Updates with respect to the previous version are marked in grey.

Anti-SARS-CoV-2 QuantiVac ELISA (IgG)

Instruction for use

For in vitro diagnostic use IVD

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2606-9601-10 G	SARS-coronavirus 2 (SARS-CoV-2)	IgG	Ag-coated microplate wells	96 x 01 (96)

CE

Intended use

The enzyme immunoassay (ELISA) provides quantitative in vitro determination of human antibodies of the immunoglobulin class IgG against SARS-CoV-2 in serum, EDTA, heparin or citrate plasma or dried blood spots (DBS) to support the diagnosis of SARS-CoV-2 infection and constitutes a supplement to the direct pathogen detection. Moreover, serology can be applied to collect epidemiological data as well as for antibody determination following vaccination with S1/RBD-based vaccines. The product is designed for use as IVD and can optionally be processed on fully automated equipment.

Clinical significance

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously called 2019-nCoV) belongs to the family of coronaviruses and, like SARS-CoV, is classified in the genus *Betacoronavirus* [1]. At the end of 2019, SARS-CoV-2 was identified as the causative pathogen of clustered cases of pneumonia of unclear origin. The virus caused an infection wave that has spread rapidly worldwide and was declared a pandemic by the WHO at the beginning of 2020 [2-5].

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected persons [3, 4, 6]. Health care personnel and family members are especially at risk [6]. The zoonotic reservoir of the virus appears to be bats [3, 4, 6].

The incubation time of SARS-CoV is three to seven, maximally 14 days [2]. The symptoms of SARS-CoV-2 infection are fever, coughing, breathing difficulties, fatigue and loss of the olfactory and taste sense [2-4, 6, 7]. In most patients the infection manifests with symptoms of a mild febrile illness with irregular lung infiltrates. Some patients, especially elderly or chronically ill patients, develop *acute respiratory distress syndrome* (ARDS) [2, 3, 5, 6]. In February 2020, the disease caused by SARS-CoV-2 was named COVID-19 by the WHO.

Suitable methods for the diagnosis of SARS-CoV-2 infections are the detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) or of virus protein by means of ELISA or rapid test primarily in sample material from the upper (nasopharyngeal or oropharyngeal swab) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, etc.) [4, 5]. Detection of viral antigens is less sensitive than RT-PCR testing.

The determination of antibodies enables confirmation of SARS-CoV-2 infections in patients with typical symptoms and in suspected cases. It also contributes to monitoring and outbreak control [4, 5]. The spike (S) and nucleocapsid (N) proteins of SARS-CoV-2 are highly immunogenic. More than 90% of the neutralising antibodies in COVID-19 patients are directed against the receptor-binding domain (RBD) of the spike protein. The spike protein is the target protein of almost all vaccines against COVID-19 [8].

Around 90% of SARS-CoV-2-infected persons develop specific antibodies until day 10 following symptom onset. IgG, IgA and IgM against the spike protein often occur simultaneously [8]. For significant serological results, two patient samples should be investigated, one from the acute phase (week 1 of the illness) and one from the convalescent phase (3 to 4 weeks later) [4, 6, 9]. SARS-CoV-2-specific T cells appear a few days after onset of symptoms. A specific T cell response is associated with a milder COVID-19 course [8].

Neutralising antibodies are associated with protective immunity against reinfection with SARS-CoV-2 or SARS-CoV. Neutralising antibodies against SARS-CoV could be detected 17 years after infection. SARS-CoV-2 reactive T cells are part of the T cell repertoire from persons who had a SARS-CoV infection in 2003. These cells proliferate following contact with SARS-CoV-2. Cross-reacting T cells were detected in around half of the investigated persons without history of SARS-CoV-2 infection and are supposedly due to prior infections with coronaviruses causing common colds. This indicates a long-lasting immunity following infection with betacoronaviruses [8, 10].

With regard to COVID-19, the immunological memory is heterogeneous: virus-specific antibodies and memory B and T cells are present in different quantities and their levels change with different dynamics. Current findings indicate that the T and B cell memory and antibodies in most cases persist over years after SARS-CoV-2 [8].

Antigen

The reagent wells of the ELISA were coated with the S1 domain of the spike protein of SARS-CoV-2 expressed recombinantly in the human cell line HEK 293.

