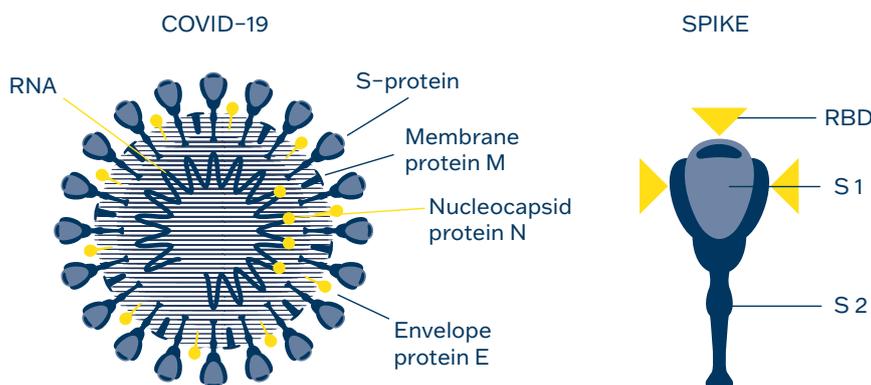# Determination of antibodies against SARS-CoV-2

Correlation of VNT and ELISA 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, Immunoblot, 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).

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.

BGI TestLine®

The correlation of the results between VNT and ELISA was performed in a comparative study of 100 samples.

The TestLine EIA COVID-19 RBD diagnostic kits were used for testing, where a significant degree of agreement was found. Both semi-quantitative evaluation of IgG results in the Positivity Index (IP) and the quantitative evaluation corresponds to the VNT titer.

VNT titer	Elisa – TESTLINE RBD						ELISA – competitor DE		
	IgG IP	IgG U/ml	IgM IP	IgM U/ml	IgA IP	IgA U/ml	IgG IP	IgM IP	IgA IP
20	3.83	97.3	0.67	15	0.72	5.57	1.29	neg.	0.93
20	0.48	11.02	0.35	10.04	0.55	3.03	neg.	neg.	neg.
20	2.82	68.18	0.45	11.56	0.78	6.55	neg.	neg.	0.94
20	2.57	61.7	0.54	12.91	0.35	9.82	neg.	neg.	neg.
20	1.26	26.81	0.64	14.51	1.67	54.16	neg.	neg.	0.94
20	1.48	47.1	0.34	9.99	1.05	21.56	1.04	neg.	0.97
20	2.45	117.27	1.59	47.85	1.33	38.22	1.35	neg.	2.84
40	2.58	61.86	0.33	9.8	0.85	7.73	1.20	neg.	1.09
40	1.48	32.8	0.22	8.11	0.69	5.18	1.35	neg.	neg.
40	4.96	131.53	1.30	34.45	0.92	18.83	2.71	neg.	1.05
40	4.11	105.57	0.43	11,23	0,39	0,4	1.51	neg.	neg.
40	4.11	105.67	0.22	7.99	1.49	44.95	1.28	neg.	1.10
40	4.79	126.35	0.49	12.21	1.94	67.97	0.99	neg.	1.00
40	7.86	219.53	0.56	13.28	5.80	320	2.72	neg.	3.60
40	1.84	42.33	0.56	13.24	0.49	2.01	neg.	neg.	neg.
40	8.74	246.24	0.39	10.61	3.11	146.71	2.35	neg.	1.92
40	3.39	83.76	0.52	12.66	1.34	37.4	1.47	neg.	neg.
40	4.03	103.22	0.26	8.73	1.59	50.19	1.43	1.98	–
40	3.34	82.44	0.52	12.62	0.68	5.03	neg.	neg.	neg.
40	2.57	61.61	0.50	12.34	1.84	62.73	neg.	1.27	0.92
40	3.81	96.74	0.51	12.5	0.84	7.53	1.47	neg.	0.99
40	6.17	168.38	0.32	9.51	1.45	43.16	1.80	neg.	1.78
40	2.39	56.77	1.64	50.41	0.83	7.42	0.95	neg.	neg.
40	4.57	320	0.85	17.72	2.59	125.8	2.63	1.41	2.10
40	3.82	248.14	1.11	25.07	0.72	15.67	1.45	neg.	1.60
80	11.25	320	0.64	14.55	1.50	45.59	4.90	–	–
80	11.38	320	0.86	17.87	4.18	222.34	4.13	–	–
80	6.19	168.76	2.25	79.43	0.67	4.91	2.12	neg.	0.98
80	11.79	320	0.88	18.16	2.35	92.49	5.43	neg.	3.99
80	4.23	109.43	0.15	7.01	3.20	152.78	1.92	neg.	7.23
80	5.78	156.44	0.47	11.93	0.76	6.2	2.56	neg.	3.38
80	6.69	184.18	0.92	18.73	2.78	122.99	2.73	neg.	2.47
80	4.91	129.93	0.45	11.6	1.04	18.79	1.39	neg.	1.03
80	2.80	67.61	0.36	10.2	1.93	67.72	1.35	neg.	neg.
80	5.02	133.5	0.35	10.04	0.33	9.54	2.41	neg.	neg.
80	4.63	121.56	0.33	9.75	1.14	27.16	1.71	neg.	0.99

