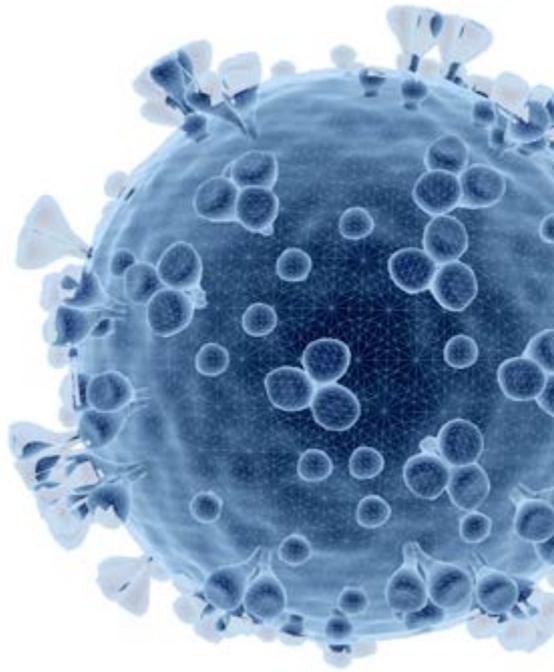




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COVID-19 International Standards & reference reagents

Microblot-Array COVID-19

SARS-CoV-2 was first reported in China in December 2019. The virus has spread worldwide, and The World Health Organization (WHO) has declared it a Pandemic in March 2020. To control the pandemic, COVID-19 vaccines are rapidly being developed and administered worldwide. Reliable assays are needed for their evaluation.

A variety of serological assays for the detection of SARS-CoV-2 antibodies (Ab) and antibody response after vaccination are used globally. WHO has highlighted the importance of the availability of International Standards and reference reagents for anti-SARS-CoV-2 antibodies as it will facilitate the development, validation, and assessment of the assays. It will allow for comparability of results from different assays. It will help diagnose the antibodies after infection, determine the antibody levels needed for efficacious vaccines and improve our understanding of virus epidemiology.

The WHO collaborating centre for biological standardization is National Institute for Biological Standards and Control (NIBSC). NIBSC has a portfolio of reference and research reagents for diagnostics, vaccine development, and research on COVID-19 to support the development of accurate and reliable diagnostic tests.

International Standards and panels, which are listed below, were tested on Microblot-Array (MBA) COVID-19 kits in IgA, IgG, and IgM antibody classes. By titrating these Standards, TestLine responded to the requirement to accurately determine the level of antibodies, sensitivity, and dilution of strongly positive results. Thanks to harmonization with International Standards, the kits can be used for monitoring the antibody concentration after vaccination.

First WHO International Standard for anti-SARS-CoV-2 immunoglobulin, human (NIBSC code: 20/136)

Intended use: Calibration and harmonisation of serological assays detecting antiSARS-CoV-2 neutralising antibodies. The preparation can also be used as an internal reference reagent for the harmonisation of binding antibody assays.

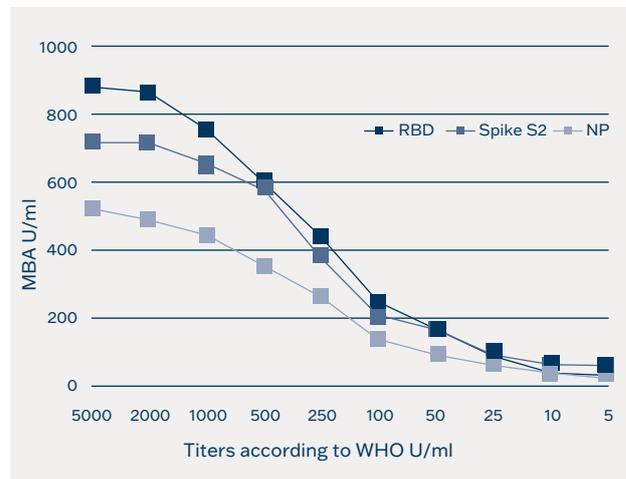
Material: Antibody, human, convalescent plasma, WHO IS

Description: Pool of convalescent plasma from recovered COVID-19 patients, containing high titer antibodies against SARS-CoV-2.

