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

SARS-CoV-2 NeutraLISA

Instructions for use

For in vitro diagnostic use IVD

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2606-9601-4	SARS-coronavirus 2 (SARS-CoV-2)	IgAGM	Ag-coated microplate wells	96 x 01 (96)

CE

Intended use

This enzyme immunoassay (ELISA) provides semiquantitative in vitro determination of neutralising antibodies in serum, EDTA or heparin plasma, which inhibit binding of the receptor binding domain (RBD) of viral SARS-CoV-2 S1 to ACE2 receptors of human cells. Neutralising antibodies against S1/RBD can inhibit the receptor-mediated entry of the virus into the host cell. The SARS-CoV-2 NeutraLISA supports the evaluation of the individual immune response following SARS-CoV-2 infection or vaccination with S1-/RBD-based vaccines. The product is designed for use as IVD and can be optionally processed on fully automated instruments.

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-2 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 some of the investigated persons without history of SARS-CoV-2 infection and are supposedly due to prior infections with coronaviruses causing common colds. This may indicate a long-lasting immunity following infection with betacoronaviruses [8, 10, 11].

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 antigens used in this ELISA were presented recombinantly in the human cell line HEK 293. The solid phase was coated with S1/RBD domain of the spike protein of SARS-CoV-2, isolate Wuhan-Hu-1. The human receptor protein ACE2 (angiotensin-converting enzyme 2) is supplied as a concentrate in solution and is biotinylated.

Test principle

The test kit contains microplate strips each with 8 break-off reagent wells coated with recombinantly presented S1/RBD domain of the spike protein of SARS-CoV-2. In the first reaction step, the controls and samples are diluted with sample buffer containing soluble biotinylated ACE2 and then incubated in the reagent wells. If neutralising antibodies are present in the sample, they compete with the receptor ACE2 for the binding sites of the SARS-CoV-2 S1/RBD proteins. Unbound ACE2 is removed in a subsequent washing step. To detect the bound ACE2, a second incubation step with peroxidase-labelled streptavidin is performed, which catalyses a colour reaction in the third reaction step. The intensity of colour formed is inversely proportional to the concentration of neutralising antibodies in the sample.

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. Positive control 5x concentrate	colourless	1 x 0.3 ml	POS CONTROL 5x
3. Negative control 5x concentrate	colourless	1 x 0.3 ml	NEG CONTROL 5x
4. Enzyme conjugate peroxidase-labelled streptavidin, ready for use	blue	1 x 12 ml	CONJUGATE
5. ACE2 concentrate 20x concentrate	colourless	1 x 2 ml	ACE2 20x
6. ACE2 dilution buffer ready for use	colourless	1 x 50 ml	ACE2 DILUENT
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Protective foil	-	2 pieces	FOIL
11. Labels for sample buffer	-	6 x 1 pieces	SAMPLE BUFFER
12. Quality control certificate	-	1 protocol	-
13. Instructions 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 and the ACE2 dilution buffer
- Stop watch
- For EUROIMMUN Analyzer I/-2P: substrate bottle 24 ml, black, with cap, EUROIMMUN order no. ZG 0106-0130 or stop solution bottle 24 ml, white, with cap, EUROIMMUN order no. ZG 0120-0120
- For EUROLabWorkstation ELISA: EUROTank 100 ml, black, set of tank + lid, EUROIMMUN order no. ZG 0870-0101 / -0180, EUROTank 100 ml, natural, set of tank + lid, EUROIMMUN order no. ZG 0871-0101 / -0180, substrate bottle 24 ml, black, with cap, EUROIMMUN order no. ZG 0106-0130 or stop solution bottle 24 ml, white, with cap, EUROIMMUN order no. ZG 0120-0120

Depending on the instrument, further materials are required for automatic processing. For more information, please refer to the instructions for use.

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Storage and stability

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

In-use stability

After initial 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 healthcare professionals in an adequate laboratory environment.
- Do not use the test kit if the packaging of the reagents is damaged.
- Before using the product, read the instructions for use carefully. Only use the valid version provided with the product.
- EUROIMMUN reagents must not be mixed with or replaced by 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 controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all test kit components should be treated as potentially infectious and handled with care.

Preparation and stability of the samples

Samples

Human serum or EDTA or heparin plasma

Sample preparation

The **patient samples** to be investigated are diluted **1:5** in sample buffer (for preparation, see below).
For example: dilute 50 µl sample in 200 µl sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

Note

- If instruments for automated incubation are used, refer to the sample handling described in the instructions for use.

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).
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.

