Software
For the automated or software-supported evaluation of antibody activities derived from OD measurement signals, the software SERION *easy*ANALYZE is recommended.

Automation
SERION ELISA *classic/agile* immunoassays are suited for automated processing on ELISA automats and are validated for use with SERION Immunomat.

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

Limits of Quantification
The test-specific limits of quantification (9) are specified on the quality control certificate. Within this measurement range the required linearity of dilution as well as a high intra- and inter-assay 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 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* Masern/Measles Virus IgG
- SERION ELISA *classic* Parvovirus B19 IgG
- SERION ELISA *classic* Röteln/Rubella Virus IgG
- SERION ELISA *classic* Tetanus IgG
- SERION ELISA *classic* Toxoplasma gondii IgG
- SERION ELISA *classic* Varicella Zoster Virus IgG
- SERION ELISA *agile* SARS-CoV-2 IgG

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

- SERION ELISA *classic* Cytomegalovirus IgG
- SERION ELISA *classic* FSME/TBE 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 immunoassays. In these cases, the resulting antibody activity is expressed in test-specific units per milliliter (U/ml).

SERION ELISA *control* - Positive Controls for SERION ELISA Immunoassays

SERION ELISA *control* Aspergillus fumigatus IgA

Artikelnr. / Article No.	BC132A		
Chargen-Nr. / Lot No.	CM0187		
IFU-Version / IFU version	6-20/11-1		
Herstelldatum / Manufacturing Date	2021-11		
Verfallsdatum / Expiry Date	2023-11-30		
Zielwert / Target value	153 U/ml		
Zielwertbereich / Target Range	76,4 U/ml	bis / to	305 U/ml
Versions-Nr. / Version No.	2021 / 11 - 1		

Bitte beachten / Please note
Aufbau der SERION ELISA Chargen-ID (Buchstabe "J" wird nicht verwendet) / Format of SERION ELISA lot ID (letter "J" not used)
Erste Stelle / First Digit: ELISA
Zweite Stelle: Jahr der Produktion beginnend mit 2010 / Second Digit: Year of Production starting with 2010
Folgende Stellen: Fortlaufende Nummerierung / Following Digits: consecutive numeration

Quality Assurance
The requirements of the guidelines of the German Medical Association (RiLiBÄK) 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 for the qualitative and quantitative determination of antibodies when using SERION ELISA immunoassays. The ready-to-use components are, in addition to the controls supplied with the SERION ELISA 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 *control* 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 *control* in combination with SERION ELISA test kits.

Application
SERION ELISA *control* are ready-to-use and must not be diluted further. Rf-absorption must also not be performed. SERION ELISA *control* are recommended to be used in each SERION ELISA test run and the results recorded on a control chart.

Reference
John W. A. Findlay and Robert F. Dillard (2007):
Appropriate Calibration Curve Fitting in Ligand Binding Assays. AAPS 9, Article 29

Highlights of SERION ELISA Immunoassays

- Comprehensive SERION ELISA product portfolio
- SERION ELISA 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 *easy*ANALYZE
- 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 SERION Immunomat and comparable automates
- CE-approved
- External positive SERION ELISA *control* according to modern quality management guidelines

Antibody Quantification - EN V6.21/12

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