SERION ELISA *agile* SARS-CoV-2 IgA / IgG / IgM

v2

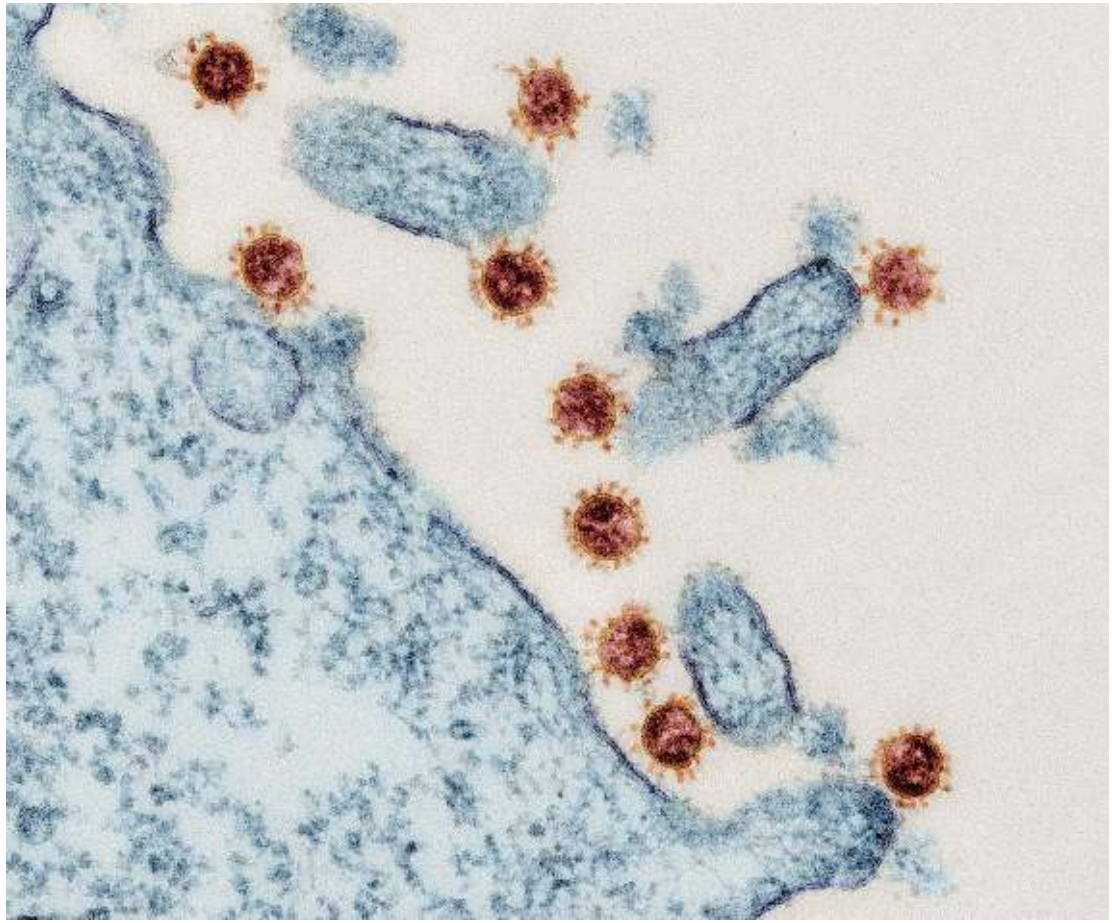
SARS-CoV-2

SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2) is a novel beta coronavirus identified in early 2020 as the causative agent of COVID-19.

Beta-coronaviruses also include SARS-CoV-1 and MERS-CoV, among others.

Coronaviruses are widespread among mammals and birds. They cause mainly mild colds in humans, but can sometimes cause severe pneumonia.

In addition to the wildtype, VOCs (Variants of Concerns) are emerging. For example the British (B.1.1.7), Brazilian (P.1) South African (B.1.351) and Delta Variants.

