Elecsys Anti-SARS-CoV-2 S

cobas®

REF		Σ	
09289275119	09289275500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
ACOV2S	10230

Intended use

Immunoassay for the in vitro qualitative and quantitative detection 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 test may also detect a response to vaccination.

The performance of the device has not been assessed on specimens from individuals who have been infected with emerging variants of SARS-CoV-2 of public health concern.

Elecsys Anti-Anti-SARS-CoV-2 S is intended to be used by trained laboratory professionals in a laboratory environment.

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

Note: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 09289275190 for the Elecsys Anti-SARS-CoV-2 S assay. The last 3 digits -190 have been replaced by -119 for logistic purposes.

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] 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.

Test principle

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

- 1st incubation: 12 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 II 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 **cobas** link.

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

Reagents - working solutions

The **cobas e** pack is labeled as ACOV2S.

M Streptavidin-coated microparticles, 1 bottle, 16.0 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 SARS-CoV-2 S-Ag~biotin, 1 bottle, 18.8 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~$Ru(bpy)_3^{2+}$, 1 bottle, 18.8 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.

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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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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
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, K2-EDTA, K3-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.

Capillary studies were conducted with capillary blood obtained by finger prick.

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 14 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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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.

- 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 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the internal Roche standard for anti-SARS-CoV-2 S.
Subsequently, it could be shown that the First WHO International Standard for anti-SARS-CoV 2 immunoglobulin (human), NIBSC code: 20/136, behaves identically to the internal Roche standard, with a Pearson correlation coefficient 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 BAU/mL are equivalent (e.g. 1 U/mL of the Elecsys Anti-SARS-CoV-2 S assay corresponds to 1 BAU/mL).
Note: Although the defined unit for the Elecsys Anti-SARS-CoV-2 S assay is identical to the binding antibody unit (BAU) defined by the WHO standard, the defined unit for the Elecsys Anti-SARS-CoV-2 S assay must not be used interchangeably with units of other assays. See also the section "Interpretation of results".
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.

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Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack 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 42 days when using the same reagent lot
- after 14 days when using the same **cobas e** pack 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 - ≤ 250 U/mL	Positive for anti-SARS-CoV-2-S, numeric value within the measuring interval
> 250 U/mL	Positive for anti-SARS-CoV-2-S, numeric value as "> 250 U/mL". Refer to the Dilution section for value above the measuring interval

Duplicate repeat results in cobas e flow ACOV2S DR	Interpretation
Both of the duplicate repeat tests < 0.80 U/mL	Negative for anti-SARS-CoV-2-S
One or both of the duplicate repeat tests ≥ 0.80 U/mL	Repeatedly reactive, 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
Albumin	≤ 70 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.[36] 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.

This assay is not intended to be used for screening patients or as an aid for diagnosis of patients with suspected COVID-19 infection.

Use in conjunction with the testing strategy outlined by public health authorities in your area.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions.

Negative results must be combined with clinical observations, patient history, and epidemiological information.

False negative results can occur in elderly and immunocompromised patients. False positive results for IgM and IgG antibodies may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

Results are for the detection of SARS-CoV-2 antibodies. IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although levels over the course of infection are not well characterized.IgG antibodies to SARS-CoV-2 become detectable later following infection. At this time, it is unknown how long IgM or IgG antibodies may persist following infection.

Positive results for both IgG and IgM could occur after infection and can be indicative of acute or recent infection [and successful immune response to a vaccine].

The presence of specific antibodies is a sign of previous or current infection, and can also be used to determine the efficacy of treatment.

The performance of this device has not been assessed in a population vaccinated against COVID-19. The performance of this device has not been validated for the detection of neutralizing antibodies.

This test identifies antibodies to the spike protein of the SARS-CoV-2 virus and is therefore unable to distinguish between previously infected individuals and vaccinated individuals.

Laboratories are required to report all positive results to the appropriate public health authorities.

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. 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.

An optimized dilution algorithm can be performed automatically (see section "cobas e flows").

cobas e flows
cobas e flows are procedures programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.

