研究用試薬

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

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

Instructions for use

For in vitro diagnostic use IVD

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

CE

Intended use

The enzyme immunoassay (ELISA) provides semiquantitative in vitro determination of human antibodies of the immunoglobulin class IgG against SARS-CoV-2 NCP 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. The product is designed for the use by healthcare professionals and can be processed and evaluated manually or on automated instruments. The results should always be interpreted together with those of further laboratory diagnostic procedures and based on the clinical picture.

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].

研究用試薬

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

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

Instructions for use

For in vitro diagnostic use IVD

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

CE

Intended use

The enzyme immunoassay (ELISA) provides semiquantitative in vitro determination of human antibodies of the immunoglobulin class IgG against SARS-CoV-2 NCP 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. The product is designed for the use by healthcare professionals and can be processed and evaluated manually or on automated instruments. The results should always be interpreted together with those of further laboratory diagnostic procedures and based on the clinical picture.

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 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 reagent wells are coated with modified nucleocapsid protein (NCP) of SARS-CoV-2.

Test principle

The test kit contains microplate strips each with 8 break-off reagent wells coated with modified nucleocapsid protein of SARS-CoV-2. Information on automated incubation is given in the instructions for use of the respective instruments. 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) catalysing a colour reaction.

Contents of the test kit

Component	Colour	Format 96 x 01	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	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. 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 deionised water
- Incubator: for incubation of the microplate at +37 °C
- Incubator or water bath: recommended to warm the wash buffer
- Stop watch

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

Storage and stability

The test kit has to be stored at +2 °C to +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, which can also be downloaded from the customer portal (https://products.euroimmun.de).
- 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 calibrators and 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, 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).

  Note: When using instruments for automated incubation, sample handling is described in the instructions for use.

  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 instructions for use required for the extraction (EUROIMMUN document number EI_2606-2G_A_UK_ZXX) are provided in the customer portal (https://products.euroimmun.de). The quality of the DBS has to be evaluated according to these instructions. Unsuitable DBS must be excluded from the analysis as they can impact the quality of the test result.
- **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.
- **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 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 calibrator and ratios 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

As no quantified international reference serum exists for antibodies against SARS-CoV-2 NCP, 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 and 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 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

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 instructions for use, 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	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 **semiquantitative analysis** of 24 patient sera (P 1 to P 24).

The calibrator (C), 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 extinction of the calibrator defines the upper limit of the reference range of non-infected persons (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8: Negative
Ratio ≥ 0.8 to <1.1: Borderline
Ratio ≥1.1: 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.

Analytical performance

The following data were collected using serum or plasma samples:

Measurement range:

Limit of blank (LoB): ratio 0.13
Limit of detection (LoD): ratio 0.21

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	Ratio 4.51		Ratio 2.39		Ratio 1.27		Ratio 1.08		Ratio 0.85		Ratio 0.26	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	0.114	2.5%	0.062	2.6%	0.051	4.1%	0.037	3.4%	0.026	3.1%	0.021	7.8%
Between-run	0.210	4.7%	0.143	6.0%	0.066	5.2%	0.108	10.0%	0.068	8.0%	0.022	8.2%
Within-day	0.239	5.3%	0.156	6.5%	0.084	6.6%	0.114	10.6%	0.073	8.6%	0.030	11.3%
Between-day	0.130	2.9%	0.077	3.2%	0.062	4.9%	0.029	2.7%	0.044	5.1%	0.014	5.2%
Within-lab	0.272	6.0%	0.175	7.3%	0.105	8.3%	0.118	10.9%	0.085	10.0%	0.33	12.5%

Cross-reactivity (analytical specificity): Due to the use of a modified nucleocapsid protein, in which significant homological regions were eliminated and diagnostically relevant epitopes combined, cross reactions with most of the human pathogens of the coronavirus family can be virtually excluded.
163 samples collected prior to 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 investigated using the Anti-SARS-CoV-2 NCP ELISA (IgG). No cross reactions with antibodies against these endemic HCoV were observed during the examinations. Cross reactions between SARS-CoV(-1) and SARS-CoV-2, however, are likely due to their relationship.