Controls

The controls are 5x concentrates and should be handled as samples. Mix and centrifuge thoroughly before use. Diluted controls must be incubated within one working day.

Enzyme conjugate

Ready for use. Mix the reagent thoroughly before use.

ACE2 dilution buffer

Ready for use. Before use, carefully warm the ACE2 dilution buffer to +37 °C and mix thoroughly to dissolve any crystals it might contain (30 minutes, e.g. in a water bath). Only then the buffer can be used for preparation of the sample buffer.

ACE2 concentrate

The ACE2 is a 20x concentrate. For preparation of the working-strength **sample buffer**, dilute the required volume 1:20 with the ACE2 dilution buffer prepared as described above.For example: 1.5 ml ACE2 concentrate plus 28.5 ml ACE2 dilution buffer

The following materials should be used: substrate bottle 24 ml, black, with cap, EUROIMMUN order no. ZG 0106- 0130, or stop solution 24 ml, white, with cap, EUROIMMUN order no. ZG 0120- 0120 (not contained in the test kit). The working-strength sample buffer must be mixed thoroughly before each use. Use new vessels for each new preparation.

Sample buffer

The sample buffer is prepared by diluting the ACE2 concentrate with ACE2 dilution buffer as described above. The working-strength sample buffer is stable for 1 week with proper handling and if stored at room temperature (+18 °C to +25 °C).

For labelling of the sample buffer, especially with fully automated processing on analysis systems, specific labels are provided in the test kit.

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.

Interchangeability of reagents

- **Coated reagent wells, controls, ACE2 concentrate and sample buffer (label)** are lot-specific and cannot be interchanged between lots. They can generally not be interchanged with reagents from other lots of the SARS-CoV-2 NeutraLISA. Within the same lot, it is possible to interchange reagents that carry identical article numbers.
- **Enzyme conjugate** and **ACE2 dilution buffer** can be used in the SARS-CoV-2 NeutraLISA and interchanged with reagents from other lots as long as they carry identical article numbers.
- **Wash buffer, chromogen/substrate solution** and **stop solution** can be used in the EUROIMMUN ELISAs independent of the lot and product. This enables optimised processing on fully automated ELISA processors.

Waste disposal

Patient samples, 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 blank and the percentage inhibitions (% IH) 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. Moreover, the single values of the blank must not deviate more than 10% from the mean value of the two single values (e.g. with a blank mean value of 2.0 OD, the tolerance range for single values of the blank is 1.8 – 2.2 OD). 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 obtained with the SARS-CoV-2 NeutraLISA (IgG) are given in percentage of inhibition (% IH). The cut-off was determined based on an internal reference material. The first international WHO standard (First WHO International Standard for anti-SARS-CoV-2 immunoglobulin NIBSC code 20/136) is available as international reference material, which contains per definition 250 IU of neutralising antibody activities per ampoule and is supplied in 250 µl of distilled water. This information refers to the total immunoglobulin content and should always be considered with reference to a specific immunoglobulin class and antigenic target structure. Direct conversion of % IH into IU/ml is not possible. However, studies were performed showing the correlation between % IH and IU/ml based on a dilution series of the first international WHO standard.

Assay procedure

(Partly) manual test performance

ATTENTION: The processing of the format EI 2606-9601-4 on the EUROLabWorkstation is based on the assay stored there. For details, please refer to the instructions for use of the instrument.

Sample incubation: (1st step)
Transfer **100 µl** of the **blank (sample buffer) or diluted controls or 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 streptavidin) into each of the microplate wells.
Incubate **30 minutes** at room temperature (+18 °C to +25 °C).

Washing:
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 **15 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

The sample buffer must be prepared manually by diluting the ACE2 concentrate in ACE2 dilution buffer (cf. Preparation and stability of the reagents). The analysis device is then loaded with the working-strength sample buffer in an additional reagent bottle, which can be ordered from EUROIMMUN (see "Additional materials and equipment (not included in the test kit))". The respective labels for marking the sample buffer are included in the test kit and allow automatic identification based on the barcode in the EUROIMMUN Analyzer I and EUROIMMUN Analyzer I-2P. For the processing on the EUROLabWorkstation ELISA, suitable labels for EUROTanks, substrate or stop solution bottles must be printed via the instrument software.

Sample dilution and subsequent test processing is performed fully automatically by an analysis instrument. The incubation conditions stored in the software authorised by EUROIMMUN may deviate slightly from the specifications in the ELISA instructions for use, but have been validated in combination with the EUROIMMUN Analyzer I, EUROIMMUN Analyzer I-2P and the EUROLabWorkstation ELISA and this EUROIMMUN ELISA. Validation documents are available on request.

