Elecsys Anti-SARS-CoV-2

cobas®

		Σ	SYSTEM
09203095119	09203095500	200	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 3000
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 737

Intended use

Elecsys Anti-SARS-CoV-2 is an immunoassay for the in vitro qualitative detection of antibodies (including IgG) to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in human serum and plasma. The test is intended as an aid in the determination of the immune reaction to SARS-CoV-2.

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

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 may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

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: 09203095190 for the Elecsys Anti-SARS-CoV-2 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 The last 3 digits -190 have been replaced by -119 for logistic purposes.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 virus of the family Coronaviridae, genus Betacoronaviruses. Viruses of this family share similarities in their genome and organization, including the 4 structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N). They cause diseases with symptoms ranging from those of a mild common cold to more severe ones such as the Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and COVID-19. Other coronaviruses known to infect humans include 229E, NL63, OC43 and HKU1. The latter are ubiquitous and infection typically causes common cold or flu-like symptoms.[1,2]

SARS-CoV-2 is mainly transmitted person-to-person primarily via respiratory droplets, but indirect transmission through contaminated surfaces is also possible.[3,4,5,6] The virus infects host cells via the angiotensin-converting enzyme 2 (ACE2), which is highly expressed in the lungs.[7,8]

The incubation period for COVID-19 is thought to be within 14 days following exposure, with median incubation period being 4-5 days.[3,9,10] The interval during which an individual with COVID-19 is infectious has not yet been clearly established, however, transmission from both symptomatic and asymptomatic individuals has been described.[1,11,12,13,14,15] Those infected often exhibit fever and respiratory symptoms.[16,17,18] The spectrum of symptomatic infection ranges from mild to critical, with severe cases occurring predominantly in adults with advanced age or underlying medical comorbidities.[17,19,20]

Definite COVID-19 diagnosis entails direct detection of SARS-CoV-2 RNA by nucleic acid amplification technology (NAAT).[21,22,23] Serological assays, which detect antibodies against SARS-CoV-2, can contribute to identify individuals, which were previously infected by the virus, and to assess the extent of exposure of a population.

Upon infection with SARS-CoV-2, the host mounts an immune response against the virus, including production of specific antibodies against viral antigens. Understanding the dynamics of the antibody response to the virus is critical in establishing a relevant time window to use serology tests. Both immunoglobulin M (IgM) and G (IgG) have been detected as early as day 5 after symptom onset.[25,26] Median seroconversion has been observed at day 10-13 for IgM and day 12-14 for IgG[27,28,29], while maximum levels have been reported at week 2-3 for IgM, week 3-6 for IgG and week 2 for total antibody.[25,26,27,28,29,30,31] Whereas IgM seems to vanish around week 6-7[32,33] high IgG seropositivity is seen at that time.[25,32,33] While IgM is typically the major antibody class secreted to blood in the early stages of a primary antibody response, levels and chronological order of IgM and IgG antibody appearance seem to be highly variable for SARS-CoV-2.

Anti-SARS-CoV-2 IgM and IgG often appear simultaneously, and some cases have been reported where IgG appears before IgM, limiting its diagnostic utility.[26,27,29,34,35]

After infection or vaccination, the binding strength of antibodies to antigens increases over time - a process called affinity maturation.[36] High-affinity antibodies can elicit neutralization by recognizing and binding specific viral epitopes.[37,38] While correlates of immunity/protection to SARS-CoV-2 still need to be identified, neutralization of the virus is presumed to be an important role of antibodies.[39] In SARS-CoV-2 infection, antibodies targeting both the spike and nucleocapsid proteins, are formed as early as day 9 onwards, which correlates with a strong neutralizing response, suggesting seroconversion may lead to protection for at least a limited time.[34,40,41,42,43] However, more scientific evidence will be necessary to determine if neutralizing antibodies against SARS-CoV-2 confer long-term immunity.

The Elecsys Anti-SARS-CoV-2 assay uses a recombinant protein representing the nucleocapsid (N) antigen in a double-antigen sandwich assay format, which favors detection of high affinity antibodies against SARS-CoV-2. Elecsys Anti-SARS-CoV-2 detects antibody titers, which have been shown to positively correlate with neutralizing antibodies in neutralization assays.[44,45]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, biotinylated SARS-CoV-2-specific recombinant antigen and SARS-CoV-2-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 automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

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

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as ACOV2.