Antibodies against	n (positive)/n (total)	Positive rate [%]
HCoV	0/163	0

Other potential interferences with autoantibodies, rheumatoid factors (Rhf) and antibodies against *Haemophilus influenza* type B (HIB), Hepatitis B (HBV), Hepatitis C (HCV), adenovirus, parainfluenza, Epstein-Barr virus (EBV) and after vaccination against influenza viruses or pneumonia infections could not be observed. Analysis of highly positive respiratory syncytial virus (RSV) samples and samples from patients with enterovirus or malaria showed 2, 3 and 6 positive results out of 35, 30 and 100 analysed samples, respectively. As there is no structural similarity between SARS-CoV-2 and RSV/enterovirus/malaria, cross-reactivities can be ruled out. Any interference is also unlikely but cannot be completely excluded.

Group	n (positive)/n (total)	Positive rate [%]
AAb	2/59	3.4
Rhf	0/40	0
HIB	0/5	0
HBV	0/6	0
HCV	0/6	0
Adenovirus	1/30	3.3
Parainfluenza	0/30	0
EBV	0/22	0
Influenza vaccination	0/40	0
Acute bacterial pneumonia	2/58	3.4
RSV	2/35	5.7
Enterovirus	3/30	10.0
Plasmodia	6/100	6.0

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. 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: 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 data were determined on 15 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	Ratio 0.25		Ratio 0.97		Ratio 1.09		Ratio 1.46		Ratio 3.51		Ratio 5.44	
	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Repeatability	0.031	12.6	0.099	10.2	0.111	10.2	0.127	8.7	0.225	6.4	0.152	2.8
Between run	0.007	3.0	0.066	6.9	0.101	9.3	0.126	8.6	0.149	4.2	0.222	4.1
Within day	0.032	12.9	0.119	12.3	0.150	13.8	0.179	12.3	0.270	7.7	0.269	4.9
Between day	0.000	0.0	0.043	4.5	0.044	4.1	0.000	0.0	0.000	0.0	0.117	2.2
Within-lab	0.032	12.9	0.126	13.1	0.157	14.4	0.179	12.3	0.270	7.7	0.293	5.4

Method comparison:

The correlation between extracts of dried blood spots (DBS) from capillary blood and serum from venous blood was determined by analysing 215 patient samples collected in Europe, using the Anti-SARS-CoV-2 NCP ELISA (IgG). For each patient, one capillary blood sample and one venous blood sample were available.
The agreement between the results of the dried capillary blood spots and the venous blood samples was 99.5% (positive agreement (PPA): 98.4%; negative agreement (NPA): 100%). Borderline samples were excluded from the calculation.

n = 215		EUROIMMUN Anti-SARS-CoV-2 NCP ELISA (IgG) Sera from venous blood		
		positive	borderline	negative
EUROIMMUN Anti-SARS-CoV-2 NCP ELISA (IgG) DBS	positive	63	4	1
	borderline	1	5	6
	negative	0	0	135

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.

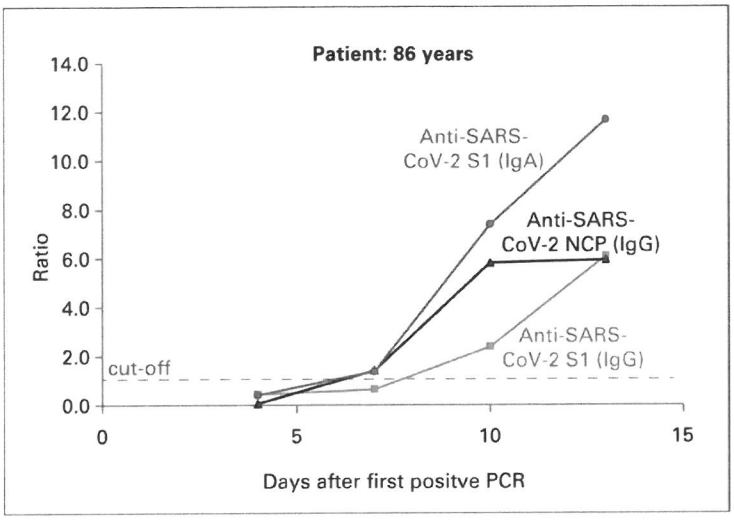
The sensitivity was determined by investigating 74 samples from 69 European patients, using the Anti-SARS-CoV-2 NCP ELISA (IgG). In these patients, infections with SARS-CoV-2 had been confirmed by RT-PCR based on a sample taken at the early phase of infection. In samples taken prior to day 10 (time point after onset of symptoms or positive direct pathogen detection), the Anti-SARS-CoV-2 NCP ELISA (IgG) showed a sensitivity of 80.0%. The sensitivity of the Anti-SARS-CoV-2 NCP ELISA (IgG) in samples collected after day 10 was 94.6%. Borderline results (n=3) were not considered in the calculation.

