SARS-CoV-2 ProtectAbility EIA

SMART WAY TO DETECT SARS-CoV-2 NEUTRALIZATION ANTIBODIES

Quantitative determination of SARS-CoV-2 neutralizing antibodies, possible substitution for conventional Virus Neutralization Test

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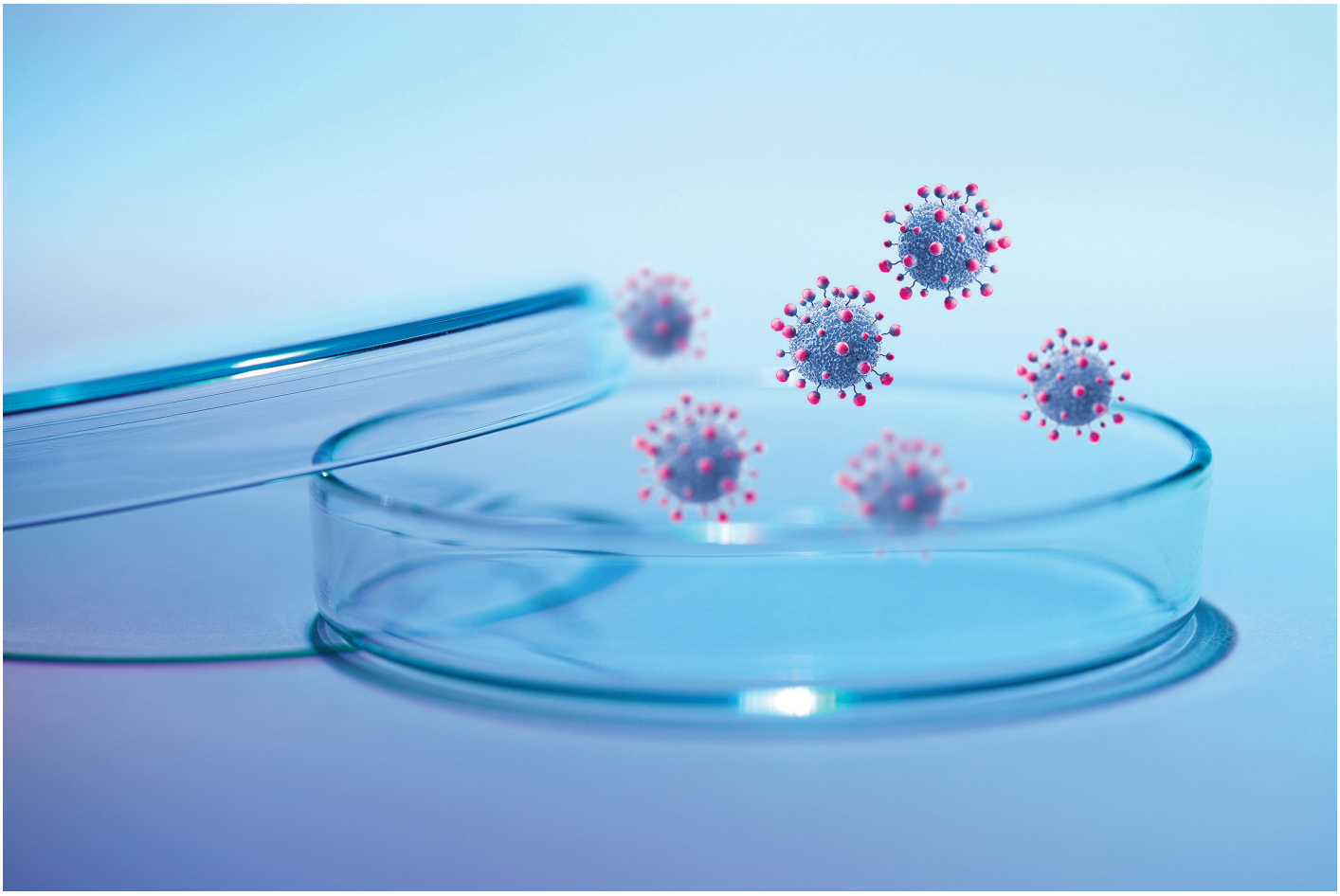
TESTING FOR SARS-CoV-2 BINDING AND NEUTRALIZING ANTIBODIES

The SARS-CoV-2 virus pandemic brought, among others, a need for testing methods that can reliably reflect the ability of organism to prevent from infection, either after previous infection or after vaccination. Fundamental constituent of adaptive immunity is the ability of immune system to produce neutralizing antibodies. Such antibodies can directly inhibit the infectivity of the virus by blocking viral structures that are needed for cell entry, in contrast to binding antibodies, i.e. antibodies that bind specifically to the pathogen but do not interfere with its infectivity.

