Elecsys® SARS-CoV-2 Antigen

Immunoassay for the qualitative detection of the SARS-CoV-2 nucleocapsid antigen

Summary

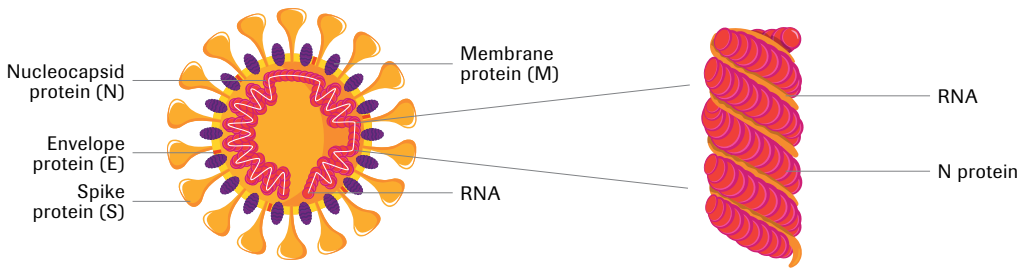
SARS-CoV-2, the causative agent of COVID-19, is an enveloped, single-stranded RNA Betacoronavirus.[1-3] 7 coronaviruses have been identified as agents of human infection, causing disease ranging from mild common cold to severe respiratory failure.[4] Coronaviruses share the 4 structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N), the latter being the most abundant.[5-8]

SARS-CoV-2 is transmitted primarily from person-to-person through respiratory droplets and aerosols.[9,10] The incubation period from infection to detectable viral load in the host commonly ranges from 2 to 14 days.[11,12] Detection of viral load can be associated with the onset of clinical signs and symptoms, although a considerable proportion of individuals remains asymptomatic or mildly symptomatic.[13-15] 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.[16-18]

An effective strategy for controlling the COVID-19 pandemic is to develop highly accurate methods for the identification and isolation of SARS-CoV-2 infected patients.[19] Diagnostic confirmation of acute SARS-CoV-2 infection can be based on the detection of unique sequences in the viral RNA or detection of viral proteins in respiratory tract samples from infected individuals.[20] Viral antigens are only expressed when the virus is actively replicating, thus making antigen tests clinically useful for identification of acute or early infection.[21,22] Current research suggests active replication of SARS-CoV-2 in the throat with high viral shedding in the first 5 days of infection, and infectious virus could be isolated from respiratory samples up to the first 7–9 days post symptom onset, indicating potential feasibility of antigen detection using throat swabs.[23-25] This time period also coincides with the time when the highest viral load is generally observed in infected individuals.[14,26-28] Therefore, the best performance of antigen tests is seen around symptom onset in symptomatic individuals and the initial phase of infection.[20] Testing of mildly symptomatic or asymptomatic individuals can be considered in the assessment of contacts of confirmed infected persons.[20] Antigen tests can also become part of regular testing regimens for identifying, isolating, and thus filtering out currently infected persons, including those who are asymptomatic.[29,30]

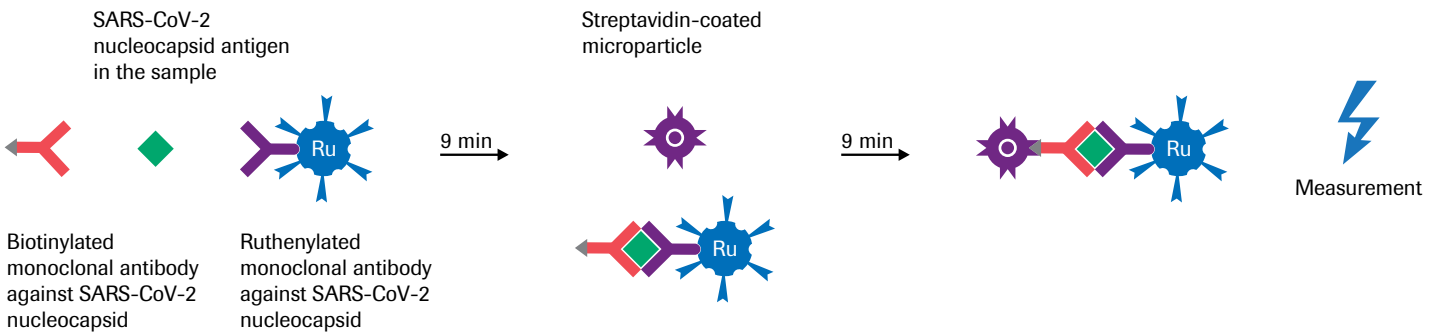
The Elecsys® SARS-CoV-2 Antigen assay uses monoclonal antibodies directed against the SARS-CoV-2 N protein in a double-antibody sandwich assay format for the qualitative detection of SARS-CoV-2 in upper respiratory tract specimens.[31]

