COVID-19

Enzyme immunoassays for the diagnostics of infection caused by SARS-CoV-2 virus (COVID-19)

ELISA and **Microblot-Array** kits are optimized and validated for detection of IgA, IgG and IgM antibodies in human serum or plasma

CE IVD

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

Introduction

Coronaviruses, which were discovered in the 1960s, belong to the family of enveloped RNA viruses. They fall in the group of zoonotic infections that cause diseases of the respiratory and digestive tracts in humans and animals (birds, mammals). Coronaviruses cause diverse clinical pictures, from common cold to severe respiratory syndromes (MERS, SARS and COVID-19). The majority of known coronaviruses circulate among animals. Alpha- and Beta-coronaviruses can infect only mammals whereas Gamma- and Delta-coronaviruses infect both birds and mammals. Alpha- and Beta-coronaviruses occur in humans. A total of 7 types of human coronaviruses are known so far – 229E, NL63, OC43, HKU1, MERS, SARS, SARS – 2. The infection can be transmitted from an infected person 1–3 days before the onset of the disease. The new coronavirus is a respiratory virus. It is primarily transmitted to an individual through a close contact with an infected person, during which infectious droplets spread to the environment, especially when the infected person talks, coughs and/or sneezes. Things freshly contaminated with secretions of an infected person can also contribute to the transmission. The virus has been successfully isolated from samples taken from the lower respiratory tract (bronchoalveolar lavage). Viral RNA has been detected in nasopharyngeal and throat swabs, serum, blood, rectal swabs, saliva, urine and faeces. The virus has been found in airway samples 1–2 days before the onset of symptoms and up to 8 days after the onset in case of a mild disease, longer in case of a more severe disease development. Susceptibility seems to be general. Existing experience suggests that the infection is as likely in children as in adults but with milder clinical manifestations. Immunity to COVID-19, if any, has not been established so far. Reported mortality ranges from 2% to 3%.

Diagnostics of Infection

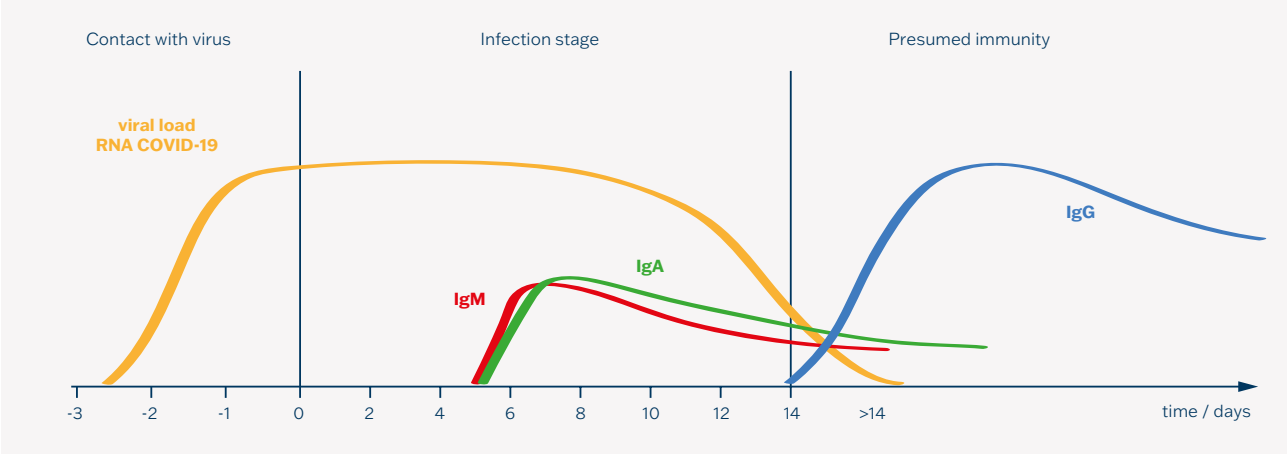
The diagnostics of the disease is based on the clinical picture, epidemiological history, and laboratory tests.