A gold standard in the detection of the neutralizing antibodies is Virus Neutralization Test (VNT). It is performed in vitro with serum sample added to cell culture in the presence of virus. The test is run in different dilutions of the sample and enables to assess the degree of inhibition of virus infectivity. However, the test is laborious, it lasts several days and can be carried out only in laboratories with high level of biosafety protection.

Rapid and cheap alternative to VNT is a use of immunoassay, which determines the amount of anti-viral antibodies. The main drawback of immunoassays is their inability to recognize between neutralizing and binding antibodies.

To overcome this disability, recent SARS-CoV-2 antibody assays employs viral structures that are engaged in host cell penetration. The SARS-CoV-2 virus enters the host cell via interaction between receptor binding domain (RBD) of SARS-CoV-2 spike protein and angiotensin-converting enzyme 2 (ACE2) receptor that is present on the surface of several human cell types. Therefore, immunoassays employ S protein or its part to determine the antibodies against these structures only, to reduce partially the amount of determined antibodies that do not have virus neutralizing effect.

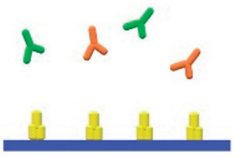


Determination of neutralization antibodies by SARS-CoV-2 Protect**Ab**ility EIA

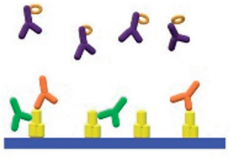
SARS-CoV-2 Protect**Ab**ility EIA assay is based on a new concept. It uses neutralizing, mouse monoclonal antibody (clone 2D11) against SARS-CoV-2 as a tool that enables selective recognition of only those antibodies that have neutralizing effect on SARS-CoV-2 binding to ACE2 receptor.

SARS-CoV-2 Protect**Ab**ility EIA is an ELISA-like assay. Samples are incubated in the wells coated with proteins containing RBD of SARS-CoV-2 S1 protein. Antibodies against the immobilized proteins, if present, bind to the surface of the wells. Then, a conjugate of monoclonal antibody 2D11 with horseradish peroxidase (HRP) is added to occupy the remaining free binding sites on RBD. The amount of bound antibody-HRP conjugate is visualized by colorimetric reaction. Optical density, measured by ELISA reader, is indirectly proportional to the amount of SARS-CoV-2 neutralization antibody in the sample.

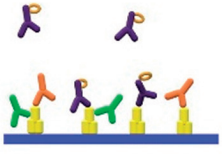
Assay Principle



Patient sample is incubated in a well coated with part of SARS-CoV-2 S1 protein containing RBD



The antibodies, if present in the sample, bind to the immobilized proteins. The well is then rinsed and conjugate of monoclonal antibody 2D11 with HRP is added.



Conjugate of monoclonal antibody 2D11 with HRP occupies the remaining free binding sites on RBD.

The well is rinsed to remove unbound conjugate. Enzymatic substrate TMB is added, enzymatic reaction stopped by HCl, and optical density read at 450 nm.

Binding antibody in sample

Neutralizing antibody in sample

Part of SARS-Cov-2 S1 protein containing RBD

Conjugate of neutralizing antibody 2D11 with HRP

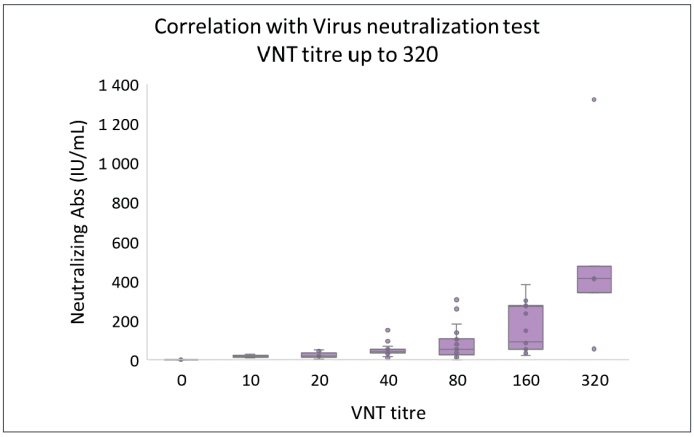
Possible substitute for conventional Virus Neutralization Test

