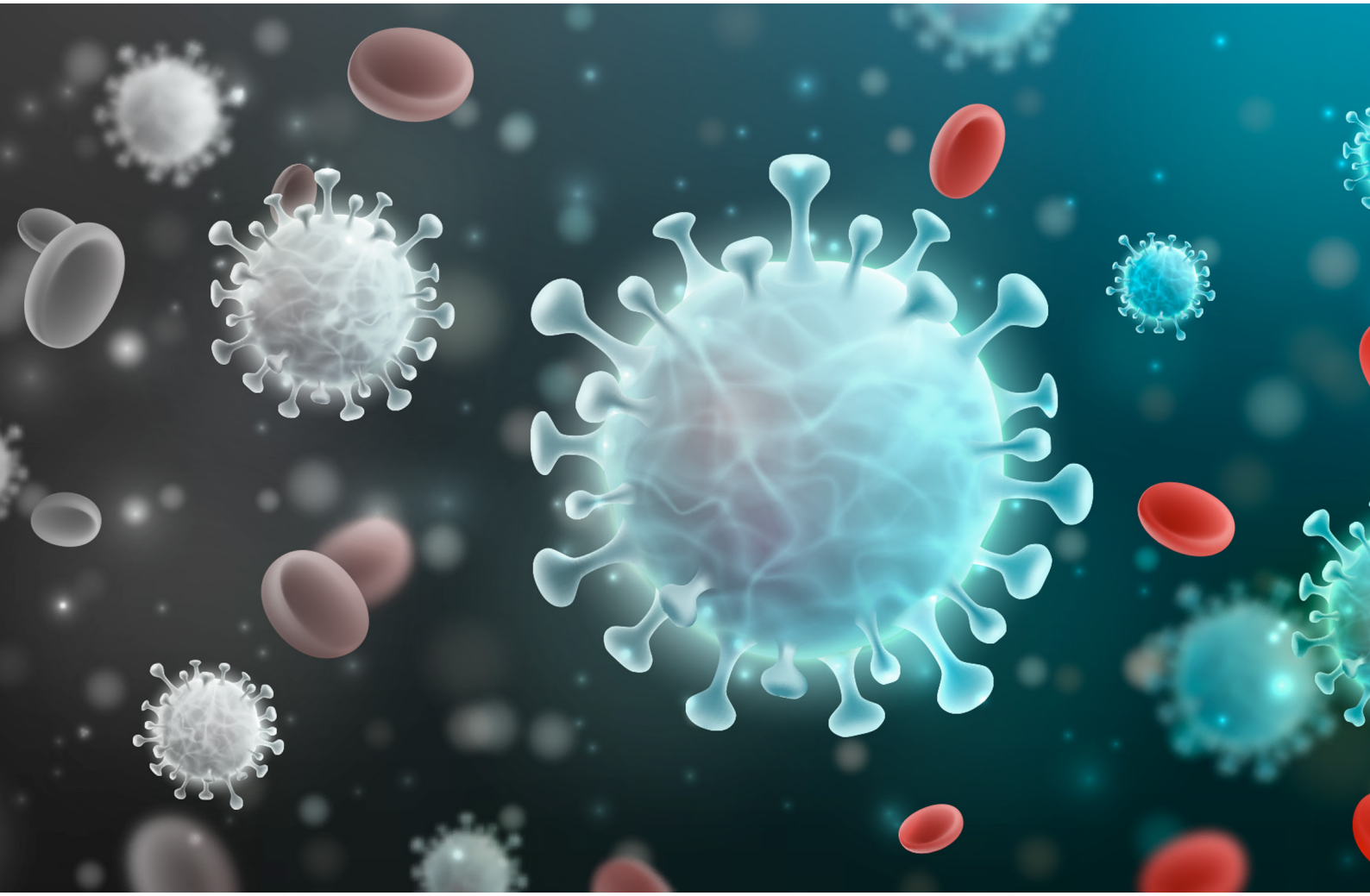
COVID-19



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

TestLine Clinical Diagnostics

INFECTIOUS SEROLOGY ▸ VIROLOGY ▸ **COVID-19**

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

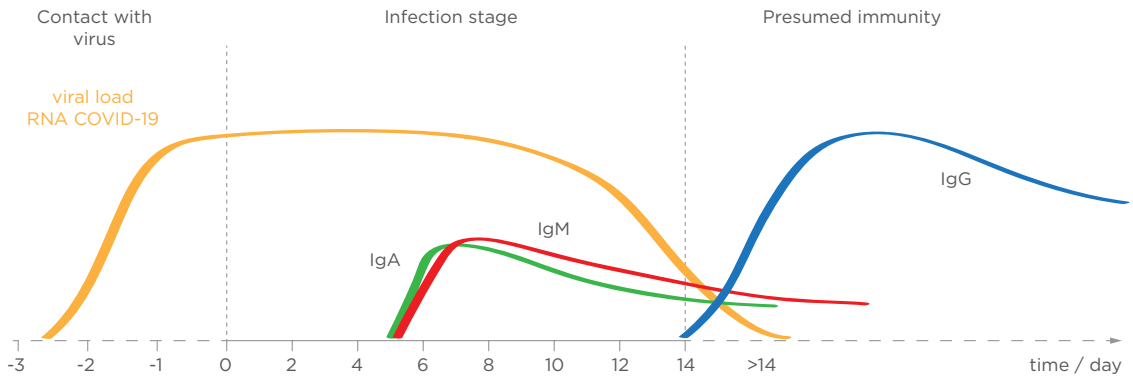
The diagnostics of the disease is based on the clinical picture, epidemiological history, and laboratory tests.

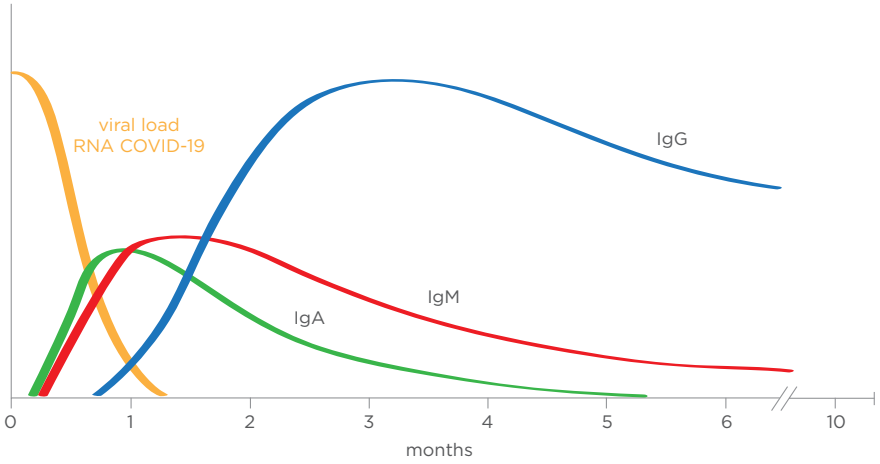
Due to the several-day-long interval between the first symptoms and the onset of the antibody response (the “window period”), serological tests play only a supporting role and, as stressed by the WHO, the results of such tests should always be verified by direct detection of the virus to diagnose an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