Test principle

The test kit contains microplate strips each with 8 break-off reagent wells coated with recombinant S1 domain of the spike protein of SARS-CoV-2. In the first reaction step, diluted samples are incubated in the wells. In the case of positive samples, specific IgG (also IgA and IgM) antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	-	12 x 8	STRIPS
2. Calibrator 1 120 RU/ml (IgG, human), ready for use	Red coloured in decreasing intensity	1 x 2.0 ml	CAL 1
3. Calibrator 2 80 RU/ml (IgG, human), ready for use		1 x 2.0 ml	CAL 2
4. Calibrator 3 40 RU/ml (IgG, human), ready for use		1 x 2.0 ml	CAL 3
5. Calibrator 4 20 RU/ml (IgG, human), ready for use		1 x 2.0 ml	CAL 4
6. Calibrator 5 10 RU/ml (IgG, human), ready for use		1 x 2.0 ml	CAL 5
7. Calibrator 6 1 RU/ml (IgG, human), ready for use		1 x 2.0 ml	CAL 6
8. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
9. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
10. Enzyme conjugate peroxidase-labelled anti-human IgG, ready for use	green	1 x 12 ml	CONJUGATE
11. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
12. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
13. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
14. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
15. Protective foil	-	3 pieces	FOIL
16. Quality control certificate	-	1 protocol	-
17. Instruction for use	-	1 booklet	-

Additional materials and equipment (not supplied in the test kit)

- Automatic microplate washer: recommended. Washing of the microplates can also be carried out manually.
- Microplate reader: wavelength of 450 nm, reference wavelength range from 620 nm to 650 nm
- Calibrated pipettes
- Pipette tips
- Stepper pipette: recommended for the pipetting of enzyme conjugate, substrate, and stop solution
- Distilled or deionised water
- Incubator: for incubation of the microplate at +37°C
- Incubator or water bath: recommended to warm the wash buffer
- Stop watch

Storage and stability

The test kit has to be stored at a temperature between +2°C and +8°C; do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

In-use stability following the first opening

After opening, the reagents are stable until the indicated expiry date when stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

Warnings and precautions

- The product must only be used by trained laboratory personnel in a clinical or research laboratory.
- If the packed reagents are visibly damaged, do not use the test kit.
- Before using the product, read the instruction for use carefully. Only the valid version is to be used.
- Do not substitute or mix the EUROIMMUN reagents with reagents from other manufacturers.
- Observe Good Laboratory Practice (GLP) and safety guidelines. Some of the reagents contain preservatives in non-declarable concentrations. Avoid eye and skin contact with samples and reagents. In case of eye or skin contact, rinse thoroughly with water. Remove and wash contaminated clothing. In case of ingestion, obtain medical advice.
- The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all reagents should be treated as being a potential infection hazard and should be handled with care.

Preparation and stability of the samples

- **Samples:** Human serum or EDTA, heparin or citrate plasma or dried capillary blood (dried blood spots, DBS), collected with the EUROIMMUN Blood Collection Card (EUROIMMUN order number ZV 9711-01100).
- **Sample preparation: Patient samples** are diluted **1:101** in sample buffer.

  For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

  When dried blood spots (DBS) are used as the sample material, these must be extracted from the membrane of the blood collection card prior to sample incubation. The test instruction required for the extraction (EUROIMMUN document number EI_2606-10G_A_UK_ZXX) is provided in the customer portal (https://products.euroimmun.de).
- **Stability of the patient samples:**
  - stored at +2°C to +8°C: up to 14 days
  - incubate diluted samples within one working day

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use.

The thermostatically adjustable ELISA incubator must be set to +37°C ± 1°C.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrators and controls:** Ready for use. Mix reagents thoroughly before use.
- **Enzyme conjugate:** Ready for use. Mix the reagent thoroughly before use.

- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before dilution. Dilute the required volume 1:10 with deionised or distilled water (1 part reagent plus 9 parts water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working-strength wash buffer is stable for 4 weeks if stored at +2°C to +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the tube immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.
- **Stop solution:** Ready to use.

Waste disposal

Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Quality control

For every group of tests performed, the extinction readings of the calibrators and the relative units determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

Reference material

The results of the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) are given in relative units (RU/ml). However, it is possible to relate them to the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code 20/136) by using a conversion factor. By definition, the preparation contains 250 IU or BAU (binding antibody units), respectively, per ampoule and was taken up in 250 µl Aqua dest. The values apply to the total immunoglobulin content and should always be observed in relation to a specific immunoglobulin class and an antigenic target structure.

Assay procedure

(Partly) manual test performance

Sample incubation: (1st step)
Transfer **100 µl** of the **calibrators, positive and negative controls or diluted patient samples** into the individual microplate wells according to the pipetting protocol. Incubate for **60 minutes** at **+37°C ± 1°C**.
For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation, follow the recommendations of the instrument manufacturer.