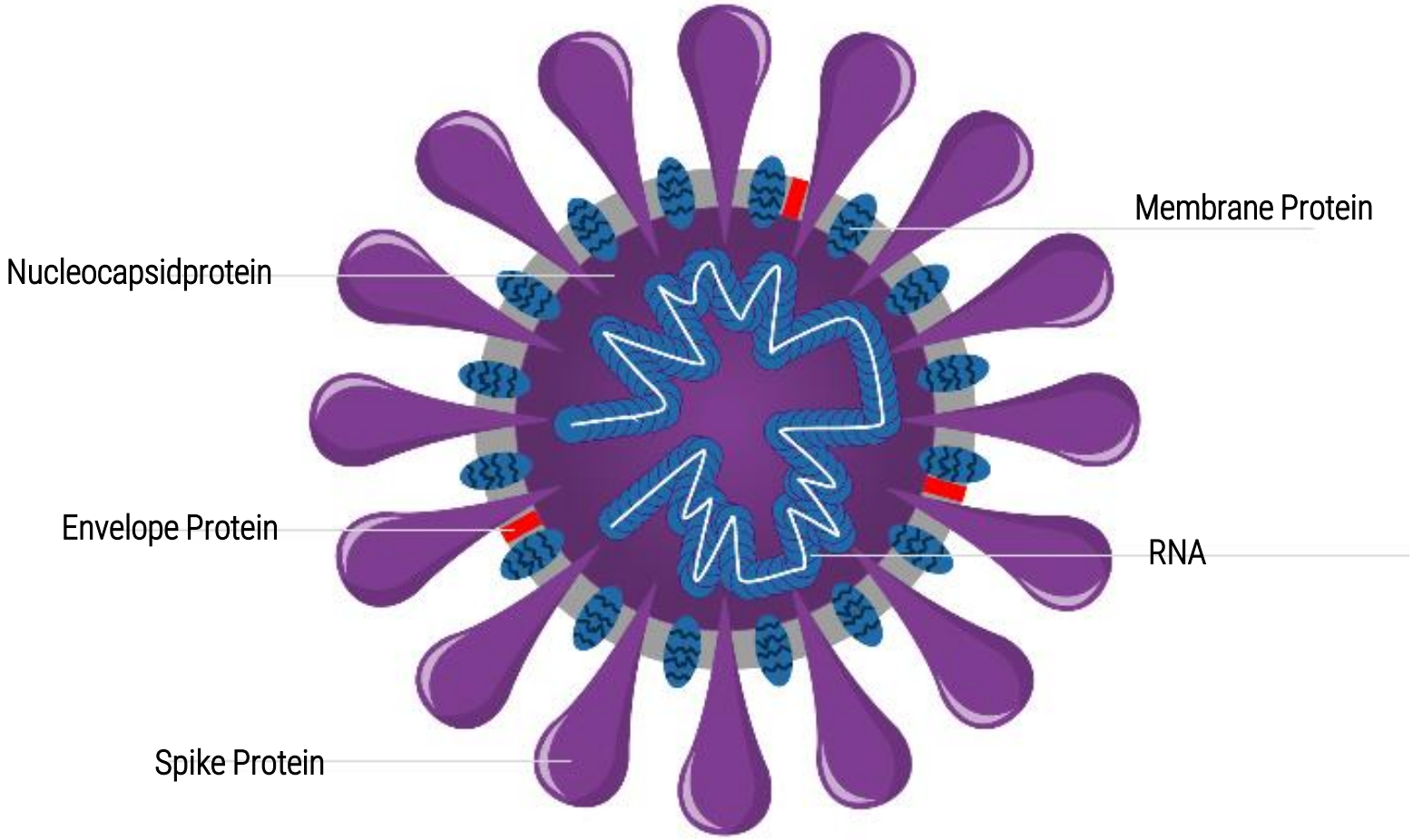


electromicroscopic image of SARS-COV-2 (source: RKI website)

SARS-CoV-2 Structure

The enveloped **RNA Virus** (29.903 nucleotides, single-stranded) SARS-CoV-2 consists of 4 structural proteins and 16 non-structural proteins. The 4 structural proteins are **spike (S)**, **envelope (E)**, **nucleocapsid (N)** and **membrane (M)**.

Like other coronaviruses SARS-CoV-2 is spherical with a diameter of **60-125 nm**.



SARS-CoV-2 Proteins

The N protein is associated with the RNA genome, the S, E and M proteins form the virus envelope.

The S protein is a glycoprotein consisting of two domains, the S1 domain, which contains the receptor binding domain (RBD), and the S2 domain including transmembrane and endodomain.

On the virus surface, the S protein forms trimeric structures, with the RBD responsible for the virus being able to attach to and fuse with the ACE2 receptor of the host cell.

N and S protein are major immunogenic proteins of the virus family *Coronaviridae* and are very well suited for serological detection of anti-SARS-CoV-2 antibodies.

Neutralizing antibodies during host immune responses are predominantly directed against the S protein.

COVID-19 Transmission

The main route of transmission for SARS-CoV-2 is **respiratory ingestion** of virus-containing particles produced by breathing, coughing, talking, singing, and sneezing.

Aerosols are excreted when breathing and speaking, but even more so when shouting and singing; coughing and sneezing also produce significantly increased numbers of larger particles.

Prolonged stays in small, poorly ventilated rooms may increase the likelihood of transmission by aerosols **even over a distance greater than 1.5 m**, especially if an infectious person emits a particularly large number of aerosols, stays in the room for a long time, and exposed persons breathe in particularly deeply or frequently.

Transmissions in **outdoor areas occur rarely** overall. If the minimum distance is maintained, the probability of transmission in outdoor areas is very low due to air movement.

Transmission via **contaminated surfaces** cannot be ruled out, especially in the immediate area of the infected person, because **replicable SARS-CoV-2 viruses can remain infectious on surfaces** for some time under laboratory conditions

COVID-19 Disease

The mean incubation period is reported to be **5-6 days** (up to 14 days) in most studies.

Women and men are affected by SARS-CoV-2 infection at about the **same rate**. However, **men** are more likely to become **severely ill** and **die twice as often** as women.

The course of the disease **varies in symptoms and severity**, from asymptomatic infections (up to 80 %) to severe pneumonia with respiratory failure and death.

The symptoms most commonly recorded in the German reporting system include **cough (40%), fever (27%), rhinitis (28%), and loss of smell and taste (21%).**

The exact period during which **contagiousness** exists is **not yet clearly defined**. It is considered certain that contagiousness is greatest in the **period around the onset of symptoms**.