The **cobas e** flow "**ACOV2S D**" is available to automatically perform an initial 1:30 sample dilution. If the result of this measurement is within the extended measuring range (12-7500 U/mL), the result is reported.
In case the initial result is found above the extended measuring range, another dilution (1:400) of the sample is automatically carried out to resolve titers up to 100000 U/mL. Results > 100000 U/mL are assigned the result message "above measuring range" with the numeric result set to 100000 U/mL.
In case the initial result is found below the measuring range associated with 1:30 dilution, another measurement is carried out without dilution of the sample and the result is reported.

The **cobas e** flow "**ACOV2S DR**" is available to measure a sample with the same automated dilution algorithm as in the cobas e flow "ACOV2S D", followed by duplicate repeat measurement for samples with an initially "reactive" result (≥ 0.8 U/mL). Confirmation of the reactive status by one or both of the repeat measurements leads to the main result "repeatedly reactive". Lack of confirmation with both of the repeats leads to the qualitative interpretation of the sample being "non-reactive" reported as the main result. Relevant results of the individual determinations are provided as sub-results in addition to the main result.

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 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
HSP[c)] 1	0.483	0.014	2.9	0.014	2.9
HSP 2	0.826	0.015	1.9	0.015	1.9
HSP 3	5.69	0.121	2.1	0.136	2.4
HSP 4	12.0	0.159	1.3	0.191	1.6
HSP 5	54.8	0.743	1.4	0.770	1.4
HSP 6	77.3	1.23	1.6	1.54	2.0
HSP 7	184	1.69	0.9	2.63	1.4
PC[d)] ACOV2S 1	< 0.40	-	-	-	-
PC ACOV2S 2	10.4	0.139	1.3	0.206	2.0

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.

Method comparison
A comparison of the Elecsys Anti-SARS-CoV-2 S assay, REF 09289275190 (**cobas e** 402 analyzer; y), with the Elecsys Anti-SARS-CoV-2 S assay, REF 09289275190 (**cobas e** 801 analyzer; x), gave the following correlations (U/mL):

Number of samples measured: 141

Passing/Bablok[32]

$y = 0.950x - 0.056$

$r = 0.998$

The sample concentrations were between 0.047 and 241 U/mL.

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.

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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
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 13 871 samples were tested with the Elecsys Anti-SARS-CoV-2 S assay. All samples were obtained before October 2019. 4 false positive samples were detected.

The resulting overall specificity was 99.97 %. The 95 % lower confidence limit was 99.93 %.

Cohort	N	Reactive	Specificity %	95 % lower confidence limit, %	95 % upper confidence limit, %
Diagnostic routine (Europe)	5352	1	99.98	99.90	100
Blood donors (USA)	2713	1	99.96	99.79	100
Blood donors (Africa)	750	0	100	99.51	100
Blood donors (Europe)	5056	3	99.94	99.83	99.99
Overall	**13871**	**4**	**99.97**	**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

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U/mL	Days after diagnosis with positive PCR					
	0-6	7-13	14-20	21-27	28-34	> 35
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], %	86.1		**98.8**			
95 % CI[h], %	80.3 - 90.7		**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	23	27	33	36	61	82	103
	20.4	22.2	30.5	47.4	51.7	73.5	87.7	114
2	21	24	31	34	37	62	83	104
	36.1	44.3	32.4	48.5	51.4	63.1	73.2	71.9
3	26	34	38	41	45	67	87	106
	139	223	186	153	150	198	147	155
4	21	30	33	36	41	62	83	107
	32.3	95.3	151	315	374	293	244	214
5	30	35	38	42	112			
	33.0	29.5	31.2	41.2	59.9			
6	20	30	38	62	71	76	86	107
	7.88	32.6	26.6	39.2	35.7	40.3	36.0	42.1
7	19	22	25	29	39	48	59	104
	20.7	40.4	101	149	115	97.7	115	175
8	15	22	30	37	40	55	79	107
	22.1	14.2	37.1	166	136	226	124	96.9
9	34	41	45	52	67	74	87	106
	181	148	148	165	152	154	125	119
10	26	29	32	35	42	52	73	103
	4.42	4.79	4.83	5.21	4.67	5.95	7.28	7.69
11	16	42	78	106				
	305	296	371	408				
12	28	31	40	44	47	62	86	103
	139	162	114	166	141	93.0	69.5	59.1
13	24	31	38	46	59	74	92	102
	33.9	45.6	63.7	53.4	47.4	41.8	41.9	42.8
14	25	28	33	41	47	59	76	109
	79.8	86.4	120	117	103	108	97.1	105
15	36	52	68	77	92	96	106	126
	255	165	126	94.8	122	107	141	162
16	30	44	51	58	73	85	90	104
	425	246	379	298	215	169	173	147
17	29	32	40	48	55	76	95	101
	220	205	177	141	136	122	116	101
18	31	39	43	53	64	68	92	102
	63.6	66.9	53.4	43.4	57.3	48.9	69.7	58.8
19	32	46	53	60	68	74	94	102
	94.5	79.5	84.3	71.8	92.1	73.6	78.9	75.8
20	38	46	68	74	82	99	106	110
	56.4	84.2	104	106	114	141	152	146
21	31	38	48	52	57	71	92	106
	9.4	10.1	8.7	9.0	8.0	8.8	10.4	10.4
22	44	49	61	70	117			
	54.3	51.0	59.2	56.9	99.8			
23	35	42	55	74	81	109		
	524	451	416	386	392	345		
24	44	48	51	58	63	73	90	104
	669	685	584	605	582	562	591	570
25	36	49	56	69	82	89	105	
	64.0	83.5	78.6	83.9	100	103	121	