Washing:
Manual: Remove the protective foil. Empty the wells and subsequently wash **3 times using 300 µl of working-strength wash buffer** for each wash.
Automatic: Remove the protective foil. Wash the reagent wells **3 times with 450 µl of working-strength wash buffer** (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note:
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette **100 µl of enzyme conjugate** (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate **30 minutes at +37°C ± 1°C**.
For manual test performance cover the reagent wells with the protective foil.

Washing:
Remove the protective foil. Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette **100 µl of chromogen/substrate solution** into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C) protected from direct sunlight.

Stopping:
Pipette **100 µl of stop solution** into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction, but have been validated in respect of the combination of the EUROIMMUN Analyzer I, the EUROIMMUN Analyzer I-2P, the EUROLabWorkstation ELISA, the Sprinter XL and the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.

Note: Processing on other fully automated systems is possible but must be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 1	P 1	P 9	P 17								
B	C 2	P 2	P 10	P 18								
C	C 3	P 3	P 11	P 19								
D	C 4	P 4	P 12	P 20								
E	C 5	P 5	P 13	P 21								
F	C 6	P 6	P 14	P 22								
G	pos.	P 7	P 15	P 23								
H	neg.	P 8	P 16	P 24								

The pipetting protocol for microplate strips 1 to 4 is an example for the **quantitative analysis** of 24 patient samples (P 1 toP 24).

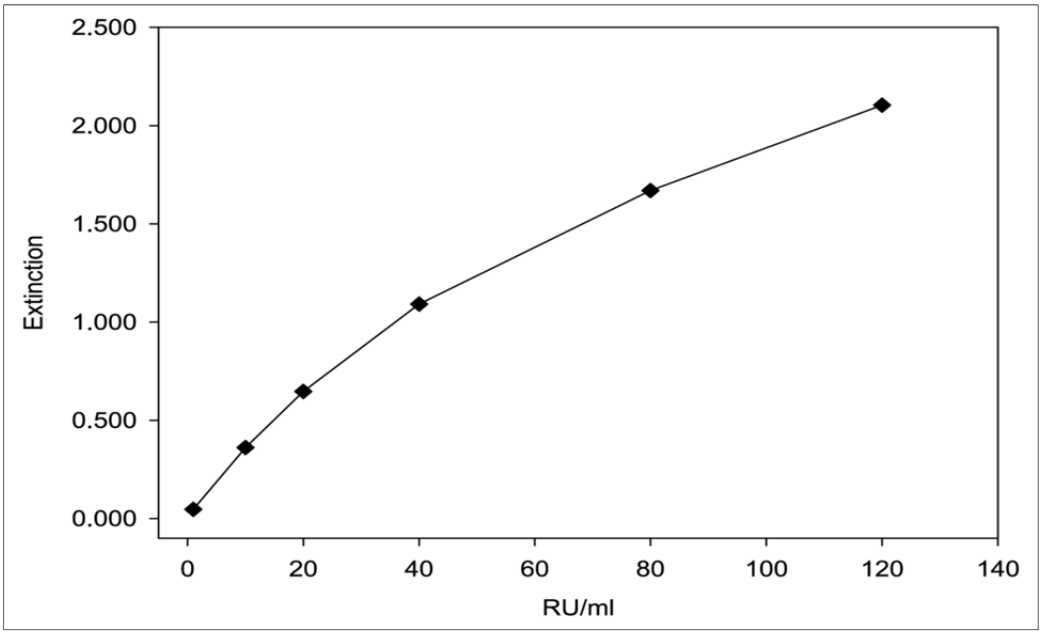
The calibrators (C 1 to C 6), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.

Test evaluation

The standard curve from which the concentration of antibodies in the samples can be taken is obtained by point-to-point plotting of the extinction readings measured for the 6 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in samples.



If the extinction for a sample lies above the extinction of calibrator 1 (corresponding to 120 RU/ml or 384 BAU/ml), the result should be reported as ">120 RU/ml or ">384 BAU/ml". It is recommended that the sample be re-measured in a new test run at a dilution of e.g. 1:1010. The result in RU/ml read from the calibration curve must then be multiplied by factor 10. Particularly after vaccination with S1-/RBD-based vaccines, the antibody concentration may be very high, which requires a significantly higher dilution in order to meet the linear measurement range.

