Detection of neutralizing antibodies to SARS-CoV-2 with the surrogate SARS neutralization test

For almost a year, it has been possible to routinely determine the antibodies to SARS-CoV-2-IgM, -IgA and/or -IgG in medical laboratories. Although this is enough to answer the question of a past infection, an assessment of the actual protective effect (following infection or vaccination) by determining antibodies is only possible to a limited extent. It has not been possible to perform plaque reduction neutralization tests (PRNT) that are often discussed in this context, i.e. tests that determine whether antibodies have a protective effect, in private practice so far. These tests require a BSL3 safety standard (handling of viruses capable of replication), which can only be guaranteed by special virological laboratories at university hospitals.

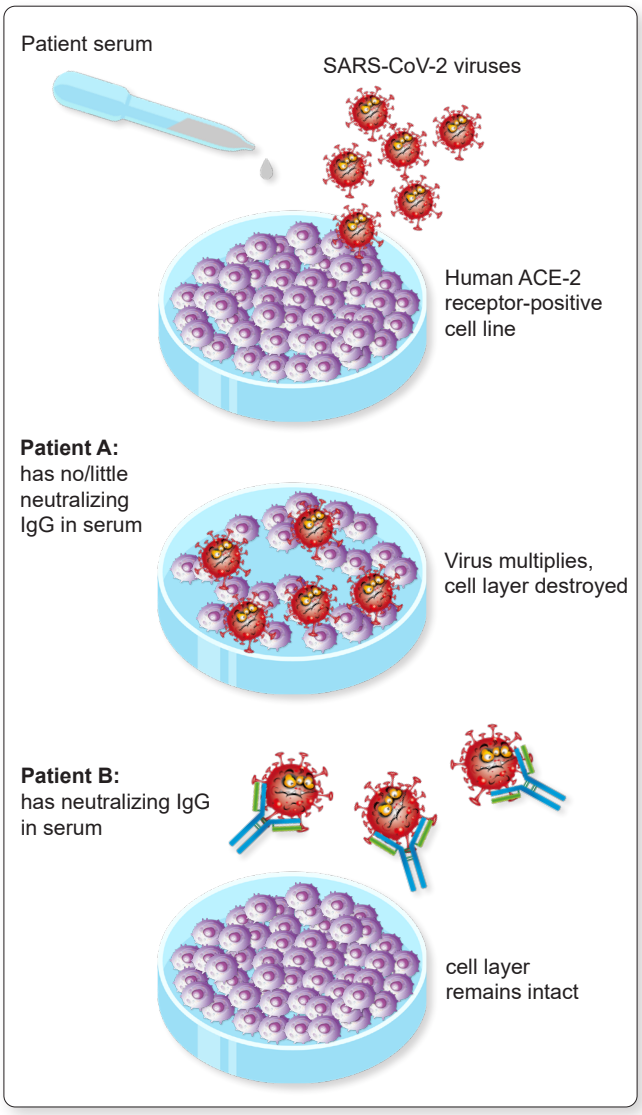

Fig. 1 Test principle of the classic plaque reduction neutralization test (PRNT).

An alternative is now available

With the surrogate virus neutralization test (sVNT), it is now possible to determine the inhibitory effect of neutralizing antibodies within a very short time using an ELISA test format (Tan et al., 2020). This test is now available in the IMD.

The test principle of the surrogate SARS neutralization test

In the SARS-CoV-2 neutralization test, the ELISA plate is coated with the human ACE-2 receptor. The patient's serum is incubated with an RBD fragment of the viral S1 protein. If the serum contains antibodies that can bind ("neutralize") the S1 protein, binding to ACE-2 receptors is inhibited in the second step. After several washing steps, the staining reaction (with TMB) and the photometric measurement, the inhibition of the RBD fragment binding is calculated in percent.

