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European Medicines Agency

Advice from the medical device Expert Panels

Mandate[1] & Advice provided to the MDCG

[1] According to the section 6.3 of the Rules of procedure of the European Commission expert panels on medical devices and in vitro diagnostic medical devices.

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Contents

1. Legal basis for the request

Article 106 (10) (a) and (b) of Regulation (EU) 2017/745 on medical devices.

2. Party requiring the advice

The Medical Device Coordination Group (MDCG).

3. Scientific context and background information

Various devices detecting antibodies against SARS-CoV-2 have been placed on the market in the EU since 2020. The design of these devices has evolved according to the increasing knowledge of the virus and of the immunological response of infected and/or vaccinated people.

Since early 2021, some manufacturers have placed on the market serological tests, for which the intended use is defined as detection of neutralising antibodies against SARS-CoV-2, after vaccination or possible infection. Manufacturers may also refer to the level of serological protection from COVID-19. The principle of these tests is most commonly based on detection of (certain) antibodies against the receptor-binding domain (RBD) of the SARS-CoV-2 S-protein.

As part of the performance study for these devices detecting (neutralising) antibody binding to RBD of the S-protein, in most cases, the manufacturers establish the diagnostic sensitivity and specificity of their devices by comparing the results obtained by their devices with the status of patients known to have been previously infected (or not) with the virus (i.e. positivity/negativity, generally established by RT-PCR). Manufacturers may also establish the diagnostic sensitivity by testing whether their device gives positive results for vaccinated patients. The justification of the intended purpose therefore rests on the design of the device, i.e. detection of the anti-RBD antibodies, and the verification that the device detects antibodies in previously infected or vaccinated patients, and does not detect them in non-infected patients. Only in rare cases have manufacturers carried out studies to confirm the intended purpose of "detection of neutralising antibodies" by evaluating the concordance of results on the same serum specimens between their device and a SARS-CoV-2 neutralisation test.

Regulation (EU) 2017/746 lays down that devices must be designed and manufactured in such a way that they are suitable for their intended purpose, as specified by the manufacturer. They must take account the generally acknowledged state of the art. They must achieve the performance intended by their manufacturer. This request concerns the question of which performance study tests could be considered as evidence that the device is suitable for its intended purpose of detecting neutralising antibodies and that it achieves the performance stated by the manufacturer

4. Relevant medical field and areas of competence required

In vitro diagnostic medical devices, virology, SARS-CoV-2.

5. Specific thematic panel or panel sub-group best suited to address the request for advice (if applicable)

In vitro diagnostic medical devices (IVD).

6. The scope of the advice is to address the following questions

Addressing the following questions:

1. Would the design of a device based on detecting antibodies against the RBD domain of the SARS-CoV2 S-protein, together with studies showing that it detects antibodies in patients known to be previously infected or not with the virus (positivity/negativity established generally by RT-PCR) or in vaccinated patients (assumed positive for the antibodies) be sufficient to claim detection of neutralising antibodies against SARS-CoV-2?

1. If not, which type of studies should the manufacturer carry out, taking into account the generally acknowledged state of the art, to provide evidence that the device designed to detect anti-RBD antibodies can be intended for detection of neutralising antibodies against SARS-CoV-2?

7. Timetable for providing the advice

Start of the advice: 11/05/2023

Advice delivered to the MDCG: 10/07/2023

8. Consultation or collaboration with other scientific bodies for the preparation of the advice (if necessary)

N/A

9. Advice provided by the IVD Expert Panel

9.1. Background information

9.1.1. Scientific context and background information of the Mandate

Since the beginning of 2021, some manufacturers have launched serological tests based on the detection of (certain) antibodies against the receptor binding domain (RBD) of the SARS-CoV-2 Spike protein, indicating as intended use the "detection of neutralising antibodies against SARS-CoV-2 after vaccination or possible infection", with some manufacturers also indicating the level of putative serological protection against COVID-19.

MDCG thus raises the questions of whether a binding assay can claim to detect neutralising antibodies and what performance studies should be considered to demonstrate that the product is fit for this claimed purpose.

