

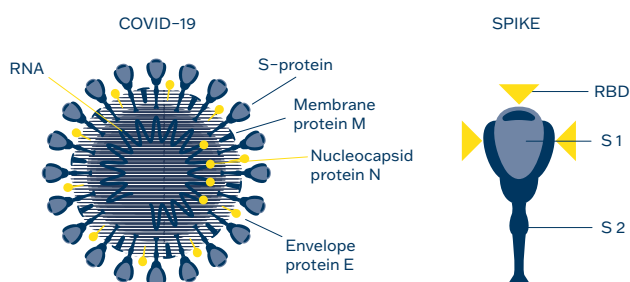
# Determination of antibodies against SARS-CoV-2

## Correlation of VNT and Microblot-Array kit results

### Compliance with WHO standard

**A standard range of methodological approaches is available for the detection of antibodies against SARS-CoV-2 antigens. For the detection of antibodies, commercial tests are available to detect IgA, IgG, and IgM based on immuno-enzymatic principles such as ELISA, Microblot-Array, Immunoblot, and CLIA.**

The antigen in the assays is usually either a structural nucleocapsid (NP) protein or a spike (S) protein (or its S1 part or only the Receptor-Binding Domain - RBD). The advantage of Microblot-Array (MBA) is that all these main antigens are in one test. Moreover, the kit contains other antigens such as the host cell receptor angiotensin-converting enzyme 2 (ACE2), PLpro protease, and Envelope protein, which can provide supplementary information.



According to the latest literature, it is most suitable to use a virus neutralization test (VNT) to determine the protective activity of antibodies present in a patient's serum. However, the determination of neutralizing antibodies (NAbs) by the VNT method is time-consuming (2-4 days) and, in addition, requires work with a live virus (BSL3 protection level laboratory). Standard commercially available assays detect anti-SARS-Cov-2 binding antibodies (BAbs) and do not have the ability to differentiate between NAbs and BAbs.

# The correlation of the results between VNT and Microblot-Array was performed in a comparative study of 100 samples.

The TestLine (TL) MBA COVID-19 diagnostic kits were used for testing, where a significant degree of agreement was found. Quantitative evaluation of IgG, IgM, and IgA results in the Units per millilitre (U/ml) corresponds to the VNT titer.

## IgG

	VNT titers	NP			RBD			Spike S2			E protein			ACE2			PLpro		
		Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max
n=7	20	588.51	220.83	1001.04	219.09	0.00	382.75	17.13	0.00	95.49	9.58	0.00	24.71	7.44	0.00	43.54	8.95	0.00	48.94
n=18	40	731.28	104.61	1007.62	497.08	192.32	897.62	98.02	6.51	244.54	3.74	0.00	30.39	4.95	0.00	58.87	4.02	0.00	26.93
n=27	80	784.09	159.69	1010.39	650.02	272.08	1000.02	101.82	0.00	229.43	10.58	0.00	90.27	12.56	0.00	86.89	9.36	0.00	86.16
n=27	160	886.26	375.34	1021.77	885.75	210.62	1023.13	206.76	0.00	967.37	11.19	0.00	61.25	8.89	0.00	62.97	9.00	0.00	65.17
n=16	320	918.94	311.75	1002.05	913.64	510.83	1001.75	224.50	0.00	561.19	10.04	0.00	39.91	6.34	0.00	55.52	9.07	0.00	40.64
n=5	640	970.01	946.69	1000.23	915.42	869.33	980.20	461.42	196.61	888.91	3.28	0.00	14.31	7.73	0.00	29.86	2.09	0.00	5.92
n=1	1280	875.36	875.36	875.36	910.07	910.07	910.07	296.57	296.57	296.57	1.41	1.41	1.41	0.00	0.00	0.00	0.00	0.00	0.00

## IgM

	VNT titers	NP			RBD			Spike S2			E protein			ACE2			PLpro		
		Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max
n=7	20	70.99	33.57	135.74	47.46	7.75	155.59	30.98	8.51	79.29	19.00	3.36	34.13	27.69	7.75	47.15	38.97	20.02	71.50
n=18	40	57.16	11.62	159.76	85.80	6.76	190.99	29.18	3.89	77.28	12.84	2.10	27.01	20.31	1.61	47.55	34.05	3.89	80.51
n=27	80	105.98	35.48	387.19	135.67	0.44	476.26	41.22	0.00	223.25	16.74	0.00	44.52	24.84	0.40	141.23	39.07	5.02	118.07
n=27	160	161.23	27.18	626.39	238.22	41.15	705.77	47.13	12.14	162.57	18.17	5.52	41.93	21.76	0.00	59.52	39.35	5.99	138.74
n=16	320	278.13	57.35	933.85	317.71	30.86	809.79	45.41	3.23	100.39	47.32	0.00	39.90	23.37	4.71	68.44	47.32	1.62	112.68
n=5	640	224.69	58.68	381.85	445.99	137.83	768.25	56.72	9.83	167.31	17.50	6.49	30.80	17.50	7.10	39.21	45.80	13.27	115.09
n=1	1280	423.07	423.07	423.07	824.62	824.62	824.62	48.51	48.51	48.51	8.94	8.94	8.94	29.78	29.78	29.78	64.86	64.86	64.86

