Elecsys Anti-SARS-CoV-2 S

cobas®

REF		Σ	SYSTEM
			cobas e 411
09289267190	09289267500	200	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 2550
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 71

Intended use

Immunoassay for the in vitro quantitative determination of antibodies (including IgG) to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) spike (S) protein receptor binding domain (RBD) in human serum and plasma. The test is intended as an aid to assess the adaptive humoral immune response to the SARS-CoV-2 S protein.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

SARS-CoV-2, the causative agent of Coronavirus Disease 2019 (COVID-19), is an enveloped, single-stranded RNA Betacoronavirus. 7 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 2 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 remains asymptomatic or mildly symptomatic.[6,7,8] 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.[9,10,11]

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), 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 acts as the virus receptor and is engaged by the receptor-binding domain (RBD) on the S1 subunit.[14,15]

Upon infection with SARS-CoV-2, the host 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 2 weeks.[17,18,19,20,21] Also, titers after a resolved infection show considerable variance from patient to patient.[22]
Antibodies against SARS-CoV-2 with strong neutralizing capacity, especially potent if directed against the RBD, have been identified.[21,23,24] Competition of antibodies with binding of the RBD to ACE2 has been established as a reliable correlate for the assessment of the presence of neutralizing antibodies.[25] Numerous vaccines for COVID-19 are in development, many of which focus on eliciting an immune response to the RBD.[26,27,28]

Serologic assays can play an important role in understanding viral epidemiology in the general population and identifying individuals who are apparently naive and thus presumably susceptible to the virus.

The Elecsys Anti-SARS-CoV-2 S assay uses a recombinant protein representing the RBD of the S antigen in a double-antigen sandwich assay format, which favors the quantitative determination of high affinity antibodies against SARS-CoV-2. Quantification of the antibody response can help to determine the specific antibody titer and aid in longitudinal monitoring of the dynamics of the antibody response in individual patients. The Elecsys Anti-SARS-CoV-2 S assay shows good agreement with direct and surrogate virus neutralization assays.

Test principle

Double-antigen sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, biotinylated SARS-CoV-2 S-RBD-specific recombinant antigen and SARS-CoV-2 S-RBD-specific recombinant antigen labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as ACOV2S.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 SARS-CoV-2 S-Ag~biotin (gray cap), 1 bottle, 16 mL: Biotinylated RBD domain of SARS-CoV-2 S as recombinant antigen < 0.4 mg/L; HEPES[b)] buffer 50 mmol/L, pH 7.4; preservative.

R2 SARS-CoV-2 S-Ag~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 16 mL: RBD domain of SARS-CoV-2 S as recombinant antigen labeled with ruthenium complex < 0.4 mg/L; HEPES buffer 50 mmol/L, pH 7.4; preservative.

b) HEPES = [4-(2-hydroxyethyl)-piperazine]-ethane sulfonic acid

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

For professional use.

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	14 days

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, 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 or K_2-EDTA sampling tubes.

Criterion: Slope 1.00 ± 0.10 + bias at 0.8 U/mL ± 20 %.

For native samples collected in sodium citrated plasma: Slope 0.84 ± 0.10.

For capillary blood derived samples: negative samples: < 0.4 U/mL, reactive samples: recovery within 70-130 % of serum value.

Sampling devices containing liquid anticoagulants have a dilution effect resulting in lower values (U/mL) for individual patient specimens. In order to minimize dilution effects it is essential that respective sampling devices are filled completely according to manufacturer's instructions.

Stable for 3 days at 15-25 °C, 14 days at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 3 times.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

The performance of the Elecsys Anti-SARS-CoV-2 S assay has not been established with cadaveric samples or body fluids other than serum and plasma.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 09289291190, CalSet Anti-SARS-CoV-2 S, for 4 x 1.0 mL
- REF 09289313190, PreciControl Anti-SARS-CoV-2 S, 4 x 1.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent or REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the internal Roche standard for anti-SARS-CoV-2-S. This standard consists of an equimolar mixture of 2 monoclonal antibodies that bind Spike-1 RBD at 2 different epitopes. 1 nM of these antibodies correspond to 20 U/mL of the Elecsys Anti-SARS-CoV-2 S assay. No international standard is available for anti-SARS-CoV-2-S.