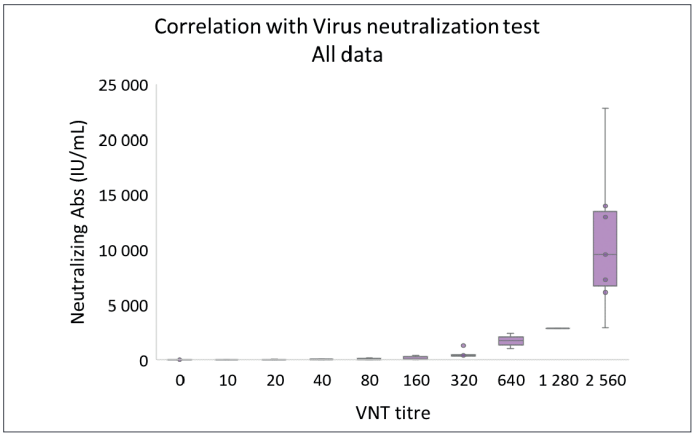
SARS–CoV–2 Protect**Ab**ility EIA assay provides rapid, easy-to-perform, cheap and reliable alternative to Virus Neutralizing Test.

Moreover, SARS–CoV–2 Protect**Ab**ility EIA can be performed in standard laboratory with common equipment, in contrast to specialized laboratory with biosafety level 3 for VNT.

Results are reported in IU/mL using a linear scale, not as individual, detached titers.

The agreement of SARS–CoV–2 Protect**Ab**ility EIA and VNT has been compared on 113 samples of individuals with or without previous SARS–CoV–2 infection and after vaccination.





Presented data show good agreement with VNT (Pearson correlation coefficient 0.857).

Quantitative determination with results reported in IU/mL

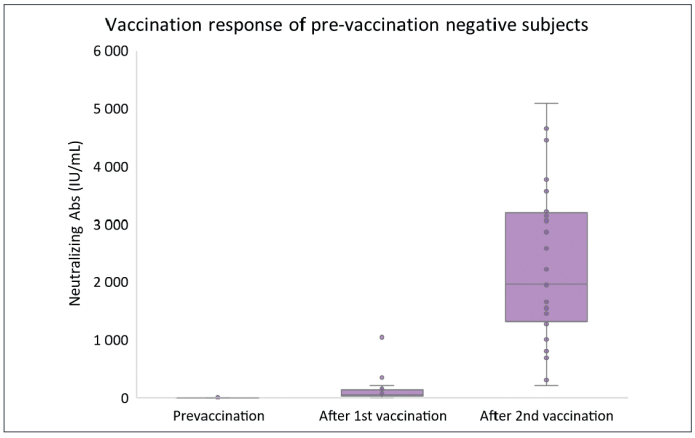
SARS–CoV–2 Protect**Ab**ility EIA assay enables quantitative determination of antibodies against SARS–CoV–2. Assay is calibrated against WHO International Standard 1st IS 20/136, and results are reported in international units to provide neutralizing antibody activity.

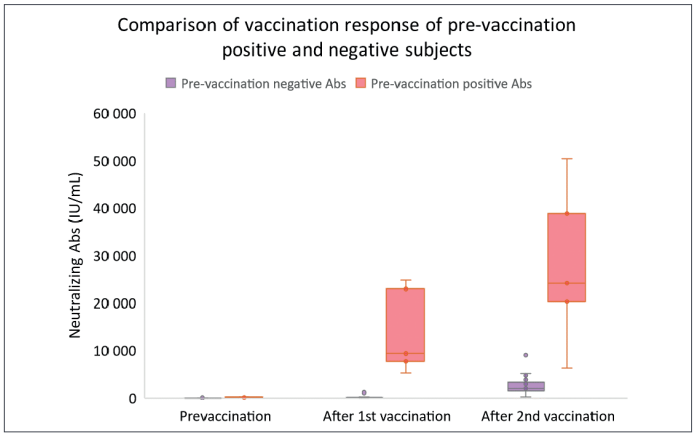
Long-term stability of calibration is warranted using pure, defined monoclonal antibody 2D11 in calibrators.

