DIA.PRO

NEUTRALIZING ANTIBODIES

to SARS-CoV-2

A brief insight

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Vaccine Follow-up

Herd Immunity

Plasma Therapy

A SHORT BACKGROUND

What is SARS-CoV-2?

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the pathogen responsible of causing the current coronavirus outbreak. On February 11th 2020, the World Health Organization (WHO) named the disease caused by this new virus "COVID-19". According to WHO, globally, as of 10 February 2021, there have been 106.321.987 confirmed cases of COVID-19 including 2.325.282 deaths.

SARS-CoV-2 structure

SARS-CoV-2 is a positive-sense single-stranded RNA virus and belongs to the Betacoronavirus Genus. Same as all other coronaviruses, the genome of SARS-CoV-2 encodes the RBD/spike protein, the envelope protein, the membrane protein and the nucleocapsid protein. The N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope (Fig. 1).

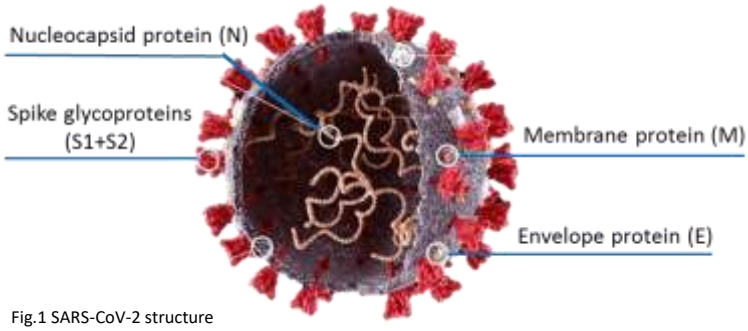

Fig.1 SARS-CoV-2 structure

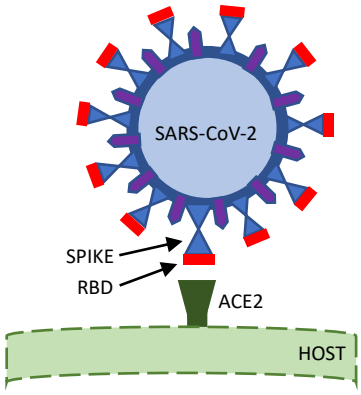

Fig 2. RBD on virus Spike protein binding to host receptor

The spike protein is composed of two subunits, S1 and S2. The S1 subunit contains a small peptide region called RBD (receptor-binding domain) that recognizes and binds to the host receptor ACE2 (angiotensin-converting enzyme 2), while the S2 subunit mediates viral cell membrane fusion. Consequently, RBD, situated in the S1 subunit, is the responsible for allowing the virus to attach to a host cell and then infect them (Fig. 2).

Immune response against SARS-CoV-2

Seroconversion after exposure to SARS-CoV-2 provides key information to understand the pandemic's past and predict its future. Serological tests help define the infection status, a previous exposure to SARS-CoV-2 and detect seroprevalence in a given population. These tests are able to detect binding antibodies (IgM, IgA, IgG) that attach to SARS-CoV-2. However only a small percentage of antibodies bind to sites on the virus that interact with host proteins inhibiting entry of that virus into the host. These are known as neutralizing antibodies. The main target for neutralizing antibodies on SARS-CoV-2 is the RBD region on spike protein.

What are Neutralizing antibodies?

Different from binding antibodies, neutralizing antibodies block the interaction between virus proteins (SPIKE1-RBD) and Human host cell receptors (ACE2) and thus stop the viral entry and further proliferation. While other **generic anti-Spike antibodies simply "disturb" the binding** of the virus, **antibodies directed specifically at RBD region on spike protein**, which are produced later during infection, **are known to have a neutralizing effect on SARS-CoV-2 by blocking the virus' binding to the ACE2 receptor** (Fig. 3).

The development of neutralizing antibodies against the RBD region is the target of many of the vaccines in development and/or approved and are considered an important biomarker of humoral immunity.

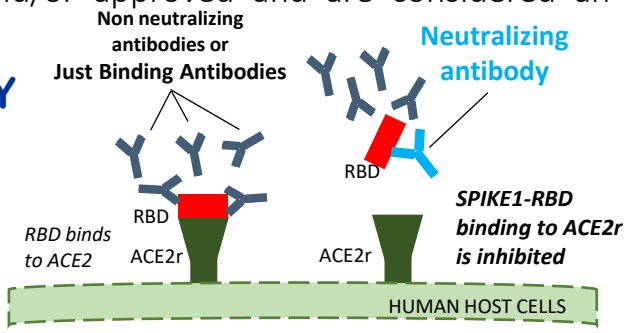

Fig.3 Neutralizing antibodies inhibiting RBD-ACE2 binding

DIA.PRO NEUTRALIZING ASSAY

Introduction

The test code ACE2-RBDNEUTR.CE developed by DIA.PRO is a CE-marked ELISA able to specifically detect the neutralizing antibodies produced to SARS-CoV-2 RBD antigen.