EUROIMMUN recommends interpreting results as follows:

<8 RU/ml:	**negative**
≥8 to <11 RU/ml:	**borderline**
≥11 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

Determination of international units (Binding Antibody Units, BAU/ml):

Due to the linear correlation of results in relative units (RU/ml) with the "First WHO International Standard", the results from the quantitative sample evaluation can be converted into standardised units. In accordance with the specifications of the WHO, when using ligand binding assays such as the Anti-SARS-CoV-2 QuantiVac ELISA (IgG), IU/ml (IU = international units) relating to the detection of neutralising antibodies or BAU/ml (BAU = binding antibody units) should be applied. Values given in IU/ml and BAU/ml are numerically identical. In order to convert test results as well as measurement and borderline ranges given in RU/ml into BAU/ml, the values are multiplied by factor 3.2. The result interpretation is as follows:

<25.6 BAU/ml:	**negative**
≥25.6 to <35.2 BAU/ml:	**borderline**
≥35.2 BAU/ml:	**positive**

The use of the conversion factor is shown as an example in the following table:

	Evaluation in RU/ml			**Evaluation in BAU/ml**	
Threshold range	**8 – 11**			**25.6 – 35.2**	
Calibrator 5 (cut-off)	**10**			**32**	
Measurement range	**1 – 120**			**3.2 – 384.0**	
	RU/ml	**Evaluation**		**BAU/ml**	**Evaluation**
Sample 1	< 1 (< min)	negative	**x 3.2**	< 3.2 (< min)	negative
Sample 2	5.5	negative		17.6	negative
Sample 3	10.1	borderline		32.3	borderline
Sample 4	25.0	positive		80.0	positive
Sample 5	108.8	positive		348.2	positive
Sample 6	> 120 (> max)	positive		> 384 (> max)	positive

Analytical performance

The following data were collected using serum or plasma samples:

Measurement range:

Limit of blank (LoB): 0.86 RU/ml
Limit of detection (LoD): 1.20 RU/ml

LoB and LoD were defined according to the requirements defined in guideline EP17-A2 of the CLSI (Clinical and Laboratory Standards Institute, https://clsi.org/).

Precision: Studies on the intra-lab precision were carried out according to CLSI guideline EP05-A3. Six samples (reactivity distributed over the entire measurement range) were measured. The precision is given as standard deviation (SD) and coefficient of variation (CV).