VNT titer	Elisa – TESTLINE RBD						ELISA – competitor DE		
	IgG IP	IgG U/ml	IgM IP	IgM U/ml	IgA IP	IgA U/ml	IgG IP	IgM IP	IgA IP
80	2.50	59.81	0.63	14.39	3.92	204.5	1.35	1.76	1.95
80	3.40	84.14	0.72	15.78	1.60	50.83	1.16	0.90	2.61
80	8.47	238.15	0.63	14.34	3.54	177.03	1.96	neg.	3.10
80	3.28	80.38	0.56	13.2	1.60	50.45	1.28	neg.	1.47
80	8.07	226.12	1.92	63.72	9.44	320	1.46	1.48	8.97
80	2.88	69.9	0.85	17.75	9.44	320	1.01	neg.	2.45
80	3.40	84.23	0.64	14.43	3.41	167.58	1.28	2.08	2.90
80	9.78	277.83	0.87	17.99	1.05	21.3	2.31	neg.	5.53
80	8.49	238.71	1.09	24.05	2.26	85.71	1.54	0.94	1.43
80	3.72	93.82	1.03	21.27	0.46	1.62	1.01	2.72	neg.
80	10.31	293.91	0.75	16.19	6.66	320	2.37	neg.	1.91
80	9.44	267.67	0.44	11.43	1.54	47.76	1.75	neg.	1.61
80	3.40	208.56	1.24	31.33	1.62	54	1.94	neg.	1.38
80	4.22	287.25	0.43	11.36	0.64	14.48	2.24	neg.	1.00
80	4.62	320	0.66	14.82	1.32	37.79	3.76	0.99	1.65
80	4.33	297.59	0.27	8.92	0.78	16.65	3.09	2.03	1.09
160	11.94	320	1.61	49.02	3.14	148.85	6.16	1.98	2.72
160	11.94	320	0.88	18.11	1.88	65.03	6.38	–	–
160	11.42	320	0.21	7.83	2.44	99.09	5.12	–	–
160	9.43	267.3	1.48	42.56	1.87	64.39	3.81	1.79	2.07
160	5.42	145.54	1.19	29.12	2.53	104.97	1.74	neg.	1.37
160	9.83	279.43	0.32	9.59	2.57	108.18	3.02	1.08	3.26
160	12.06	320	1.22	30.64	6.51	320	5.02	0.92	7.55
160	9.86	280.46	0.61	14.1	1.51	45.97	3.21	neg.	3.59
160	11.76	320	0.54	12.95	3.29	159.2	3.56	neg.	2.69
160	11.55	320	0.53	12.75	1.37	38.68	3.64	neg.	1.54
160	10.23	291.65	0.68	15.16	1.09	21.61	3.35	neg.	1.69
160	11.41	320	0.44	11.48	1.36	38.29	3.90	neg.	2.50
160	11.08	317.32	0.92	18.81	2.18	80.36	2.39	neg.	2.07
160	2.23	52.59	1.22	30.52	5.98	320	0.95	neg.	3.62
160	9.11	257.61	0.78	16.68	1.38	39.45	2.16	neg.	1.01
160	12.01	320	1.06	22.79	4.17	221.63	2.68	2.89	4.97
160	12.04	320	0.81	17.09	1.80	60.68	4.42	neg.	1.93
160	11.55	320	2.40	90.06	1.44	42.39	2.39	neg.	0.95
160	11.69	320	0.93	18.93	3.36	320	4.34	3.43	2.47
160	10.83	309.89	0.68	15.08	2.57	107.83	1.61	neg.	1.90

Elisa – TESTLINE RBD / ELISA – competitor DE (left set)