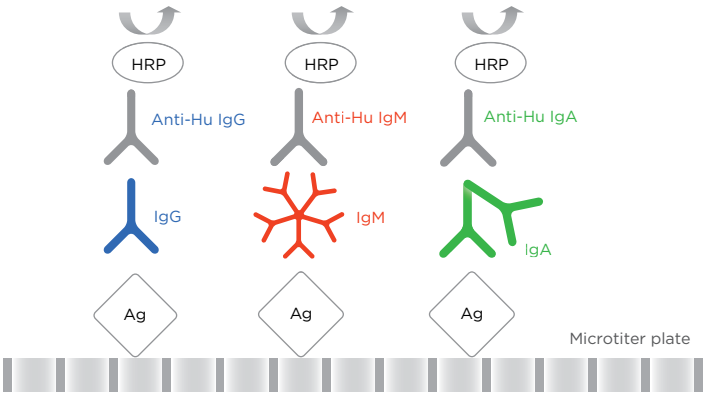




TEST PRINCIPLE

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

Sandwich ELISA



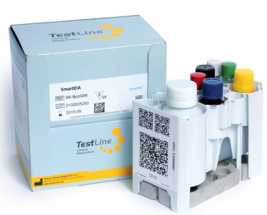
SUMMARY PROTOCOL

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 minutes at 37 °C
4	Aspirate and wash the wells 5 times
5	Add Conjugate 100 µl • Including blank
6	Incubate 30 minutes at 37 °C
7	Aspirate and wash the wells 5 times
8	Add 100 µl Substrate (TMB-Complete) • Including blank
9	Incubate 30 minutes at 37 °C
10	Add 100 µl Stopping solution • Including blank
11	Read colour intensity at 450 nm