M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1	SARS-CoV-2-Ag~biotin (gray cap), 1 bottle, 16 mL: Biotinylated SARS-CoV-2-specific recombinant antigen (E. coli) < 0.5 mg/L; HEPES[b)] buffer 50 mmol/L, pH 7.7; preservative.
R2	SARS-CoV-2-Ag ~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 16 mL: SARS-CoV-2-specific recombinant antigen labeled with ruthenium complex < 0.5 mg/L; HEPES buffer 50 mmol/L, pH 7.7, preservative.

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

ACOV2 Cal1	Negative calibrator 1 (white cap), 1 bottle of 0.67 mL: Human serum, non-reactive for anti-SARS-CoV-2 antibodies; buffer; preservative.
ACOV2 Cal2	Positive calibrator 2 (black cap), 1 bottle of 0.67 mL: Human serum, reactive for anti- SARS-CoV-2 antibodies; buffer; preservative.

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:

Elecsys Anti-SARS-CoV-2

cobas®

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

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-SARS-CoV-2 (ACOV2 Cal2) was heat-inactivated for 30 minutes at 56 °C.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[46,47]

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

Reagent handling

For professional use.

The reagents in the kit are ready-for-use and are supplied in bottles compatible with the system.

cobas e 411 analyzer: The calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 4 calibration procedures per calibrator bottle set should be performed.

cobas e 601 and **cobas e** 602 analyzers: Perform only one calibration procedure per bottle.

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

Please note: Both the vial labels contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so that the barcode between the yellow markers can be read by the system. Place the vial on the analyzer as usual.

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 of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	21 days
on the analyzers	14 days

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
or after opening at 2-8 °C	72 hours
on **cobas e** 411 at 20-25 °C	up to 3 hours
on **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

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, K_2-EDTA and K_3-EDTA plasma.

Li-heparin and K_2-EDTA plasma tubes containing separating gel can be used.

Stable for 7 days at 15-25 °C, 7 days at 2-8 °C, 28 days at -20 °C (± 5 °C).The samples may be frozen 3 times.

Capillary blood collected in serum, Li-heparin or K_2-EDTA sampling tubes. Capillary blood for plasma preparation should be collected in standard blood collecting microtubes containing Li-heparin or K_2-EDTA. Due to the risk of the blood volume variation in the microtubes and deviation in the blood: anticoagulant ratio, plasma samples prepared from capillary blood should be tested as soon as possible. Stability and shipment conditions of the plasma samples containing elevated levels of anticoagulants were not evaluated.
Capillary blood for serum preparation should be collected in standard blood collecting microtubes with or without clot activator.
Criterion: Absolute deviation of negative samples ± 0.3 COI (cutoff index) from serum value; reactive samples: recovery within 70-130 % of serum value.
Serum or plasma prepared from whole venous blood are stable for 7 days at 15-25 °C, 7 days at 2-8 °C, 28 days 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.

Pooled samples and other artificial material may have different effects on different assays and thus may lead to discrepant findings.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls 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 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 09216928190, PreciControl Anti-SARS-CoV-2, 4 x 1.0 mL
- REF 03609987190, Diluent MultiAssay, 2 x 16 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.

Calibrators:

Place the calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (**cobas e** 601 and **cobas e** 602 analyzers).

Calibration

No international standard is available for Anti-SARS-CoV-2.

Calibration frequency: Calibration must be performed once per reagent lot using ACOV2 Cal1, ACOV2 Cal2 and fresh reagent (i.e. not more than 24 hours since the 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 25 days when using the same reagent lot
- after 7 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.

In addition, other suitable control material can be used.

Alternatively, controls can be prepared as follows:

Negative control: Determine the COI of ACOV2 Cal1 by measuring it as a routine sample. Pool serum samples with a COI result of ≤ 150 % compared to the COI result of ACOV2 Cal1 (pooling of ≥ 5 non-reactive samples in this range is recommended). Mix carefully, avoiding foam formation. Prepare aliquots of at least 250 µl from this sample pool and store frozen at -20 °C (± 5 °C) or colder. Use these aliquots to perform regular quality control.

This negative control has a target value range of COI < 0.8 (qualitative assay result "non-reactive")

Positive control: Determine the COI of ACOV2 Cal2 by measuring it as a routine sample. Pool serum samples with a COI result that is higher than the COI result of ACOV2 Cal2 (pooling of ≥ 3 reactive samples in this range is recommended). Dilute the sample pool by adding pooled negative serum (pooling criterion see negative control) or Diluent MultiAssay to obtain a COI between 3 and 15. Mix carefully, avoiding foam formation. It is recommend to confirm calculated reactivity after dilution by a measurement. Prepare aliquots of at least 250 µl from this sample pool and store frozen at -20 °C (± 5 °C) or colder. Use these aliquots to perform regular quality control. Upon first use of this control, determine the COI of the control by measurement of the control in triplicate and using a freshly opened reagent rack pack.