VNT titer	IgG IP	IgG U/ml	IgM IP	IgM U/ml	IgA IP	IgA U/ml	IgG IP	IgM IP	IgA IP
160	11.81	320	2.18	76.01	4.52	246.6	5.12	1.17	3.06
160	12.01	320	4.16	227.17	9.23	320	1.97	0.99	8.45
160	3.70	93.35	5.75	320	7.80	320	neg.	3.51	3.69
160	4.81	320	7.22	320	3.65	222.5	3.63	neg.	5.07
160	4.68	320	0.37	10.41	1.72	59.44	4.14	neg.	3.94
160	4.46	309.84	2.03	68.61	2.51	118.1	3.86	0.98	5.41
160	4.62	320	0.42	11.09	2.30	98.91	5.13	neg.	4.42
320	12.01	320	3.62	185	8.88	320	7.52	–	–
320	5.80	157.62	1.30	34.45	8.93	320	2.74	1.21	5.54
320	5.20	138.86	1.62	49.52	1.38	39.57	1.49	neg.	1.35
320	11.49	320	0.98	19.63	3.65	184.7	5.29	neg.	5.17
320	12.23	320	0.90	18.4	8.72	320	4.28	neg.	2.34
320	11.88	320	0.29	9.14	3.04	141.72	4.12	neg.	3.78
320	11.88	320	0.67	15	2.24	84.64	5.08	1.10	3.54

Elisa – TESTLINE RBD / ELISA – competitor DE (right set)

VNT titer	IgG IP	IgG U/ml	IgM IP	IgM U/ml	IgA IP	IgA U/ml	IgG IP	IgM IP	IgA IP
320	12.23	320	1.43	40.4	9.26	320	3.98	1.12	4.81
320	11.01	315.25	6.40		4.16	221.45	3.69	3.80	2.57
320	11.42	320	0.85	17.7	7.17	320	3.89	neg.	7.47
320	11.69	320	0.45	11.56	2.66	114.25	2.41	neg.	2.11
320	12.06	320	1.83	59.16	5.30	302.25	5.72	2.01	5.24
320	11.69	320	7.06	320	9.32	320	5.53	4.70	4.40
320	12.23	320	0.78	16.56	3.63	183.28	6.52	neg.	4.84
320	11.81	320	1.91	63.08	2.69	116.39	2.83	3.32	2.04
640	11.94	320	0.99	19.88	8.73	320	6.93	neg.	5.31
640	11.76	320	1.25	31.66	7.65	320	6.16	neg.	7.66
640	10.08	286.95	2.72	114.9	0.32	9.35	4.26	2.14	0.98
640	11.94	320	2.25	79.18	5.79	320	5.40	2.10	4.20
640	12.01	320	9.57	320	4.74	262.3	4.29	3.09	4.02
1280	11.40	320	7.93	320	9.39	320	5.36	3.30	7.51

Results interpretation

	negative	borderline	positive
TL IP:	negative: < 0,9	borderline: 0,9 – 1,1	positive: > 1,1
TL U/ml:	negative: < 18	borderline: 18 – 22	positive: > 22
competitor DE:	negative: < 0,8	borderline: 0,8 – 1,1	positive: > 1,1

The correlation of results with VNT method

VNT vs EIA TESTLINE IgG

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

All classes of VNT antibodies vs EIA TESTLINE

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

VNT vs EIA competitor IgG (DE)

		EIA pos	EIA neg
VNT	pos	92	8
	neg	0	0
Agreement		**92 %**	

All classes of VNT antibodies vs EIA competitor (DE)

		EIA pos	EIA neg
VNT	pos	96	4
	neg	0	0
Agreement		**96 %**	

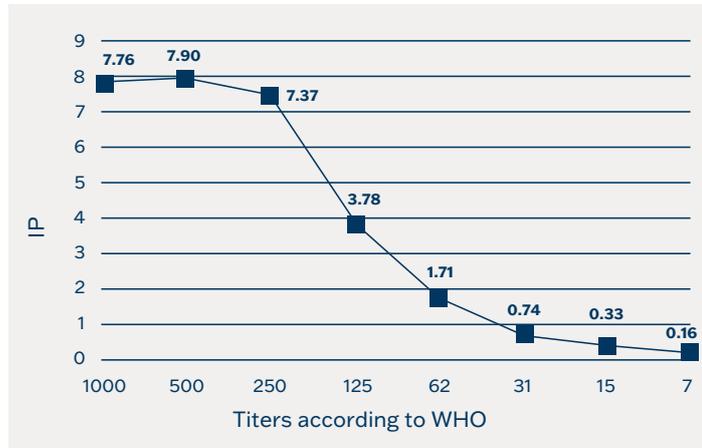### Mean Positive Index (IP) values of IgG anti-RBD antibodies (TestLine) in relation to individual VNT titers