TYPES OF KITS

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

EIA



SmartEIA

ANTIGENS

EIA COVID-19 NP
Nucleocapsid recombinant antigen (NP)

EIA COVID-19 RBD
Recombinant antigen Receptor-binding domain (RBD), a subunit of the Spike S1 protein

CLINICAL APPLICATION

- Diagnostics of the disease (additional examination)
- Negative result allows termination of a quarantine
- Prevalence study

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) or quantitative evaluation of results (IU/ml)

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

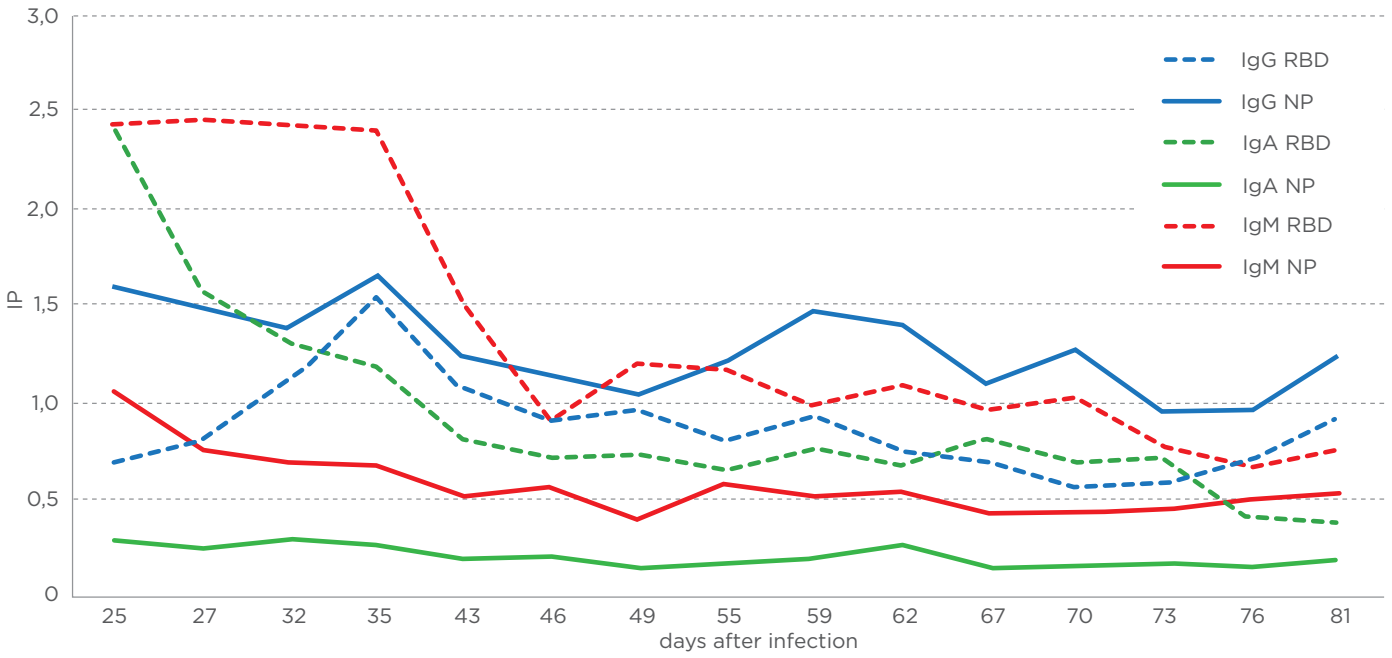
ELISA	Diagnostic sensitivity	Diagnostic specificity
EIA COVID-19 NP IgA	97.4%	97.7%
EIA COVID-19 NP IgG	95.1%	99.0%
EIA COVID-19 NP IgM	95.7%	97.7%
EIA COVID-19 RBD IgA	96.6%	98.9%
EIA COVID-19 RBD IgG	99.9%	99.1%
EIA COVID-19 RBD IgM	97.5%	95.1%