Structure of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)



Electro-chemiluminescence immunoassay (ECLIA)

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



Step 1 (9 minutes)
50 µL*/30 µL** of the patient sample are incubated with a mix of biotinylated and ruthenylated monoclonal antibodies to the SARS-CoV-2 antigen. Double-antibody sandwich (DABS) immune complexes are formed in the presence of corresponding antigen.

* **cobas e** 411 analyzer and **cobas e** 601/602 modules
** **cobas e** 402 and 801 analytical units

Step 2 (9 minutes)
After addition of streptavidin-coated microparticles, the DABS 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.

Elecsys® SARS-CoV-2 Antigen 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 antibody sandwich assay	
Calibration	2-point	
Interpretation	CO1 <1.0 = non-reactive, COI ≥1 = reactive	
Specimen types	Nasopharyngeal, oropharyngeal and nasal specimens, collected using flocked or polyester-tipped swabs, placed in 3 mL Copan Universal Transport Medium (UTM-RT™), BD™ Universal Viral Transport (UVT), Viral Transport Media (VTM)*, or sterile saline (0.9 % NaCl)	
Sample volume	50 µL	30 µL
Sample extraction	dry swabs: SARS-CoV-2 Extraction Solution; swabs in UTM/VTM: SARS-CoV-2 Extraction Solution C	
Onboard stability	8 weeks	16 weeks
Intermediate** precision in positive samples	**cobas e** 411 analyzer: CV# 2.2 – 5.8 % **cobas e** 601 / 602 modules: CV 1.9 – 3.5 %	CV 1.7 – 5.7 %

* prepared according to CDC SOP DSR-052-05; ** between runs; # coefficient of variation

Detection limit[31]

The detection limit in different transport media was determined by limiting dilution studies using an inactivated viral lysate (USA-WA1/2020). Detection limit is defined as the lowest detectable concentration of SARS-CoV-2 at which a minimum of 19 from 20 replicates per concentration generate a reactive test result (≥1.0 COI), and expressed as $TCID_{50}$*/mL.

Transport Medium	$TCID_{50}$/mL
UTM-RT; VTM; SARS-CoV-2 Extraction Solution	22.5
Sterile Saline (0.9 % NaCl)	37.5