## IgA

	VNT titers	NP			RBD			Spike S2			E protein			ACE2			PLpro		
		Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max	Mean value	range min	max
n=7	20	265.56	54.69	997.49	110.05	27.18	230.94	53.28	5.47	85.24	11.00	0.70	17.37	28.08	9.73	87.76	30.85	14.61	58.76
n=18	40	207.59	37.13	599.09	158.10	38.36	708.26	88.10	7.41	573.70	12.75	0.00	36.55	31.13	5.56	67.61	37.44	5.00	66.84
n=27	80	376.92	49.98	1007.33	287.79	67.49	964.00	69.55	20.59	413.92	20.41	3.32	102.48	28.90	4.00	55.87	35.06	7.68	156.18
n=27	160	408.42	96.52	1004.90	338.79	139.33	872.08	19.61	1.82	878.82	26.30	0.45	227.84	31.68	1.57	69.22	49.41	10.41	385.50
n=16	320	475.23	128.78	1002.15	537.26	135.00	926.34	141.74	36.73	281.92	15.70	1.19	37.09	78.89	8.01	763.13	52.05	21.27	175.63
n=5	640	455.06	151.85	996.45	484.06	108.39	689.63	91.73	12.16	193.16	7.92	4.34	14.29	25.92	13.02	53.52	25.92	7.40	103.34
n=1	1280	999.54	999.54	999.54	999.54	999.54	999.54	644.50	644.50	644.50	32.35	32.35	32.35	11.44	11.44	11.44	559.35	559.35	559.35

### Interpretation of MBA results – Units per millilitre (U/ml)

- negative: < 185
  - borderline: 185 to 210
  - positive: > 210
- n = number of samples

## The correlation of results with VNT method

VNT vs MBA TL IgG

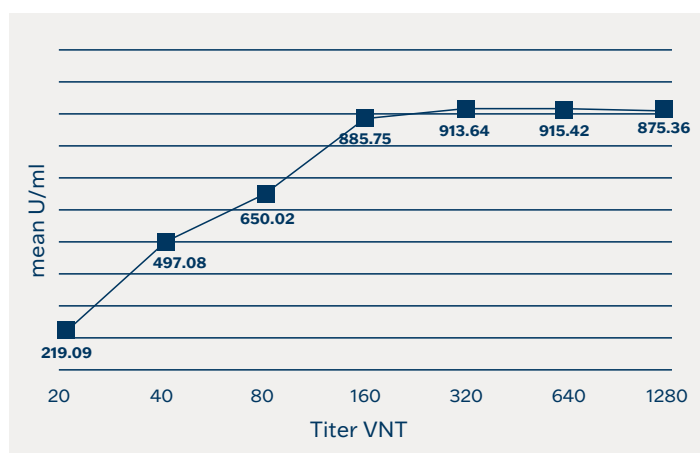
		TL	
		pos	neg
VNT	pos	100	0
	neg	0	0
Agreement		100%	

All classes of VNT antibodies vs MBA

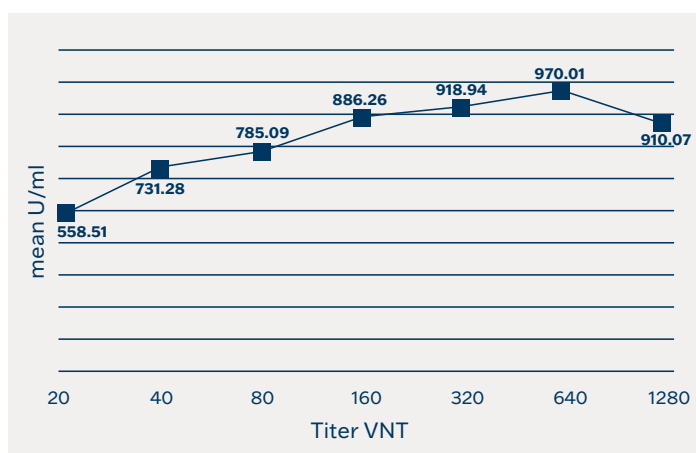
		TL	
		pos	neg
VNT	pos	100	0
	neg	0	0
Agreement		100%	

