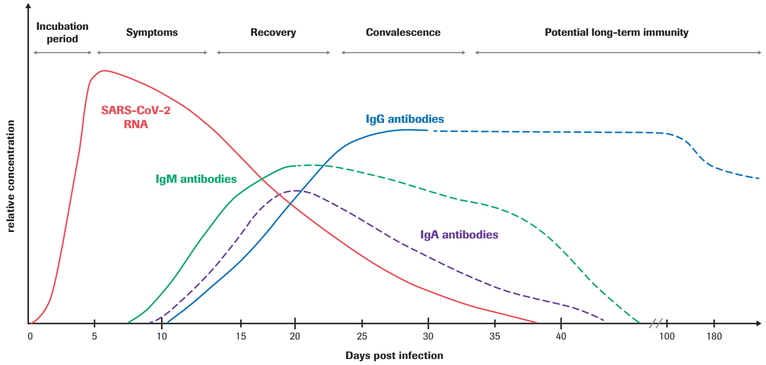
Estimated course of markers in SARS-CoV-2 infection[32]



Ordering information

Product	Material configuration	Material number
Elecsys® Anti-SARS-CoV-2 S [a)]	200 tests	09 289 267 190
Elecsys® Anti-SARS-CoV-2 S [b)]	300 tests	09 289 275 190
CalSet Anti-SARS-CoV-2 S	4 × 1.0 mL	09 289 291 190
PreciControl Anti-SARS-CoV-2 S	4 × 1.0 mL	09 289 313 190

a) for use on the **cobas e** 411 analyzer and the **cobas e** 601/602 modules
b) for use on the **cobas e** 402 and **cobas e** 801 analytical units

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Elecsys® Anti-SARS-CoV-2 S

Immunoassay for the quantitative determination of antibodies to the SARS-CoV-2 spike protein

Summary

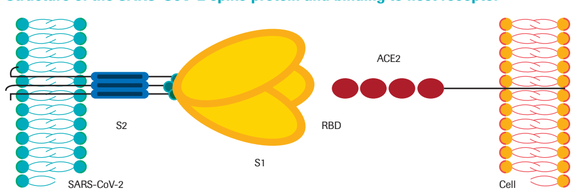
SARS-CoV-2, the causative agent of Coronavirus Disease 2019 (COVID-19), is an enveloped, single-stranded RNA Beta-coronavirus. Seven coronaviruses have been identified as agents of human infection, causing disease ranging from mild common cold to severe respiratory failure.[1]

SARS-CoV-2 is transmitted primarily from person-to-person through respiratory droplets and aerosols.[2,3] The incubation period from infection to detectable viral load in the host commonly ranges from two to 14 days.[4,5] Detection of viral load can be associated with the onset of clinical signs and symptoms, although a considerable proportion of individuals remain asymptomatic or mildly symptomatic.[6–8] The interval during which an individual with COVID-19 is infectious has not yet been clearly established, however, transmission from symptomatic, asymptomatic, and pre-symptomatic individuals has been well described.[9–11]

Coronavirus genomes encode 4 main structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N). The S protein is a very large transmembrane protein that assembles into trimers to form the distinctive surface spikes of coronaviruses. Each S monomer consists of an N-terminal S1 subunit and a membrane-proximal S2 subunit. The virus gains entry to the host cell through binding of the S protein to the angiotensin-converting enzyme 2 (ACE2) receptor, which is present on the surface of numerous cell types including the alveolar type II cells of the lung and epithelial cells of the oral mucosa.[12,13] Mechanistically, ACE2 is engaged by the receptor-binding domain (RBD) on the S1 subunit.[14,15]

Upon infection with SARS-CoV-2, the host usually mounts an immune response against the virus, typically including production of specific antibodies against viral antigens. IgM and IgG antibodies against SARS-CoV-2 appear to arise nearly simultaneously in blood.[16] There is significant inter-individual difference in the levels and chronological appearance of antibodies in COVID-19 patients, but median seroconversion has been observed at approximately two weeks.[17–20]

Structure of the SARS-CoV-2 spike protein and binding to host receptor

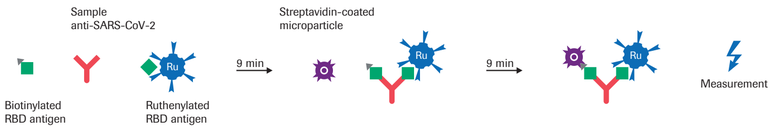


After infection or vaccination, the binding strength of antibodies to antigens increases over time – a process called affinity maturation[21]. High-affinity antibodies can elicit neutralization by recognizing and binding specific viral epitopes[22,23]. Antibodies against SARS-CoV-2 with strong neutralizing capacity, especially potent if directed against the RBD, have been identified.[24–27] Numerous vaccines for COVID-19 are in development, many of which focus on eliciting an immune response to the RBD.[28–30]

Elecsys® Anti-SARS-CoV-2 S is an immunoassay for the in vitro quantitative determination of total antibodies to the SARS-CoV-2 S protein RBD in human serum and plasma. The assay uses a recombinant RBD protein in a double-antigen sandwich assay format, which favors the quantitative determination of high affinity antibodies against SARS-CoV-2. The test is intended as an aid to assess the adaptive humoral immune response, including neutralizing antibodies, to the SARS-CoV-2 S protein after natural infection with SARS-CoV-2 or in vaccine recipients.