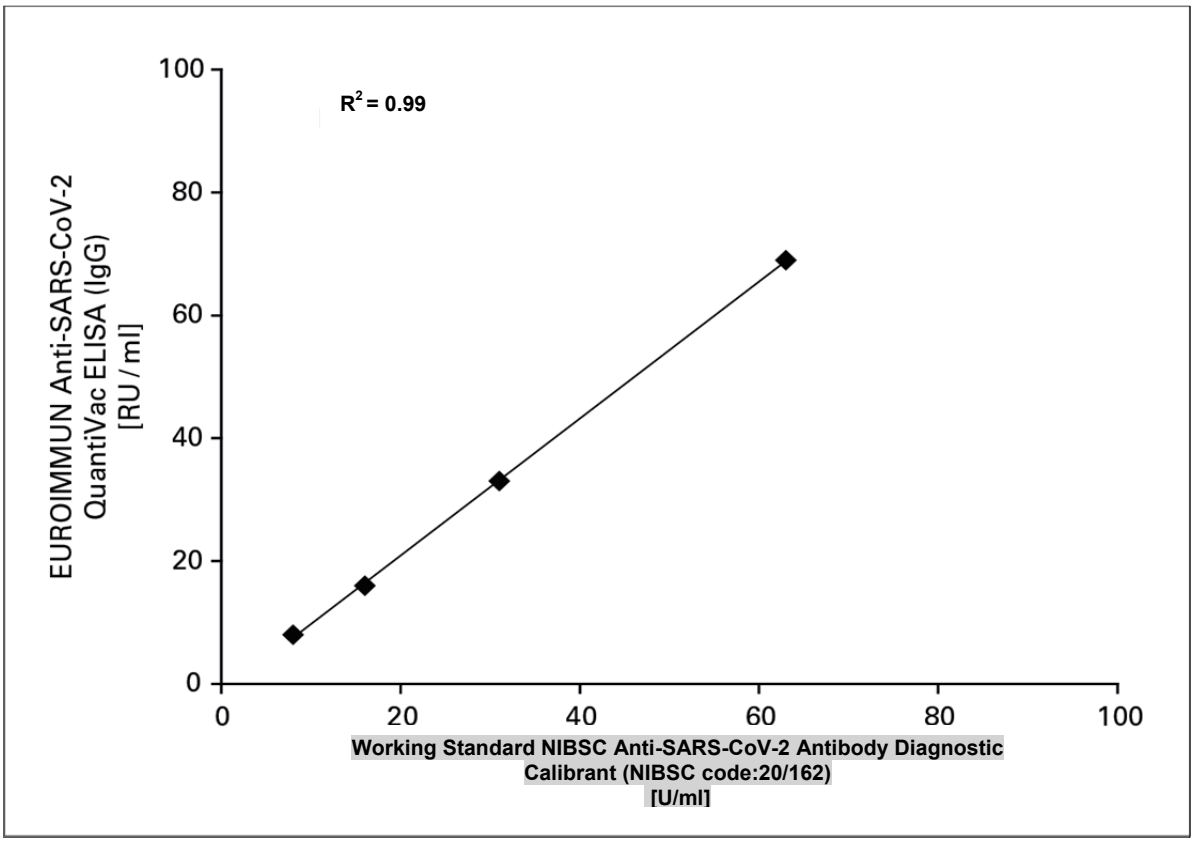
Intra-lab precision

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
Mean	2.53 RU/ml		7.63 RU/ml		10.24 RU/ml		10.23 RU/ml		30.03 RU/ml		91.93 RU/ml	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	0.226	9.0%	0.266	3.5%	0.326	3.2%	0.270	2.6%	0.709	2.4%	4.220	4.6%
Between-run	0.350	13.9%	0.578	7.6%	0.749	7.3%	0.983	9.6%	2.533	8.4%	5.311	5.8%
Within-day	0.417	16.5%	0.637	8.3%	0.817	8.0%	1.020	10.0%	2.630	8.8%	6.784	7.4%
Between-day	0.311	12.3%	0.000	0.0%	0.578	5.6%	0.000	0.0%	0.000	0.0%	2.142	2.3%
Within-lab	0.520	20.6%	0.637	8.3%	1.000	9.8%	1.020	10.0%	2.630	8.8%	7.114	7.7%

Linearity: The linearity of the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) was investigated according to CLSI guideline EP06-A. The Anti-SARS-CoV-2 QuantiVac ELISA (IgG) is linear at least in the tested concentration range (6 RU/ml to 120 RU/ml).

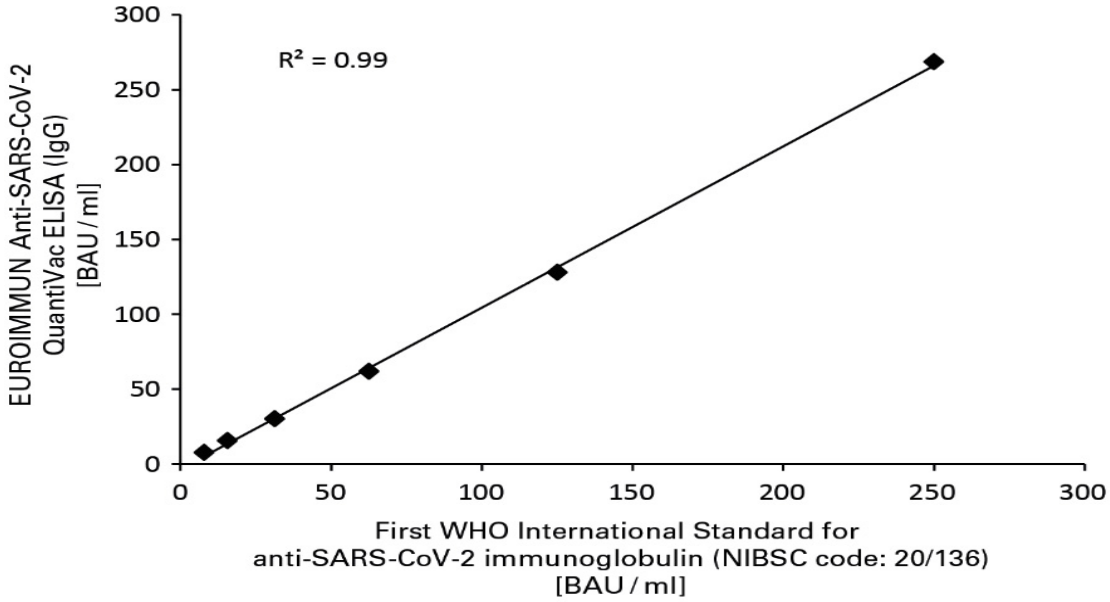
Study I: Correlation with the Working Standard NIBSC Anti-SARS-CoV-2 Antibody Diagnostic Calibrant (NIBSC code: 20/162)

Serial dilutions of the Working Standard NIBSC Anti-SARS-CoV-2 Antibody Diagnostic Calibrant (NIBSC code: 20/162) were investigated using the Anti-SARS-CoV-2 QuantiVac ELISA (IgG). The correlation analysis yielded a correlation coefficient of r = 0.99.



