Anti-SARS-CoV-2 ELISA (IgG)
Instruction for use

For in vitro diagnostic use. For prescription use only. For emergency use authorization only.

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

CE

Intended use

The EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) is an enzyme-linked immunosorbent assay intended for the qualitative detection of IgG class antibodies to SARS-CoV-2 in human serum or plasma (K+-EDTA, Li+-heparin, Na+-citrate). The EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) should not be used to diagnose acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 32 U.S.C. 263a, to perform high complexity tests.

Results are for the detection of SARS CoV-2 antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) is only for use under the Food and Drug Administration's Emergency Use Authorization.

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]. The new coronavirus originated in China in the city of Wuhan, Hubei province. It caused an infection wave, which has spread rapidly within the country and worldwide [2, 3]. Just a few days after the first report about patients with pneumonia of unclear origin, the causative pathogen was identified as SARS-CoV-2 [2-5].

SARS-CoV-2 is predominantly transmitted by droplet infection via coughing or sneezing and through close contact with infected persons [2-4, 6]. Health care personnel and family members are especially at risk of infection [6, 7]. The zoonotic reservoir of the virus appears to be bats [2, 4, 6].

The incubation time of SARS-CoV is three to seven, maximally 14 days [2]. The most common symptoms of SARS-CoV-2 infection are fever, coughing, breathing difficulties and fatigue [2-4, 6]. 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]. Reported case fatality rates depend on geographic location, age, and comorbidities. 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 detection of viral RNA by reverse transcriptase polymerase chain reaction (RT-PCR) or of viral protein by ELISA primarily in sample material from the upper (nasopharyngeal or oropharyngeal smear) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, nasopharyngeal secretion, oropharyngeal secretion, etc). The determination of antibodies enables confirmation of recent or prior SARS-CoV-2 infection in patients with typical symptoms and in suspected cases. It also contributes to monitoring and outbreak control [4, 5].

Cross reactions with antibodies within the genus Betacoronavirus have been described [4, 5]. Currently, there is no medication or vaccine available against infection with this new virus [2, 7].

Antigen

The reagent wells of the ELISA were coated with an 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 structural protein of SARS-CoV-2. In the first reaction step, diluted patient 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) catalyzing a color reaction.

Contents of the test kit

Component	Color	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 (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgG, ready for use	green	1 x 12 ml	CONJUGATE
6. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colorless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colorless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colorless	1 x 12 ml	STOP SOLUTION
10. Protective foil	-	3 pieces	FOIL
11. Quality control certificate	-	1 protocol	-
12. 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 deionized 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.
- For in vitro diagnostic use under emergency use authorization only. Use of this product is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.
- If the packed reagents are visibly damaged, do not use the test kit.
- Before using the product, read the instruction for use carefully. Use only the valid version provided with the product.
- The pipetting volumes, incubation times, temperatures, and preparation steps given in the instruction for use must be adhered to.
- Do not substitute or mix the EUROIMMUN reagents with reagents from other manufacturers.
- Observe Good Laboratory Practice (GLP) and safety guidelines. The stop solution contains sulfuric acid. 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, flush with copious amounts of water. Remove and wash contaminated clothing. In case of ingestion, obtain medical advice.
- The calibrator 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.
- **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).
- **Stability of the patient samples:**
  The Clinical and Laboratory Standards Institute (CLSI GP44-A4) recommends the following storage conditions for samples: Samples should be stored at room temperature no longer than 8 hours. If the assay will not be completed within 8 hours, the samples should be refrigerated at +2°C to +8°C. If the assay will not be completed within 48 hours, or if the samples will be stored beyond 48 hours, samples should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed. Frozen samples must be mixed well after thawing and prior to testing. Diluted samples should be incubated within 8 hours. Do not use bacterially contaminated samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine its own specific stability criteria.

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, but not longer than the indicated expiry date.
- **Calibrator 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 crystallization occurs in the concentrated buffer, warm it to +37°C and mix well before dilution. The amount required should be removed from the bottle using a clean pipette and diluted 1:10 with deionized 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 up to 28 days if stored at +2°C to +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the bottle 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 colored.
- **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

The controls and calibrator included in the test kit must be used with each run. Results cannot be validated if the control values deviate from the expected values stated on the quality control certificate. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated. The positive control and negative control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay cut-off. Additional controls may be required according to guidelines or local, state, and/or federal regulations (such as 42 CFR 493.1256) or accrediting organizations.