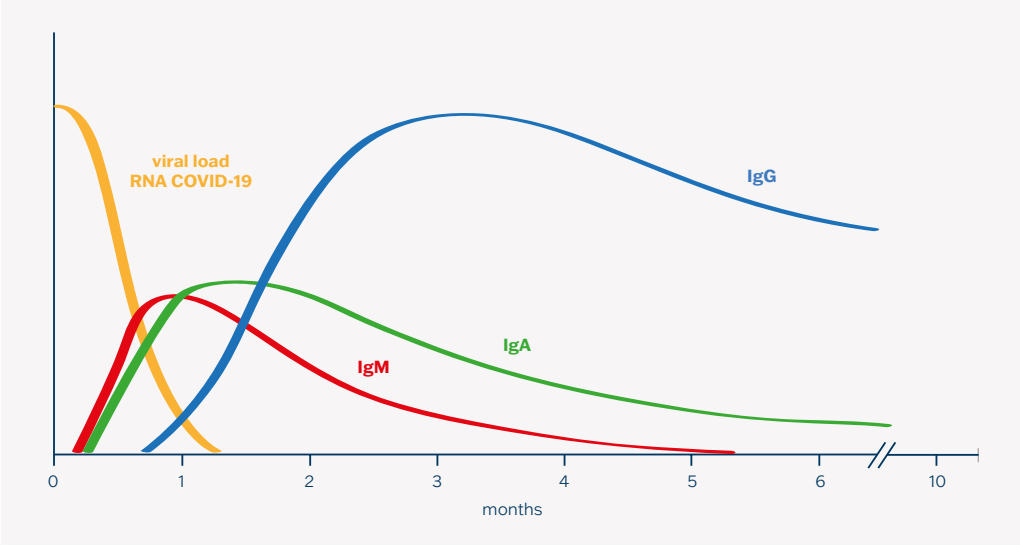
Due to the several-day-long interval between the first symptoms and the onset of the antibody response (the “window period”), serological tests play only a supporting role and, as stressed by the WHO, the results of such tests should always be verified by direct detection of the virus to diagnose an acute COVID-19 disease.

An increase in antibody levels occurs in most patients at 2nd week after the onset of symptoms. Positivity of IgA and IgM class antibodies is usually detected on days 3–6, IgG class antibodies subsequently on days 10–18 after the onset of symptoms.

Serological tests are also used in prevalence studies and their negative result allows termination of a quarantine. The development of antibodies and their persistence after natural infection is a subject of further research.

Antibody post-infection response



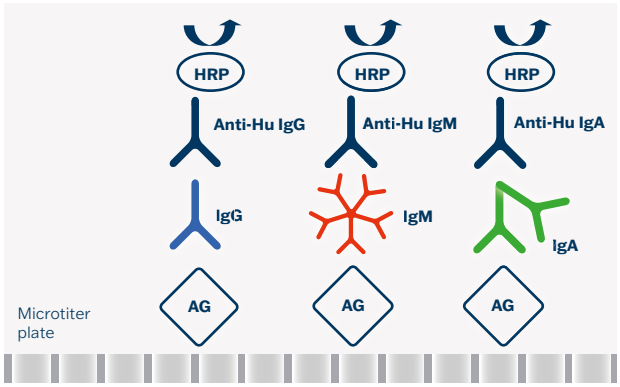


ELISA

Test Principle

The assays are based on a sandwich type of ELISA method.

Sandwich ELISA



Summary Protocol

Step		Test steps
	1.	Dilution of samples – serum/plasma 1:101 (10 µl + 1 ml)
	2.	Pipette Controls and diluted samples 100 µl – Including blank
	3.	Incubate 30 min. at 37 °C
	4.	Aspirate and wash the wells 5 times
	5.	Add Conjugate 100 µl – Including blank
	6.	Incubate 30 min. at 37 °C
	7.	Aspirate and wash the wells 5 times
	8.	Add 100 µl Substrate (TMB-Complete) – Including blank
	9.	Incubate 15 min. at 37 °C
	10.	Add 100 µl Stopping solution – Including blank
	11.	Read colour intensity at 450 nm

Antigens

EIA COVID-19 NP
Nucleocapsid recombinant antigen (NP)

EIA COVID-19 RBD
Recombinant Receptor-binding domain (RBD) antigen and combination of S1 Spike relevant SARS-CoV-2 mutations

Clinical Application

- Diagnostics of the disease (additional examination)
- Prevalence study
- Detection of post-vaccination antibodies (RBD)

User Comfort

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF and calibrators included
- Semiquantitative evaluation of results (Index of Positivity, IP) or quantitative evaluation of results (U/ml)
- U are equal to BAU units, based on titration and evaluation of international standards issued by WHO

Advantages

- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Identical assay procedure
- Short total assay time
- Ready for automation
- Customer support