Note: the defined unit is specific for the Elecsys Anti-SARS-CoV-2 S assay and must not be used interchangeably with units of other assays.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the same reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 31 days when using the same reagent lot
- after 14 days when using the same reagent kit on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Anti-SARS-CoV-2 S.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in U/mL.

Interpretation of the results

Result	Interpretation
< 0.80 U/mL	Negative for anti-SARS-CoV-2-S
≥ 0.80 U/mL	Positive for anti-SARS-CoV-2-S

Note: Due to the diversity of the antibodies, the measured anti-SARS-CoV-2-S value can vary depending on the testing procedure used and the applied standard. Results obtained from a single sample using tests from different manufacturers can therefore differ. If there is a change in the assay procedure used during the monitoring of antibody titers, then the anti-SARS-CoV-2-S values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods. For citrated plasma (1 part citrate solution + 9 parts blood), the dilution effect must be taken into account.

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 1000 mg/dL or ≤ 10 g/L
Intralipid	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7.0 g/dL or ≤ 70 g/L
IgA	≤ 1.6 g/dL or ≤ 16 g/L
IgM	≤ 1.0 g/dL or ≤ 10 g/L

Criterion: For concentrations of 1.0-20 U/mL, the deviation is ≤ 20 %. For concentrations > 20 U/mL, the deviation is ≤ 30 %. For concentrations < 1.0 U/mL, the deviation is ≤ 0.2 U/mL.

No false negative results due to a high-dose hook effect were found with the Elecsys Anti-SARS-CoV-2 S assay but occurrence of high-dose hook effect cannot be completely excluded.

Pharmaceutical substances

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

Interference of itraconazole was tested up to the listed concentration and no impact on results was observed.

Drug	Concentration tested
Itraconazole	15 mg/L

In addition, the following special drugs were tested. No interference with the assay was found.

Antivirals

Drug	Concentration tested
Interferon-alpha-2a	14400 IU/mL
Interferon-alpha-2b	1000 IU/mL
Zanamivir	0.002 mg/mL
Ribavirin	0.247 mg/mL
Oseltamivir	0.030 mg/mL
Peramivir	0.120 mg/mL
Lopinavir	0.240 mg/mL
Ritonavir	0.160 mg/mL
Arbidol	0.040 mg/mL
Remdesivir	0.040 mg/mL
Actemra (Tocilizumab)	0.128 mg/mL

Antibiotics

Drug	Concentration tested
Levofloxacin	0.1 mg/mL
Azithromycin	0.1 mg/mL
Ceftriaxone	0.8 mg/mL
Meropenem	1.20 mg/mL
Tobramycin	0.120 mg/mL

Others

Drug	Concentration tested
Hydroxychloroquine	0.16 mg/mL

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

A negative test result does not completely rule out the possibility of an infection with SARS-CoV-2. Serum or plasma samples from the very early (pre-seroconversion) phase can yield negative findings. Therefore, this test cannot be used to diagnose an acute infection. It has also been reported that certain patients with confirmed infection do not develop SARS-CoV-2 antibodies.[21] Furthermore, waning of antibody titers has been reported in some individuals within a range of months after infection, a feature which has also been reported for other coronaviruses.[29,30,31]

Limits and ranges

Measuring range

0.40-250 U/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 0.40 U/mL. Values above the measuring range are reported as > 250 U/mL (or up to 2500 U/mL for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.30 U/mL

Limit of Detection = 0.35 U/mL

Limit of Quantitation = 0.40 U/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantified with a CV ≤ 20 %. It has been determined using samples with low concentration of anti-SARS-CoV-2-S.

