

Sensitive immunodetection of SARS-CoV-2 variants-of-concern 501Y.V2 and 501Y.V1

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Summary: Antigen-based point-of-care tests are implemented worldwide for COVID-19 detection. A recently developed S1-based test, enabled sensitive and accurate detection of emerging SARS-CoV-2 variants-of-concern from nasopharyngeal swabs.

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Abstract:

Emerging SARS-CoV-2 variants potentially influence the effectiveness of existing laboratory diagnostics. In this study we determined whether the British (20I/501Y.V1) and South-African (20H/501Y.V2) SARS-CoV-2 variants-of-concern (VOC) are detected by an in-house S1-based antigen-detection assay. Analysis was performed in spiked pools of qRT-PCR negative nasopharyngeal swab specimens. The assay, composed of a combination of four monoclonal antibodies, allowed sensitive detection of both the wild-type and the analyzed VOCs, despite the accumulation of several mutations in the variants' S1 region. We suggest that the combination of four monoclonal antibodies, targeting distinct epitopes, maintained both the specificity and the universality of the assay.

Key words: SARS-CoV-2, Variants-of-Concern, 501Y.V1, 501Y.V2, Antigen-detection, spike protein, Antibodies

Background:

Antigen-based rapid-tests are used worldwide in various screening schemes for COVID-19 detection [1]. For example, China, Vietnam and Slovakia apply mass repeat testing to stop community transmission. In a similar manner, Great-Britain and Austria, apply regular testing in high risk settings (care homes, prisons etc.), to protect clinically vulnerable populations. Japan, on the other hand, uses rapid tests for cluster identification, focusing on symptomatic individuals. Due to their low sensitivity compared to qRT-PCR tests [1, 2], antigen-based tests are mostly used as rapid indicators of disease infectivity rather than infection (high viral loads) for both symptomatic and asymptomatic individuals [1].

The recent emergence of various SARS-CoV-2 variants, raises concerns regarding the ability of these variants to evade natural or vaccine-induced immunity as-well-as detection by specific diagnostic tests. Of special interest are the British (501Y.V1) and South-African (501Y.V2) variants-of-concern (VOC) which were first identified in the United Kingdom and South Africa respectively, between October and mid-December, 2020. Due to increased transmission, these variants have since been detected in many countries around the world. Both variants have multiple mutations and/or deletions in the spike protein (<https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#Consequence>). There is some evidence indicating that one of the spike protein mutations present in the 501Y.V2 VOC, E484K, may affect neutralization by some polyclonal and monoclonal antibodies [3] as opposed to the 501Y.V1 variant, where evidence indicates that the mutations do not impact vaccine efficacy [3-6].

While the widely used qRT-PCR enables detection and differentiation between different SARS-CoV-2 variants [7, 8], it is of interest to determine whether antigen-based detection, specifically spike-based, enables detection of SARS-CoV-2 emerging variants. We have

recently isolated a panel of high-affinity monoclonal antibodies directed towards the SARS-CoV-2 spike protein [9, 10]. These antibodies were characterized and shown to target seven discreet epitopes on the Receptor-Binding-Domain (RBD) and the N-Terminal-Domain (NTD) of the spike protein [9, 10]. The development of a TRF-ELISA assay, based on a combination of four of these antibodies, was reported recently [11]. In this diagnostic assay, two of the anti-RBD antibodies (MD29 and MD65) were applied as reporter antibodies and an additional anti-RBD (BL6) coupled with an anti-NTD antibody (BL11) as capture antibodies. The sensitivity and specificity of the developed assay for SARS-CoV-2 detection were successfully demonstrated in a large cohort of qRT-PCR positive and negative nasopharyngeal swab specimens from symptomatic and asymptomatic individuals. In the present work we employ the developed S1-based antigen assay to compare the detection capability of both the wild-type (W.T.) SARS-CoV-2 and the 501Y.V1 and 501Y.V2 VOC.

Materials and Methods:

Antigens and antibodies: The 501Y.V1 and 501Y.V2 variants were isolated by the Israeli Ministry of Health and sequenced by NGS (IIBR). W.T. SARS-CoV-2 (GISAID accession EPI_ISL_406862) and variants were cultivated as described [12]. Antibodies were isolated, purified and biotinylated as described [9-11].

Binding profile: SARS-CoV-2, 501Y.V1 and 501Y.V2 viruses (1×10^5 pfu/ml) were captured by a rabbit polyclonal antibody fraction raised against recombinant SARS-CoV-2 spike protein [9] and reacted with serially diluted (10-1500 ng/ml) biotinylated monoclonal antibodies (MD29, MD65, BL6 and BL11) and Human-Fc ACE-2. Reporter antibodies were then detected with StreptAvidin-Europium^{III}, followed by enhancement solution (Delfia, Perkin-Elmer). S/N ratios were calculated as the signal (S) of antibody and virus containing wells vs virus-only wells (N).