COVID-19 Hospitalization

Some patients become so severely ill that hospitalization and in some cases even intensive medical treatment are necessary. According to data from the German reporting system, approximately **7% of cases** reported during the first wave in 2020 in Germany were **hospitalized** and **14% of them received intensive medical care.**

A study of 10,021 hospitalized patients from Germany showed that **17% were ventilated**, and the risk of requiring ventilation was twice as high among hospitalized men as among women.

The ratio of infection rates to hospital admissions is highly **dependent on vaccination rates**. In intensive care units, the proportion of **unvaccinated people is disproportionately high**; however, more and more **vaccination breakthroughs** are also being recorded in **older people** because the immune system is no longer as efficient and/or a booster vaccination has been missed.

COVID-19 Therapy and Vaccination

Only some of the COVID-19 diseases are severe. The treatment of the infection currently focuses on the optimal supportive measures according to the severity of the clinical picture (e.g. oxygen administration).

Two antibody therapies (Roche/Regeneron, Celltrion) are currently (November 2021) approved by the EMA (European Medicines Agency) for use in early stages of infection.

The EMA has granted emergency approval to MSD's Corona drug molnupiravir. A second drug (Paxlovid from Pfizer) for COVID-19 patients is currently under review by the EMA.

Since December 2020, vaccination against COVID-19 is available in Germany and many other countries. To date (November 2021), two mRNA vaccines (Biontech/Pfizer, Moderna) and two vector-based vaccines (AstraZeneca, J+J) have been approved by the EMA.

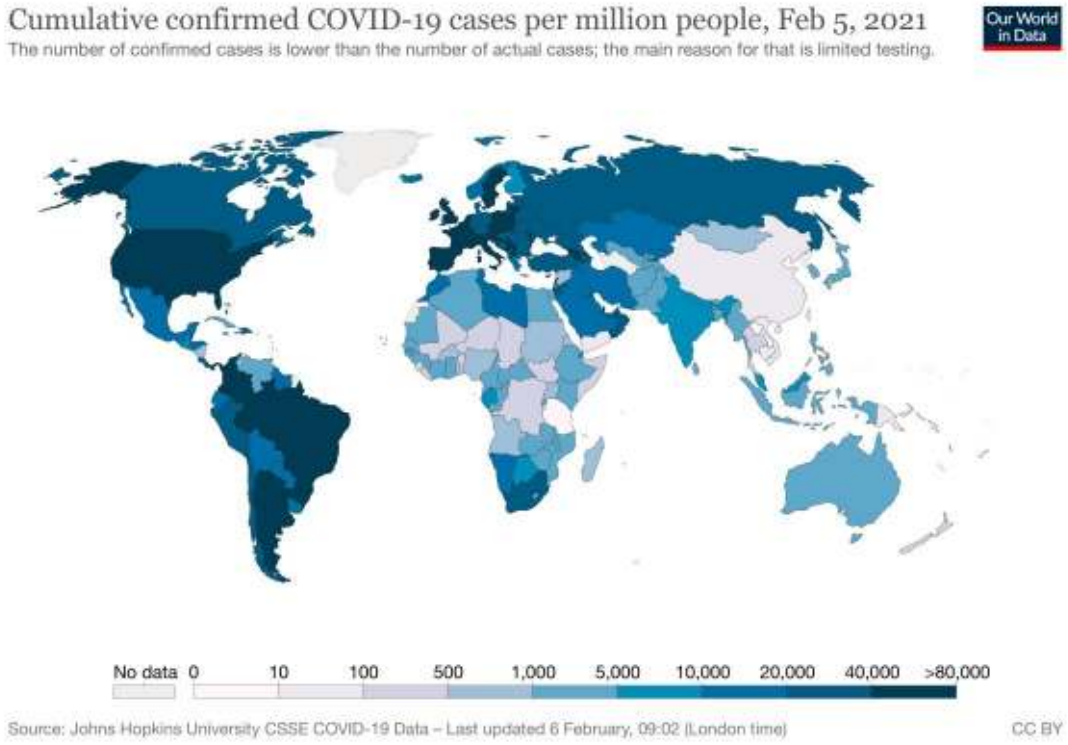
Currently, four additional vaccines (Novavax, CureVac, Sputnik V, Sinovac) are in the EMA approval process.

COVID-19 Epidemiology

Currently (23rd November 2021) the WHO (https://covid19.who.int/) reports globally **~ 257 millions confirmed cases** of COVID-19, including **~ 5.16 millions deaths**.