Study II: Correlation with the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136)

Serial dilutions of the “First WHO International Standard for anti-SARS-CoV-2 immunoglobulin” (NIBSC code: 20/136) were investigated with the Anti-SARS-CoV-2 QuantiVac ELISA (IgG). The resulting concentrations in RU/ml were converted into BAU/ml by multiplying them by the factor 3.2. The correlation analysis yielded a correlation coefficient of r= 0.99.



Cross-reactivity (analytical specificity): Due to low homologies of the S1 protein within the coronavirus family, cross-reactions to most of the human pathogenic representatives of this virus family are virtually excluded. 163 samples collected prior the occurrence of SARS-CoV-2 (before January 2020) and positive for antibodies against at least one human pathogenic coronavirus (HCoV HKU1; HCoV OC43; HCoV NL63; HCoV 229-E) were analysed using the Anti-SARS-CoV-2 QuantiVac ELISA IgG. Positive reactions were only found in 1.2% of investigations (2 samples). Therefore, cross-reactions with these endemic HCoV are unlikely.However, cross-reactions between SARS-CoV(-1) and SARS-CoV-2 are likely since they are closely related.

Antibodies against	n (positive) / n (total)	EUROIMMUN-Anti-SARS-CoV-2-QuantiVac-ELISA IgG Positive rate [%]
HCoV	2/163	1,2

Other potential interferences with autoantibodies such as anti-mitochondrial antibodies (AMA), with rheumatoid factors (Rhf) and antibodies against non-related structures like *Haemophilus influenza* type B (HIB), hepatitis B virus (HBV), hepatitis C virus (HCV), respiratory syncytial virus (RSV), parainfluenza virus, Epstein-Barr virus (EBV) as well as with antibodies resulting from vaccination against influenza virus and tick-borne encephalitis (TBE) virus could not be observed. In patient samples with high antibody titers against adenovirus and enterovirus, very high antibody titers against different autoantigens and with severe bacterial pneumonia, weakly positive reactions were determined in very few individual cases. However, a general interference is excluded due to the very low frequency of occurrence.

Group	n (positive) / n (total)	Positive rate [%]
HIB	0/5	0
HBV	0/6	0
HCV	0/6	0
Influenza vaccination	0/40	0
TBE	0/25	0
RSV	0/35	0
Adeno	1/30	3.3
Parainfl.	0/30	0
Entero	1/30	3.3
EBV	0/22	0
AAb	1/40	2.5
AMA	0/19	0
RF	0/40	0
Acute bacterial pneumonia	1/58	1.7

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to concentrations of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA. Samples with other possible interfering substances also showed no influence on the result in this ELISA up to concentrations of 60 g/l albumin, 13 mmol/l cholesterol, 3510 ng/ml biotin and 2.0 mg/ml human IgA, human IgG and human IgM, respectively. Common drugs showed no influence on the result up to concentrations of 200 mg/l paracetamol, 900 mg/l aspirin, 500 mg/l ibuprofen, 150 mg/l acetylcysteine in this ELISA.

The following data were collected using DBS samples:

Precision: Six samples (reactivity distributed over the entire measurement range) were measured. The data were determined on 20 days in two runs per day with two replicates each. Each replicate was yielded by independent extraction from a dried blood spot. The precision is given as standard deviation (SD) and coefficient of variation (CV).

Intra-lab precision

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
Mean	88.29 RU/ml		55.78 RU/ml		8.90 RU/ml		9.97 RU/ml		1.68 RU/ml		11.48 RU/ml	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	8.888	10.1%	5.332	9.6%	0.832	9.4%	0.787	7.9%	0.340	20.3%	1.163	10.1%
Between-run	7.652	8.7%	3.709	6.6%	0.625	7.0%	0.481	4.8%	0.096	5.7%	0.487	4.2%
Within-day	11.728	13.3%	6.495	11.6%	1.041	11.7%	0.922	9.2%	0.354	21.1%	1.261	11.0%
Between-day	0.589	0.7%	2.023	3.6%	0.409	4.6%	0.599	6.0%	0.197	11.7%	0.867	7.5%
Within-lab	11.743	13.3%	6.803	12.2%	1.118	12.6%	1.099	11.0%	0.405	24.1%	1.530	13.3%

Linearity: The linearity of the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) was investigated according to CLSI guideline EP06-A. The Anti-SARS-CoV-2 QuantiVac ELISA (IgG) is linear at least in the tested concentration range (6 RU/ml to 80 RU/ml).

Method comparison:

Study I: To determine the correlation of results obtained using extracts from dried blood spots (DBS) and plasma from venous blood, 20 spiked whole blood samples and six patient samples (origin: Europe, USA) were investivated with the Anti-SARS-CoV-2 QuantiVac ELISA (IgG). For the six patients, one capillary blood sample and one venous blood sample were available. With the spiked samples, venous whole blood was investigated in comparison with plasma from venous blood.