SARS-CoV-2 neutralizing antibodies – response to vaccination

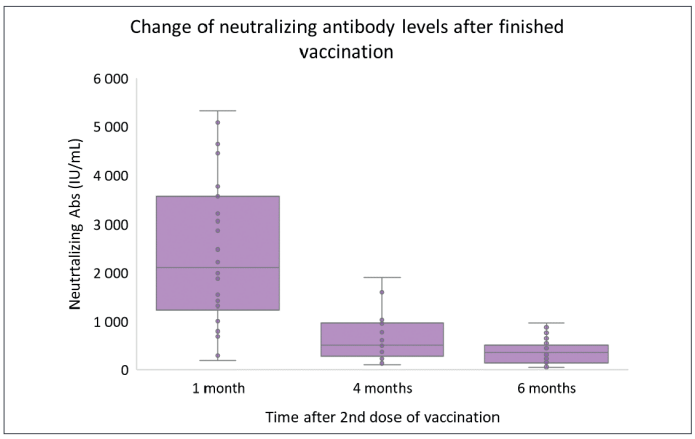
Samples from 35 volunteers were collected before vaccination, 20 days after the first dose, and 30 days after the second dose of mRNA vaccine. The samples were determined with SARS–CoV–2 Protect**Ab**ility EIA to assess the response of neutralizing antibodies to vaccination.

Significant increase of antibody levels appeared after the second dose of vaccine. The response to vaccination was much more pronounced on those with positive pre-vaccination level of antibodies (n = 5) than in those individuals who were negative (n=30) before vaccination.





SARS-CoV-2 neutralizing antibodies – changes with time



Samples from 30 volunteers were collected 1 month, 4 months and 6 months after finished vaccination with mRNA vaccine to assess the duration of neutralizing antibodies with time.

Clinical sensitivity and specificity

Cut-off value of SARS-CoV-2 Protect**Ab**ility EIA has been set at 18 IU/mL.

Values < cut-off	Values ≥ cut-off
125	0
Specificity = 100.0%	

125 pre-Covid samples were determined with SARS-CoV-2 ProtectAbility EIA to assess clinical specificity.

Values ≥ cut-off	Values < cut-off
115	10
Sensitivity = 92.0%	

125 samples were collected from individuals who had been tested positive for SARS-CoV-2 by PCR. Samples were collected not sooner than 14 days and not later than 6 months after positive PCR test, from both symptomatic and asymptomatic individuals.

SARS-CoV-2 Protect**Ab**ility EIA – summary of assay characteristics

REF #	C77994
Principle	Two step EIA
Target	SARS-CoV-2 neutralization antibodies, all Ab classes
Sample type	Serum, EDTA plasma
Sample volume	25 µL
Incubation	2 x 1 hr/shaking + 10 min
Traceability	WHO International Standard 1st IS 20/136
Calibration	5 point calibration, range 0, 30 – 400 IU/mL Based on defined monoclonal antibody 2D11
Controls	2 levels (low, high)
LoD	16.8 IU/mL
Cut-off	18.0 IU/mL
Clinical specificity	100.0%
Clinical sensitivity	92.0%

POSSIBLE USE OF SARS-CoV-2 Protect**Ab**ility EIA

Stand-alone test

To aid in diagnosis of previous SARS-CoV-2 infection and for the assessment of immune response efficacy after vaccination and previous infection

Confirmatory test

To confirm equivocal or dubious results obtained by the assay based on another principle, e.g. binding antibody assay

SARS-CoV-2 Protect**Ab**ility EIA benefits

Neutralizing Abs

Assay determines selectively neutralizing Abs which block SARS-CoV-2 binding to ACE2 receptor of human target cells

Substitute for VNT

Rapid, easy-to-perform and cheap alternative to Virus Neutralization Test

Calibration to WHO

Quantitative determination with calibration against WHO 1st IS 20/136

Literature

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2. Muruato, A. E., Fontes-Garfias, C. R., Ren, P. et al.: A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. Nat Commun 11, 4059 (2020). https://doi.org/10.1038/s41467-020-17892-0 [Accessed September 2, 2021].

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6. Beckman Coulter SARS-CoV-2 Protect**Ab**ility EIA Instructions For Use, version PI-C77994–01 of 31 August 2021.

For additional information, please contact your local Beckman Coulter representative or e-mail us to imunochem@beckman.com