Reference material

As no quantified international reference serum exists for antibodies against SARS-CoV-2, the calibration is performed in ratios which are a relative measure for the concentration of antibodies in serum or plasma.

Assay procedure

(Partly) manual test performance

Sample incubation: (1st step)	Transfer **100 µl** of the **calibrator, 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. For manual test performance cover the reagent wells with the protective foil. Incubate for **30 minutes at +37°C ± 1°C**.
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 color 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, carefully shake the microplate to ensure a homogeneous distribution of the solution.

Pipetting protocol

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

The pipetting protocol for microplate strips 1 to 4 is an example for the **ratio-based analysis** of 24 patient sera (P 1 to P 24).

In this example the calibrator (C), the positive (pos.) and negative (neg.) controls as well as the patient samples have been incubated in one well each.

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 minimizes reagent wastage.

Test evaluation

Ratio-based analysis: Results can be evaluated by calculating a ratio of the OD of the control or patient sample over the OD of the calibrator. Calculate the ratio according to the following formula:

$$\frac{\textbf{OD of the control or clinical sample}}{\textbf{OD of calibrator}} = \textbf{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8: negative
Ratio ≥ 0.8 to <1.1: borderline
Ratio ≥1.1: positive

In case of a borderline result, a secure evaluation is not possible. It is recommended the patient may be re-drawn one to two weeks later and re tested with the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) assay.

Analytical performance

Precision: Studies on the intra-lab precision were carried out according to CLSI guideline EP05-A3. Four 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

n= 30	**Sample 1**		**Sample 2**		**Sample 3**		**Sample 4**	
Mean ratio	0.07		1.12		2.37		5.23	
Range of ratio values	0.04 – 0.09		1.00 – 1.32		2.00 – 2.71		4.59 – 6.02	
Overall result	negative		borderline/positive		positive		positive	
% positive	0.0%		56.7%		100.0%		100.0%	
% borderline	0.0%		43.3%		0.0%		0.0%	
% negative	100.0%		0.0%		0.0%		0.0%	
	SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Repeatability	0.012	16.0	0.060	5.4	0.091	3.9	0.231	4.4
Between run	0.000	0.0	0.021	1.9	0.058	2.4	0.168	3.2
Within day	0.012	16.0	0.063	5.7	0.108	4.6	0.285	5.5
Between day	0.002	2.3	0.060	5.4	0.174	7.4	0.089	1.7
Within lab	0.012	16.2	0.087	7.8	0.205	8.7	0.299	5.7

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. However, due to the close relationship of SARS-CoV(-1) and SARS-CoV-2, cross-reactions between these two viruses are likely. Sera from patients with SARS-CoV(-1), MERS-CoV, HCoV-229E, HCoV-NL63, HCoV-HKU1 or HCoV-OC43 infections were investigated to examine this further. Pronounced cross-reactions occur mainly with Anti-SARS-CoV(-1) IgG antibodies. Cross-reactions to other human pathogenic coronaviruses were not observed.

An additional cross-reactivity study was performed by a national reference laboratory in the US Mid-west using clinical samples from human pathogenic representatives of this virus family and some non-coronavirus populations with similarities to this virus family. Low cross-reactivity was observed in some species of pneumonia and to the respiratory syncytial virus, but that is common. Cross-reactions to other human pathogenic viruses were not observed. The overall cross-reactivity for both the studies showed the following results:

No.	**Panel**	**n**	**Anti-SARS-CoV-2 ELISA (IgG)**	
			Negatives	**%Negative**
1	SARS-CoV(-1) Infection (2005)	2	2	100.0%
2	SARS-CoV(-1) Infection (2020)	3	0	0.0%*
3	HCoV-NL63 Infection	4	4	100.0%
4	HCoV-229E Infection	2	2	100.0%
5	HCoV-OC43 Infection	3	3	100.0%
6	HCoV-HKU1 Infection	7	7	100.0%
7	MERS-CoV Infection	2	2	100.0%
8	Acute EBV infections with heterophile antibodies	22	22	100.0%
9	ANA	30	29	96.7%
10	Other autoantibodies	10	9	90.0%*
11	Rheumatoid factors	40	40	100.0%
12	Fresh vaccination against influenza and follow-ups	40	40	100.0%
13	Influenza Antibody Positive	18	18	100.0%
14	Influenza PCR Positive	14	14	100.0%