* Days after initial positive PCR

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.[33] 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 %

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.[35] 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.

Clinical sensitivity

In addition, an external study at three European sites was performed. One or more consecutive samples were collected at various timepoints after PCR confirmation from 272 subjects (including 54 hospitalized patients). The time span of samples collected after positive PCR was between day 0 and day 120. For subjects with sequential blood draws with more than one sample per time interval, solely the last valid value per time interval was enrolled for sensitivity calculation. As such, a total of 240 samples from different patients had a sampling date of 14 days or later after diagnosis with PCR.. 235 of 240 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 97.92% (95% CI: 95.21 - 99.32%).

Elecsys Anti-SARS-CoV-2 S

U/ml	Jours après diagnostic par résultat de PCR positif*						
	0-6	7-13	14-20	21-27	28-34	≥35	≥14
<0.4	18	6	1	0	0	5	5
0.4 - <0.8	1	1	0	0	0	0	0
0.8 - <1.5	0	1	0	0	0	2	2
1.5 - <2.5	2	0	2	2	0	1	5
2.5 - <5	2	3	0	0	0	2	2
5 - <10	0	2	2	6	2	7	11
10 - <20	2	2	2	3	2	13	17
20 - <50	3	3	4	5	4	23	32
50 - <100	4	5	2	3	4	27	32
100 - <150	2	3	1	7	1	12	17
150 - <200	1	3	1	1	5	12	18
200 - ≤250	1	1	4	4	2	8	16
>250	8	19	28	27	23	57	83
≥0.8	25	42	46	58	43	164	240
Total	44	49	47	58	43	169	235
Sensibilité, % [95% CI], %	**56.82** [41.03 - 71.65]	**85.71%** [72.76 - 94.06%]	**97.87%** [88.71 - 99.95%]	**100.00%** [93.84 - 100.00%]	**100.00%** [91.78 - 100.00%]	**97.04%** [93.23 - 99.03%]	**97.92%** [95.21 - 99.32%]

* Les intervalles de temps incluent uniquement le dernier échantillon par intervalle de chaque sujet (voir la ligne marquée en gris pour le nombre d'échantillons inclus).

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36 The 17 commonly used pharmaceuticals tested are:

Drug	Concentration tested
Acetylcysteine	150 mg/L
Acetylsalicylic acid	30 mg/L
Ampicillin	75 mg/L
Ascorbic acid	52.5 mg/L
Cefoxitin	750 mg/L
Doxycycline	18 mg/L
Heparin	3300 IU/L
Levodopa	7.5 mg/l
Methyldopa	22.5 mg/L
Metronidazole	123 mg/L
Rifampicin	48 mg/L
Acetaminophen	156 mg/L
Cyclosporine	1.8 mg/L
Ibuprofen	219 mg/L
Theophylline	60 mg/l
Phenylbutazone	321 mg/L
Itraconazole	15 mg/L

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

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