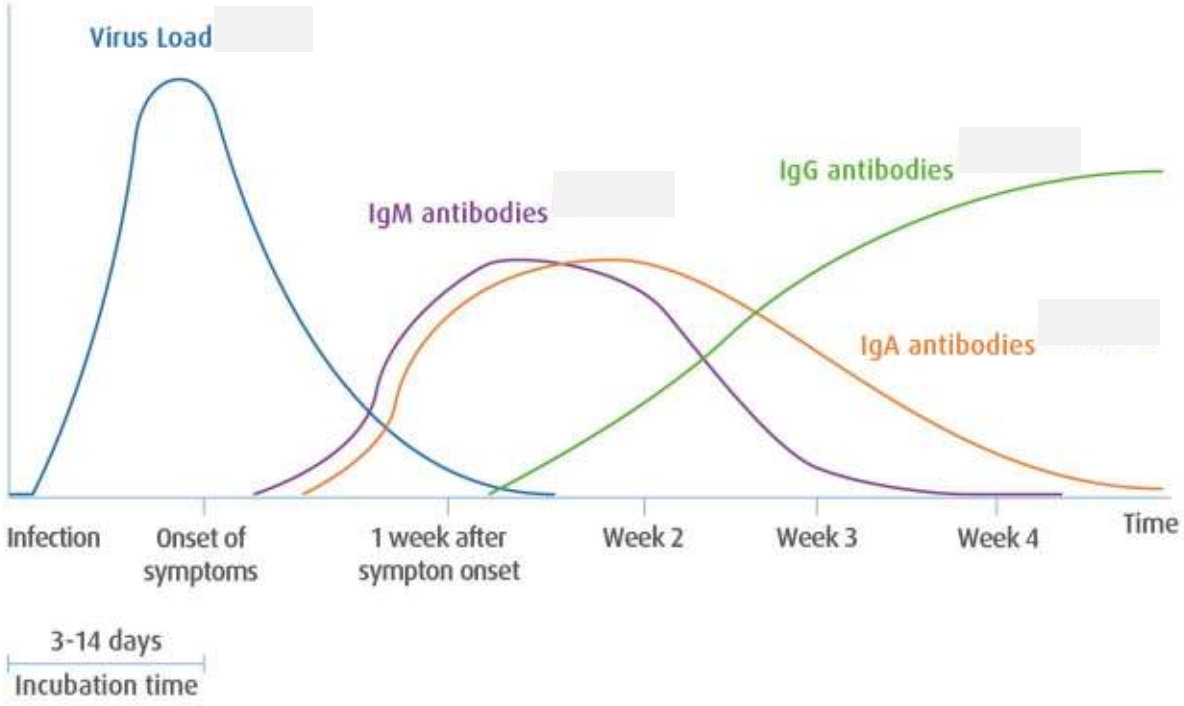
Situation by WHO region
(confirmed cases in millions), February 2021:

- Americas: ~ 96.1
- Europe: ~ 84.1
- South-East Asia: ~ 44.4
- Eastern Mediterranean: ~16.7
- Africa: ~6.2
- Western Pacific: ~10



COVID-19 Immunity

Infection with SARS-CoV-2 induces the secretion of various antibodies that are detectable at a median of the second week after symptom onset.

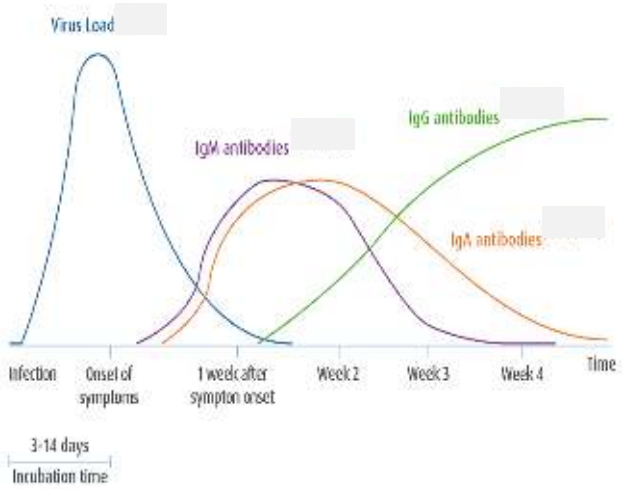


COVID-19 Immunity

Antibodies of the **IgA and IgM** class are detectable in the median at the earliest from about **6 days** after the onset of symptoms, increase in the further course of the disease and decrease again between **18 and 35 days**.

Antibodies of the **IgG** class are detectable in the median **10-18 days** after onset of symptoms and are detectable over **several months**. Studies with SARS and MERS indicate that IgG antibodies are detectable even for years.

As the significance of studies depends very much on the test system used and the patient material, there is currently **no definitive consensus on the exact timing of seroconversion.**



Diagnosis of COVID-19 – Direct Pathogen Detection

Currently, direct pathogen detection by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) is the most commonly used acute diagnostic method. Critical factors are the time point and the procedure for taking the samples. Furthermore, the virus is often no longer available in sufficient quantity for a positive detection after activation of the immune system.

Rapid antigen strip tests (usually lateral flow) are able to detect SARS-CoV-2-specific antigen from upper respiratory tract examination material. The tests often use monoclonal antibodies directed against the N protein and/or the S1 domain. The tests usually provide a qualitative result within 15 minutes and is suited to discriminate between non-infectious and infectious persons. The sensitivity is below that of the PCR method.

Diagnosis of COVID-19 – Indirect Pathogen Detection

Various test systems are available for the serological detection of antibodies against SARS-CoV-2 for the detection of a recent SARS-CoV-2 infection, and for the determination of the SARS-CoV-2 seroprevalence. In addition antibody tests can be used for the determination of acute infections as complement to direct pathogen detection.

To detect neutralizing (protective) antibodies against SARS-CoV-2, a neutralization test must be performed. The neutralization test has a high specificity, but is very work-intensive, cannot be automated or can only be automated to a very limited extent.

SERION ELISA *agile* SARS-CoV-2

The SERION ELISA *agile* SARS-CoV-2 IgA, IgG and IgM tests are qualitative and quantitative immunoassays for the demonstration of human antibodies directed against SARS-CoV-2 in serum, plasma and CSF (for IgG testing) samples.

The sensitivity of the qRT-PCR alone is not sufficient to adequately exclude false-negative findings.