Days after symptom onset or positive direct pathogen detection	EUROIMMUN Anti-SARS-CoV-2 NCP ELISA (IgG)		
	positive	negative	Sensitivity
≤ 10	12	3	**80.0%**
> 10	53	3	**94.6%**

The time course of antibody secretion 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 graphs show individual immune responses in COVID-19 patients measured with the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgA) and the EUROIMMUN Anti-SARS-CoV-2 ELISA (IgG), based on the recombinant S1 domain of the spike protein, and the EUROIMMUN Anti-SARS-CoV-2 NCP ELISA (IgG), for which a modified nucleocapsid protein (NCP) is used as antigen.

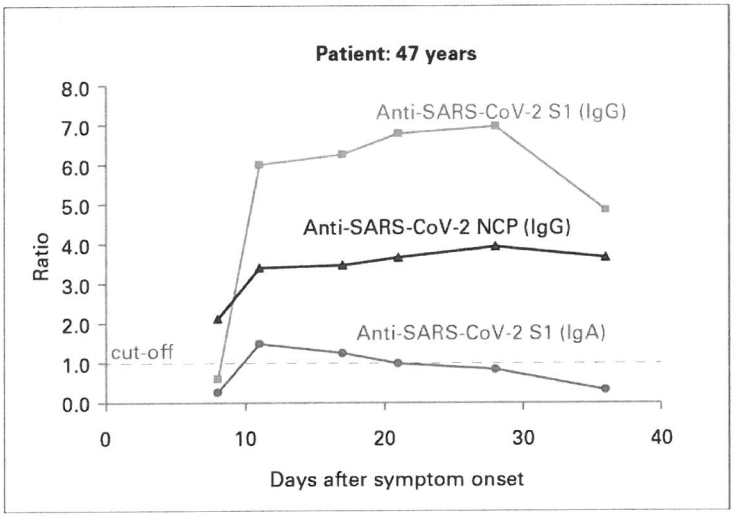
Patient 1 (86 years old)

Anti-S1 IgA and anti-NCP IgG antibodies were detectable as early as seven days after RT-PCR. The level of anti-S1 IgG antibodies was still negative seven days after positive RT-PCR, but was increased in the subsequent sample taken on day 10.



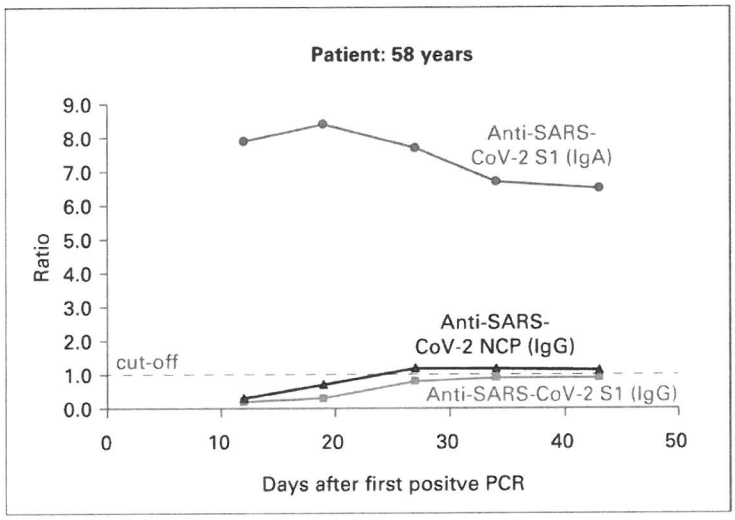
Patient 2 (47 years old)

The anti-NCP IgG antibody level was elevated as early as day 8 after the onset of symptoms. Anti-S1 IgA and IgG antibodies were not yet detectable. A follow-up sample taken on day 11 after the onset of symptoms showed an increase in the antibody levels for both Ig classes.