Dilution

Samples with anti-SARS-CoV-2-S concentrations above the measuring range can be diluted with Diluent Universal or Diluent Universal 2. The recommended dilution range is 1:10 up to 1:100.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Note: Antibodies to SARS-CoV-2 are heterogeneous. In some isolated cases, this may lead to non-linear dilution behavior.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 1 run per day with 5 replicates of each sample for 5 days (n = 25). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
HSP[c] 1	0.483	0.014	2.8	0.016	3.4
HSP 2	0.826	0.023	2.8	0.023	2.8
HSP 3	5.74	0.131	2.3	0.150	2.6
HSP 4	12.3	0.266	2.2	0.304	2.5
HSP 5	54.6	1.58	2.9	1.58	2.9
HSP 6	77.9	1.78	2.3	2.07	2.7
HSP 7	190	3.03	1.6	3.69	1.9
PC[d] ACOV2S 1	< 0.40	-	-	-	-
PC ACOV2S 2	10.8	0.207	1.9	0.230	2.1

c) HSP = human specimen (serum/plasma)

d) PC = PreciControl: PC ACOV2S 1 is free of analyte and therefore consistently resulted below measuring range (< 0.40 U/mL) throughout the experiment, standard deviation and coefficient of variance could therefore not be determined.

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
HSP 1	0.441	0.007	1.6	0.016	3.7
HSP 2	0.933	0.014	1.5	0.022	2.3
HSP 3	5.60	0.102	1.8	0.181	3.2
HSP 4	12.0	0.189	1.6	0.334	2.8
HSP 5	53.2	0.761	1.4	1.46	2.7

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
HSP 6	75.5	1.55	2.1	2.70	3.6
HSP 7	183	3.31	1.8	5.13	2.8
PC ACOV2S 1	< 0.40	-	-	-	-
PC ACOV2S 2	10.5	0.118	1.1	0.341	3.3

Analytical specificity

1468 samples containing potentially cross-reacting analytes were tested with the Elecsys Anti-SARS-CoV-2 S assay. All samples were obtained before October 2019. No cross-reactivity was found. The resulting overall specificity was 100 %. Results are shown in the following tables:

SARS-CoV-2 related

Indication	**N**	**Reactive**	**Specificity %**
MERS CoV (anti-S1 IgG+)	51	0	100
Common Coronavirus panel[e]	151	0	100

e) Pre-pandemic samples which showed serologic reactivity to at least 1 of the endemic Coronaviruses HKU1, NL63, 229E or OC43.

Infectious respiratory diseases

Indication	**N**	**Reactive**	**Specificity %**
Bordetella pertussis	39	0	100
Chlamydia pneumoniae	36	0	100
Common cold panel[f]	21	0	100
Enterovirus	35	0	100
Haemophilus influenzae B	75	0	100
Influenza A	40	0	100
Influenza B	45	0	100
Influenza vaccinees	25	0	100
Mycoplasma pneumoniae	46	0	100
Parainfluenza	82	0	100
Respiratory syncytial virus	51	0	100

f) 21 potentially cross-reactive samples from individuals with common cold symptoms, collected before October 2019

Other infectious diseases

Indication	**N**	**Reactive**	**Specificity %**
Adenovirus	25	0	100
Borrelia	6	0	100
Candida albicans	13	0	100
Chlamydia trachomatis	12	0	100
CMV acute (IgM+, IgG+)	86	0	100
E. coli (anti-E. coli-reactive)	10	0	100
EBV acute (IgM+, VCA IgG+)	106	0	100
Gonorrhea (tripper)	5	0	100
HAV acute (IgM+)	10	0	100
HAV late (IgG+)	15	0	100
HAV vaccinees	15	0	100
HBV acute	12	0	100

Elecsys Anti-SARS-CoV-2 S

cobas®

Indication	N	Reactive	Specificity %
HBV chronic	12	0	100
HBV vaccinees	15	0	100
HCV	50	0	100
HEV	12	0	100
HIV	10	0	100
HSV acute (IgM+)	24	0	100
HTLV	6	0	100
Legionella (IgGAM+)	7	0	100
Listeria	6	0	100
Measles	10	0	100
Mumps	14	0	100
Parvovirus B19	30	0	100
Plasmodium falciparum (malaria)	8	0	100
Rubella acute (IgM+, IgG+)	12	0	100
Toxoplasma gondii (IgM+, IgG+)	8	0	100
Treponema pallidum (syphilis)	62	0	100
VZV (varicella-zoster virus)	30	0	100

Autoimmune diseases

Indication	N	Reactive	Specificity %
AMA (anti-mitochondrial antibodies)	30	0	100
ANA (anti-nuclear antibodies)	17	0	100
Hemophiliacs	15	0	100
RA (rheumatoid arthritis)	10	0	100
SLE (systemic lupus erythematosus)	10	0	100

Hepatic diseases

Indication	N	Reactive	Specificity %
Alcohol induced hepatitis/cirrhosis	13	0	100
Drug induced hepatitis/cirrhosis	10	0	100
Fatty liver	10	0	100
Liver cancer	10	0	100
Non-viral liver disease	15	0	100

Clinical specificity

A total of 5991 samples were tested with the Elecsys Anti-SARS-CoV-2 S assay. All samples were obtained before October 2019. 1 false positive sample was detected.