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

The pipetting protocol is an example for the **semiquantitative analysis** of 24 patient sera (P 1 to P 24).

The blank (sample buffer) is analysed in duplicate determination and serves as a reference. The positive (pos.) and negative (neg.) controls and the patient samples are each investigated in single determination. The reliability of the analysis can be further 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 upper threshold of the reference range (**cut-off**) recommended by EUROIMMUN is 25% IH. Values above the percentage of inhibition are considered as positive and values below as negative.

Semiquantitative

Calculation of the % IH based on the mean value of the blank measurement allows semiquantitative evaluation of the results. This involves calculating a ratio of the extinction values of the controls or patient samples over the extinction value of the blank duplicate determination for which the highest extinction is expected. Calculate the % IH according to the following formula:

$$100\ \% - \frac{\text{Extinction of patient sample} \times 100\ \%}{\text{Extinction of blank}} = \%\ \text{IH}$$

Example: **Blank measured: 2.0 OD**
Patient sample: 1.0 OD
Calculation: 100% - (1.0 OD x 100 % / 2.0 OD) = 50% inhibition

EUROIMMUN recommends interpreting results as follows:

% IH < 20: negative
% IH ≥ 20 to < 35: borderline
% IH ≥ 35: positive

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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.

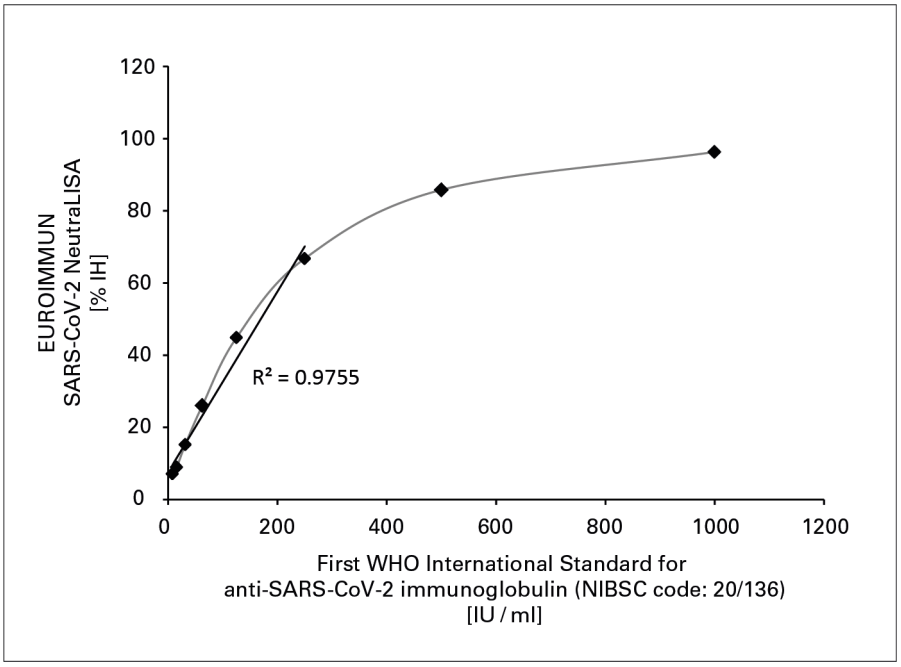
Negative values of % IH can occur due to slight variations of the extinction and should be interpreted as described above. In the case of negative values < - 20% IH, which may indicate technical problems during the incubation, the sample should be retested. If the negative control yields negative values of < -20% IH, the test run is invalid and should be repeated.

Analytical performance

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 in triplicate determination using the SARS-CoV-2 NeutraLISA. The linear regression analysis yielded a coefficient of determination of $R^2 = 0.98$ up to a value of 67% IH.

First WHO International Standard (NIBSC code: 20/136)		EUROIMMUN SARS-CoV-2 NeutraLISA
Dilution	IU/ml	Mean value % IH
1/5	1000.0	96
1/10	500.0	86
1/20	250.0	67
1/40	125.0	45
1/80	62.5	26
1/160	31.3	15
1/320	15.6	9
1/640	7.8	7



Precision

Studies on the intra-lab precision were carried out according to CLSI guideline EP05-A3. Seven 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	
Mean	99.66 % IH		68.62 % IH		53.79 % IH		24.67 % IH	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	0.046	0.0	1.143	1.7	0.999	1.9	1.474	6.0
Between run	0.027	0.0	1.614	2.4	0.715	1.3	1.775	7.2
Within day	0.053	0.1	1.978	2.9	1.229	2.3	2.307	9.4
Between day	0.018	0.0	1.710	2.5	1.645	3.1	2.060	8.4
Within lab	0.056	0.1	2.615	3.8	2.053	3.8	3.093	12.5