A combination of IgA/IgM antibody detection with qRT-PCR increases the diagnostic sensitivity compared to the sole detection of COVID-19 disease by qRT-PCR.

Furthermore, it is possible to perform antibody tests for epidemiological studies, e.g. to determine the immune status after natural infection or vaccination of the population and to investigate the development of the pandemic.

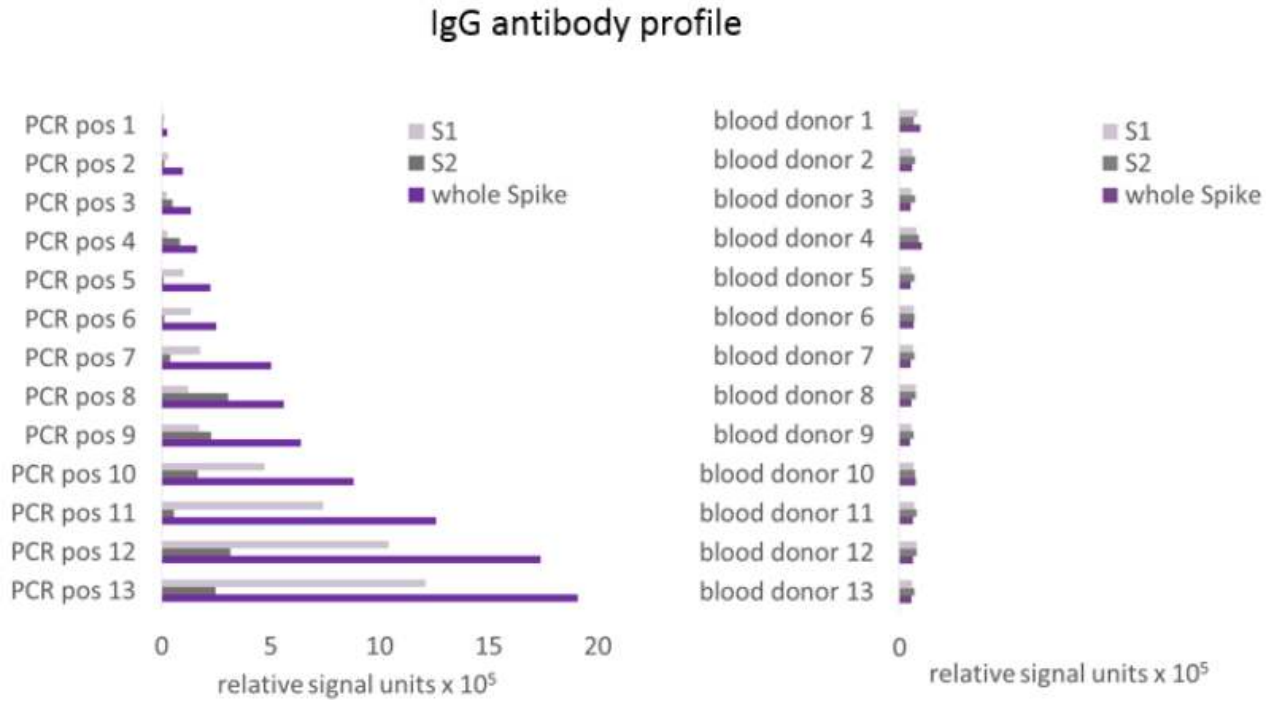
Antibody tests can also help to clarify infection chains.

SERION ELISA *agile* SARS-CoV-2 IgG

The utilization of the **whole spike protein** ensures an early and sensitive detection of SARS-CoV-2 IgG. Currently the SERION ELISA *agile* SARS-CoV-2 is the only commercially available ELISA test based on the whole spike protein (S1/S2 ectodomain).

The usage of the whole spike protein can **enhance the test sensitivity** compared to S1, S2 (or RBD) alone.

A **prolonged detection of IgG antibodies** compared to tests of competitors is possible (see study Strömer et al.).



SERION ELISA *agile* SARS-CoV-2 IgG

Specific (99.2 %) and sensitive (96.2) IgG test for epidemiological studies, determination of the immune status and to analyze pandemic progression.

Quantitative evaluation of IgG antibody titers, also in standardized BAU/ml based on the "First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human): NIBSC code: 20/136".

No detection of antibodies against other pathogens with similar symptoms (EBV VCA, Adenovirus, Influenza A Virus, EBV EA, *Malaria falciparum/ovale* and Dengue Virus) causing similar symptoms, also from the corona virus family.

Very good precision data:

SERION ELISA *agile* SARS-CoV-2 IgG

Sample	Mean Value (OD)	Intraassay (CV%)	Mean Value (OD)	Interassay (CV%)
Serum 1	0.185	4.1	0.180	3.6
Serum 2	0.787	2.3	0.755	3.5
Serum 3	1.931	1.7	1.840	1.2

SERION ELISA *agile* SARS-CoV-2 IgG – Published Data

The outstanding performance of SERION ELISA *agile* SARS-CoV-2 IgG has been confirmed in an external study (publication Strömer et al., 2020).

With SERION ELISA *agile* SARS-CoV-2 IgG, the highest values for **sensitivity** as well as for **specificity and accuracy** have been evaluated. To determine sensitivity also a panel with defined titers of >1:10 of neutralizing antibodies has been used. As neutralizing antibodies are mainly produced against the spike protein, the usage of whole spike protein as antigen is advantageous in the context of immunity studies or vaccination titer studies.