The resulting overall specificity in the internal study was 99.98 %. The 95 % lower confidence limit was 99.91 %.

Cohort	N	Reactive	Specificity %	95 % lower confidence limit, %	95 % upper confidence limit, %
Diagnostic routine (Europe)	2528	0	100	99.85	100
Blood donors (USA)	2713	1	99.96	99.79	100
Blood donors (Africa)	750	0	100	99.51	100
Overall	**5991**	**1**	**99.98**	**99.91**	**100**

Sensitivity

A total of 1610 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. 1 or more sequential samples from these patients were collected at various time points after PCR confirmation.

1423 of the tested samples had a sampling date of 14 days or later after diagnosis with PCR. 1406 of these 1423 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.

U/mL	Days after diagnosis with positive PCR					
	0-6	7-13	14-20	21-27	28-34	> 35
< 0.4	4	16	7	3	0	0
0.4 - < 0.8	0	6	7	0	0	0
0.8 - < 1.5	2	3	4	1	0	0
1.5 - < 2.5	0	2	6	2	0	0
2.5 - < 5	3	10	9	12	10	40
5 - < 10	1	7	7	15	25	49
10 - < 20	0	11	19	32	25	62
20 - < 50	1	13	19	40	38	183
50 - < 100	3	9	11	34	48	232
100 - < 150	1	4	11	11	21	135
150 - < 200	2	4	2	5	11	95
200 - ≤ 250	3	8	0	1	5	47
> 250	15	59	28	20	14	77
≥ 0.8	31	130	116	173	197	920
Total	35	152	130	176	197	920
Sensitivity, %	88.6	85.5	89.2	98.3	100	100
CS[g], % 95 % CI[h], %	86.1 80.3 - 90.7		**98.8** **98.1 - 99.3**			

g) CS = Cumulated sensitivity

h) CI = confidence intervall

Titer development was investigated with sequential samples from individual patients ranging up to 126 days following a reactive PCR result. None of the samples showed a decline of titer below the reactive range.

Titer development over time for patient samples ranging ≥ 100 days following a reactive PCR result is shown below.

Donor	D* U/mL	D U/mL	D U/mL	D U/mL	D U/mL	D U/mL	D U/mL	D U/mL
1	20 20.4	23 22.2	27 30.5	33 47.4	36 51.7	61 73.5	82 87.7	103 114
2	21 36.1	24 44.3	31 32.4	34 48.5	37 51.4	62 63.1	83 73.2	104 71.9
3	26 139	34 223	38 186	41 153	45 150	67 198	87 147	106 155