Data points (Titer VNT, Mean IP): 20 → 2.13; 40 → 4.14; 80 → 6.11; 160 → 9.39; 320 → 10.84; 640 → 11.55; 1280 → 11.40

Mean values of U/ml IgG anti-RBD antibodies (TestLine) in relation to individual VNT titers

Data points (Titer VNT, Mean U/ml): 20 → 61.34; 40 → 127.39; 80 → 191.09; 160 → 286.11; 320 → 295.12; 640 → 313.39; 1280 → 320.00
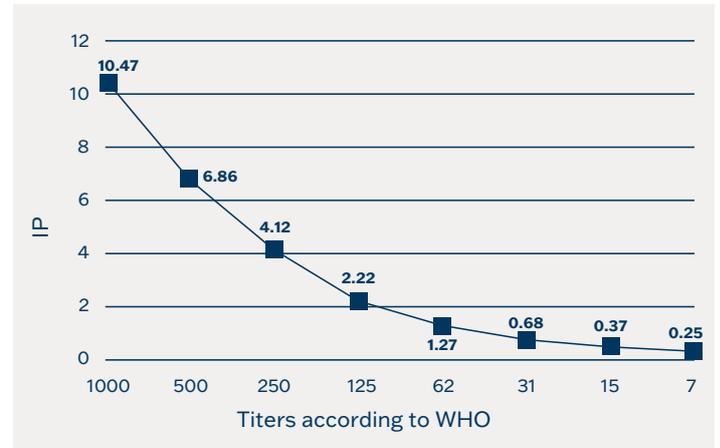
Compliance with WHO standard

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.
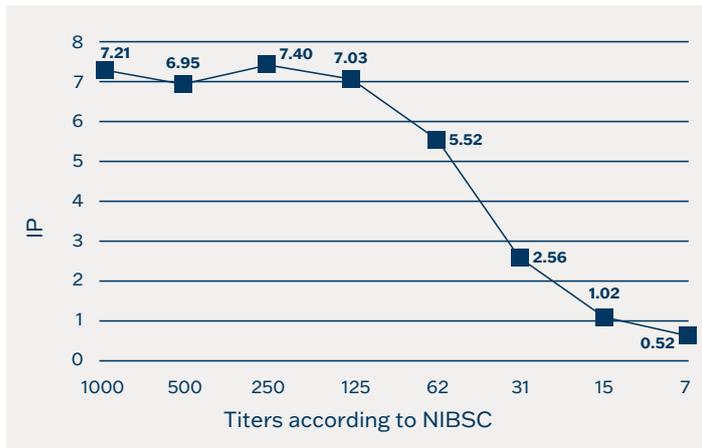

Titration of the WHO 20/136 IgA standard
A group of convalescent plasma from patients recovered from COVID-19, containing high titers of antibodies against SARS-CoV-2.
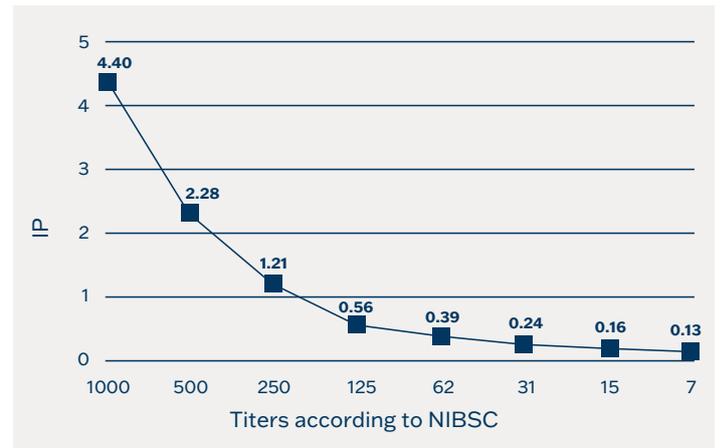

Titration of the NIBC 20/162 IgG standard
High titer anti-SARS-CoV-2 antibody material was used to assess and compare relative sensitivities for Anti-Sars CoV-2 determination of antibodies by dilution at the endpoint.

Titration of the 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.

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. According to the achieved titer of the Standard was recommended further dilution to determine the level of antibody decline in strongly positive results over time. 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 high level of agreement between results obtained by Virus Neutralization Test and TL EIA COVID-19 RBD IgG kit. The level of neutralizing antibodies corresponds with level of anti-RBD IgG antibodies and therefore EIA kits developed by TestLine can be used to determine if concentration of antibodies has the 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
CoRA96	EIA COVID-19 RBD IgA	96
CoRG96	EIA COVID-19 RBD IgG	96
CoRM96	EIA COVID-19 RBD 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

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

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