The obtained median of these measurements serves as target value for this positive control. Subsequent measurements of all aliquots of this control material must match this target value ± 45 % (3SD = 45 %, 1SD = 15 %; qualitative assay result "reactive"). In case the quality control fails, thaw a new aliquot and re-assess the performance of the assay.

The target value of the positive control is lot specific and target value assessment as described above has to be performed for every assay lot.

After measurement, discard aliquots with a remaining volume of 250 µl or less. Aliquots with a remaining volume of more than 250 µl can be re-used if sealed tightly and stored immediately at 2-8 °C for max. 3 days.

In case quality control fails for any reason, thaw a new control aliquot and re-assess the performance of the assay.

Also pools of plasma samples with similar reactivity can be used, however re-clotting frequently occurs with plasma after thawing. If this occurs, either discard or centrifuge the aliquot before use. Do not mix serum samples and plasma samples to prepare a sample pool.

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.

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

If necessary, repeat the measurement of the samples concerned.

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

Note: The controls should be run like external controls. All values and ranges have to be entered manually. Please refer to the section "QC" in the operator's manual or to the online help of the instrument software. Only one target value and range for each control level can be entered in the analyzer. The reagent lot-specific target values have to be re-entered each time a specific reagent lot with different control target values and ranges is used. Two reagent lots with different control target values and ranges cannot be used in parallel in the same run.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of ACOV2 Cal1 and ACOV2 Cal2.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (COI; signal sample/cutoff).

Interpretation of the results

Results obtained with the Elecsys Anti-SARS-CoV-2 assay can be interpreted as follows:

Numeric result	Result message	Interpretation
COI < 1.0	Non-reactive	Negative for anti-SARS-CoV-2 antibodies
COI ≥ 1.0	Reactive	Positive for anti-SARS-CoV-2 antibodies

The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present in the sample.
The individual immune response following SARS-CoV-2 infection varies considerably and might give different results with assays from different manufacturers. Results of assays from different manufacturers should not be used interchangeably.

Elecsys Anti-SARS-CoV-2

cobas®

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 samples with a COI ≥ 1.0, the deviation is ≤ 20 %. For samples with a COI < 1.0, the deviation is ≤ 0.2 COI.

Potential interferences by pharmaceutical compounds other than biotin have not been tested and an interference cannot be excluded.

Limitations

- 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

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

-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.

- Potential for cross reactivity with SARS positive samples.
- Lower sensitivity may be observed in samples tested in early infection (<14 days).
- Use in conjunction with the testing strategy outlined by public health authorities in your area.
- Positive results could occur after infection and can be indicative of acute or recent infection.
- False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
- The presence of specific antibodies are a sign of previous or current infection, and can also be used to determine the efficacy of treatment.
- Laboratories are required to report all positive results to the appropriate health authorities.
- The performance of Elecsys Anti-SARS-CoV-2 has not been assessed in a population vaccinated against COVID-19.

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. Also, over time, titers may decline and eventually become negative.

cobas e 601 and **cobas e** 602 analyzers:

Note: Only required if the Elecsys Anti-SARS-CoV-2 assay runs on the same analyzer module as the Elecsys SARS-CoV-2 Antigen assay (REF 09345272190).

Make sure that in the Special Wash List (Screen → Utility → Special Wash → Immune) the Elecsys Anti-SARS-CoV-2 assay is combined with all assays performed on the analyzer - including the Elecsys Anti-SARS-CoV-2 assay itself:

From test	Step	To test	Step 0	Step 1	Step 2
Anti-SARS-CoV-2	1	Anti-SARS-CoV-2	x	x	x
Anti-SARS-CoV-2	1	each other assay	x	x	x

If new tests are installed, make sure that the Special Wash List is updated accordingly.

The described additions to the Special Wash List have to be entered manually. Please refer to the operator's manual.