Test Characteristics

ELISA	Diagnostic sensitivity	Diagnostic specificity
EIA COVID-19 NP IgA	97.4%	97.7%
EIA COVID-19 NP IgG	95.1%	99.0%
EIA COVID-19 NP IgM	95.7%	97.7%
EIA COVID-19 RBD IgA	96.6%	98.9%
EIA COVID-19 RBD IgG	99.9%	99.1%
EIA COVID-19 RBD IgM	97.5%	95.1%

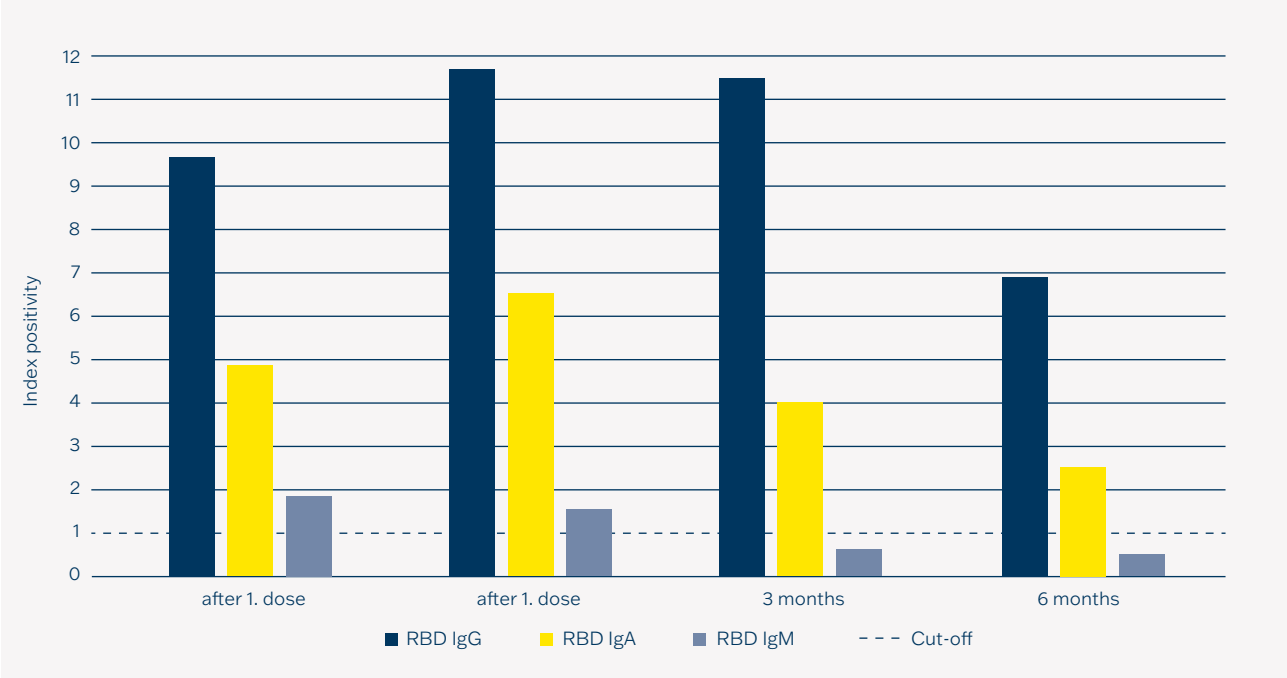
Types of Kits

SmartEIA kits are designed for automated processing using the Agility® analyser.

EIA

SmartEIA

Overview of post-vaccination reactivity of ELISA kits



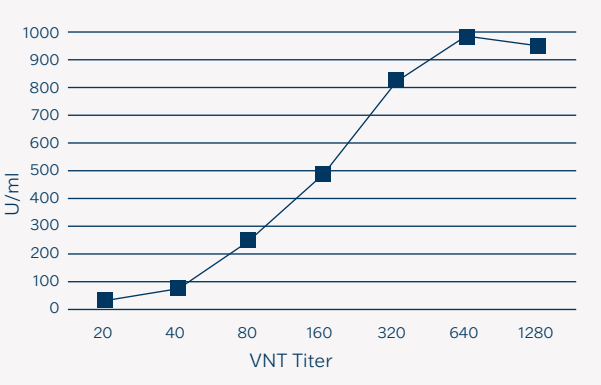
40 individuals were tested after the 1st dose, 2nd of the vaccine (Pfizer/BioNTech) and then after 3 months and 6 months after the vaccination was completed. Values in the graph are the arythmetic mean of obtained values.