Unitage: The assigned potency is 250 IU/ampoule for neutralising antibody activity. After reconstitution in 0.25 mL of distilled water, the final concentration of the preparation is 1000 IU/ml. For binding antibody assays, an arbitrary unitage of 1000 binding antibody units (BAU)/ml, can be used to assist the comparison of assays detecting the same class of immunoglobulins with the same specificity.

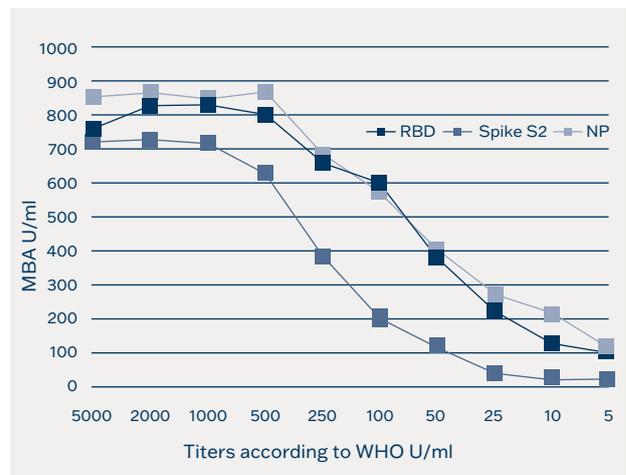
Titration of the First WHO International standard IgA (20/136)

IgA		MBA COVID-19		
WHO titers		NP	RBD	Spike S2
U/ml	VNT	U/ml		
5000	1000	716.88	881.85	517.33
2000	500	714.07	864.57	489.47
1000	250	648.56	755.19	440.92
500	125	575.03	595.43	350.76
250	62.5	376.57	435.95	259.01
100	25	198.70	241.86	132.02
50	12.5	160.87	165.41	91.74
25	6.25	91.03	83.91	56.98
10	2.5	56.20	32.31	25.46
5	1.25	51.92	27.53	23.74



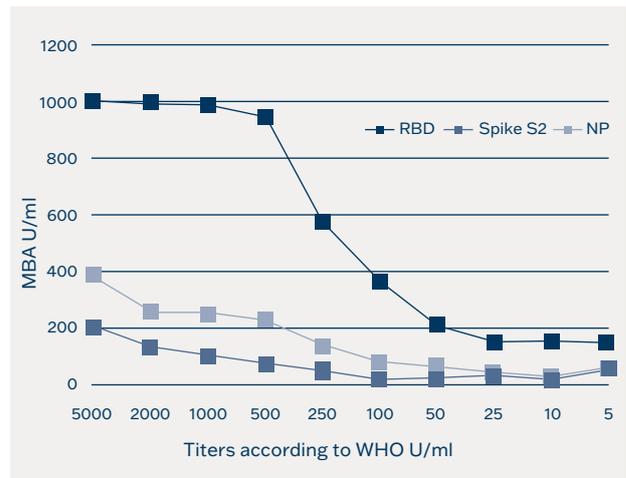
Titration of the First WHO International standard IgG (20/136)

IgG		MBA COVID-19		
WHO titers		NP	RBD	Spike S2
U/ml	VNT	U/ml		
5000	1000	856.87	760.36	720.43
2000	500	865.14	832.26	731.43
1000	250	847.09	835.34	719.41
500	125	872.48	804.24	631.49
250	62.5	684.08	666.48	388.55
100	25	581.57	599.56	204.38
50	12.5	405.16	384.97	121.67
25	6.25	273.34	232.46	41.98
10	2.5	217.20	128.46	27.72
5	1.25	120.76	103.64	24.42



Titration of the First WHO International standard IgM (20/136)

IgM		MBA COVID-19		
WHO titers		NP	RBD	Spike S2
U/ml	VNT	U/ml		
5000	1000	388.78	996.44	204.92
2000	500	255.94	996.05	129.52
1000	250	249.10	990.67	96.48
500	125	228.58	947.72	70.52
250	62.5	131.85	569.85	44.47
100	25	68.69	360.09	8.57
50	12.5	55.82	206.26	18.02
25	6.25	45.18	144.75	29.19
10	2.5	33.59	157.99	15.11
5	1.25	53.11	139.72	48.10



Interpretation

= negative
 = borderline
 = positive

Summary: By titrating First WHO International Standard for Anti-SARS-CoV-2 immunoglobulin (20/136) TestLine responded to the requirement to accurately determine the level of antibodies after vaccination. Thanks to harmonization with International Standards, the kits can be used for monitoring the antibody concentration after vaccination.