The agreement between the results of the dried blood spots and the venous blood samples was 100% (positive agreement (PPA): 100%; negative agreement (NPA): 100%). Borderline samples were excluded from the calculation.

n = 26		EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG) Plasma from venous blood		
		positive	**borderline**	**negative**
EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG) DBS	**positive**	14	0	0
	borderline	0	1	2
	negative	0	0	9

Clinical performance

Diagnostic sensitivity (Prevalence):

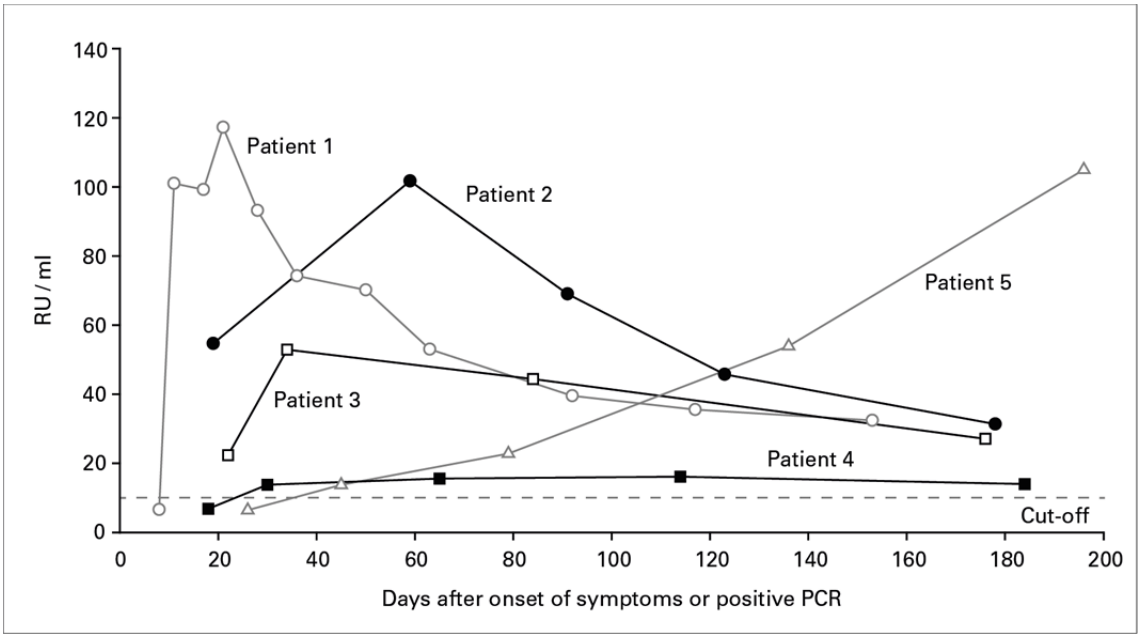
To determine the diagnostic sensitivity, samples from patients with confirmed SARS-CoV-2 infection were analysed. The following sensitivity therefore corresponds to the prevalence of antibodies against SARS-CoV-2 in COVID-19 infected persons.
The sensitivity was determined by investigating 202 samples from 194 patients (origin: Europe, USA), using the Anti-SARS-CoV-2 QuantiVac ELISA (IgG). In these patients, infections with SARS-CoV-2 had been confirmed by RT-PCR test based on a sample taken at the early phase of infection. In samples taken up to day 10 (time point after onset of symptoms or positive direct pathogen detection), the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) showed a sensitivity of 56.7%. The sensitivity of the Anti-SARS-CoV-2 ELISA (IgG) in samples collected after day 10 was 90.3%. Borderline results (n = 7) were not considered in the calculation.

Days after symptom onset or positive direct pathogen detection	EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG)		
	Positive	**Negative**	**Sensitivity**
≤ 10	17	13	**56.7%**
> 10	149	16	**90.3%**

For the 46 patients in whom a SARS-CoV-2 infection was confirmed by RT-PCR test in one sample each from an early infection phase, several consecutive samples were available. The antibody detection yielded a positive result over the progression in 93.2% of the patients after ≥ 21 days past symptom onset or positive direct pathogen detection.

Days after symptom onset or positive direct pathogen detection	EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG)			
	Positive	**Borderline**	**Negative**	**Sensitivity**
≥ 21	41	2	3	**93.2%**

The time course of antibody formation and the antibody activity at specific time points can vary significantly. In most patients, antibodies are detectable after day 10 after symptom onset or positive direct pathogen detection. In individual cases, a significantly delayed synthesis of IgG (> 4 weeks after onset of symptoms or positive direct pathogen detection) has been reported. The graphic shows individual immune responses in COVID-19 patients which were determined using the EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG).