Correlation of VNT and ELISA kit results

VNT vs EIA TESTLINE IgG

		EIA	
		pos.	neg.
VNT	pos.	99	1
	neg.	0	0
Agreement		**99%**	

Mean Index of Positivity (IP) values of IgG anti-RBD antibodies (TestLine) in relation to individual VNT titers



MICROBLOT-ARRAY

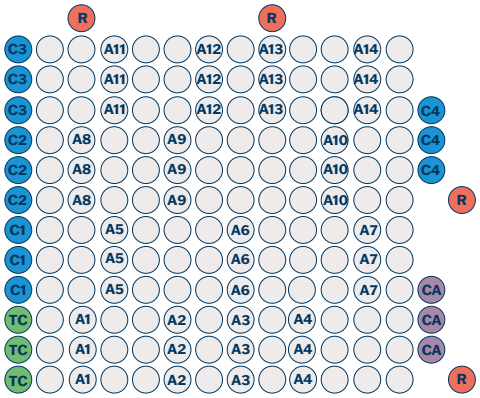
Distribution of antigens and control spots

Distribution of antigens

A1 – Nucleocapsid NP
A2 – RBD
A3 – Spike S1
A4 – Spike S2
A5 – Spike S1 α-variant (UK)
A6 – Spike S1 γ-variant (Brazil)
A7 – Spike S1 δ-variant (Indian)
A8 – Envelope protein (E)
A9 – ACE2
A10 – PLPro protein
A11 – MERS-CoV
A12 – SARS-CoV
A13 – HCoV 229E Np
A14 – HCoV NL63 Np

Distribution of control spots

R – Reference
TC – Test control
CG – Conjugate control IgG
CM – Conjugate control IgM
C1 – Calibration 1
C2 – Calibration 2
C3 – Calibration 3
C4 – Calibration 4



Overview of specific antigens

Antigens	Description	Meaning, function
Nucleocapsid NP	Nucleocapsid NP	A potent immunodominant antigen of coronavirus that contains diagnostically important epitopes for the diagnosis of SARS-CoV-2 Sensitive detection of anti-SARS-CoV-2 IgG antibodies
RBD	Receptor binding domain S1 subunit spike (S) SARS-CoV-2 protein	Anti-RBD SARS-CoV-2 antibodies are highly subtype specific and protective The presence of anti-RBD antibodies significantly correlates with the formation of neutralizing antibodies IgA – for monitoring the immune response after a positive PCR reaction; indicator of the onset of the immune response IgM, IgG – detection of antibodies from 2 to 4 weeks after infection
Spike S1	Spike Glycoprotein S1 (Wuhan-Hu-1)	The S1 subunit of the spike protein SARS-CoV-2, contains a receptor-binding domain (RBD), it is through this that the virus binds to the surface of the host cell Anti-S1 antibodies are highly subtype specific, showing high sensitivity to SARS-CoV-2 and have a protective character
Spike S2	S2 subunit spike SARS-CoV-2 protein	Plays an important role in the fusion of the virus with the cell membrane
Spike S1 α-variant	British mutation	Spike Glycoprotein S1 (B.1.1.7)
Spike S1 γ-variant	Brazilian mutation	Spike Glycoprotein S1 (P.1)
Spike S1 δ-variant	Indian mutation	Spike Glycoprotein S1 (B1.617.2)
Envelope protein (E)	The smallest major structural protein	Important for different stages of viral infection and replication, important role in the life cycle of the virus
ACE2	Angiotensin Converting Enzyme (transmembrane glycoprotein)	A key component of the renin-angiotensin system Expressed in vascular endothelial cells in the heart, kidneys, but also the testes, liver, intestines, lungs and also the brain Involved in the regulation of cardiovascular and renal functions
PLpro	Papain-like protease	One of the basic proteins of SARS-CoV-2, essential for virus replication; deubiquitination activity Essential for proteolysis of the viral polyprotein
MERS-CoV S1	Middle East Respiratory Syndrome Coronavirus S1 protein	Exclusion of cross-reactivities with other endemic coronaviruses
SARS-CoV Np	Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid protein	Exclusion of cross-reactivities with other endemic coronaviruses
HCoV 229E Np	Human coronavirus 229E Nucleocapsid protein	Exclusion of cross-reactivities with other endemic coronaviruses
HCoV NL63 Np	Human coronavirus NL63 Nucleocapsid protein	Exclusion of cross-reactivities with other endemic coronaviruses