9.1.2. Definitions

Antibody binding assays and neutralisation assays are two different test types to analyse the interaction between specific antibodies and a virus, such as SARS-CoV-2.

Antibody Binding Assay, is a ligand-receptor test that detects the presence of antibodies to a specific antigen, such as the receptor binding domain (RBD) of the SARS-CoV-2 spike (S) protein. These tests can be used to determine whether the immune system has produced antibodies directed against the specific antigen(s) of the SARS-CoV-2 virus in response to infection or vaccination. In the antibody

binding assay, the test sample (e.g., serum or plasma) is incubated with one or more viral target antigens (e.g., RBD) immobilised on a solid phase. If antibodies to the target antigen(s) are present in the sample, they bind to the antigen to form an antibody-antigen complex. The complex is then detected with a secondary antibody labelled with a chromogen or fluorescence marker or conjugated to an enzyme that then catalyses a substrate for a colour reaction or other signal measurement reactions. The development of colour/fluorescence corresponds to the presence of antibodies. The test result is usually expressed as qualitative (positive/negative) or quantitative (antibody titre). Most commercially available antibody tests, such as enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), immunofluorescence assay (IFA), or rapid antibody test, are designed to detect binding antibodies. However, they do not provide direct information on the neutralising capabilities of the detected antibodies.

Neutralisation Assay, or virus neutralisation test (VNT), measures the ability of antibodies to neutralise the infectivity of a virus. These tests measure the functional aspect of certain antibodies targeting specific sites (epitopes) in the SARS-CoV-2 virus that can block viral infectivity, e.g., receptor binding, fusion of the virus with the cell membrane, entry of the virus into the cell, or virus replication. In a virus neutralisation assay, the test sample (e.g., serum or plasma) is incubated with a defined amount of replication-competent virus (e.g., SARS-CoV-2) and transferred to susceptible target cells. If antibodies in the sample are able to neutralise the virus, they will bind to the specific viral epitopes that prevent infection of susceptible cells. The degree of reduction in viral infection or replication compared to control samples indicates the presence and potency of neutralising antibodies. The potency of neutralising antibodies is quantitated by dilution series of individual sera, and the positive end point indicated as "neutralisation titre". Neutralisation tests using live virus are plaque neutralisation assay, microneutralisation assay (or CPE reduction assay), and focus neutralisation test; contrived neutralisations tests are pseudotype virus or virus-like particles carrying the SARS-CoV-2 S or RBD protein; and there are surrogate neutralisation tests. Live neutralisation assays such as the plaque neutralisation and microneutralisation assay are considered gold standards for measurement of neutralisation activity, estimated as closest to natural conditions based on the use of native infectious virus in combination with susceptible human cells. A variant-dependent neutralisation activity is determined by employing the corresponding virus variant.

1. Plaque reduction neutralisation test (PRNT): The antibody sample to be tested is diluted and mixed with a virus suspension to allow the antibody to react with the virus. The solution is poured over appropriate host cells in a semi-solid medium (e.g., agar or methylcellulose) so that the virus can spread only over adjacent cells to form plaques (areas of infected cells). The reduction of plaque-forming units indicates the neutralising activity of the antibodies.

2. Microneutralisation test (MNT): A defined quantity of virus (e.g., 100 tissue culture infectious dose 50 (TCID50)) is mixed with serial dilutions of the test serum. Following incubation to enable potential neutralisation activity, virus/serum mixtures are inoculated into susceptible culture cells in microplate wells and are examined for the production of virus-induced cytopathic effect (CPE) [1] or other indicator of viral growth. The cells are examined for the presence or absence of viral replication, by visual reading or by using a specific staining or detection method.

3. Focus reduction neutralisation test (FRNT): Serum is serially diluted, incubated with the virus and added to susceptible cells. After incubation, the cells are fixed and permeabilised. Infected foci (groups of infected cells) may be visualised by staining (e.g. crystal violet) or probed with specific antibodies to detect viral antigens or markers. Reduction of the viral foci compared to control samples (e.g. highest dilution that inhibit the foci by 50 %) indicates neutralising activity of the antibodies.