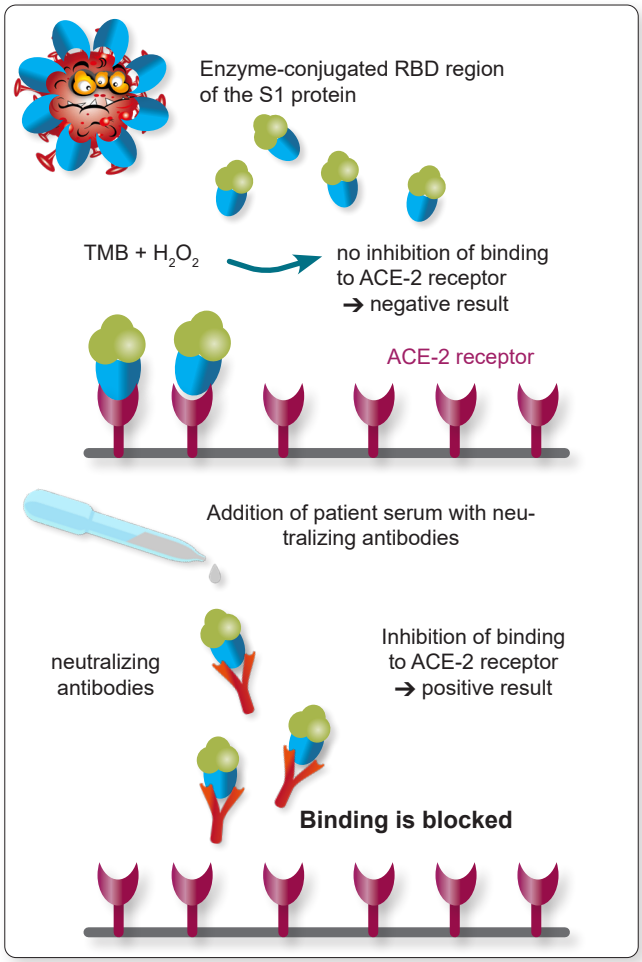

Fig. 2 Test principle of the new surrogate SARS neutralization test. If the patient's serum contains antibodies that recognize and "cover" the binding region (RBD) of the S1 protein, binding to the ACE-2 receptor is prevented in the test.

Do you have any questions? Our service team will be happy to answer them at +49 (0)30 770 01-220.

IMD Berlin MVZ
Nicolaistraße 22
12247 Berlin (Steglitz)
Tel. +49 (0)30 77001-220
Fax +49 (0)30 77001-236
Info@IMD-Berlin.de
IMD-Berlin.de

Studies show excellent correlation with plaque reduction neutralization tests (PRNT)

As shown in the figures from the cited publications, results of plaque reduction neutralization tests (PRNT) correlate very well with the surrogate SARS neutralization test. Figure 3b shows that an excellent differentiation between positive and negative serum samples can be obtained at a cut-off value of 30%.

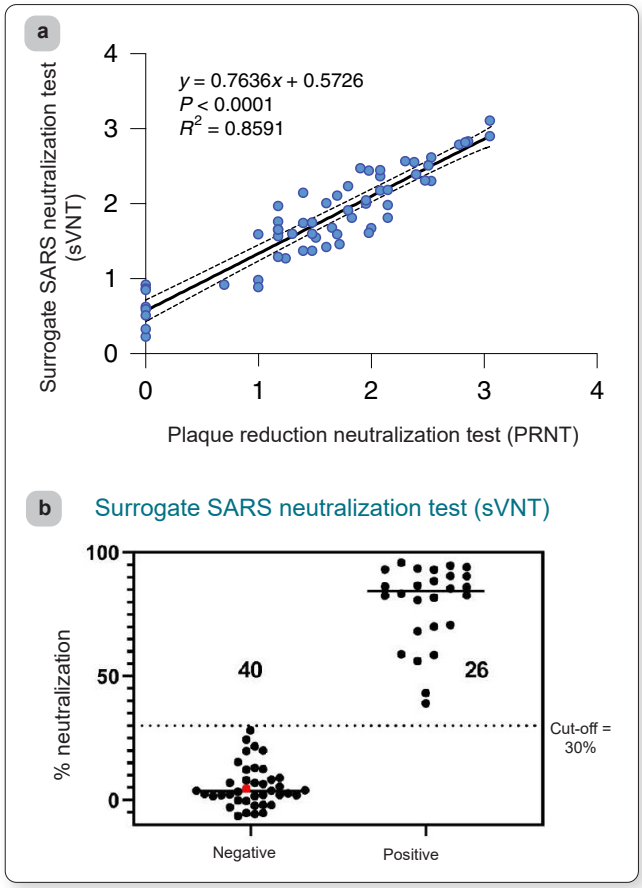

Fig. 3a from Chee Wah Tan et. al. Nature Biotechnology 2020. The figure shows the correlation between the classic virus neutralization test (cVNT), and the new surrogate VNT (sVNT), **Fig. 3b** from Sean C. Taylor et. al. shows that a clear distinction can be made between cVNT-positive and negative patients at a cut-off value of 30%.