Summary Protocol

Step		Test steps
	1.	Pipette Universal solution 150 µl
	2.	Strips soaking 10 min. at room temperature
	3.	Incubate 30 min. at room temperature
	4.	Dilute samples – serum/plasma 1:51 (10 µl + 500 µl)
	5.	Pipette Controls and diluted samples 100 µl
	6.	Incubate 30 min. at room temperature
	7.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	8.	Pipette Conjugate 100 µl
	9.	Incubate 30 min. at room temperature
	10.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	11.	Pipette Substrate solution (BCIP/NBT) 100 µl
	12.	Incubate 15 min. at room temperature
	13.	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
	14.	Dry and evaluate strips

The processing of Microblot-Array (MBA) kits is identical to standard performance of other immunoenzymatic tests with the possibility of using ELISA instrumentation (automatic analyzer, washer).

Advantages

Efficiency

- Analysis of up to 96 patient samples per plate
- Low sample consumption
- Parallel testing of multiple markers simultaneously

Automation

- Possibility of automated processing using an ELISA instrument
- Intuitive software for test evaluation
- Remote troubleshooting
- LIS connectivity

User comfort

- Ready-to-use components
- Color-coded brekable wells
- Identical assay procedure (30/30/15 min.)
- Antigens spotted in triplicate – minimizing statistical variation
- Controls and calibration spots in each well

Test Characteristics

Microblot-Array	Diagnostic sensitivity	Diagnostic specificity
COVID-19 IgA	98.3%	99.2%
COVID-19 IgG	98.7%	99.3%
COVID-19 IgM	97.7%	99.3%

Prevalence of antibodies during infection

MBA COVID-19 IgA (n=207)		Days from initial symptoms		
		< 14	15-25	> 25
Positive	RBD	14	10	110
	NP	14	9	43
Negative	RBD	9	3	62
	NP	9	4	130
Prevalence of antibodies	RBD	60.87%	76.92%	63.95%
	NP	60.87%	69.23%	24.86%
	MBA COVID-19 IgA	**69.57%**	**84.62%**	**66.67%**

MBA COVID-19 IgG (n=208)		Days from initial symptoms		
		< 14	15-25	> 25
Positive	RBD	11	10	145
	NP	15	12	164
Negative	RBD	10	3	9
	NP	6	1	8
Prevalence of antibodies	RBD	52.38%	94.16%	94.16%
	NP	71.43%	95.35%	95.35%
	MBA COVID-19 IgG	**71.43%**	**98.28%**	**98.28%**

MBA COVID-19 IgM (n=188)		Days from initial symptoms		
		< 14	15-25	> 25
Positive	RBD	8	9	75
	NP	11	8	40
Negative	RBD	14	3	78
	NP	11	4	108
Prevalence of antibodies	RBD	36.36%	75.00%	49.02%
	NP	50.00%	66.67%	27.03%
	MBA COVID-19 IgM	**50.00%**	**75.00%**	**51.30%**

Specificity on panels with possible cross-reactivity

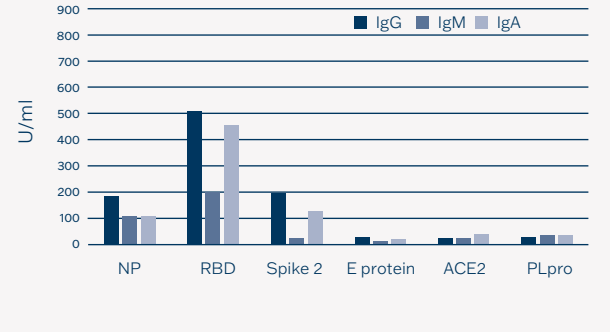
MBA COVID-19 IgA		Panel		
		blood donors (n=593)	potential cross-reactivities (n=196)	endemic coronaviruses (n=56)
Positive	RBD	1	0	0
	NP	4	5	1
Negative	RBD	592	196	56
	NP	589	191	55
Specificity	RBD	99.83%	100.00%	100.00%
	NP	99.33%	97.45%	98.21%
	MBA COVID-19 IgA	**99.16%**	**97.45%**	**98.21%**

MBA COVID-19 IgG		Panel		
		blood donors (n=600)	potential cross-reactivities (n=198)	endemic coronaviruses (n=62)
Positive	RBD	0	2	0
	NP	4	6	1
Negative	RBD	600	196	62
	NP	596	192	61
Specificity	RBD	100.00%	98.99%	100.00%
	NP	99.33%	96.97%	98.39%
	MBA COVID-19 IgG	**99.33%**	**96.46%**	**98.39%**