No.	Panel	n	Anti-SARS-CoV-2 ELISA (IgG)	
			Negatives	%Negative
15	Influenza PCR Positive (Paired Convalescent)	2	2	100.0%
16	Adenovirus	6	6	100.0%
17	Anti-CMV (IgM/IgG)	15	15	100.0%
18	Metapneumovirus	4	4	100.0%
19	Mycoplasma (IgM/IgG)	15	14	93.3%*
20	Respiratory Syncytial Virus (RSV)	3	2	66.7%
21	Rhino/Enterovirus	1	1	100.0%
22	Streptococcus pneumoniae urine antigen	12	10	83.3%
23	Acute, severe bacterial pneumonia with high concentrations of procalcitonin	58	56	96.6%
24	Chlamydophila pneumoniae (IgG)	15	14	93.3%
25	Chlamydophila pneumoniae (IgM/IgG)	4	4	100.0%

*Borderlines were counted as positive (SARS CoV-1: 2/3 borderline; Other autoantibodies: 1/10 borderline; Mycoplasma: 1/15 borderline).

Interference: Potential interference from high levels of hemoglobin, triglycerides and bilirubin was evaluated. Samples at different anti-SARS-CoV-2 IgG antibody concentrations across the assay measuring range were spiked with potential interfering substances and incubated with the test system according to the package insert. Hemolytic, lipemic and icteric samples showed no influence on the result up to concentrations of 10 mg/ml hemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA. Class specificity was evaluated by means of robustness of the SARS-CoV-2 specific IgG antibody binding reaction in the presence of high levels of human total IgG, IgM and IgA. Samples spiked at different anti-SARS-CoV-2 IgG antibody concentrations across the assay measuring range were used. No significant effect was observed for concentrations of up to 10.4 mg/ml for human IgG, 1.4 mg/ml for human IgM and 2.1 mg/ml for human IgA.

Matrix comparison: The usability of plasma was investigated using 11 sample pairs each of serum and corresponding plasma (EDTA, heparin, citrate). Passing-Bablok regression was performed for the comparison of plasma to serum and showed the following results:

	EDTA plasma	Heparin plasma	Citrate Plasma
n	11	11	11
Assay ratio range (serum)	0.11 - 8.45	0.11 - 8.45	0.11 - 8.45
Assay ratio range (plasma)	0.16 - 8.59	0.16 - 8.61	0.02 - 7.55
Regression equation (y = plasma, x = serum)	0.057 + 1.130 x	0.021 + 1.012 x	0.110 + 0.941 x
95% C.I. of intercept	-0.169 - 0.199	-0.241 - 0.063	-0.195 - 0.190
95% C.I. of slope	1.008 - 1.311	0.954 - 1.194	0.879 - 1.135
Coefficient of determination R^2	0.9936	0.9963	0.9947

Clinical performance

Positive Agreement to PCR: The positive agreement to PCR was evaluated for the Anti-SARS-CoV-2 ELISA (IgG) in 4 different studies. One study was performed in cooperation with 5 different clinical hospitals and laboratories from Germany and the other 3 studies were performed at various research institutions/reference labs within United States. Serum and plasma samples were tested. The samples were grouped for analysis into two sets based on the information available to estimate the stage of disease at the time of serology sample collection: Set 1 included information on days post onset of symptoms (n=78 from 47 different patients), and Set 2 included information on days post PCR confirmation (n=519). The summary of the studies is presented below.

Counting borderline results as negative, the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) displayed a 13.9% positive agreement to PCR before **10 days post onset of symptoms** (n = 36), from **11 to 20 days post onset of symptoms**, the positive agreement was seen to be 61.1% (n = 36) and after **21 days post onset of symptoms** the positive agreement to PCR was 100.0% (n = 6)

Counting borderline results as negative, the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) displayed a 21.7% positive agreement to PCR before **5 days post PCR confirmation** (n = 240), from **5 to 10 days post PCR confirmation**, the positive agreement was seen to be 69.4% (n = 242) and after **11 days post PCR confirmation** the positive agreement to PCR was 81.1% (n = 37)

Summary of results in relation to days **post onset of symptoms**:

Days Post Onset of Symptoms	n*	Anti-SARS-CoV-2 ELISA (IgG)		
		Negative	Borderline	Positive
0	3	2	0	1
1	1	1	0	0
2	5	5	0	0
3	2	2	0	0
4	4	4	0	0
5	5	5	0	0
6	2	1	0	1
7	6	4	1	1
8	2	2	0	0
9	2	1	0	1
10	4	3	0	1
11	3	1	0	2
12	3	0	0	3
13	8	5	0	3
14	5	2	0	3
15	1	1	0	0
16	3	1	0	2
17	5	2	0	3
18	2	1	0	1
19	4	0	1	3
20	2	0	0	2
21	1	0	0	1
23	1	0	0	1
26	1	0	0	1
32	2	0	0	2
36	1	0	0	1
Total	**78**	**43**	**2**	**33**

* n=78 from 47 different patients

Days Post Onset of Symptoms	EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG)														
	1st Serial Measurement			2nd Serial Measurement			3rd Serial Measurement			4th Serial Measurement			5th Serial Measurement		
	n	bord	pos	n	bord	pos	n	bord	pos	n	bord	pos	n	bord	pos
≤ 10 days	32	1	4	4	0	1	0	0	0	0	0	0	0	0	0
11-20 days	14	0	7	12	0	9	8	0	5	1	1	0	1	0	1
≥ 21 days	1	0	1	0	0	0	2	0	2	2	0	2	1	0	1
Total Subjects	47	1	12	16	0	10	10	0	7	3	1	2	2	0	2

Days post onset of symptoms	n	Anti-SARS-CoV-2 ELISA (IgG)			
		Neg.	Bord.	Pos.	Positive % Agreement to PCR (95% C.I.) borderline counted as negative
≤ 10	36	30	1	5	13.9% (6.1% - 28.7%)
11 – 20	36	13	1	22	61.1% (44.9% - 75.2%)
≥ 21	6	0	0	6	100.0% (61.0% - 100%)

Summary of results in relation to days **post PCR confirmation**:

Days Post PCR Confirmation	n	Anti-SARS-CoV-2 ELISA (IgG)		
		Negative	Borderline	Positive
0	58	54	1	3
1	27	25	0	2
2	42	32	4	6
3	45	28	2	15
4	68	35	7	26
5	43	18	1	24
6	46	16	0	30
7	53	14	0	39
8	39	10	2	27
9	36	7	0	29
10	25	6	0	19
11	19	2	0	17
12	8	1	0	7
13	4	1	0	3
14	4	3	0	1
15	1	0	0	1
17	1	0	0	1
Total	**519**	**252**	**17**	**250**

Days post PCR confirmation	n	Anti-SARS-CoV-2 ELISA (IgG)			
		Neg.	Bord.	Pos.	Positive % Agreement to PCR (95% C.I.) borderline counted as negative
< 5	240	174	14	52	21.7% (16.9% - 27.3%)
5 - 10	242	71	3	168	69.4% (63.4% - 74.9)
≥ 11	37	7	0	30	81.1% (65.8% - 90.5%)

Independent Clinical Agreement Validation Study: The EUROIMMUN SARS-COV-2 ELISA (IgG) was tested under this validation program on 2020-04-21 at the Frederick National Laboratory for Cancer Research (FNLCR), a Federally Funded Research and Development Center (FFRDC) sponsored by the National Cancer Institute (NCI).
The test was validated against a panel of previously frozen samples consisting of 30 SARS-CoV-2 antibody-positive serum samples and 80 antibody-negative serum and plasma samples. Each of the 30 antibody-positive samples were confirmed with a nucleic acid amplification test (NAAT) and both IgM and IgG antibodies were confirmed to be present in all 30 samples. The presence of antibodies in the samples was confirmed by several orthogonal methods prior to testing with the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG). The presence of IgM and IgG antibodies specifically was confirmed by one or more comparator methods. Antibody-positive samples were selected at different antibody titers. All antibody-negative samples were collected prior to 2020 and include i) Seventy (70) samples selected without regard to clinical status, “Negatives” and ii) Ten (10) samples selected from banked serum from HIV+ patients, “HIV+”. Testing was performed by one operator using one lot of the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG). Confidence intervals for sensitivity and specificity were calculated per a score method described in CLSI EP12-A2 (2008). For evaluation of cross-reactivity with HIV+, it was evaluated whether an increased false positive rate among antibody-negative samples with HIV was statistically higher than the false positive rate among antibody-negative samples without HIV (for this, a confidence interval for the difference in false positive rates was calculated per a score method described by Altman). The results and data analysis are shown in the tables below.

The EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG) displayed a positive agreement of 90% (95% C.I. 73.5% - 97.9%), counting borderlines as negative,and a negative agreement of 100.0% (95% C.I. 95.5% - 100.0%).