Elecsys Anti-SARS-CoV-2 S

cobas®

Donor	D* U/mL	D U/mL	D U/mL	D U/mL	D U/mL	D U/mL	D U/mL	D U/mL
4	21 32.3	30 95.3	33 151	36 315	41 374	62 293	83 244	107 214
5	30 33.0	35 29.5	38 31.2	42 41.2	112 59.9			
6	20 7.88	30 32.6	38 26.6	62 39.2	71 35.7	76 40.3	86 36.0	107 42.1
7	19 20.7	22 40.4	25 101	29 149	39 115	48 97.7	59 115	104 175
8	15 22.1	22 14.2	30 37.1	37 166	40 136	55 226	79 124	107 96.9
9	34 181	41 148	45 148	52 165	67 152	74 154	87 125	106 119
10	26 4.42	29 4.79	32 4.83	35 5.21	42 4.67	52 5.95	73 7.28	103 7.69
11	16 305	42 296	78 371	106 408				
12	28 139	31 162	40 114	44 166	47 141	62 93.0	86 69.5	103 59.1
13	24 33.9	31 45.6	38 63.7	46 53.4	59 47.4	74 41.8	92 41.9	102 42.8
14	25 79.8	28 86.4	33 120	41 117	47 103	59 108	76 97.1	109 105
15	36 255	52 165	68 126	77 94.8	92 122	96 107	106 141	126 162
16	30 425	44 246	51 379	58 298	73 215	85 169	90 173	104 147
17	29 220	32 205	40 177	48 141	55 136	76 122	95 116	101 101
18	31 63.6	39 66.9	43 53.4	53 43.4	64 57.3	68 48.9	92 69.7	102 58.8
19	32 94.5	46 79.5	53 84.3	60 71.8	68 92.1	74 73.6	94 78.9	102 75.8
20	38 56.4	46 84.2	68 104	74 106	82 114	99 141	106 152	110 146
21	31 9.4	38 10.1	48 8.7	52 9.0	57 8.0	71 8.8	92 10.4	106 10.4
22	44 54.3	49 51.0	61 59.2	70 56.9	117 99.8			
23	35 524	42 451	55 416	74 386	81 392	109 345		
24	44 669	48 685	51 584	58 605	63 582	73 562	90 591	104 570
25	36 64.0	49 83.5	56 78.6	69 83.9	82 100	89 103	105 121	

* Days after initial positive PCR

Correlation of assay results to detection of SARS-CoV-2 inhibitory antibodies

534 samples from patients with PCR confirmed SARS-CoV-2 infection covering a range of 7 to 210 days post reactive PCR were used. The samples included cohorts from patients with severe disease requiring hospitalization (n = 122) and mild disease following quarantine at home (n = 412).

Assay results were compared to the result obtained with a commercially available qualitative IVD to detect SARS-CoV-2 inhibitory antibodies (cPass, Genescript, Netherlands).
Application of the medical decision point of the Elecsys Anti-SARS-CoV-2 S assay at 0.8 U/mL (differentiating non-reactive and reactive results) led to the following correlation:

		cPass SARS-CoV-2 Surrogate Virus Neutralization Test		
		Neutralizing (≥ 20 % inhibition)	Non-neutralizing (< 20 % inhibition)	Total
Elecsys Anti-SARS-CoV-2 S assay	≥ 0.8 U/mL (reactive)	490	19	509
	< 0.8 U/mL (non-reactive)	4	21	25
	Total	494	40	534

	Point estimate	95 % CI[i)]
PPA (positive percent agreement)	99.19 %	97.94 - 99.78 %
NPA (negative percent agreement)	52.50 %	36.13 - 68.49 %
PPV (positive predictive value)	96.27 %	94.90 - 97.28 %
NPV (negative predictive value)*	n.a.	n.a.

i) CI = confidence interval
* The analysis focused on PPV only, all included samples were derived from patients with PCR-confirmed SARS-CoV-2 infection. Therefore, NPV is not applicable.

The application of a threshold of 15 U/mL to the results of the Elecsys Anti-SARS-CoV-2 S assay further improved the PPV:

		SARS-CoV-2 Surrogate Virus Neutralization Test		
		Neutralizing (≥ 20 % inhibition)	Non-neutralizing (< 20 % inhibition)	Total
Elecsys Anti-SARS-CoV-2 S assay	≥ 15 U/mL	439	4	443
	< 15 U/mL	55	36	91
	Total	494	40	534

	Point estimate	95 % CI
PPA	88.87 %	85.76 - 91.50 %
NPA	90.00 %	76.34 - 97.21 %
PPV	99.10 %	97.74 - 99.64 %
NPV*	n.a.	n.a.

* The analysis focused on PPV only, all included samples were derived from patients with PCR-confirmed SARS-CoV-2 infection. Therefore, NPV is not applicable.

This study showed that samples with a result of ≥ 15 U/mL had a likelihood of 99.10 % to contain SARS-CoV-2 inhibitory antibodies as determined with the reference assay for detection of inhibitory antibodies.