Anti-SARS-CoV-2 Antibody Diagnostic Calibrant (NIBSC code: 20/162)

Intended use: Monitoring and estimation of the sensitivity of assay for Anti-SARS-CoV-2 by end-point dilution and assessing any variation in the sensitivity of different lots of assay kits by comparing changes in the end-point dilution. Data obtained with this reagent can be used to confirm that the chosen assay is acceptable for clinical use.

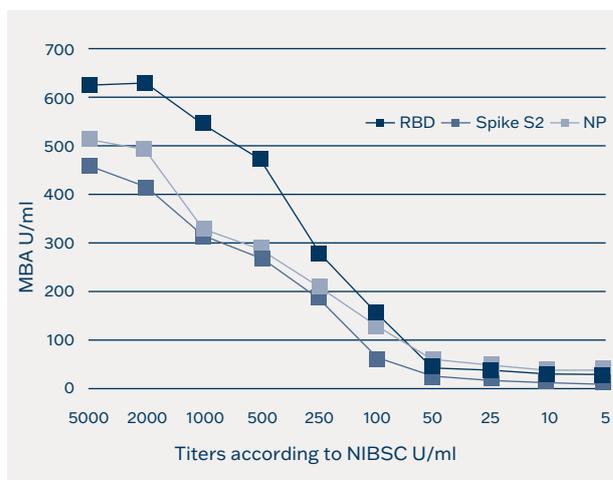
Material: Antibody, human, convalescent plasma

Description: High titer Anti-SARS-CoV-2 antibody positive material used to assess and compare the relative sensitivities for Anti-Sars-CoV-2 antibody assays by end-point dilution.

Unitage: This reagent has been assigned an arbitrary unitage of 1000U.

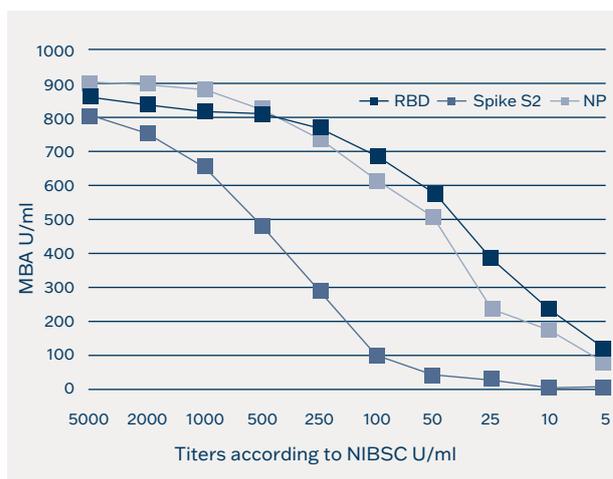
Titration of Diagnostic Calibrant IgA (20/162)

IgA			
MBA COVID-19			
NIBCS titers	NP	RBD	Spike S2
U/ml	U/ml		
5000	513.88	624.26	457.57
2000	490.68	628.53	413.89
1000	326.91	543.73	308.13
500	285.87	471.45	264.78
250	199.95	276.66	186.16
100	126.03	154.41	55.45
50	52.14	39.62	18.49
25	41.69	34.50	10.13
10	34.56	24.06	5.77
5	27.47	15.93	5.08



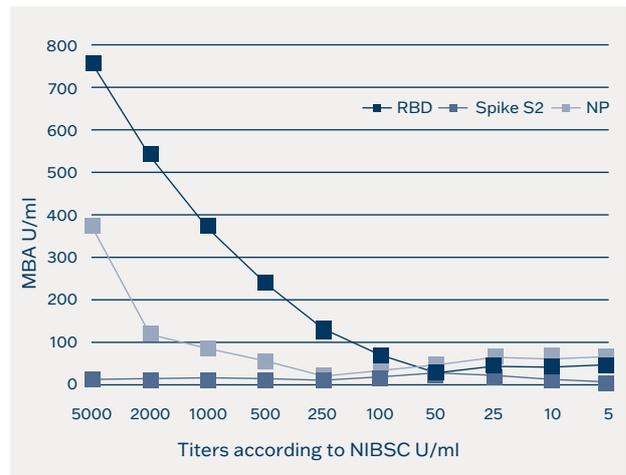
Titration of Diagnostic Calibrant IgG (20/162)