	Sample 5		Sample 6		Sample 7	
Mean	25.67 % IH		18.54 % IH		-1.07 % IH	
	SD	% CV	SD	% CV	SD	% CV*
Repeatability	1.565	6.1	1.490	8.0	2.194	-
Between run	2.099	8.2	2.007	10.8	1.088	-
Within day	2.618	10.2	2.499	13.5	2.449	-
Between day	1.354	5.3	2.228	12.0	1.678	-
Within lab	2.947	11.5	3.348	18.1	2.968	-

* %CV for negative sample not applicable, negative samples must remain negative.

Reproducibility

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

Inter-lot-Precision

	Sample 1		Sample 2		Sample 3		Sample 4	
Mean	99.68 % IH		71.04 % IH		55.04 % IH		27.89 % IH	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	0.086	0.1	2.153	3.0	1.257	2.3	3,219	11.5
Between run	0.000	0.0	0.960	1.4	1.454	2.6	0.000	0.0
Between day	0.017	0.0	1.062	1.5	1.120	2.0	3.068	11.0
Within lot	0.088	0.1	2.585	3.6	2.225	4,0	4.447	15.9
Between lot	0.035	0.0	1.210	1.7	1.144	2.1	1.896	6.8
Reproducibility	0.095	0.1	2.855	4.0	2.502	4.5	4.834	17.3

	Sample 5		Sample 6		Sample 7	
Mean	26.13 % IH		17.79 % IH		0.88 % IH	
	SD	% CV	SD	% CV	SD	% CV*
Repeatability	2.129	8.1	2.046	11.5	4.054	-
Between run	1.746	6.7	2.551	14.3	1.643	-
Between day	1.641	6.3	2.370	13.3	2.471	-
Within lot	3.205	12.3	4.039	22.7	5.023	-
Between lot	2.049	7.8	2.278	12.8	0.199	-
Reproducibility	3.804	14.6	4.637	26.1	5.027	-

* %CV for negative sample not applicable, negative samples must remain negative.

Cross-reactivity (analytical specificity)

Due to low homologies of the S1 protein within the family of coronaviruses, cross-reactions with most human pathogenic representatives of this virus family are virtually excluded. 55 samples taken prior to the occurrence of SARS-CoV-2 (before January 2020) and that were antibody-positive for at least one human pathogenic coronavirus (HCoV HKU1; HCoV OC43; HCoV NL63; HCoV 229-E) were investigated with the SARS-CoV-2 NeutraLISA. No cross reactions with antibodies against the endemic HCoV were observed in the analyses.

Cross-reactions between SARS-CoV(-1) and SARS-CoV-2, however, are likely to occur due to their close relationship.

Antibodies against	**n (positive) / n (total)**	**EUROIMMUN SARS-CoV-2 NeutraLISA Positive rate [%]**
HCoV	0/55	0.0

Other possible interference with rheumatoid factors (RF) and antibodies against unrelated structures such as Epstein-Barr-Virus (EBV) and *Haemophilus influenzae* type B (HIB) or antibodies formed after vaccinations against influenza viruses and *Streptococcus* were not observed.

Antibodies against	n (positive) / n (total)	EUROIMMUN SARS-CoV-2 NeutraLISA Positive rate [%]
Rheumatoid factors	0/40	0.0
EBV	0/22	0.0
HIB	0/6	0.0
Influenza	0/39	0.0
Streptococcus	0/10	0.0

Interference

Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.1 mg/ml bilirubin in this ELISA. Samples with other possibly interfering substances did not show any influence on the result up to a concentration of 60 g/l albumin, 13 mmol/l cholesterol and 3510 ng/ml biotin on the results of this ELISA.

Clinical performance

Diagnostic sensitivity

The diagnostic sensitivity was determined by investigating 124 samples from convalescent COVID-19 patients with the EUROIMMUN SARS-CoV-2 NeutraLISA. These samples were taken ≥ 15 days after symptom onset and showed a positive result in the neutralisation test ($PRNT_{50}$ and NT_{50}). The SARS-CoV-2 NeutraLISA achieved a sensitivity of 95.9%. Borderline results (n = 1) were excluded from the calculation of sensitivity.