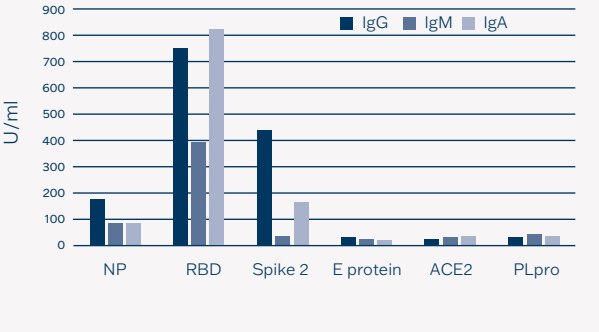
MBA COVID-19 IgM		Panel		
		blood donors (n=598)	potential cross-reactivities (n=197)	endemic coronaviruses (n=57)
Positive	RBD	0	2	0
	NP	4	2	0
Negative	RBD	598	195	57
	NP	594	195	57
Specificity	RBD	100.00%	98.98%	100.00%
	NP	99.33%	98.98%	100.00%
	MBA COVID-19 IgM	**99.33%**	**97.97%**	**100.00%**

Overview of post-vaccination reactivity of Microblot-Array kits

Mean values after the 1st dose of vaccination against SARS-CoV-2



Mean values after the 2nd dose of vaccination against SARS-CoV-2



Correlation of VNT and Microblot-Array kit results

VNT vs MBA TL IgG

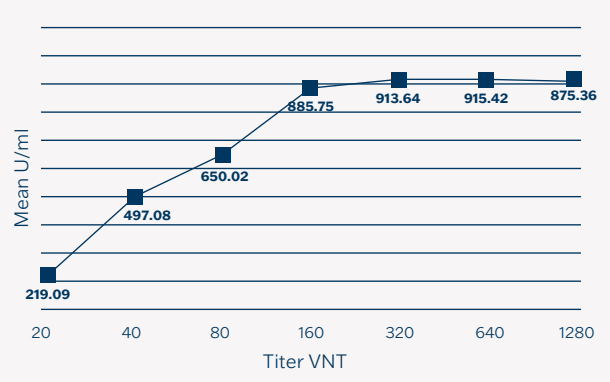
		TL	
		pos	neg
VNT	pos	100	0
	neg	0	0
Agreement		**100%**	

All classes of VNT antibodies vs MBA

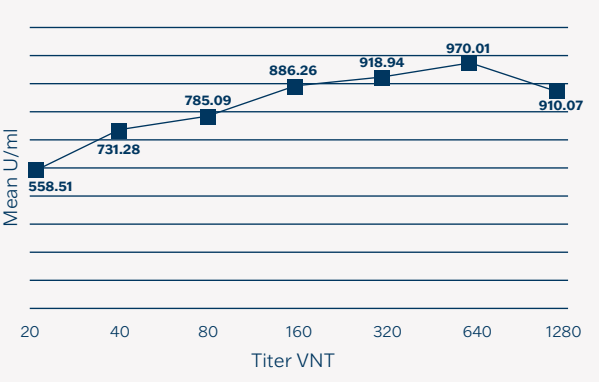
		TL	
		pos	neg
VNT	pos	100	0
	neg	0	0
Agreement		**100%**	

Mean values of units per millilitre IgG anti-RBD antibodies and IgG anti-NP antibodies (TestLine) in relation to individual VNT titers

Mean values of units per millilitre IgG anti-RBD antibodies (TestLine) in relation to individual VNT titers



Mean values of units per millilitre IgG anti-NP antibodies (TestLine) in relation to individual VNT titers



Ordering information

ELISA

Cat. No.	Product	Units
CoNA96	EIA COVID-19 NP IgA	96 wells
CoNG96	EIA COVID-19 NP IgG	96 wells
CoNM96	EIA COVID-19 NP IgM	96 wells
CoRA96	EIA COVID-19 RBD IgA	96 wells
CoRG96	EIA COVID-19 RBD IgG	96 wells
CoRM96	EIA COVID-19 RBD IgM	96 wells

MICROBLOT-ARRAY

Cat. No.	Product	No. of tests
CoVAMA96	Microblot-Array COVID-19 IgA	96
CoVGMA96	Microblot-Array COVID-19 IgG	96
CoVMMA96	Microblot-Array COVID-19 IgM	96

TestLine®

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CERTIFIED ISO 9001 · ISO 13485

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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