IgG			
MBA COVID-19			
NIBCS titers	NP	RBD	Spike S2
U/ml	U/ml		
5000	900.60	864.34	809.50
2000	902.98	839.47	755.61
1000	882.25	818.60	654.50
500	825.89	816.20	479.40
250	742.58	769.97	286.57
100	612.82	686.11	93.89
50	507.96	575.89	46.40
25	234.06	385.43	33.19
10	175.16	234.85	4.57
5	81.29	116.81	1.11



Titration of Diagnostic Calibrant IgM (20/162)

IgM			
MBA COVID-19			
NIBSC titers	NP	RBD	Spike S2
U/ml	U/ml		
5000	372.00	751.23	11.58
2000	118.27	538.91	14.19
1000	84.17	371.94	14.27
500	54.36	238.57	10.00
250	21.18	128.13	10.49
100	37.03	66.00	17.35
50	38.34	20.58	19.94
25	62.00	41.99	19.33
10	55.77	40.33	12.47
5	59.23	45.64	16.73



Interpretation

= negative
 = borderline
 = positive

Summary: The NIBSC Anti-SARS-Cov-2 standard is designated as Quality Control Reagent and monitors the sensitivity of serological tests. The sensitivity and dilution management of strongly positive antibody levels were verified by titration of NIBSC 20/162.

Anti-SARS-CoV-2 Verification Panel for Serology Assays (NIBSC code: 20/B770)

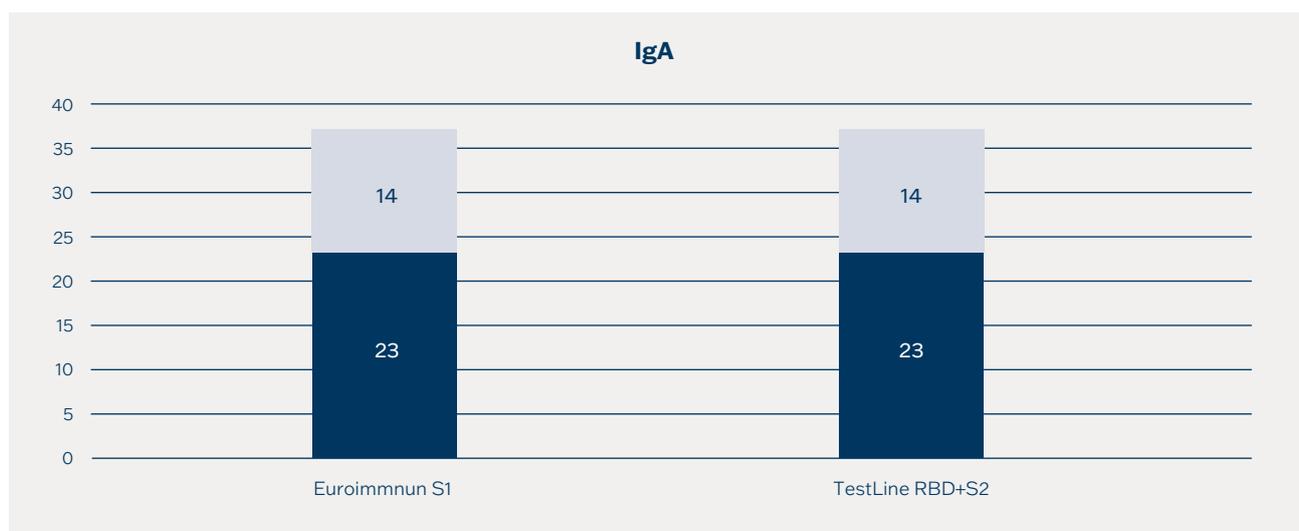
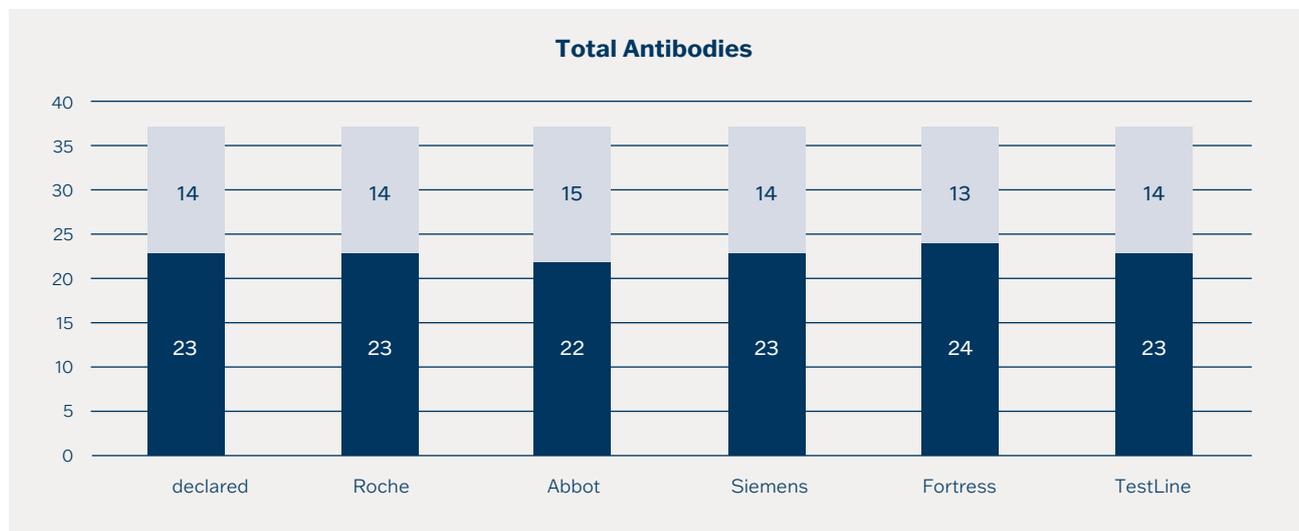
Intended use: For use as a verification panel to detect claims by manufacturers on the detection of SARS-CoV-2 antibodies. It can be used as part of a laboratory's verification process when adopting a new AntiSARS-CoV-2 assay.