Electro-chemiluminescence immunoassay (ECLIA)

Test principle: double-antigen sandwich assay (testing time: 18 minutes)[31]



Step 1 (9 minutes)
20 µL* / 12 µL** of the patient sample are incubated with a mix of biotinylated and ruthenylated RBD antigen. Double-antigen sandwich immune complexes are formed in the presence of corresponding antibodies.

Step 2 (9 minutes)
After addition of streptavidin-coated microparticles, the DAGS complexes bind to the solid phase via interaction of biotin and streptavidin.

Step 3 (measurement)
The reagent mixture is transferred to the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are subsequently removed. Electrochemiluminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield increases with the antibody titer.

* **cobas e** 411 analyzer and **cobas e** 601/602 modules
** **cobas e** 801 module

Elecsys® Anti-SARS-CoV-2 S assay characteristics[31]

Systems	**cobas e** 411 analyzer **cobas e** 601 / **cobas e** 602 modules	**cobas e** 402 analytical unit **cobas e** 801 analytical unit
Testing time	18 minutes	
Test principle	One-step double antigen sandwich assay	
Traceability	Standardized against the internal Roche standard for anti-SARS-CoV-2-S. The 1st WHO International Standard for anti-SARS-CoV 2 immunoglobulin (NIBSC code: 20/136) behaves identically to the internal Roche standard (Pearson r = 0.9996 between Limit of Quantitation and 1000 BAU/mL). Hence, the numeric results in U/mL of the Elecsys® Anti-SARS-CoV-2 S assay and WHO BAU/mL are equivalent.	
Linear range	0.4 to 250 U/mL	
Calibration	2-point (separate CalSet)	
Interpretation	<0.8 U/mL = non-reactive, ≥0.8 U/mL = reactive	
Specimen types	Serum collected using standard sampling tubes or tubes containing separating gel; Li-heparin, K_2-EDTA-, K_3-EDTA-, and sodium citrate plasma. Li-heparin and K_2-EDTA plasma tubes containing separating gel can be used. Capillary blood collected in serum, Li-heparin plasma or K_2-EDTA plasma sampling tubes.	
Sample volume	20 µL	12 µL
Onboard stability	28 days	16 weeks
Intermediate precision in positive samples	**cobas e** 411 analyzer: CV* 1.9 – 2.9 % **cobas e** 601 / **cobas e** 602 modules: CV 2.7 – 3.6 %	CV 1.4 – 2.4 %

* coefficient of variation

Clinical sensitivity[31]

A total of 1,610 samples from 402 symptomatic patients (including 297 samples from 243 hospitalized patients) with a PCR confirmed SARS-CoV-2 infection were tested with the Elecsys® Anti-SARS-CoV-2 S assay. One or more sequential samples from these patients were collected at various time points after PCR confirmation.

1,423 of the tested samples had a sampling date of 14 days or later after diagnosis with PCR. 1,406 of these 1,423 samples were determined with ≥0.8 U/mL in the Elecsys® Anti-SARS-CoV-2 S assay and hence considered positive, resulting in a sensitivity of **98.8 % (95 % CI: 98.1 – 99.3 %)** in this sample cohort.

Days post PCR confirmation	N	Non-reactive	Sensitivity (95 % CI*)
0 – 6 days	35	4	**88.6 %** (73.3 – 96.8 %)
7 – 13 days	152	22	**85.5 %** (78.9 – 90.7 %)
14 – 20 days	130	14	**89.2 %** (82.6 – 94.0 %)
21 – 27 days	176	3	**98.3 %** (95.1 – 99.7 %)
28 – 34 days	197	0	**100 %** (98.1 – 100 %)
≥35 days	920	0	**100 %** (99.6 – 100 %)

* confidence interval

Analytical specificity[31]

A total of 1,100 potentially cross-reactive samples collected before October 2019, including anti-MERS-CoV positive samples, samples from individuals with common cold symptoms, and samples from individuals confirmed to be infected with one of the four common cold coronaviruses were tested with the Elecsys® Anti-SARS-CoV-2 S assay. Overall specificity in this cohort of potentially cross-reactive samples was **100 % (95 % CI: 99.7 – 100 %).**