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 for 5 days and 5 determinations per sample. The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
Human serum 1*	0.063	0.002	2.4	0.003	4.4
Human serum 2*	0.052	0.001	2.5	0.003	5.7
Human serum 3**	1.16	0.021	1.8	0.052	4.5
Human serum 4**	1.22	0.034	2.8	0.057	4.7
Human serum 5***	5.02	0.137	2.7	0.209	4.2
Human serum 6***	13.4	0.219	1.6	0.663	5.0
Human serum 7***	22.4	0.447	2.0	0.986	4.4
Human serum 8****	0.664	0.015	2.3	0.038	5.7
Human serum 9****	0.689	0.013	1.9	0.049	7.2
PC[c)] ACOV2 1	0.059	0.002	2.6	0.003	5.0
PC ACOV2 2	2.97	0.038	1.3	0.065	2.2

c) PC = PreciControl

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
Human serum 1*	0.062	0.001	1.8	0.003	4.8
Human serum 2*	0.051	0.001	2.5	0.003	6.5
Human serum 3**	1.07	0.009	0.8	0.025	2.3
Human serum 4**	1.15	0.013	1.1	0.031	2.7
Human serum 5***	4.76	0.050	1.1	0.110	2.3
Human serum 6***	12.9	0.112	0.9	0.303	2.4
Human serum 7***	22.3	0.147	0.7	0.562	2.5
Human serum 8****	0.636	0.009	1.4	0.032	5.0
Human serum 9****	0.700	0.008	1.1	0.039	5.6
PC ACOV2 1	0.059	0.001	1.9	0.003	5.1
PC ACOV2 2	2.96	0.018	0.6	0.079	2.7

* negative
** low positive
*** positive
**** negative, near cutoff

Analytical specificity

Out of 792 tested samples with potential cross-reactive, 4 samples showed reactivity in the Elecsys Anti-SARS-CoV-2 assay resulting in an overall specificity in this cohort of 99.5 %. Results are shown in the following table:

Indication	N	Non-reactive	Reactive	Specificity %
Common cold panel[d)]	40	40	0	100
Coronavirus panel[e)]	40	40	0	100
CMV acute (IgM+, IgG+)	85	84	1	98.8
EBV acute (IgM+, IgG+)	105	103	2	98.1
Borrelia burgdorferi	6	6	0	100
Chlamydia pneumoniae	8	8	0	100

Indication	N	Non-reactive	Reactive	Specificity %
E. coli (anti-E. coli-reactive)	10	10	0	100
Neisseria gonorrhoeae	5	5	0	100
HAV acute (IgM+)	10	10	0	100
HAV late (IgG+)	15	15	0	100
HAV vaccinees	15	15	0	100
HBV early acute (HBsAg+, HBeAg+)	12	12	0	100
HBV acute (anti-HBs+)	7	7	0	100
HBV acute (anti-HBc IgM+)	8	8	0	100
HBV chronic	12	12	0	100
HBV vaccinees	15	15	0	100
HCV acute (anti-HCV IgM+)	6	6	0	100
HCV (anti-HCV IgG+)	60	60	0	100
HEV	12	12	0	100
HIV	10	10	0	100
HSV acute (IgM+)	24	24	0	100
HTLV	6	6	0	100
Influenza vaccinees	25	25	0	100
Listeria	6	6	0	100
Measles	10	10	0	100
Mumps	14	14	0	100
Parvovirus B19	30	30	0	100
Plasmodium falciparum (Malaria)	8	8	0	100
Rubella acute (IgM+, IgG+)	12	12	0	100
Toxoplasma gondii (IgM+, IgG+)	8	8	0	100
Treponema pallidum (Syphilis)	62	62	0	100
VZV (Varicella Zoster)	30	30	0	100
AMA (anti-mitochondrial antibodies)	30	30	0	100
ANA (anti-nuclear antibodies)	26	26	0	100
SLE (systemic lupus erythematosus)	10	9	1	90.0
RA (rheumatoid arthritis)	10	10	0	100

d) 40 potentially cross-reactive samples from individuals with common cold symptoms, collected before December 2019

e) 40 potentially cross-reactive samples from individuals following an infection with Coronavirus HKU1, NL63, 229E or OC43, confirmed by PCR

Clinical specificity

A total of 10453 samples were tested with the Elecsys Anti-SARS-CoV-2 assay. All samples were obtained before December 2019. 21 false positive samples were detected.

The resulting overall specificity in the internal study was 99.80 %. The 95 % lower confidence limit was 99.69 %.