4. Pseudovirus neutralisation test (pVNT): Pseudoviruses (e.g., vesicular stomatitis viruses or lentiviruses) recombinantly expressing the S or RBD protein of SARS-CoV-2 are incubated in the presence of various concentrations of antibody. The pseudovirus also contains a reporter gene, e.g. luciferase that is expressed only after entry into the cell. The more pseudoviruses, the higher the intensity of the emitted light. In the case of neutralising antibodies entry into the cells is blocked, and a reduction in light emission is measured, indicated by an inhibitory concentration (e.g., IC50, IC80, or other) [2]. The pVNT can be packaged with S or the RBD protein of any variant as needed.
5. Virus-like particles neutralisation (VLP) neutralisation test: Artificially produced VLPs carrying SARS-CoV-2 S protein enter susceptible cells via binding to the receptor ACE2 and proteolytic cleavage of the viral fusion S protein. The VLPs contain an activator peptide that allows for immediate enzymatic measurement. This assay can also be adapted to different variants of the virus [3].
6. Surrogate virus neutralisation test (sVNT): This test measures the ability of antibodies in sera to inhibit (by a competitive EIA test format) the binding of recombinant SARS-CoV-2 RBD to plate-bound human ACE2 protein [4] as the major target of neutralising antibodies. Because the sVNT is not based on measuring actual infectivity reduction on virus-susceptible cells, it is referred to as a surrogate test. The sVNT can be adapted for different variants [5], though most commercial sVNTs do not seem to be designed to differentiate variants.

9.2. Questions and Answers

9.2.1. Would the design of a device based on detecting antibodies against the RBD domain of the SARS-CoV2 S-protein, together with studies showing that it detects antibodies in patients known to be previously infected or not with the virus (positivity/negativity established generally by RT-PCR) or in vaccinated patients (assumed positive for the antibodies) be sufficient to claim detection of neutralising antibodies against SARS-CoV-2?

An antibody binding assay targeting SARS-CoV-2 RBD or S will not be able to differentiate between binding antibodies and neutralising antibodies, nor can it determine the function of neutralising antibodies like a neutralisation assay. Therefore, a binding test cannot be referred to as a "neutralisation test". Also, simple equation of the outcome of a binding test with a claim for the detection of neutralising antibodies is not applicable.

Nevertheless, it is known from the scientific literature that S or the RBD protein based binding tests may show strong correlation between binding antibodies and the presence of neutralising antibodies [6,7,8,9]. All SARS-CoV-2 neutralising antibodies target the S protein [10], with RBD within the S protein as the major target [11,12,13]. For this reason, detection of binding antibodies to S or the RBD protein is reported to be useful for predicting the presence and amount of neutralising antibodies [14,15,16].

However, to justify the claim of neutralisation in a binding assay, additional studies are required. This can be done by comparative studies in which the results of antibody binding assays can be correlated with neutralisation assays [17,18,19]. This involves comparative testing of a number of samples in both the antibody binding tests and in an acknowledged neutralisation test. For this purpose, quantitative antibody binding assays based on the S or the RBD protein are most promising. This must be done for the individual binding assays because the results of the various SARS-CoV-2 antibody binding assays are not interchangeable across assays, nor is there a uniform ratio of binding to neutralising antibodies in the various assays [17,18,19,20,21].

9.2.2. If not, which type of studies should the manufacturer carry out, taking into account the generally acknowledged state of the art, to provide evidence that the device designed to detect anti-RBD antibodies can be intended for detection of neutralising antibodies against SARS-CoV-2?

Certain performance evaluation studies may show a correlation between binding and neutralising antibodies, taking into account potential limitations due to different SARS-CoV-2 variants (VOC) and antibody kinetics.

The following types of studies can be referred to for this purpose (not exhaustive):

1. Convalescent plasma studies: Using convalescent plasma from individuals who have recovered from SARS-CoV-2 infection or COVID-19, evaluate the correlation between the presence of anti-RBD- or anti-S-protein antibodies detected by the binding test and the neutralising capacity of the convalescent plasma [22].