Currently available serological tests on market only detect the presence of antibodies to SARS-CoV-2 proteins, regardless of whether or not they are really efficient in neutralizing the virus.

DIA.PRO neutralizing assay, in addition to the determination of the presence of total antibodies to RBD, measures the real biological activity of the antibodies in inhibiting the binding of RBD of SARS-CoV-2 to its receptor ACE2, thus preventing the virus from entering into the target cells (Fig. 3). ACE2-RBDNEUTR.CE become the assay for excellence in the determination of the effectiveness of naturally produced or vaccine-induced anti RBD antibodies.

Principle of the test

The inhibition of the binding between ACE2 and RBD is determined by means of an ELISA carried out on plasma/serum whose antibodies neutralizing action wants to be measured. Microplates are coated with SARS-CoV-2 specific recombinant glycosylated RBD produced in mammalian cells. The sample is incubated allowing anti RBD/Spike antibodies, if present, to bind to such antigen. After washing free RBD/Spike are determined by the addition of recombinant ACE2 biotinilated antigen and then in sequence of SAV-HRP. A color will be generated by TMB/H2O2 if no antibodies have bound to RBD while a strong inhibition on the color development will be observed in case antibodies to RBD have blocked the binding of the biotin-labelled ACE2 to it. The presence of such antigen on the solid phase is finally determined by the addition of SAV-HRP, which will bind to ACE2 if no neutralizing antibodies are present or not in case antibodies have blocked the coated RBD.

Assay principle

The assay provides the possibility to perform two different tests:

1. Screening Assay: The screening assay is recommended as a first test for antibodies to RBD/Spike in COVID-19 recovered people and vaccinated individuals;
2. Neutralizing Titration Assay: The titration of the neutralizing bioactivity of a sample, positive in the previously described screening assay, is recommended to determine the strength of such antibody to neutralize the binding between RBD and its receptor ACE2;

Assay features

DIA.PRO assay is a surrogate Virus Neutralizing Test (sVNT), a method that mimic the virus-host interaction in an ELISA plate well. It uses SARS-CoV-2 specific recombinant glycosilated RBD instead of the live virus providing a safer solution since no cells, biosafety containment facilities nor highly skilled operators are required. Results are faster (less than 2 hours) and the test is easily adaptable on ELISA automation making it an ideal solution for high-throughput.

Comparison to competitors

	DIA.PRO	Competitors
TEST PROCEDURE	**NO pre-analytical step.** **FULL** process in the microplate for reverse binding	Pre-analytical step needed. Neutralization step followed by microplate reverse binding
PROCESS AUTOMATION	**Easy adaptable to any ELISA workstation**	Difficult and time consuming. Not easily adaptable on ELISA workstation
STRATEGIC MATERIALS	Microplate coated with RBD ACE2-biot-SAVHRP as conjugate	Microplate coated with ACE2 RBD-HRP as conjugate
TIMING	Max 2 hours **Perfectly ADAPTABLE with full automation massive screening**	Pre analytical step + test procedure Incubation time NOT adaptable to a massive screening

Diagnostic performance

100% SENSITIVITY
SPECIFICITY

Sensitivity was evaluated by testing, in two studies, total 150 IgG samples tested positive in the DiaPro's ELISA code COV19GSPIKE.CE. Specificity was evaluated by testing 440 sera prescreened negative for IgG to RBD/SPIKE with the DiaPro's specific ELISA.
Additional data available on request.

Potential uses

Determination of vaccination effectiveness by assuring a reliable positive and efficient immunization with development of high titer neutralizing anti RBD/Spike IgG antibodies. The test can be also used to monitor durability of neutralizing antibody response

Assessing natural immunity by confirmation of anti RBD antibodies neutralizing activity in patients positively recovered from COVID-19 showing antibodies to RBD/Spike

Identification of individuals who developed a strong neutralizing antibodies response and could serve as donors of convalescent serum (or hyperimmune plasmas) as a therapeutic in COVID-19 patients

Kit specifics & ordering information

Method: ELISA

Sample: Plasma or Serum

Shelf life: 15 months

Storage temperature: +2°…+8°C

NAME	CODE	FORMAT
ACE2-RBD Neutralization	ACE2-RBDNEUTR.CE	96 TEST

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