Specificity: The specificity of the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) was determined by analysing 210 patient samples that were positive for antibodies against other human pathogenic coronaviruses, other pathogens, or for rheumatoid factors, as well as 230 samples collected from asymptomatic and symptomatic donors during the Zika virus outbreak in Colombia. Additionally, 1018 samples from blood donors, children and pregnant women obtained before the occurrence of SARS-CoV-2 (before January 2020) were analysed. The results in the borderline range (n = 7) were not considered in the calculation. The specificity of the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) amounted to 99.8%.

Panel	**n**	**EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG)**
		Specificity
Blood donors	849	99.9%
Pregnant women	99	99.0%
Children	70	100.0%
Elderly people	97	100.0%
Asymptomatic donors during the Zika virus outbreak 2015/16 (Colombia)	150	100.0%
Symptomatic donors during the Zika virus outbreak 2016/17 (Colombia)	80	98.7%
Infections with other human pathogenic coronaviruses	11	100.0%
Influenza (freshly vaccinated, including courses)	40	100.0%
Acute EBV infections & heterophile antibodies	22	100.0%
Rheumatoid factors	40	100.0%
Total	**1458**	**99.8%**

Correlation with neutralisation tests (NT):

Study I:

109 samples from a mixed panel (25 samples from healthy blood donors, collected before January 2020, and 84 samples from patients with confirmed past SARS-CoV-2 infections) were investigated with the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) from EUROIMMUN and a commercially available Surrogate NT. The qualitative results obtained with the two tests agreed to 98.2%.

n = 109		Anti-SARS-CoV-2 QuantiVac ELISA (IgG)	
		positive	negative
Commercial Surrogate NT	positive	81	2
	negative	0	26

Study II:

74 samples from patients with confirmed past SARS-CoV-2 infections were investigated with the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) from EUROIMMUN and a $PRNT_{50}$ (plaque reduction neutralisation test according to Wölfel et al. 2020). The qualitative results obtained with the two tests agreed to 97,3%.

n = 74		Anti-SARS-CoV-2 QuantiVac ELISA (IgG)	
		positive	negative
SARS-CoV-2 $PRNT_{50}$	positive	71	0
	negative	2	1

Limitations of the procedure

- For a medical diagnosis, the serological test result should always be interpreted together with the clinical symptoms of the patient and other results, e.g. those of the direct pathogen detection.
- A negative serological result does not exclude an infection. Particularly in the early phase of infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In the case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative or borderline test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significantly higher specific IgG antibody levels (increase by more than factor 2) and/or seroconversion in a follow-up sample taken after at least 7 to 10 days can indicate an acute infection. Sample and follow-up sample should be incubated in parallel in adjacent wells of the ELISA microplate within the same test run.
- The pipetting volumes, incubation times, temperatures, and preparation steps given in the instruction for use must be adhered to.
- Correct performance of sample collection and storage is crucial for the test results.
- The test system is validated for the determination of anti-SARS-CoV-2 IgG in human serum, plasma or dried blood spots only.
- The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostatically adjusted ELISA incubator in all incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction. The same variations also apply to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

- Insufficient washing (e.g. less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false extinction readings.
- Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings.
- The partial or complete adjustment of the test system to the use of instruments for automated sample processing or other liquid handling devices may result in differences between the results obtained with automated processing and those obtained with manual procedure. It is the responsibility of the user to validate the instruments used so that they yield test result within the reliable range.

Literature

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Technical support

In case of technical problems you can obtain assistance via the EUROIMMUN website (https://www.euroimmun.de/en/contact/).

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
STRIPS	Microplate strips	STOP SOLUTION	Stop solution
CAL 1-6	Calibrators 1 to 6	FOIL	Protective foil
CAL 1	Calibrator 1	IVD	In vitro diagnostic medical device
CAL 2	Calibrator 2	LOT	Lot description
CAL 3	Calibrator 3		Protect from sunlight
CAL 4	Calibrator 4		Storage temperature
CAL 5	Calibrator 5		Unopened usable until (YYYY-MM-DD)
CAL 6	Calibrator 6	CE	CE-labelled
POS CONTROL	Positive control		Manufacturing date (YYYY-MM-DD)
NEG CONTROL	Negative control		Manufacturer
CONJUGATE	Conjugate		Observe instructions for use
SAMPLE BUFFER	Sample buffer	REF	Order number
WASH BUFFER 10x	Wash buffer, 10x concentrate		Contents suffice for <n> analyses
SUBSTRATE	Substrate		Biological risks