2. Vaccine response studies: Evaluate the performance of the binding test in vaccinated individuals by comparing the results of the antibody detection with neutralising antibody activity induced by vaccination measured through established neutralisation assays [15,23,24,25].

3. In addition, antibody binding assays can be compared with the results of clinical and/or virological parameters associated with protection against SARS-CoV-2 by neutralising antibodies to support a correlation of binding assays with neutralisation. This may include, for example, clinical studies on a cohort of patients who had previously tested positive for SARS-CoV-2 infection or who had been vaccinated. Correlation between the presence of binding antibodies, as determined by the test, and clinical characteristics such as risk for symptomatic infection, severity of COVID-19 disease and hospitalisation rate [26] or duration of viral shedding [27] may indicate neutralising significance of the detected binding antibodies [28,29].
4. Competitive antibody binding assay format. Using the above sVNT (see section definitions, 6.), a test can detect binding antibodies in the sample that inhibit RBD/ACE2 interaction, mimicking neutralisation. These assays are now available from several manufacturers. With this assay design, the requirements for (i) sensitivity and specificity of a binding assay and (ii) inhibitory surrogate neutralising activity can be evaluated in the same test.

For the above studies, the WHO International Standard for anti-SARS-CoV-2 immunoglobulin [20,30] may be helpful. This standard distinguishes between activity for functional neutralising antibodies expressed in international units (IU/ml) and binding antibody units (BAU/ml) specific for the individual viral antigens and immunoglobulin class detected by the respective test, e.g., anti-RBD IgG. Using serial dilutions of the WHO standard, a calibration curve can be generated for each binding and neutralisation test, and the antibody concentrations in sera from vaccinated and/or convalescent individuals for the detection of binding antibody and neutralisation activity can be determined and the correlation analysed.

Any correlation identified in these studies should be statistically analysed, e.g., by regression analysis or other statistical means to demonstrate correlation with a neutralisation test. Furthermore, a sufficiently large sample size should be considered to ensure statistical significance.

The following potential limitations may need to be considered:

SARS-CoV-2 variants of concern (VOC): Current commercially available antibody binding tests are, as far as known, in most cases coated with proteins from wild-type SARS-CoV-2. It has been reported that the performance of binding tests may be reduced in samples derived from the currently predominant Omicron variant [31,32,33,34]. According to other reports, the basic performance of binding antibody assays does not appear to have been significantly affected by SARS-CoV-2 VOCs, including Omicron [20]. Neutralisation by viral variants with mutations in the S protein may be reduced or, as with Omicron, may escape the majority of neutralising antibodies [34,35,36,37,38]. As a result, both the basic performance of binding and neutralisation tests and their correlation to each other may vary depending on specific VOCs. On the other hand, published reports suggest that binding tests correlated well with neutralisation titres across multiple VOCs [14,39,40] due to pre-existing immunity (induction of antibodies specific to the first-encountered variant) and the formation of cross-reactive neutralising antibodies. Nevertheless, published results show that the VNT remains the method of choice for unequivocal detection of VOC-specific neutralising antibodies [41]. The sVNT does not detect neutralising antibodies outside of the RBD/ACE2 interaction, which may be important with escape variants increasingly mutated in RBD [41]. In addition, most sVNTs do not appear to be adapted to viral variants.

The manufacturer should address potential performance limitations due to viral variants. If a correlation with neutralisation is claimed, the impact of VOCs on correlation with the binding assay should be justified and evaluated. This needs to be updated with the emergence of new viral variants involving mutations in the S protein.

Time dependence: Persistence and concentration of binding antibodies and neutralising antibodies may reveal different kinetics over time depending on the design of the binding test [42], and possibly individual patient conditions [43]. This may change the correlation between binding and neutralising test, and longitudinal studies may be needed to determine whether a test consistently detects binding and neutralising antibodies over time. The manufacturer should therefore justify the specific claim of the test and respective potential limitations.

10. Literature

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