Cohort	N	Non-reactive	Reactive	Specificity, % (95 % CI[f])
Diagnostic routine	6305	6293	12	99.81 (99.67-99.90)
Blood donors	4148	4139	9	99.78 (99.59-99.90)
Overall	**10453**	**10432**	**21**	**99.80 (99.69-99.88)**

f) CI = confidence interval

Sensitivity

A total of 496 samples from 102 symptomatic patients with a PCR confirmed SARS-CoV-2 infection were tested with the Elecsys Anti-SARS-CoV-2 assay. 1 or more consecutive samples from these patients were collected after PCR confirmation at various time points.

Days post PCR confirmation	N	Reactive	Non-reactive	Sensitivity, % (95 % CI)
0-6	161	97	64	60.2 (52.3-67.8 %)
7-13	150	128	22	85.3 (78.6-90.6 %)
≥ 14	185	184	1[g]	99.5 (97.0-100 %)

g) 1 patient was non-reactive at day 14 (0.696 COI) but reactive at day 16 (4.48 COI)

After recovery from infection, confirmed by a negative PCR result, 26 consecutive samples from 5 individuals were tested with the Elecsys Anti-SARS-CoV-2 assay.

Patient	Day of negative PCR*		Days after diagnosis with positive PCR						
			21-23	24-26	27-29	30-32	33-35	36-38	39-40
1	9	COI	24.7	-	27.4	31.7	38.9	56.0	-
2	12		28.8	29.8	30.6	32.7	35.7	-	-
3	17		-	46.5	53.6	-	67.1	73.7	77.0
4	21		24.1	29.8	40.7	51.2	61.5	67.5	-
5	24		-	0.990	1.12	1.55	-	1.66	1.97

* Day 0 represents initial positive PCR.

Correlation of assay results to serum neutralization capacity

The Elecsys Anti-SARS-CoV-2 assay was compared to a VSV[h]-based pseudo-neutralization assay.[48] The results for 46 clinical samples from individual patients are summarized in in the following table:

		Pseudo-neutralization assay	
		Positive	**Negative**
Elecsys Anti-SARS-CoV-2 assay	**Positive**	38	0
	Negative	6	2

Percent positive agreement: 86.4 % (95 % CI: 73.3-93.6 %)
Percent negative agreement: 100 % (95 % CI: 34.2-100 %)
Percent overall agreement: 87.0 % (95 % CI: 74.3-93.9 %)
A titer of 1:20 was used as the positive cutoff for the pseudo-neutralization assay.

h) VSV = Vesicular Stomatitis Virus

References

1 Su S, Wong G, Shi W, et al. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol 2016;24(6):490-502.

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

3 Chan JF, Yuan S, Kok KH, 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;395:514-523.

4 U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. How Coronavirus Spreads. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html. Published April 2, 2020. Accessed April 15, 2020.

5 World Health Organization. Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations. https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations. Published March 29, 2020. Accessed April 15, 2020.

6 Kampf G, Todt D, Pfaender S, et al. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J Hosp Infect 2020;104(3):246-251.

7 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;5:562–569.

8 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;181:271-80.e8.

9 World Health Organization. Coronavirus disease 2019 (COVID-19) Situation Report – 74. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200403-sitrep-74-covid-19-mp.pdf. Published April 3, 2020. Accessed April 15, 2020.

10 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;172(9):577-82.

11 Rothe C, Schunk M, Sothmann P, et al. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N Engl J Med 2020;382(10):970-971.

12 Kupferschmidt K. Study claiming new coronavirus can be transmitted by people without symptoms was flawed. Science. https://www.sciencemag.org/news/2020/02/paper-non-symptomatic-patient-transmitting-coronavirus-wrong. Published February 4, 2020. Accessed April 15, 2020.

13 Bai Y, Yao L, Wei T, et al. Presumed Asymptomatic Carrier Transmission of Covid-19. JAMA 2020;323(14):1406-1407.

14 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;25(10):2000180.

15 Hu Z, Song C, Xu C, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci 2020;63(5):706-711.

16 U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Symptoms of Coronavirus. https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html. Published March 20, 2020. Accessed April 15, 2020.

17 Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA. 2020. 10.1001/jama.2020.1585.

18 Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395(10223):15-21.

19 Arentz M, Yim E, Klaff L. Characteristics and outcomes of 21 critically Ill patients with COVID-19 in Washington state. JAMA 2020;323(16):1612-1614.

20 Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease Control and prevention JAMA 2020;323(13):1239-1242.