(%) Reference test	Sensitivity PCR[1]	Sensitivity PRNT[2]	Specificity[3]	Accuracy PCR[1]	Accuracy PRNT[2]
Assay\n	26	27	100	126	127
Abbott	96.2	96.3	99.0	98.4	98.4
Diasorin	84.6	88.9	96.0	93.7	94.5
Epitope	80.8	92.6	100.0	96.0	98.4
Euroimmun	80.8	88.9	97.0	93.7	95.3
Mikrogen	88.5	96.3	98.0	96.0	97.6
Roche	88.5	96.3	99.0	96.8	98.4
Viramed	88.5	96.3	99.0	96.8	98.4
Virion-Serion	96.2	96.3	100.0	99.2	99.2

[1]SARS-CoV-2 RNA was previously detected in the PCR test. [2]SARS-CoV-2-neutralizing antibodies with a titer >1:10 were demonstrated in the plaque reduction neutralization test (PRNT). [3]100 stored sera from 2018/2019.

SERION ELISA *agile* SARS-CoV-2 IgG – Excellent external study results

Publication	Type and scope	Results
Strömer *et al.*, 2020	Comparative analysis of eight different anti-SARS-CoV-2 immuno-assays **Sample panel:** 26 SARS-CoV-2 RT-PCR samples 27 sera with neutralizing antibodies against SARS-CoV-2 (neutralization assay: PRNT[1]) 100 pre-pandemic sera	SERION ELISA *agile* SARS-CoV-2 IgG showed highest sensitivity and specificity values **Sensitivity vs. PCR: 96.2%** **Sensitivity vs. PRNT: 96.3%** **Specificity: 100%**
Werner *et al.*, 2021	Comparative analysis of 16 ELISA/ECLIA-based and 16 LFA-based tests. **Sample panel:** 101 SARS-CoV-2 RT-PCR sera with defined collection time after positive RT-PCR 60 SARS-CoV-2 negative sera 22 sera with defined SARS-CoV-2 virus-neutralization titers (Neutralization assay: IC50[2])	SERION ELISA *agile* SARS-CoV-2 IgA und IgG tests showed very good sensitivities and specificities compared to other manufacturers. **With an R^2 value of 0.87, the SERION ELISA *agile* SARS-CoV-2 IgG achieved the best correlation with SARS-CoV-2 virus neutralization titers**

[1]PRNT = Plaque reduction neutralization assay [2]IC50= half maximal inhibitory concentrations

SERION ELISA *agile* SARS-CoV-2 IgG – Excellent external study results

Publication	Type and scope	Results
Krone *et al.*, 2021	Comparative analysis of six anti-SARS-CoV-2 assays with a neutralization assay. **Sample panel:** 63 samples that are positive in a neutralization assay (neutralization assay: PRNT) 50 SARS-CoV-2 negative sera	The SERION ELISA *agile* SARS-CoV-2 IgG showed the best performance: **Sensitivity: 98.3%** **Specificity: 100%** The SERION ELISA *agile* SARS-CoV-2 IgA achieved very good results: **Sensitivity: 82.5%** **Specificity: 100%** A combination of SERION ELISA *agile* SARS-CoV-2 IgA and IgG increases sensitivity without loss of specificity. **Sensitivity: 100%** **Specificity: 100%**
Perkmann *et al.*, 2021	Comparative analysis of five quantitative spike protein-based assays against a surrogate virus neutralization test (sVNT) **Sample panel:** 69 sera from initially SARS-CoV-2 naive individuals after first vaccination with BNT162b2 (Pfizer/BioNTech)	**Overall, a very good correlation between SERION ELISA *agile* SARS-CoV-2 IgG and the sVNT was demonstrated.**

SERION ELISA *agile* SARS-CoV-2 IgG – Immune Status

The **standardization of IgG antibody activities** (NIBSC code: 20/136) is an important step to be able to evaluate the **immune status** in a standardized way in the future.

SERION ELISA *agile* SARS-CoV-2 IgG is based on the **whole spike protein** (S1/S2 ectodomain), providing the largest possible number of epitopes.

By using the whole spike protein as antigen SERION ELISA *agile* SARS-CoV-2 IgG was shown to **detect neutralizing antibodies with high sensitivity** (Strömer et al., 2020).

Currently available vaccines are also based on the **full-length spike protein**.

SERION ELISA *agile* SARS-CoV-2 IgA and IgM

Sensitive IgA and IgM antibody detection with high specificity ideal for determination of acute infections as complement to direct pathogen detection.

Quantitative evaluation of IgA antibody titers; qualitative evaluation of IgM antibodies.