Correlation of assay results to serum neutralization capacity

The Elecsys Anti-SARS-CoV-2 S assay was compared to a VSV (Vesicular Stomatitis Virus)-based pseudo-neutralization assay.[32] The results for 15 clinical samples from individual patients are summarized in the following table:

		Pseudo-neutralization assay		
		Positive	Indeterminate	Negative
Elecsys Anti-SARS-CoV-2 S assay	≥ 0.8 U/mL	12	0	0
	< 0.8 U/mL	1	1	1

Positive agreement rate: 92.3 %

Calculation of predictive values was not performed due to low sample numbers and resulting lack of statistical significance.

In a randomized, placebo controlled clinicial trial on use of Tocilizumab in hospitalized patients with severe COVID-19 pneumonia[33], samples were analyzed for virus neutralization capacity by a functional in vitro whole virus neutralization assay (Viroclinics, Netherlands) and antibody titers to the

RBD of SARS-CoV-2 S1 (Elecsys Anti-SARS-CoV-2 S assay). The obtained neutralization results were compared to the results of the Elecsys Anti-SARS-CoV-2 S assay. The comparison was performed for the placebo group only to avoid any potential confounding with putative treatment effects.

The sample cohort comprised 206 samples from 111 hospitalized patients with PCR confirmed SARS-CoV-2 infection and severe COVID-19 pneumonia. Up to 3 samples were collected from each patient covering baseline visit (median 11 days from symptom onset, range 2 to 30 days) and 28 or 60 days after enrollment. Presence of 80 % neutralization (NT80) at a sample dilution of 1:8 or higher identified functional virus neutralization in vitro. Comparison to results of the Elecsys Anti-SARS-CoV-2 S assay was realized by application of two different qualitative thresholds, one representing the decision point to identify presence of RBD specific antibodies (0.8 U/mL, medical decision point of the assay to define reactive results) and one based on optimized correlation with detection of inhibitory effects (15 U/mL).

		Whole virus NT		
		Neutralizing (NT80 ≥ 1:8)	Non-neutralizing	Total
Elecsys Anti-SARS-CoV-2 S assay	≥ 0.8 U/mL (reactive)	187	1	188
	< 0.8 U/mL (non-reactive)	6	12	18
	Total	193	13	206

	Point estimate	95 % CI
PPA	96.9 %	93.4 - 98.9 %
NPA	92.3 %	64.0 - 99.8 %
PPV	99.5 %	97.1 - 100 %
NPV**	n.a.	n.a.

** Predicting absence of neutralization based on absence of RBD-specific antibodies is not recommended because neutralizing antibodies may also be directed to other proteins besides the RBD. Therefore, NPV is not applicable.

The application of a threshold of 15 U/mL to the results of the Elecsys Anti-SARS-CoV-2 S assay resulted in a PPV of 100 %:

		Whole virus NT		
		Neutralizing (NT80 ≥ 1:8)	Non-neutralizing	Total
Elecsys Anti-SARS-CoV-2 S assay	≥ 15 U/mL	164	0	164
	< 15 U/mL	29	13	42
	Total	193	13	206

	Point estimate	95 % CI
PPA	85.0 %	79.1 - 89.7 %
NPA	100 %	75.3 - 100 %
PPV	100 %	97.8 - 100 %
NPV**	n.a.	n.a.

** Predicting absence of neutralization based on absence of RBD-specific antibodies is not recommended because neutralizing antibodies may also be directed to other proteins besides the RBD. Therefore, NPV is not applicable.

In this study, samples with a result of ≥ 15 U/mL had a likelihood of 100 % to confer in vitro neutralization to SARS-CoV-2 as determined with the applied whole virus NT method.

In a study of the Vitalant Research Institute (CA, USA) investigating COVID-19 convalescent plasma for neutralization capacity, plasma donations from convalescent donors after SARS-CoV-2 infection were analyzed for whole virus neutralizing potential in vitro (BROAD Institute plaque reducing neutralization assay (PRNT), USA). Presence of 50 % neutralization (NT50) at a sample dilution of > 1:20 identified functional virus neutralization in vitro.

390 donations, including cross-sectional and longitudinal sample panels, were analyzed and compared to the obtained Elecsys Anti-SARS-CoV-2 S assay results. Comparison to results of the Elecsys Anti-SARS-CoV-2 S assay was realized by application of two different thresholds, one representing the decision point to identify presence of RBD specific antibodies (0.8 U/mL, medical decision point of the assay to define reactive results) and one based on optimized correlation with detection of inhibitory effects (15 U/mL).