EUROIMMUN Anti-SARS-COV-2 ELISA (IgG)	Comparator Method			Total
	Positive (IgM/IgG)+	Negative (IgM/IgG)-	Negative HIV+	
Positive IgG+	27			27
Negative IgG-	1	70	10	81
Borderline	2			2
Total	30	70	10	110

Measure	Estimate	Confidence Interval
IgG Sensitivity (PPA)	90% (27/30)	(74.4%; 96.5%)
IgG Specificity (NPA)	100% (80/80)	(95.4%; 100%)
PPV for prevalence = 5%	100%	(46.1%; 100%)
NPV for prevalence = 5%	99.5%	(98.6%; 99.8%)
Cross-reactivity with HIV+	0% (0/10), not detected	

Negative Agreement: To evaluate the negative percent agreement of the Anti-SARS-CoV-2 ELISA (IgG) for presumed negative samples, samples from unselected apparently healthy blood donors from the US, Europe and China prior to November, 2019 were used. A second panel with samples taken from unselected healthy blood donors during the COVID-19 outbreak was also used. The agreement of the Anti-SARS-CoV-2 ELISA (IgG) results with the expected negative results combined from both panels corresponds to 99.0% (95% C.I. 98.4% - 99.4%; borderlines counted as negative.

Panels before COVID-19 pandemic	**n**	**Anti-SARS-CoV-2 ELISA (IgG)**			
		Pos.	**Bord.**	**Neg.**	**NPA borderline counted as negative**
US Blood donors (2017)	400	3	1	396	99.3%
US Blood donors (2018)	146	1	1	144	99.3%
European Blood donors (2010)	150	1	3	146	99.3%
European Blood donors (2017)	250	0	3	247	100.0%
European Pregnant women (Jun. 2019)	100	0	0	100	100.0%
European Children (3 - 10 years, Oct. 2019)	100	1	4	95	99.0%
European Children (0 - 3 years, Oct. 2019)	100	5	3	92	95.0%
Chinese Blood donors (2013)	49	0	0	49	100.0%
Chinese Pregnant women (2013)	100	1	0	99	99.0%
Chinese Children (2013)	50	2	1	47	96.0%
Overall	**1,445**	**14**	**16**	**1,415**	**98.6%**

Panels during COVID-19 pandemic	**n**	**Anti-SARS-CoV-2 ELISA (IgG)**			
		Pos.	**Bord.**	**Neg.**	**NPA borderline counted as negative**
European Blood donors (2020)	200	3	3	194	98.5%
European Routine controls (samples sent in for procalcitonin testing, Mar. 2020)	111	1	1	109	99.1%
Overall	**311**	**4**	**4**	**303**	**98.7%**

Limitations of the procedure

- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. The sensitivity of the test early after infection is unknown. False positive results for IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made.
- Cross-reactivity was observed with anti-SARS-CoV-1 IgG antibodies.
- A negative result can occur if the quantity of antibodies for the SARS-CoV-2 virus present in the specimen is below the detection limit of the assay, or if the virus has undergone minor amino acid mutation(s) in the epitope recognized by the antibody used in the test.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
- Not for the screening of donated blood.
- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
- Correct performance of sample collection and storage is crucial for the test results.
- The test system is validated for the qualitative determination of anti-SARS-CoV-2 IgG 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 adjusted ELISA incubator in all incubation steps. The higher the room temperature during the incubation steps, the greater will be the OD. 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 high OD readings.
- Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low OD 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.

CONDITONS OF AUTHORIZATION FOR THE LABORATORY

The Anti-SARS-CoV-2 ELISA (IgG) Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Recipients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

Authorized laboratories using the Anti-SARS-CoV-2 ELISA (IgG) ("your product" in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

1. Authorized laboratories* using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media
2. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
3. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
4. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/ CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and EUROIMMUN US, Inc. (support@euroimmun.us) any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics of your product of which they become aware.
6. All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product
7. Authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

*The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

Literature

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6. Xiao SY, Wu Y, Liu H. **Evolving status of the 2019 novel coronavirus Infection: proposal of conventional serologic assays for disease diagnosis and infection monitoring.** J Med Virol. 2020; 1-4
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Meaning of the symbols

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

Technical support

In case of technical problems, you can obtain assistance via the EUROIMMUN website (www.euroimmun.com/contact).

Contact information for the U.S.:

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