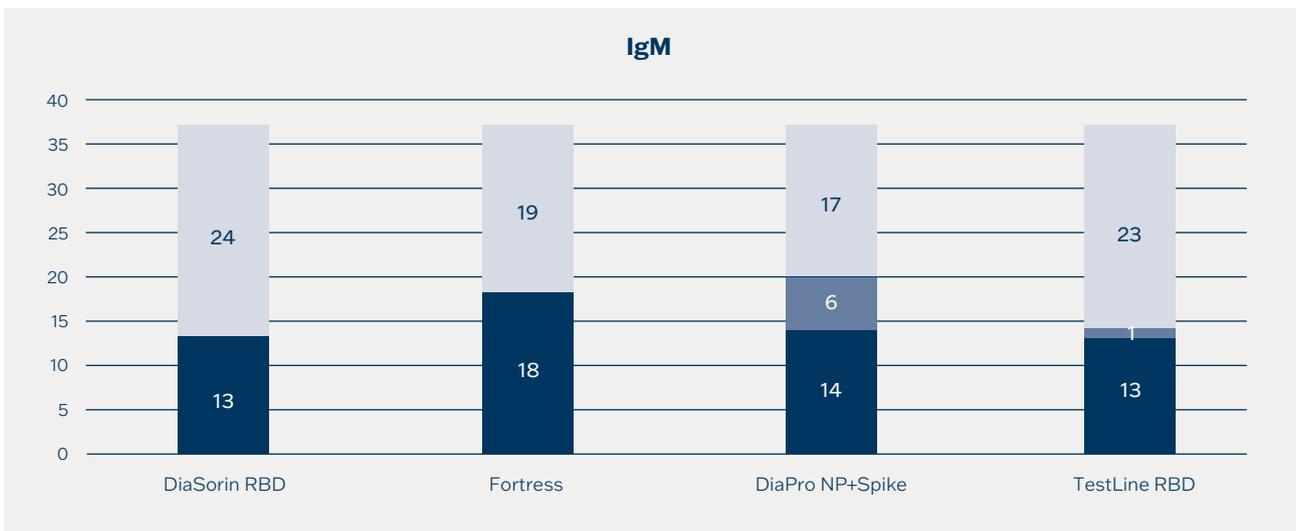
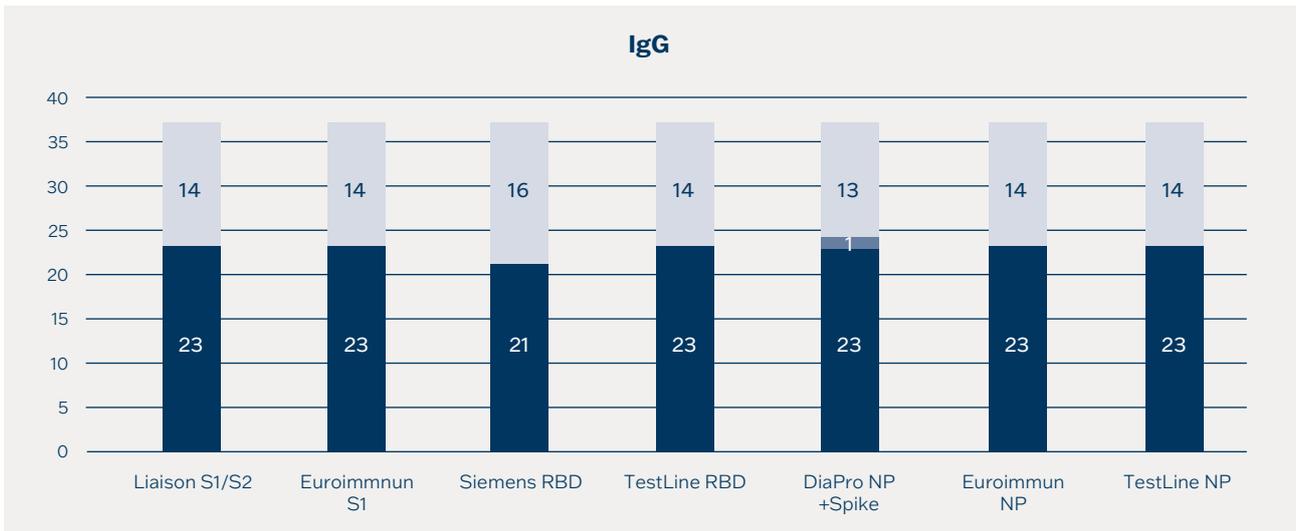
Material: Antibody, human, convalescent plasma Anti-SARS-CoV-2 negative plasma

