SARS-CoV-2 Antigen ELISA

- **Laboratory diagnostic test for the direct detection of SARS-CoV-2 by semiquantitative determination of the virus-specific nucleocapsid protein in swab samples from the upper respiratory tract**
- **To support acute diagnostics especially during a COVID-19 outbreak**
- **Established ELISA method – suitable for every diagnostic laboratory and fully automatable**

Technical data

Antibody	The reagent wells were coated with a monoclonal anti-SARS-CoV-2 antibody
Calibration	Semiquantitative; calculation of a ratio from the extinction of the sample and the extinction of the calibrator
Result interpretation	EUROIMMUN recommends interpreting results as follows (observe two decimal places): Ratio < 0.50: negative Ratio ≥ 0.50 to < 0.60: borderline Ratio ≥ 0.60: positive
Sample dilution	Nasopharyngeal swabs (e.g. in VTM, UTM, NaCl); 1 : 3 in sample buffer (with prior inactivation in lysis buffer 1 : 1.5)
Reagents	Ready for use, with the exception of the wash buffer (10x); colour-coded; sample, lysis and wash buffers as well as chromogen/substrate and stop solutions are exchangeable between lots
Test procedure	60 min / 60 min / 60 min / 15 min (samples / biotin / conjugate / substrate incubations),
Measurement	450 nm, reference wavelength between 620 nm and 650 nm
Test kit format	96 break-off wells; kit includes all necessary reagents
Order number	**EQ 2606-9601**

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*. At the end of 2019, SARS-CoV-2 was identified as the causative pathogen of clustered cases of pneumonia of unclear origin. It caused an infection wave that has spread rapidly worldwide and was declared a pandemic by the WHO at the beginning of 2020. The disease caused by SARS-CoV-2 is called COVID-19.

SARS-CoV-2 is predominantly transmitted by respiratory uptake of virus-containing droplets and aerosols produced during speaking, breathing, coughing or sneezing. The incubation time of SARS-CoV-2 is three to seven, maximally 14 days. The infection may manifest asymptomatically or with symptoms of a febrile illness with irregular lung infiltrates. Some patients, especially elderly or chronically ill patients, develop acute respiratory distress syndrome (ARDS).

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 primarily in sample material from the upper (nasopharyngeal or oropharyngeal swab) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal secretion, sputum, etc.). The determination of antibodies enables confirmation of SARS-CoV-2 infection in patients with typical symptoms and in suspected cases. It also contributes to monitoring and outbreak control.

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Test principle

The test kit contains microplate strip coated with monoclonal anti-SARS-CoV-2 antibody. The patient samples are first inactivated by lysis of the virus before they are transferred into the reagent wells and incubated in the first reaction step. In a second reaction step, the biotin-labelled anti-SARS-CoV-2 antibody is added, which is detected enzymatically by means of streptavidin-bound horseradish peroxidase. The intensity of the colour formed is proportional to the SARS-CoV-2 antigen concentration in the samples.

Cross reactivity

SARS-CoV-2-negative nasopharyngeal swab samples were mixed with 1 µg/ml of recombinant nucleocapsid protein from different human pathogenic coronaviruses and analysed with the EUROIMMUN SARS-CoV-2 Antigen ELISA. The result was only positive in the samples containing the SARS-CoV(-1) antigen.

Cross-reactant	229E	NL63	OC43	HKU1	SARS-CoV(-1)	MERS
EUROIMMUN SARS-CoV-2 Antigen ELISA	negative	negative	negative	negative	positive	negative

Interference

SARS-CoV-2-negative and -positive samples from nasopharyngeal swabs were mixed with potentially interfering viruses and reagents and analysed using the EUROIMMUN SARS-CoV-2 Antigen ELISA. Based on the tested samples, neither microbial, nor endogenous interference was detected.

		Concentration	Interference with EUROIMMUN SARS-CoV-2 Antigen ELISA
Microbial interference	Influenza virus type A	n. d.*	negative
	Influenza virus type B	n. d.*	negative
	RSV type B (ATCC)	10^4 PFU/ml	negative
Endogenous interference	Biotin	120 µg/ml	negative
	Whole blood	0.5% (v/v)	negative

* Panel confirmed by molecular diagnostic tests

Clinical performance

The clinical performance of the EUROIMMUN SARS-CoV-2 Antigen ELISA was determined in 98 nasopharyngeal swab samples. 48 samples originated from symptomatic patients (sample collection < 10 days after onset of symptoms) with SARS-CoV-2 infections detected by molecular diagnostic testing. 50 samples originated from patients with influenza infection detected by molecular diagnostic testing. The comparison of the test results yielded a positive agreement (sensitivity) of 93.6% and a negative agreement (specificity) of 100% (borderline values excluded).

n = 98		Molecular diagnostic detection	
		positive	negative
EUROIMMUN SARS-CoV-2 Antigen ELISA	positive	44	0
	borderline	1	0
	negative	3	50

A total of 63 naso- and oropharyngeal swab samples were investigated in parallel using the real-time PCR test EUROReal-Time SARS-CoV-2 and the SARS-CoV-2 Antigen ELISA from EUROIMMUN. The method comparison yielded a high positive agreement of the test results also with Ct values over 30. Borderline results from the Antigen ELISA were not taken into account.

EURORealTime SARS-CoV-2		EUROIMMUN SARS-CoV-2 Antigen ELISA	Positive agreement
positive at a Ct value up to	n	positive	
25	24	24	100%
28	34	34	100%
30	44	43	97.7%
31	48	45	93.8%
32	54	49	90.7%
34	63	52	82.5%

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