Patient 3 (58 years old)
The anti-S1 IgA antibody level is already highly elevated 12 days after positive RT-PCR. In contrast, the levels of anti-S1 and anti-NCP IgG antibodies increased only slowly until day 43 after positive RT-PCR.

In individual cases, delayed antibody secretion may occur, so that antibodies can only be detected after a period of several weeks after the onset of symptoms.



Specificity

The specificity of the Anti-SARS-CoV-2 NCP ELISA (IgG) was determined by analysing 226 patient samples that were positive, for instance, for antibodies against other human pathogenic coronaviruses, other pathogens or for rheumatoid factors. Additionally, 1022 samples from blood donors, children and pregnant women obtained before the first occurrence of SARS-CoV-2 were analysed. In these panels, no SARS-CoV-2-specific antibodies should be detectable. The results in the borderline range (n = 4) were not included in the calculation, resulting in a specificity of the Anti-SARS-CoV-2 NCP ELISA (IgG) of 99.8%.

Panel	n	EUROIMMUN Anti-SARS-CoV-2 NCP ELISA (IgG)
		Specificity
Blood donors	849	99.8%
Pregnant woman	99	100.0%
Children	74	100.0%
Elderly people	97	99.0%
Infections with other human pathogenic coronaviruses	27	100.0%
Influenza (freshly vaccinated, including courses)	40	100.0%
Acute EBV infection & heterophile antibodies	22	100.0%
Rheumatoid factors	40	100.0%
Total	**1248**	**99.8%**

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 an infection. Particularly in the early phase of an 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 serological 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. A significant increase in the specific IgG antibody level (by more than twofold) and/or seroconversion in a follow-up sample taken after at least 7 to 10 days may be interpreted as an indication of acute infection. The sample and the follow-up sample should be incubated in the same test run.
- 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 reliability of the results.
- The test system is validated for the determination of anti-SARS-CoV-2 NCP 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 to use 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 results within the permissible range.
- Since interferences with samples from patients with acute *Plasmodium* spp. infections cannot be excluded, malaria should always be considered in differential diagnostics.

Literature

1. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. **The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2.** Nat Microbiol. 2020; 5(4): 536-44
2. Borges do Nascimento IJ, von Groote TC, O'Mathúna DP, Abdulazeem HM, Henderson C, et al. International Task Force Network of Coronavirus Disease 2019 (InterNetCOVID-19). **Clinical, laboratory and radiological characteristics and outcomes of novel coronavirus (SARS-CoV-2) infection in humans: A systematic review and series of meta-analyses.** PLoS One. 2020; 15(9): e0239235
3. Gralinski LE, Menachery VD. **Return of the Coronavirus: 2019-nCoV.** Viruses 2020, 12(2), 135
4. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, et al. **Diagnosing COVID-19: The Disease and Tools for Detection.** ACS Nano. 2020 Apr 9.
5. Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, et al. **Diagnostic Testing for Severe Acute Respiratory Syndrome-Related Coronavirus-2: A Narrative Review.** Ann Intern Med. 172(11): 726-34

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; 92(5): 464-7
7. Lechien JR, Chiesa-Estomba CM, De Siati DR, Horoi M, Le Bon SD, Rodriguez A, et al. **Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study.** Eur Arch Otorhinolaryngol. 2020; 277(8): 2251-61
8. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. Cell. 2021; 184(4): 861-80
9. Okba NMA, Müller MA, Li W, Wang C, Geurts van Kessel CH, Corman VM, et al. **Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients.** Emerg Infect Dis. 2020; 26(7)
10. Swadling L, Maini MK. **T cells in COVID-19 - united in diversity.** Nat Immunol. 2020 ; 21(11): 1307-8
11. Ortega N, Ribes M, Vidal M, Rubio R, Aguilar R, Williams S, et al. **Seven-month kinetics of SARS-CoV-2 antibodies and role of pre-existing antibodies to human coronaviruses.** Nat Commun. 2021; 12(1): 4740

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/).

Additional information

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
CAL	Calibrator		Protect from sunlight
POS CONTROL	Positive control		Storage temperature
NEG CONTROL	Negative control		Unopened usable until (YYYY-MM-DD)
CONJUGATE	Conjugate	CE	CE-labelled
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
FOIL	Protective foil	Σ	Contents suffice for <n> analyses
CAP	Cap		Biological risks
IVD	In vitro diagnostic medical device	UDI	Unique Device Identifier