Description: This product is CE marked for use as an IVD within the EU member states and EEA countries. In all other territories, it can be used for research purposes only. The Verification Panel consists of 37 samples (23 positive and 14 negative).

Unitage: Tables below summarise results obtained for the Anti-SARS-CoV-2 Verification panel for Serology Assays on 13 commercially available assays and one in-house assay. These results are intended only as a guide to the approximate levels of reactivity to be expected and may not be exactly reproduced in other laboratories.

Titration of Verification Panel IgA, IgG, and IgM (20/B770) on MBA COVID-19 kits and comparison with other commercially available assays and declared samples.





Interpretation

= negative
 = borderline
 = positive

Summary: The verification Panel was tested on Microblot-Array COVID-19. Results from testing show that MBA has comparable results with other 13 commercially available assays and one in-house assay. In some cases, the results are even better. These results are intended only as a guide to the approximate levels of reactivity to be expected.

First WHO International Reference Panel for anti-SARS-CoV-2 immunoglobulin, human (NIBSC code: 20/268)

Intended use: Detection and quantification of anti-SARS-CoV-2 antibodies in an assessment and development of assays.

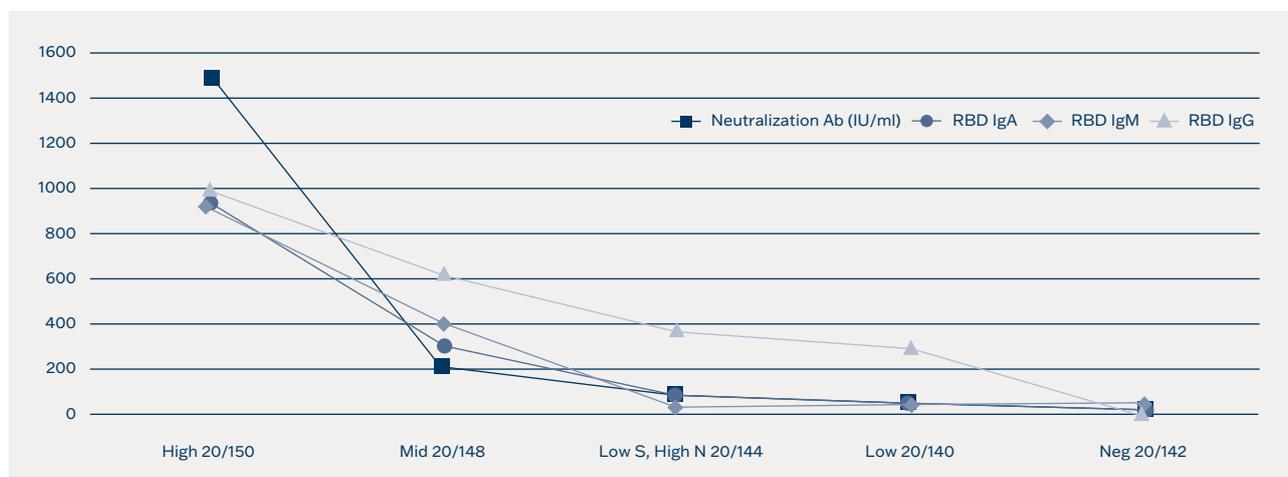
Material: Antibody, human, convalescent plasma, WHO reference panel

Description: It comprises of 5 panel members; four pools of convalescent plasma from recovered COVID-19 patients, containing high, medium, low anti-S but relatively high anti-N, low antibodies against SARS-CoV-2, and negative control (healthy donors collected before 2019).

Unitage: No unitage has been assigned.

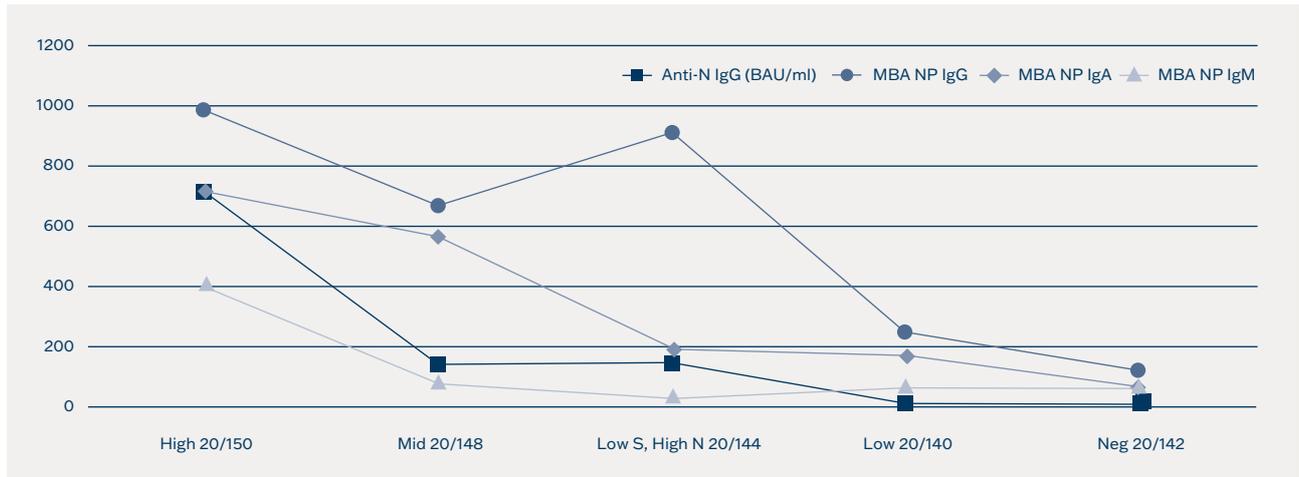
Reactivity of anti-RBD antibodies – correlation with VNT Ab 20/268 WHO Reference Panel

NIBSC sample	Neutralization Ab IU/ml	MBA COVID-19		
		RBD IgA	RBD IgG	RBD IgM
High 20/150	1473	933.58	989.07	945.07
Mid 20/148	210	310.62	618.16	406.67
Low S, high N 20/144	95	78.42	368.88	51.52
Low 20/140	44	74.40	287.03	56.15
Neg 20/142	0	45.81	3.15	32.64