OVERVIEW OF REACTIVITY OF TESTLINE ELISA KITS ON A DEFINED SAMPLE

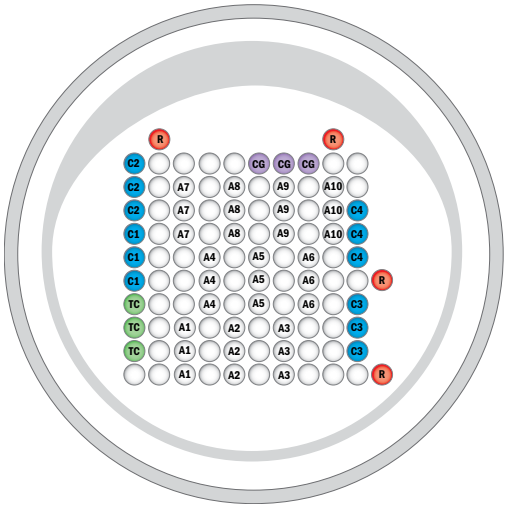
Woman, 27 years old, PCR positive	EIA COVID-19 RBD			EIA COVID-19 NP		
Day after infection	IgA	IgG	IgM	IgA	IgG	IgM
25	**2,40**	0,69	**2,42**	0,29	**1,58**	1,04
27	**1,58**	0,79	**2,45**	0,25	**1,48**	0,75
32	**1,31**	**1,10**	**2,43**	0,28	**1,38**	0,69
35	**1,16**	**1,55**	**2,40**	0,25	**1,65**	0,66
43	0,82	1,07	**1,49**	0,18	**1,23**	0,51
46	0,71	0,90	0,90	0,19	**1,14**	0,55
49	0,72	0,94	**1,19**	0,15	1,05	0,39
55	0,66	0,80	**1,16**	0,19	**1,20**	0,56
59	0,75	0,93	0,99	0,19	**1,45**	0,52
62	0,67	0,74	1,08	0,25	**1,39**	0,52
67	0,81	0,68	0,96	0,13	1,08	0,43
70	0,69	0,58	1,02	0,15	**1,26**	0,43
73	0,71	0,57	0,75	0,16	0,95	0,45
76	0,40	0,70	0,66	0,15	0,96	0,50
81	0,36	0,91	0,74	0,18	**1,22**	0,53

positive cut off negative

DYNAMICS OF INDIVIDUAL ANTIBODIES RESPONSE



DISTRIBUTION OF ANTIGENS AND CONTROL SPOTS



Description of antigens

A1 – Nucleocapsid NP
A2 – RBD
A3 – Spike S2
A4 – Envelope protein (E)
A5 – ACE2
A6 – PLPro protein
A7 – MERS-CoV
A8 – SARS – CoV
A9 – HCoV 229E Np
A10 – HCoV NL63 Np

Description of control spots

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

SUMMARY PROTOCOL

Step	Test steps
1	Pipette Universal solution 150 µl
2	Strips soaking 10 min. at room temperature
3	Aspirate
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

USER COMFORT

- Low sample consumption
- Antigens spoted in triplicate - minimizing statistical verification
- Fully automatic assay processing and results evaluation using spot intensity (AU), IP or quantitative (U/ml)
- Parallel testing of multiple markers simultaneously
- High sensitivity

TEST CHARACTERISTICS

Parameter	Diagnostic sensitivity	Diagnostic specificity
Microblot-Array COVID-19 IgA	98.3%	99.2%
Microblot-Array COVID-19 IgG	98.7%	99.3%
Microblot-Array 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	**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	**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	**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	**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	**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	**99.33%**	**97.97%**	**100.00%**

ORDERING INFORMATION

ELISA

Cat. No.	Product	No. of Wells
CoNA96	EIA COVID-19 NP IgA	96
CoNG96	EIA COVID-19 NP IgG	96
CoNM96	EIA COVID-19 NP IgM 96	96
CoRA96	EIA COVID-19 RBD IgA	96
CoRG96	EIA COVID-19 RBD IgG	96
CoRM96	EIA COVID-19 RBD IgM	96
SK-CoNA96	SmartEIA COVID-19 NP IgA	96
SK-CoNG96	SmartEIA COVID-19 NP IgG	96
SK-CoNM96	SmartEIA COVID-19 NP IgM	96
SK-CoRA96	SmartEIA COVID-19 RBD IgA	96
SK-CoRG96	SmartEIA COVID-19 RBD IgG	96
SK-CoRM96	SmartEIA COVID-19 RBD IgM	96

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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

CONTACT

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

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