Cohort	N	Reactive	Specificity (95 % CI)
MERS-CoV*	7	0	**100 %** (59.0 – 100 %)
Common cold panel**	21	0	**100 %** (83.4 – 100 %)
Coronavirus panel***	94	0	**100 %** (96.2 – 100 %)
Other potentially cross-reactive samples****	978	0	**100 %** (99.6 – 100 %)
Overall	**1,100**	**0**	**100 % (99.7 – 100 %)**

* positive for IgG antibodies against the Middle East respiratory syndrome-related coronavirus (MERS-CoV) spike protein subunit S1
** 40 samples from individuals with common cold symptoms, collected before October 2019
*** from individuals with past infection with coronavirus HKU1, NL63, 229E, or OC43, confirmed by antigen testing
**** pre-pandemic samples with reactivity for various other indications, which could have an elevated potential for unspecific interference

Clinical specificity[31]

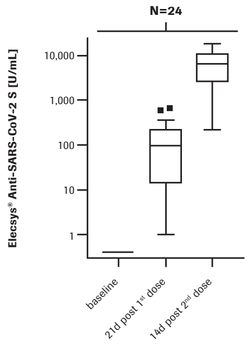
A total of 5,991 samples from diagnostic routine and blood donors drawn before October 2019 were tested with the Elecsys® Anti-SARS-CoV-2 S assay. Overall specificity in this cohort of pre-pandemic samples was **99.98 % (95 % CI: 99.91 – 100 %).**

Cohort	N	Reactive	Specificity (95 % CI)
Diagnostic routine	2,528	0	100 % (99.85 – 100 %)
US blood donors	2,713	1	99.96 % (99.79 – 100 %)
African blood donors	750	0	100 % (99.51 – 100 %)
Overall	**5,991**	**1**	**99.98 % (99.91 – 100 %)**

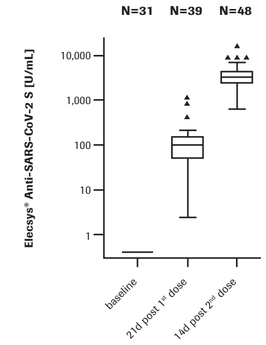
Detection of antibodies induced by active immunization with vaccines against SARS-CoV-2[31]

After vaccination with the Moderna vaccine Spikevax® (mRNA-1273) and the Pfizer-BioNTech vaccine Comirnaty® (BNT162b2), applying the respectively approved 2-dose vaccination scheme, the antibody response in vaccinated, infection-naïve individuals was assessed using the Elecsys® Anti-SARS-CoV-2 S assay at three time-points: pre-vaccination (baseline), 21 days post 1st vaccination dose, and 14 days post 2nd vaccination dose. Following vaccination, rapidly rising antibody titers, indicating a strong humoral immune response to vaccination, were observed. All individuals that had been seronegative at baseline seroconverted after vaccination.

Anti-RBD titer after vaccination with Spikevax®



Anti-RBD titer after vaccination with Comirnaty®



Correlation of assay results to serum neutralization capacity[31]

In a study investigating COVID-19 convalescent plasma for virus neutralization capacity, plasma donations from convalescent donors after a SARS-CoV-2 infection were analyzed for whole virus neutralizing potential using an in vitro plaque reducing neutralization (PRNT) assay (BROAD Institute, USA). Presence of 50 % neutralization (NT_{50}) at a sample dilution of >1:20 identified functional virus neutralization in vitro.

390 donations, including cross-sectional and longitudinal sample panels, were analyzed by PRNT and compared to Elecsys® Anti-SARS-CoV-2 S assay results by applying two different thresholds: one representing the assay's cutoff for detecting presence of RBD-specific antibodies (0.8 U/mL), and one based on optimized correlation with detection of virus neutralizing effects (15 U/mL).

		Virus Neutralization Test (PRNT)		
		Neutralizing (NT_{50} ≥ 1:20)	**Non-neutralizing**	**Total**
Elecsys® Anti-SARS-CoV-2 S	**≥ 0.8 U/mL**	356	4	**360**
	< 0.8 U/mL	2	28	**30**
	Total	**358**	**32**	**390**
Percent Positive Agreement	99.4 % (98.0 – 99.9 %)			
Percent Negative Agreement	87.5 % (71.0 – 96.5 %)			
Positive Predictive Value	98.9 % (97.2 – 99.7 %)			

Elecsys® Anti-SARS-CoV-2 S	**≥ 15 U/mL**	331	0	**331**
	< 15 U/mL	27	32	**59**
	Total	**358**	**32**	**390**
Percent Positive Agreement	92.5 % (89.2 – 95.0 %)			
Percent Negative Agreement	100 % (89.1 – 100 %)			
Positive Predictive Value	100 % (98.9 – 100 %)			