Days after symptom onset	EUROIMMUN SARS-CoV-2 NeutraLISA		
	positive	negative	Sensitivity
≥ 15	118	5	**95.9%**

Specificity

The specificity of the SARS-CoV-2 NeutraLISA was evaluated based on 159 samples that were positive for antibodies against other human pathogenic coronaviruses, other pathogens or rheumatoid factors or showed a negative result in the SARS-CoV-2 neutralisation test (NT_{50}). Moreover, 600 samples from blood donors and children, taken prior to the occurrence of SARS-CoV-2 (before January 2020) were investigated. The evaluation of results yielded a specificity of the SARS-CoV-2 NeutraLISA of 99.7%. Borderline results (n = 2) were excluded from the calculation of specificity.

n	EUROIMMUN SARS-CoV-2 NeutraLISA		
	positive	negative	Specificity
759	2	755	**99.7%**

Correlation with neutralisation tests (NT):

Study I:

74 samples from patients with past confirmed SARS-CoV-2 infection were investigated with the SARS-CoV-2 NeutraLISA from EUROIMMUN and a $PRNT_{50}$ (plaque reduction neutralisation test according to Wölfel et al. 2020 [11]). The agreement of the qualitative results from both tests was 98.6%.

n = 74		EUROIMMUN SARS-CoV-2 NeutraLISA	
		positive	negative
SARS-CoV-2-$PRNT_{50}$	**Positive**	71	0
	Negative	1	2

Study II:
52 samples from patients with past confirmed SARS-CoV-2 infection were investigated with the SARS-CoV-2 NeutraLISA from EUROIMMUN and another commercially available surrogate NT. The agreement of the qualitative results from both tests was 96.2%.

n = 52		EUROIMMUN SARS-CoV-2 NeutraLISA	
		positive	negative
Other commercial surrogate NT	positive	50	2*
	negative	0	0

*For these samples, neither information on the clinical precharacterisation nor follow-up samples were available. Both samples were negative in the $PRNT_{50}$.

Correlation with IgG antibody detection:

111 samples from patients with past confirmed SARS-CoV-2 infection were investigated with the SARS-CoV-2 NeutraLISA and the Anti-SARS-CoV-2 QuantiVac ELISA (IgG) from EUROIMMUN. The agreement of the qualitative results from both tests was 99.1%. Borderline results (n = 2) were excluded from the calculation.

n = 111		EUROIMMUN SARS-CoV-2 NeutraLISA		
		positive	borderline	negative
EUROIMMUN Anti-SARS-CoV-2 QuantiVac ELISA (IgG)	positive	107	2	1
	negative	0	0	1

Limitations of the procedure

- The results should always be interpreted together with those of further laboratory diagnostic procedures and based on the clinical picture. A negative serological result does not exclude a disease.
- The specifications in the instructions for use, e.g. pipetting volumes, incubation times, temperatures and preparation steps must be observed to avoid incorrect results.
- Correct sample collection and storage are crucial for the correctness of the results.
- The test system is validated for the determination of neutralising antibodies against SARS-CoV-2 in human serum or plasma only.
- The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostatically controlled ELISA incubator in all incubation steps.
- 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 conversion and lead to false low extinction readings.
- Partial or complete adaptation of the test system for use with automated sample processors or other liquid handling devices may lead to differences between the results obtained with the automated and manual procedure. It is the responsibility of the user to validate the automated instruments used for the analysis to ensure that they yield test result within the permissible range.

Literature

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Liability

The test kit, including original accessories, must only be used in accordance with the intended use. EUROIMMUN accepts no liability for any other use (e.g. non-compliance with the instructions for use and improper use) or for resulting damages.

Technical support

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

研究用試薬

Additional information

Further information on the product can be found in the short report on safety and performance in the European Database on Medical Devices (EUDAMED), once it is available.

Regulatory information for customers in the European Union: Please observe the obligation to report any serious incidents occurring in connection with this product to the competent authorities and to EUROIMMUN.

Meaning of the symbols

Symbol	Meaning	Symbol	Meaning
STRIPS	Microplate strips	LOT	Lot description
FOIL	Protective foil		Protect from sunlight
POS CONTROL 5x	Positive control, 5x concentrate		Storage temperature
NEG CONTROL 5x	Negative control, 5x concentrate		Unopened usable until (YYYY-MM-DD)
CONJUGATE	Conjugate	CE	CE-marking
SAMPLE BUFFER	Sample buffer		Manufacturing date (YYYY-MM-DD)
WASH BUFFER 10x	Wash buffer, 10x concentrate		Manufacturer
SUBSTRATE	Substrate		Observe instructions for use
STOP SOLUTION	Stop solution	REF	Order number
IVD	In vitro diagnostic medical device		Contents suffice for <n> analyses
ACE2 20x	ACE2 concentrate, 20x concentrate		Biological risks
ACE2 DILUENT	ACE2 dilution buffer		