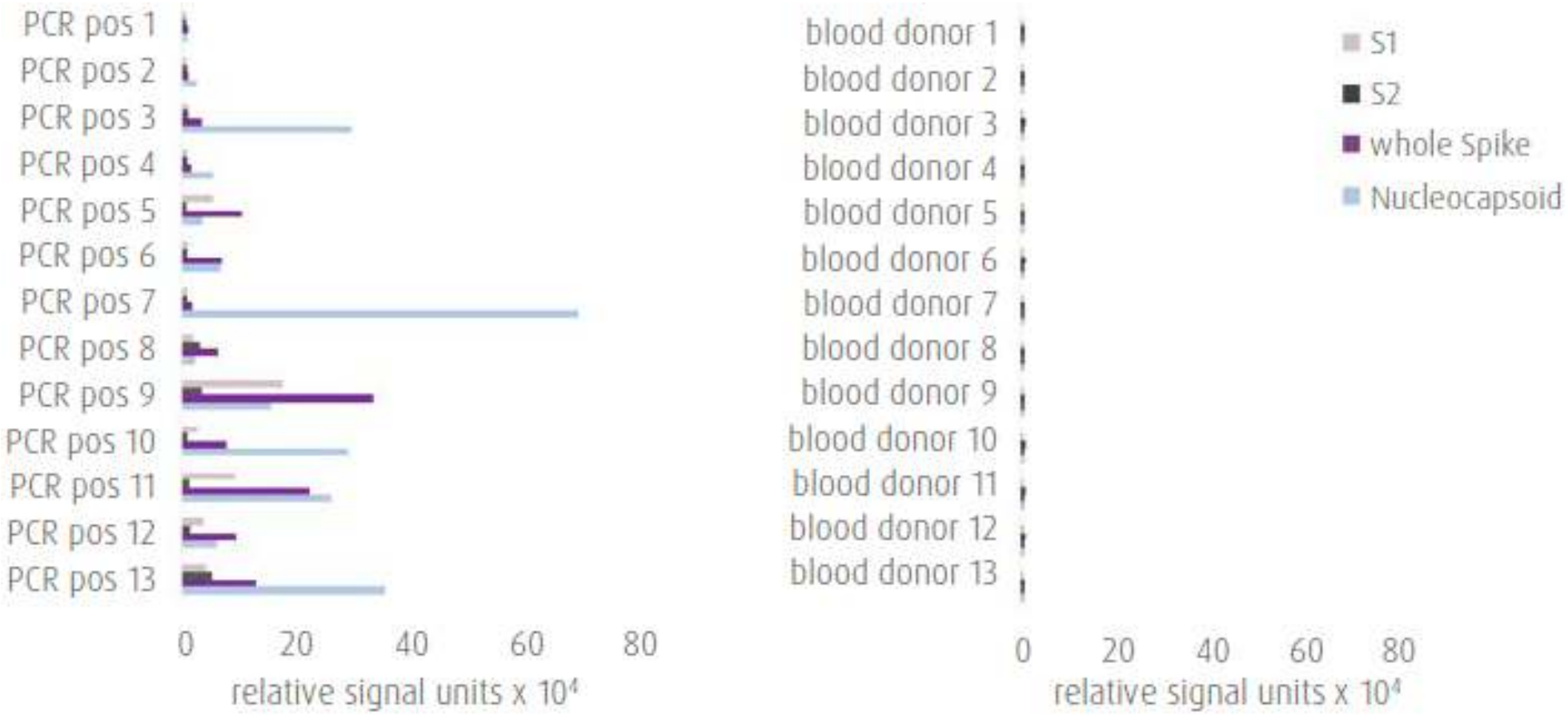
No detection of antibodies against other pathogens with similar symptoms (Epstein-Barr Virus VCA, Adenovirus, Influenza A Virus, Cytomegalovirus and *Chlamydia pneumoniae*), also from the corona virus family.

To achieve the best possible diagnostic properties, the IgA and IgM tests are based on a mixture of nucleocapsid protein and the whole spike protein (S1/S2 ectodomain) of SARS-CoV-2, recombinantly expressed in insect cells and highly purified.

High sensitivity and specificity:

Product	Sensitivity	Specificity
SERION ELISA *agile* SARS-CoV-2 IgA	96.3 %	> 99 %
SERION ELISA *agile* SARS-CoV-2 IgM	96.2 %	> 99 %

SERION ELISA *agile* SARS-CoV-2 IgA and IgM



SERION ELISA *agile* SARS-CoV-2 IgA and IgM

Very good precision data:

SERION ELISA *agile* SARS-CoV-2 IgA

Sample	Mean Value (OD)	Intraassay (CV%)	Mean Value (OD)	Interassay (CV%)
Serum 1	0.234	2.7	0.238	4.7
Serum 2	0.441	3.2	0.418	7.6
Serum 3	1.052	2.3	0.758	13.4

SERION ELISA *agile* SARS-CoV-2 IgM

Sample	Mean Value (OD)	Intraassay (CV%)	Mean Value (OD)	Interassay (CV%)
Serum 1	0.122	4.1	0.113	10.8
Serum 2	0.233	3.6	0.221	8.3
Serum 3	2.463	2.6	2.801	6.3

SERION ELISA *agile* SARS-CoV-2 IgG, IgA and IgM: Cross reactivity study confirms high specificity

Category	SERION ELISA *agile* SARS-CoV-2 IgA		SERION ELISA *agile* SARS-CoV-2 IgG		SERION ELISA *agile* SARS-CoV-2 IgM	
	Number of samples tested	Equivocal or positive results	Number of samples tested	Equivocal or positive results	Number of samples tested	Equivocal or positive results
Other Coronaviruses	9	0	9	0	8	0
Epstein-Barr Virus VCA	10	0	10	0	10	0
Epstein-Barr Virus EA	–	–	10	0	-	-
Adenovirus	10	0	10	0	7	0
Influenza A Virus	10	0	10	0	7	0
Plasmodium falciparum	–	–	15	0	15	3
Plasmodium ovale	–	–	5	0	5	0
Dengue Virus (type 1-4)	–	–	10	0	10	1
HIV	10	0	10	0	10	0
ANA	10	0	10	0	10	0
RF	10	0	10	0	10	1

SERION ELISA *control* SARS-CoV-2 IgA, IgG, IgM

Ready-to-use **positive controls** for the qualitative and quantitative antibody determination.