* Median Tissue Culture Infectious Dose

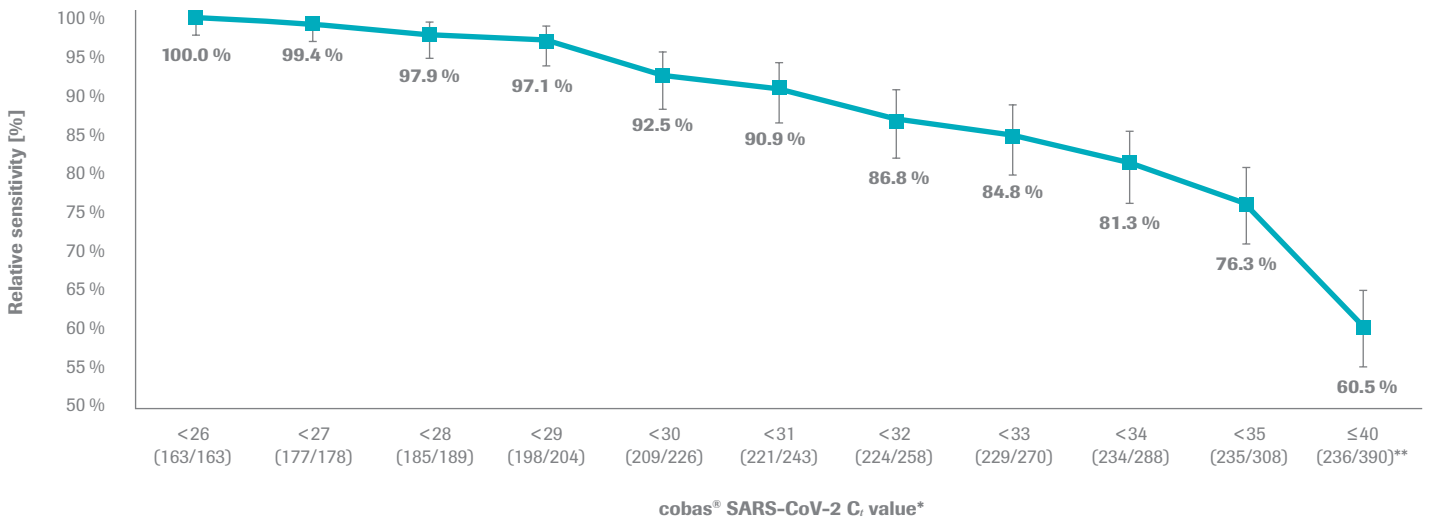
Relative sensitivity[31]

Relative sensitivity was evaluated using
A) 232 nasopharyngeal and 158 oropharyngeal swab specimens, collected from individuals with signs and symptoms suggestive of COVID-19, with known or suspected exposure to SARS-CoV-2, and from individuals undergoing pre-admission screening before hospitalization for surgical intervention unrelated to an infectious disease.
B) 116 nasal swab specimens, collected from individuals with signs and symptoms suggestive of COVID-19.

All subjects included in the analysis were tested positive in the **cobas**® SARS-CoV-2 RT-PCR test[32]. RT-PCR-positive samples were further stratified using Target 2 (structural protein envelope E-gene/pan-Sarbecovirus detection) cycle threshold (C_t) values.

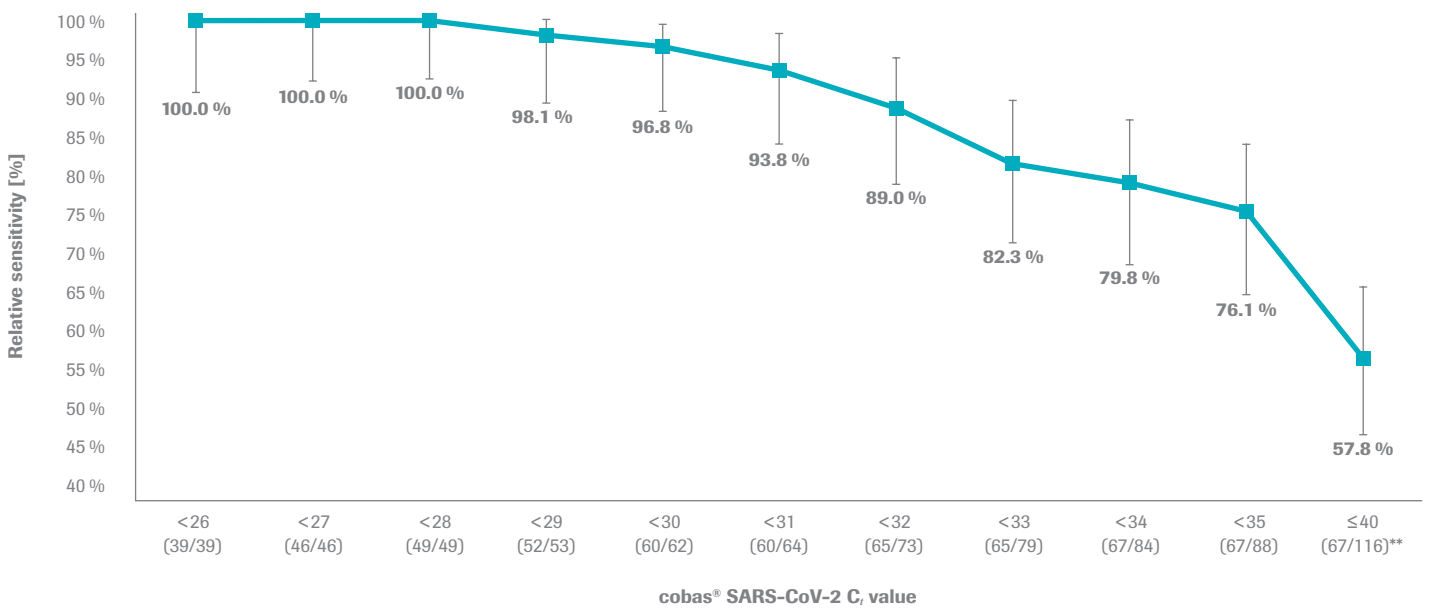
The figure below correlates the performance of the Elecsys® SARS-CoV-2 Antigen assay in all RT-PCR-positive naso-/oropharyngeal swab samples (A) or nasal swab samples (B), respectively, to the **cobas**® SARS-CoV-2 C_t values.