## 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



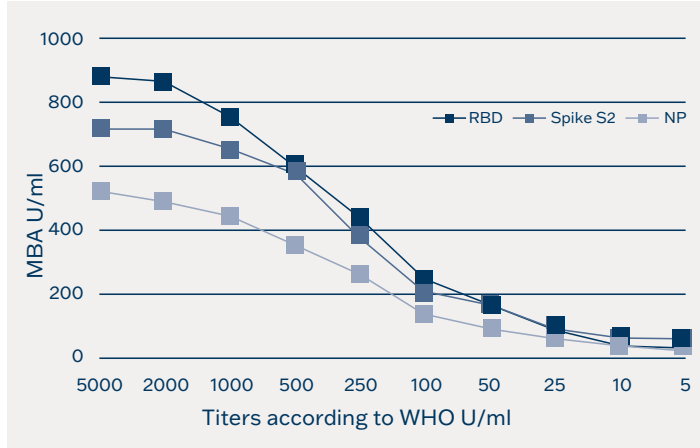
Mean values of units per millilitre IgG anti-NP antibodies (TestLine) in relation to individual VNT titers



# Compliance with WHO standard

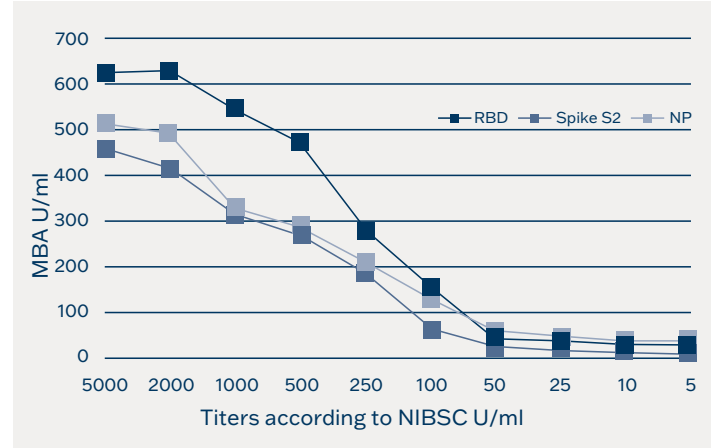
## Titration of the WHO 20/136 IgA standard

A group of convalescent plasma from patients recovered from COVID-19, containing high titers of antibodies against SARS-CoV-2.



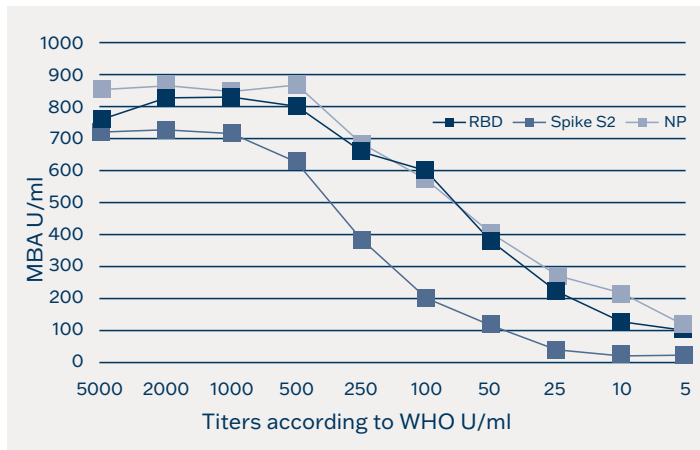
## Titration of the NIBC 20/162 IgA standard

High titer anti-SARS-CoV-2 antibody material was used to assess and compare relative sensitivities for Anti-Sars CoV-2 determination of antibodies by dilution at the endpoint.