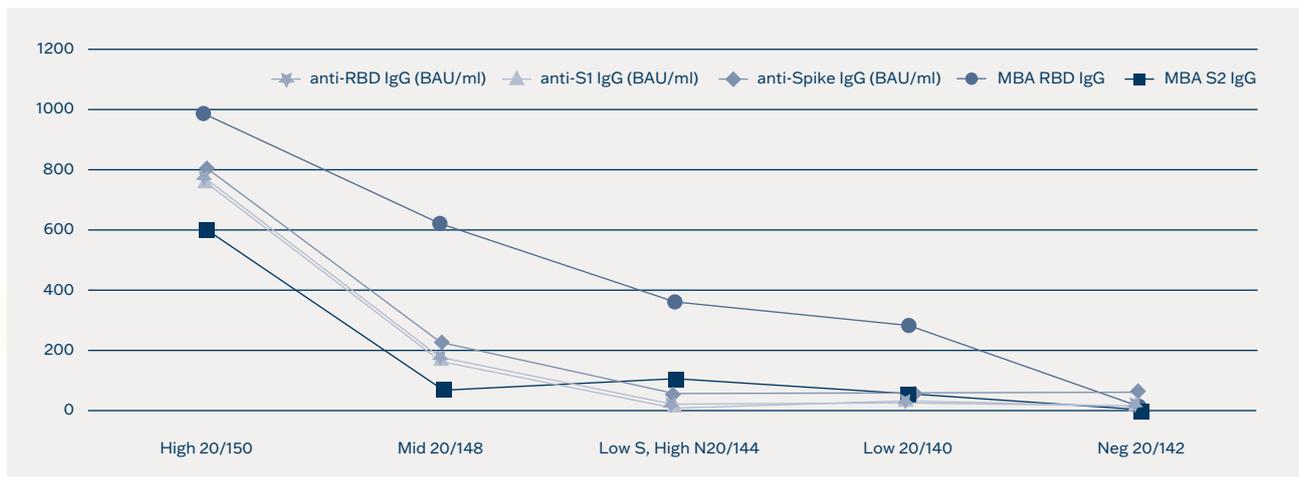
Reactivity of anti-Nucleocapsid antibodies – correlation with 20/268 WHO Reference Panel

NIBSC sample	Anti-N IgG BAU/ml	MBA COVID-19		
		NP IgA	NP IgG	NP IgM
		U/ml		
High 20/150	713	800.38	1002.44	412.35
Mid 20/148	146	573.35	671.26	76.20
Low S, high N 20/144	146	80.01	925.61	32.19
Low 20/140	12	172.02	251.87	52.95
Neg 20/142	0	67.02	63.65	118.27



Reactivity of anti-RBD and anti-S2 antibodies – correlation with 20/268 WHO Reference Panel

NIBSC sample	IgG			MBA COVID-19 IgG		
	anti-RBD	anti-S1	anti-Spike	RBD	Spike S2	
		BAU/ml			U/ml	
Imunoglobulin High 20/150	817	766	832	989.07	614.02	
Imunoglobulin Mid 20/148	205	246	241	618.16	68.84	
Imunoglobulin low S, high N 20/144	66	50	86	368.88	108.74	
Imunoglobulin Low 20/140	45	46	53	287.03	54.09	
	0	0	0	3.15	0.00	



**Interpretation**

■ = negative ■ = borderline ■ = positive

Summary: Testing Reference Panel for anti-SARS-CoV-2 immunoglobulin on MBA COVID-19 is used for assessment and quantitation between antibodies from WHO reference panel and TestLine MBA COVID-19 kits.

Ordering information

<u>Cat. No.</u>	<u>Product</u>	<u>No. of wells</u>
CoVAMA96	Microblot-Array COVID-19 IgA	96
CoVGMA96	Microblot-Array COVID-19 IgG	96
CoVMA96	Microblot-Array COVID-19 IgM	96

References

[1] World Health Organization (2021, January 12). Standardization of vaccines for coronavirus disease (COVID-19).

Available from: <https://www.who.int/news-room/feature-stories/detail/standardization-of-vaccines-for-coronavirus-disease-covid-19>.

[2] The National Institute for Biological Standards and Control. Coronavirus (COVID-19)-related research reagents available from the NIBSC.

Available from: https://www.nibsc.org/science_and_research/idd/cfar/covid-19_reagents.aspx.



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