A) Naso- and oropharyngeal swab samples



*structural protein envelope E-gene/pan-Sarbecovirus detection; **N (cumulative): reactive in the Elecsys® SARS-CoV-2 Antigen assay/total

B) Nasal swab samples



The tables below show additional analyses based on days post-symptom onset (DPSO) and stratification by a **cobas®** SARS-CoV-2 C_t value of 30. (A) The resulting overall relative sensitivity in naso-/oropharyngeal swab samples from symptomatic individuals with a **cobas®** SARS-CoV-2 Target 2 C_t value <30 was **94.5 % (95 % CI, two-sided: 90.4 – 97.2 % [189/200])**. B) The resulting relative sensitivity in nasal swab samples from symptomatic individuals at 5 DPSO with a **cobas®** SARS-CoV-2 Target 2 C_t value <30 was **96.8 % (95 % CI, two-sided: 88.8 - 99.6 % [60/62])**.

A) Naso- and oropharyngeal swab samples

Cohort	cobas® SARS-CoV-2 C_t <30			cobas® SARS-CoV-2 C_t ≥30		
	N	Non-reactive	Sensitivity (95 % CI[§])	N	Non-reactive	Sensitivity (95% CI[§])
Symptomatic; ≤5 DPSO	119	3	**97.5 %** (92.8 – 99.5 %)	30	22	**26.7 %** (12.3 – 45.9 %)
Symptomatic; ≤10 DPSO	158	8	**94.9 %** (90.3 – 97.8 %)	78	60	**23.1 %** (14.3 – 34.0 %)
Symptomatic; >10 DPSO	4	1	**75.0 %** (19.4 – 99.4 %)	18	15	**16.7 %** (3.6 – 41.4 %)
Symptomatic; unknown DPSO	38	2	**94.7 %** (82.3 – 99.4 %)	17	13	**23.5 %** (6.8 – 49.9 %)
Known or suspected exposure	27	3	**88.9 %** (70.8 – 97.6 %)	51	50	**1.96 %** (0.05 – 10.4 %)
Screening	21	4	**81.0 %** (58.1 – 94.6 %)	30	29	**3.33 %** (0.84 – 17.2 %)

B) Nasal swab samples

Cohort	cobas® SARS-CoV-2 C_t <30			cobas® SARS-CoV-2 C_t ≥30		
	N	Non-reactive	Sensitivity (95 % CI[§])	N	Non-reactive	Sensitivity (95% CI[§])
≤1 DPSO	2	0	**100 %** (15.8 – 100 %)	1	1	**0 %** (–-)
≤2 DPSO	9	0	**100 %** (66.4 – 100 %)	8	7	**12.5 %** (0.32 – 52.7 %)
≤3 DPSO	27	0	**100 %** (87.2 – 100 %)	18	14	**22.2 %** (6.41 – 47.6 %)
≤4 DPSO	61	2	**96.7 %** (88.7 – 99.6 %)	49	42	**14.3 %** (5.94 – 27.2 %)
≤5 DPSO	62	2	**96.8 %** (88.8 – 99.6 %)	54	47	**13.0 %** (5.37 – 24.9 %)