21 World Health Organization. Laboratory testing for coronavirus disease (COVID-19) in suspected human cases. https://apps.who.int/iris/bitstream/handle/10665/331501/WHO-COVID-19-laboratory-2020.5-eng.pdf. Published March 19, 2020. Accessed April 15, 2020.

22 U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Interim Guidance: Healthcare Professionals 2019-nCoV. https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-criteria.html. Published March 14, 2020. Accessed April 15, 2020.

23 An overview of the rapid test situation for COVID-19 diagnosis in the EU/EEA. European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/sites/default/files/documents/Overview-rapid-test-situation-for-COVID-19-diagnosis-EU-EEA.pdf. Published April 1, 2020. Accessed April 15, 2020.

25 Liu W, Liu L, Kou G, et al. Evaluation of Nucleocapsid and Spike Protein-based ELISAs for detecting antibodies against SARS-CoV-2 [published online ahead of print, 2020 Mar 30]. J Clin Microbiol 2020 pii: JCM.00461-20. doi: 10.1128/JCM.00461-20.

26 To K, Tsang O, Leung W, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study [published online ahead of print, 2020 Mar 23]. Lancet Infect Dis. 2020. pii: S1473-3099(20)30196-1. doi:10.1016/S1473-3099(20)30196-1.

27 Long Q, Deng H, Chen J, et al. Antibody responses to SARS-CoV-2 in COVID-19 patients: the perspective application of serological tests in clinical practice. medRxiv 2020. https://doi.org/10.1101/2020.03.18.20038018.

28 Lou B, Li TD, Zheng SF, et al. Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset. Eur Resp J 2020. https://doi.org/10.1183/13993003.00763-2020.

29 Zhao J, Yuan Q, Wang H, et al.. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019 [published online ahead of print, 2020 Mar 28]. Clin Infect Dis 2020. pii: ciaa344. doi: 10.1093/cid/ciaa344.

30 Zhang B, Zhou X, Zhu C, et al. Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. medRxiv. 2020. doi: https://doi.org/10.1101/2020.03.12.20035048.

31 Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465-469.

32 Xiao DAT, Gao DC, Zhang DS. Profile of Specific Antibodies to SARS-CoV-2: The First Report [published online ahead of print, 2020 Mar 21]. J Infect 2020;S0163-4453(20)30138-9. doi:10.1016/j.jinf.2020.03.012.

33 Tan W, Lu Y, Zhang J, et al. Viral Kinetics and Antibody Responses in Patients with COVID-19. Tan et al. 2020. medRxiv. 2020. preprint doi: https://doi.org/10.1101/2020.03.24.20042382.

34 Okba N, Müller M, Li W, et al. SARS-CoV-2 specific antibody responses in COVID-19 patients. medRxiv. 2020. doi: https://doi.org/10.1101/2020.03.18.20038059.

35 Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. B Cells and Antibodies. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26884/

36 Klasse PJ. How to assess the binding strength of antibodies elicited by vaccination against HIV and other viruses. Expert Rev Vaccines 2016;15(3):295-311.

37 Payne S (2017). Viruses: Chapter 6 - Immunity and Resistance to Viruses, Editor(s): Susan Payne, Academic Press, Pages 61-71, ISBN 9780128031094.

38 Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. Nat Rev Immunol 2020. https://doi.org/10.1038/s41577-020-0321-6.

39 Tay MZ, Poh CM, Rénia L, et al. The trinity of COVID-19: immunity, inflammation and intervention. Nat Rev Immunol 2020;20:363-374.

40 Amanat F, Stadlbauer, D, Strohmeier, S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med (2020). https:// doi.org/10.1038/s41591-020-0913-5.

41 Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579(7798):270-273.

42 Haveri A, Smura T, Kuivanen S, et al. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro Surveill 2020;25(11):2000266.

43 Poh C, Carissimo G, Wang B, et al. Potent neutralizing antibodies in the sera of convalescent COVID-19 patients are directed against conserved linear epitopes on the SARS-CoV-2 spike protein. bioRxiv. 2020. preprint doi: https://doi.org/10.1101/2020.03.30.015461.

44 Kohmer N, Westhaus S, Ruehl C, et al. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. J Clinl Virol 2020;129:104480.

45 Mueller L, Ostermann PN, Walker A, et al. Sensitivity of commercial Anti-SARS-CoV-2 serological assays in a high-prevalence setting. medRxiv 2020.06.11.20128686.

46 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

47 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

48 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.

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 after reconstitution or mixing
GTIN	Global Trade Item Number

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

Roche