		BROAD PRNT		
		Neutralizing (NT50 ≥ 1:20)	Non-neutralizing	Total
Elecsys Anti-SARS-CoV-2 S assay	≥ 0.8 U/mL (reactive)	356	4	360
	< 0.8 U/mL (non-reactive)	2	28	30
	Total	358	32	390

	Point estimate	95 % CI
PPA	99.4 %	98.0 - 99.9 %
NPA	87.5 %	71.0 - 96.5 %
PPV	98.9 %	97.2 - 99.7 %
NPV**	n.a.	n.a.

** Predicting absence of neutralization based on absence of RBD-specific antibodies is not recommended because neutralizing antibodies may also be directed to other proteins besides the RBD. Therefore, NPV is not applicable.

The application of a threshold of 15 U/mL to the results of the Elecsys Anti-SARS-CoV2 S assay resulted in a PPV of 100 % (95 % CI: 98.9-100 %):

		BROAD PRNT		
		Neutralizing (NT50 ≥ 1:20)	Non-neutralizing	Total
Elecsys Anti-SARS-CoV-2 S assay	≥ 15 U/mL	331	0	331
	< 15 U/mL	27	32	59
	Total	358	32	390

	Point estimate	95 % CI
PPA	92.5 %	89.2 - 95.0 %
NPA	100 %	89.1 - 100 %
PPV	100 %	98.9 - 100 %
NPV**	n.a.	n.a.

** Predicting absence of neutralization based on absence of RBD-specific antibodies is not recommended because neutralizing antibodies may also be directed to other proteins besides the RBD. Therefore, NPV is not applicable.

In this study on convalescent plasma, samples with a result of ≥ 15 U/mL had a likelihood of 100 % (95 % CI: 98.9-100 %) to confer in vitro neutralization to SARS-CoV-2 as determined with the applied PRNT method.

Screening for convalescent plasma for the treatment of hospitalized patients with COVID-19

The Elecsys Anti-SARS-CoV-2 S assay has been included in the emergency use approval (EUA) granted by US FDA for the emergency use of convalescent plasma for the treatment of hospitalized patients with COVID-19.[34] The assay has been approved to be used for the purpose of qualifying high titer COVID-19 convalescent plasma in the manufacture of COVID-19 convalescent plasma. US FDA defined ≥ 132 U/mL as the titer cutoff for qualification of high titer COVID-19 convalescent plasma.

This EUA will be effective until the declaration that circumstances exist justifying the authorization of the emergency use of drugs and biological products during the COVID-19 pandemic is terminated under Section 564(b)(2) of the Act or the EUA is revoked under Section 564(g) of the Act. Please refer to the US FDA website for current status.

References

1 Ye Z-W, Yuan S, Yuen K-S, et al. Zoonotic origins of human coronaviruses. Int J Biol Sci 2020 Mar 15;16(10):1686-1697.

2 Transmission of SARS-CoV-2: implications for infection prevention precautions [Internet]. 2020 [cited 2020 Jul 14]. Available from: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions

3 Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020 20;382(8):727-733.

4 Chan JF-W, Yuan S, Kok K-H, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020 15;395(10223):514-523.

5 Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 2020 Mar 10.

6 Zhou R, Li F, Chen F, et al. Viral dynamics in asymptomatic patients with COVID-19. International Journal of Infectious Diseases 2020 Jul 1;96:288-290.

7 He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nature Medicine 2020 May;26(5):672-675.

8 Mizumoto K, Kagaya K, Zarebski A, et al. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. Euro Surveill 2020 Mar 12;25(10).

9 Gao M, Yang L, Chen X, et al. A study on infectivity of asymptomatic SARS-CoV-2 carriers. Respir Med 2020 Aug;169:106026.

10 Yu P, Zhu J, Zhang Z, et al. A Familial Cluster of Infection Associated With the 2019 Novel Coronavirus Indicating Possible Person-to-Person Transmission During the Incubation Period. J Infect Dis 2020 11;221(11):1757-1761.