- **Easy handling** due to predilution
- Additional **quality assurance** by determination of methodology precision
- Laboratory internal error limits can be determined for increased quality control performance

Product	Order No.
SERION ELISA *control* SARS-CoV-2 IgA	BC400A
SERION ELISA *control* SARS-CoV-2 IgG	BC400G
SERION ELISA *control* SARS-CoV-2 IgM	BC400M

Publications

Amanat F. et al., “A Serological Assay to Detect SARS-CoV-2 Seroconversion in Humans“, July 2020, Nature Medicine, 26(7):1033 - 1036.

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Publications

Peeri NC. et al., “The SARS, MERS and Novel Coronavirus (COVID-19) Epidemics, the Newest and Biggest Global Health Threats: What Lessons Have We Learned?“, June 2020, International Journal of Epidemiology 49(3):717 - 726.

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Publications

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Perkmann et al., 2021, Anti-Spike Protein Assays to Determine SARS-CoV-2 Antibody Levels: a Head-to-Head Comparison of Five Quantitative Assays; Microbiol Spectr. 2021 Sep 3;9(1):e0024721

Krone et al., 2021, Performance of Three SARS-CoV-2 Immunoassays, Three Rapid Lateral Flow Tests, and a Novel Bead-Based Affinity Surrogate Test for the Detection of SARS-CoV-2 Antibodies in Human Serum; J Clin Microbiol. 2021 Jul 19;59(8):e0031921

SERION ELISA *agile* SARS-CoV-2

- The utilization of the whole spike protein ensures an early and sensitive detection of SARS-CoV-2 IgG
- Quantitative IgG test allows monitoring of immune response after infection or vaccination
- Expression of SARS-CoV-2 IgG antibody activity in standardized BAU/ml possible
- Detection of intrathecally synthesized IgG antibodies for CSF diagnostics
- Excellent correlation of the IgG test with neutralization assays
- Sensitive IgA and IgM antibody detection with high specificity ideal for determination of acute infections as complement to direct pathogen detection
- Quantitative evaluation of IgA antibody activities
- In house produced antigens guarantee reliable and reproducible results

Comparison with other manufacturers: Euroimmun

Test	SERION ELISA *agile* SARS-CoV-2 IgG	Anti-SARS-CoV-2 QuantiVac ELISA IgG (Euroimmun)
Antigen	Whole Spike Protein	S1
Calibrators	1-Point Calibration; standard serum: duplicate	6-point calibration curve
1st WHO Int Std (BAU/mL)	Conversion factor 2.1	Conversion factor 3.2
Measurement range	6.3 - 525 BAU/mL	3.2 - 384 BAU/mL
CSF	yes	no

Comparison with other manufacturers: Novatec

Test	SERION ELISA *agile* SARS-CoV-2 IgG	NovaLisa SARS-CoV-2 IgG ELISA
Antigen	Whole Spike Protein	Nucleocapsid Protein
Calibrators	1-Point Calibration; standard serum: duplicate	qualitative
1st WHO Int Std (BAU/mL)	Conversion factor 2.1	no
Measurement range	6.3 - 525 BAU/mL	qualitative
CSF	yes	no

Comparison with other manufacturers: Novatec

Test	SERION ELISA *agile* SARS-CoV-2 IgG	**GSD NovaLisa® SARS-CoV-2 (COVID-19) Quantitative IgG**
Antigen	Whole Spike Protein	Trimeric Spike
Calibrators	1-Point Calibration; standard serum: duplicate	Info not available
1st WHO Int Std (BAU/mL)	Conversion factor 2.1	Calibrators correlate to First WHO International Standards
Measurement range	6.3 - 525 BAU/mL	Info not available
CSF	yes	No; But validated for finger prick

Comparison with other manufacturers: Roche (ECLIA)

Test	SERION ELISA *agile* SARS-CoV-2 IgG	Elecsys Anti-SARS-CoV-2 S (total antibody)
Antigen	Whole Spike Protein	RBD
Calibrators	1-Point Calibration; standard serum: duplicate	2-point (separate Cal Set)
1st WHO Int Std (BAU/mL)	Conversion factor 2.1	1 U/mL = 1 BAU/mL (no conversion factor)
Measurement range	6.3 - 525 BAU/mL	0.4 - 250 U/mL
CSF	yes	no

Comparison with other manufacturers: Diasorin (CLIA)

Test	SERION ELISA *agile* SARS-CoV-2 IgG	Liaison SARS-CoV-2 Trimerics IgG
Antigen	Whole Spike Protein	Recombinant trimeric Spike Protein
Calibrators	1-Point Calibration; standard serum: duplicate	2-Point Calibration
1st WHO Int Std (BAU/mL)	Conversion factor 2.1	Information not available
Measurement range	6.3 - 525 BAU/mL	4.81 - 2080 BAU/mL
CSF	yes	no

Thank you!

Institut Virion\Serion GmbH
Friedrich-Bergius-Ring 19
D-97076 Würzburg, Germany
Tel. +49 931 30 45 0
Fax +49 931 30 45 100

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Inspired by Dedication