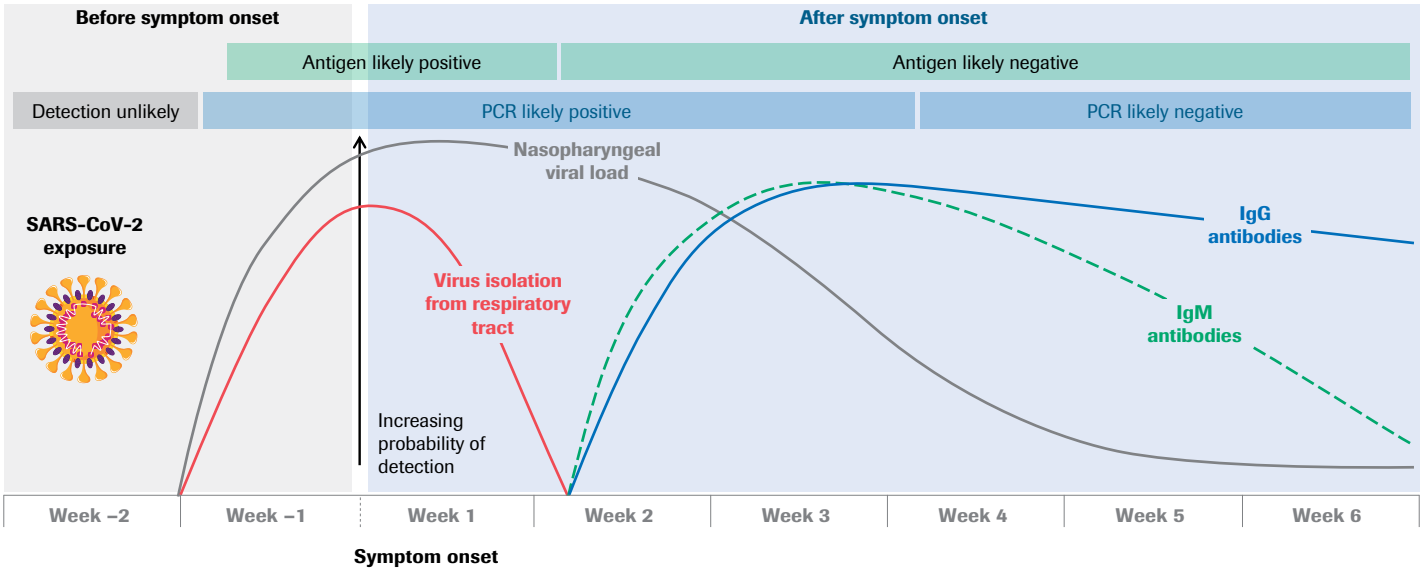
Relative specificity[31]

Relative specificity of the Elecsys® SARS-CoV-2 Antigen assay was evaluated using 2,747 RT-PCR negative naso-/oropharyngeal swab specimens, collected from individuals with signs and symptoms suggestive of COVID-19, with known or suspected exposure to SARS-CoV-2, and from individuals undergoing pre-admission screening before hospitalization for surgical intervention unrelated to an infectious disease.

Cohort	N	Reactive	Specificity (95 % CI)
Symptomatic	548*	0	**100 %** (99.3 - 100 %)
Known/suspected exposure and screening	2,199**	4	**99.8 %** (99.5 - 100 %)
Overall	**2,747**	**4**	**99.9 % (99.6 – 100 %)**

*3; **12 samples invalid with **cobas®** SARS-CoV-2 RT-PCR, but negative with another SARS-CoV-2 RT-PCR test

Estimated course of markers in SARS-CoV-2 infection[27,30]



Ordering information

Product	Material configuration	Material number
Elecsys® SARS-CoV-2 Antigen [a)]	200 tests	09 345 272 190
Elecsys® SARS-CoV-2 Antigen [b)]	300 tests	09 345 299 190
PreciControl SARS-CoV-2 Antigen	6 × 2.0 mL	09 345 302 190
SARS-CoV-2 Extraction Solution	1,000 mL	09 370 064 190
SARS-CoV-2 Extraction Solution C	500 mL	09 370 099 190

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 801 analytical units

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