11 Liu Z, Chu R, Gong L, et al. The assessment of transmission efficiency and latent infection period on asymptomatic carriers of SARS-CoV-2 infection. International Journal of Infectious Diseases 2020 Jun 13.

12 Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol 2020;5(4):562-569.

13 Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020 Feb 24;12(1):1-5.

14 Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020 13;367(6483):1260-1263.

15 Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020 16;181(2):271-280.e8.

16 Centers for Disease Control and Prevention. Interim Guidelines for COVID-19 Antibody Testing [Internet]. Centers for Disease Control and Prevention. 2020 [cited 2020 Jun 4]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html

17 Long Q-X, Liu B-Z, Deng H-J, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020 Apr 29.

18 Lou B, Li T-D, Zheng S-F, et al. Serology characteristics of SARS-CoV-2 infection since exposure and post symptom onset. Eur Respir J 2020 May 19;2000763.

19 Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020 Mar 28.

20 Tuaillon E, Bolloré K, Pisoni A, et al. Detection of SARS-CoV-2 antibodies using commercial assays and seroconversion patterns in hospitalized patients. Journal of Infection 2020 Jun 3.

21 Luchsinger LL, Ransegnola B, Jin D, et al. Serological Analysis of New York City COVID19 Convalescent Plasma Donors [Internet]. Infectious Diseases (except HIV/AIDS); 2020 Jun [cited 2020 Jul 23]. Available from: http://medrxiv.org/lookup/doi/10.1101/2020.06.08.20124792

22 Salazar E, Kuchipudi SV, Christensen PA, et al. Relationship between Anti-Spike Protein Antibody Titers and SARS-CoV-2 In Vitro Virus Neutralization in Convalescent Plasma [Internet]. Immunology; 2020 Jun [cited 2020 Jun 13]. Available from: http://biorxiv.org/lookup/doi/10.1101/2020.06.08.138990

23 Klasse P, Moore JP. Antibodies to SARS-CoV-2 and their potential for therapeutic passive immunization. Giamarellos-Bourboulis EJ, van der Meer JW, editors. eLife. 2020 Jun 23;9:e57877.

24 Premkumar L, Segovia-Chumbez B, Jadi R, Martinez DR, Raut R, Markmann AJ, et al. The receptor-binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. Science Immunology 2020 Jun 11;5(48).

25 Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat. Biotechnol 2020 doi:10.1038/s41587-020-0631-z.

26 Mukherjee R. Global efforts on vaccines for COVID-19: Since, sooner or later, we all will catch the coronavirus. J Biosci 2020;45.

27 Graham BS. Rapid COVID-19 vaccine development. Science 2020 29;368(6494):945-946.

28 Hotez PJ, Corry DB, Bottazzi ME. COVID-19 vaccine design: the Janus face of immune enhancement. Nature Reviews Immunology 2020 Jun;20(6):347-348.

29 Liu A, Wang W, Zhao X, et al. Disappearance of antibodies to SARS-CoV-2 in a Covid-19 patient after recovery. Clinical Microbiology and Infection 2020 Jul 8;0(0).

30 Long Q-X, Tang X-J, Shi Q-L, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nature Medicine 2020 Jun 18;1-5.

31 Wu L-P, Wang N-C, Chang Y-H, et al. Duration of Antibody Responses after Severe Acute Respiratory Syndrome - Volume 13, Number 10 - October 2007 - Emerging Infectious Diseases journal - CDC. [cited 2020 Jul 16]; Available from: https://wwwnc.cdc.gov/eid/article/13/10/07-0576_article

32 Meyer B, Torriani G, Yerly S, et al. Validation of a commercially available SARS-CoV-2 serological Immunoassay. medRxiv. 2020. https://doi.org/10.1101/2020.05.02.20080879.

33 Rosas IO, Bräu N, Waters M, et al. 2020, Tocilizumab in Hospitalized Patients With COVID-19 Pneumonia, medRxiv 2020.08.27.20183442; doi: https://doi.org/10.1101/2020.08.27.20183442

34 FDA Updates Emergency Use Authorization for COVID-19 Convalescent Plasma to Reflect New Data Convalescent Plasma EUA Letter of Authorization

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

Additions, deletions or changes are indicated by a change bar in the margin.

Elecsys Anti-SARS-CoV-2 S

cobas®

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Roche