IMD Labor Berlin — **Medical report**

Test	Result	Unit	Reference range
SARS-CoV-2 IgG Ab (S1) in serum (ELISA)	49.3	BAU/ml	< 25.6

Detection of IgG Ab against SARS-CoV-2 spike protein (S1).
This finding either suggests that an infection with SARS-CoV-2 has taken place or, in the case of a previous vaccination, that a desired vaccination reaction has been obtained.

Fig. 4 Measurement of IgG antibodies against the spike protein 1 (S1) 4 weeks after vaccination

IMD Labor Berlin — **Medical report**

Test	Result	Unit	Reference range
neutral Ab SARS-CoV-2 in serum. (ELISA)	85	%	> 30

The test measures the inhibition of the virus compound (RBD region) at the host cell receptor (ACE2) by specific SARS-CoV-2 antibodies present in the serum.

% Neutralization	test result
< 30%	no neutralization capacity
30-50%	low neutralization capacity
51-74%	moderate neutralization capacity
from 75%	high neutralization capacity

Fig. 5 Despite a relatively low IgG (S1) titer (4 weeks after vaccination; see Fig. 4), this patient has a clearly positive neutralization test.

What does the test reveal?

The following figure shows the first results from IMD Berlin. The classic IgG(S1) antibody sufficiently differentiates between patients with and without the neutralization effect of their IgG antibodies. However, qualitative differences can be observed with one and the same IgG(S1) titer, which in individual cases reach 20-30%. In no case did patients with isolated positive IgG (Nc) antibodies show positive results in the neutralization test (4 patients shown in red).

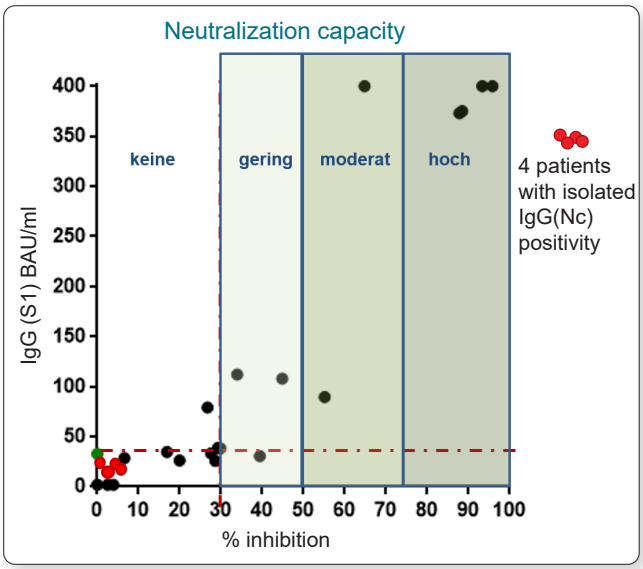

Fig. 6 First data from the IMD on the neutralization capacity of IgG-S1 antibodies

Material

1 ml of serum

Transport to the laboratory is not time-critical, and samples can be sent by post.

Invoicing

Costs of the test are 35.44 €.

Important note:

We would like to point out that, according to current jurisdiction, immunological findings, i.e. SARS-CoV-2 antibodies or a SARS-specific T cell response, do not amount to "proof of immunity" which, according to the current legal situation, equates you with vaccinated or convalescent people.

Literature

- Addetia, A. et al. Neutralizing Antibodies Correlate with Protection from SARS-CoV-2 in Humans during a Fishery Vessel Outbreak with a High Attack Rate. JCM. Oct 2020, 58 (11)
- Tan, C.W. et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat Biotechnol 38, 1073–1078 (2020)
- Wu, J. et al. SARS-CoV-2 infection induces sustained humoral immune responses in convalescent patients following symptomatic COVID-19. medRxiv. 2020
- Sean C Taylor et al. A New SARS CoV-2 Dual Purpose Serology Test: Highly Accurate Infection Tracing and Neutralizing Antibody Response Detection. J Clin Microbiol 2021 26;JCM.02438-20. doi: 10.1128/JCM.02438-20.