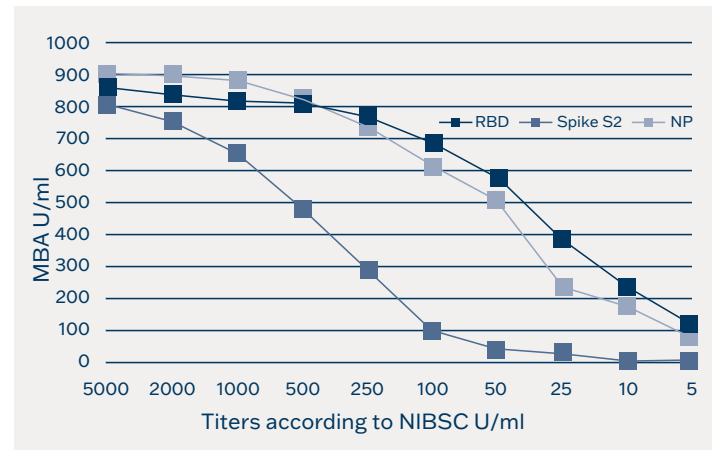
## Titration of the WHO 20/136 IgG standard

A group of convalescent plasma from patients recovered from COVID-19, containing high titers of antibodies against SARS-CoV-2



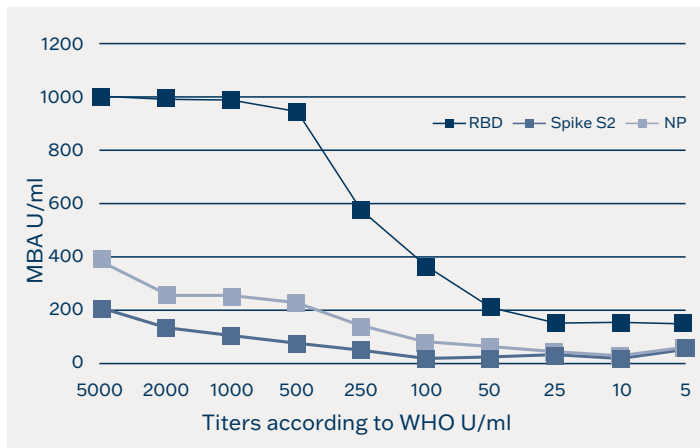
## Titration of the NIBC 20/162 IgG standard

High titer anti-SARS-CoV-2 antibody material was used to assess and compare relative sensitivities for Anti-Sars CoV-2 determination of antibodies by dilution at the endpoint.



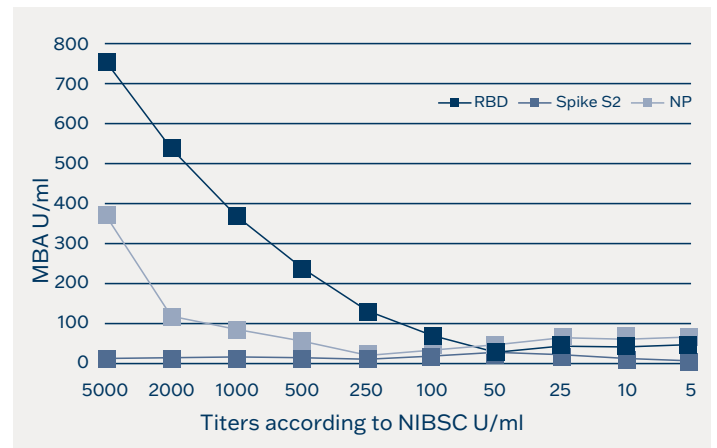
## Titration of the WHO 20/136 IgM standard

A group of convalescent plasma from patients recovered from COVID-19, containing high titers of antibodies against SARS-CoV-2



## Titration of the NIBC 20/162 IgM standard

High titer anti-SARS-CoV-2 antibody material was used to assess and compare relative sensitivities for Anti-Sars CoV-2 determination of antibodies by dilution at the endpoint.



The WHO 20/136 International Standard for Anti-SARS-CoV-2 is intended for the calibration and harmonization of serological tests detecting the anti-SARS-CoV-2 neutralizing effect and for determining the validated level of binding antibodies. The NIBSC Anti-SARS-Cov-2 standard is designated as Quality Control Reagent and monitors the sensitivity of serological tests.

By titrating WHO 20/136 Standard, TestLine responded to the requirement to accurately determine the level of antibodies after vaccination. The sensitivity and dilution management of strongly positive antibody levels were verified by titration of NIBSC 20/162.

## Summary

By the correlation study, TestLine confirmed the high level of agreement between results obtained by Virus Neutralization Test and TL Microblot-Array COVID-19 kit. The level of neutralizing antibodies corresponds with the level of anti-RBD IgG antibodies and therefore MBA kits developed by TestLine can be used to determine if the concentration of antibodies has a protective effect. Moreover, by harmonization with International Standards, the kits can be used for monitoring the antibody concentration after vaccination by the time.

## Ordering information:

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



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