S1 -based TRF ELISA detection assay: The S1-based assay, schematically depicted in Fig 2A, was performed as described [11]. In short, BL11 (α NTD Ab) and BL6 (α RBD Ab) were adsorbed to MaxiSorp ELISA plates. SARS-CoV-2 W.T and VOCs were serially diluted in pooled negative clinical samples or sterile swab buffer and incubated with the adsorbed antibodies, followed by consecutive incubations with biotinylated reporter antibodies MD29 and MD65 (α RBD Abs), StreptAvidin-Europium^{III} and finally, enhancement solution. Assay's limit of detection was calculated as described previously [11].

Clinical samples: Negative qRT-PCR nasopharyngeal swab samples from symptomatic and asymptomatic individuals were collected as part of routine scanning of nursing homes. Ethical review and approval were waived, since the samples used for this study were leftovers of anonymized samples.

Results:

Antibody binding profile: The binding profiles of the anti-RBD (BL6, MD29 and MD65) and anti-NTD (BL11) antibodies employed in the S1-based assay, were determined on both the W.T. SARS-CoV-2 and the 501Y.V1 and 501Y.V2 genetic variants (Fig 1). These antibodies bind discrete epitopes on the spike's RBD and NTD domains as was demonstrated previously [9-11]. The interaction of the viruses with ACE-2 - SARS-CoV-2 human receptor, was also determined, in order to evaluate the overall reactivity of the variants' spike protein with its intended target. As demonstrated, the interaction of ACE-2 (Fig 1C) with both variants was not affected by the variants accumulated mutations. However, the antibodies utilized in the diagnostic assay displayed either slightly enhanced (BL6), significantly enhanced (MD29 and MD65 mostly for the 501Y.V1 variant) or reduced (BL11) interactions. It is important to note that the interaction with BL11, while diminished for both variants, was not completely abolished.

Application of the S1-based antigen assay for COVID-19 detection: Our in-house TRF-ELISA S1-based assay [11] employs antibodies BL6 and BL11 as capture antibodies and MD65 and MD29 as reporter antibodies, as schematically depicted in Fig 2A. As demonstrated (Fig. 1), the interaction of these antibodies with both variants was indeed altered. To determine whether the assay in its current composition is still able to detect both VOC, the propagated SARS-CoV-2 and 501Y.V1 and 501Y.V2 VOC were diluted either in sterile nasopharyngeal swab buffer (COPAN®) or in a pool of qRT-PCR negative nasopharyngeal swab specimens from non-COVID-19 symptomatic and asymptomatic individuals (Fig 2B and C). Both variants have been associated with higher viral loads compared with the W.T. SARS-CoV-2, resulting in lower cycle threshold (Ct) values for PCR testing [13]. We therefor determined pfu/ml to Ct dependence (E-gene) and applied a correction factor in-order to plot our assay's result as W.T. SARS-CoV-2 genome-equivalent values. Results indicate that the developed assay detects both the W.T. SARS-CoV-2 and the 501Y.V1 variant with equal sensitivity (1×10^4 pfu/ml W.T genome-equivalents) and the 501Y.V2 variant with a 2-3-fold lower sensitivity ($2-3 \times 10^4$ pfu/ml W.T genome-equivalents). This result highlights the benefits of employing a combination of several monoclonal antibodies in immunoassays, maintaining both specificity and universality. It appears that the presence of BL6 as a capture antibody and the enhancement of the interaction with MD29 and/or MD65 reporter antibodies compensated for the decline in BL11 interaction.

Discussion:

With the emergence of SARS-CoV-2 variants around the world, rapid tests are liable to be influenced, resulting in the alteration of antibodies' recognition sites leading to false negative results. FDA-approved antigen-tests target either the nucleocapsid or the spike proteins. While mutations in the spike protein are of major concern, as all vaccines are based on this antigen, it was established that both the spike and the nucleocapsid proteins accumulate

mutations at higher rates than most of the other SARS-CoV-2 proteins [14, 15], thus raising concerns regarding the use of both proteins for antigen detection.

Rapid antigen tests are composed of polyclonal and monoclonal antibodies directed against a desired antigen. While polyclonal antibodies might ensure a wider recognition spectrum, they are prone to non-specific interactions and are thus not ideal. In this work we utilized a S1-based assay, comprised of a combination of four monoclonal antibodies targeting different epitopes on the S1 domain of the spike protein, for detection of the 501Y.V1 and 501Y.V2 VOC. The 501Y.V2 variant is currently not prevalent in Israel and therefore could not be detected directly from nasopharyngeal swab specimens of infected individuals. In order to compare detection performance, all the viruses were spiked in a pool of qRT-PCR negative nasopharyngeal swab specimens. While the interaction of individual antibodies with the variants was indeed influenced by the mutations accumulated by the spike protein of each strain, the overall test performance was not significantly affected. This result is due in part to the fact that the interaction of some of the antibodies was actually enhanced, but was mostly due to the use of oligo-clonal (as a substitute for poly-clonal) capture and reporter monoclonal antibody combinations. The use of a mixture of monoclonal antibodies enables compensation for reduced interaction of individual antibodies incorporated in the test, maintaining on the one hand the specificity of detection while on the other hand preserving recognition. The assay presented herein can be easily performed in clinical laboratories and in our hands proved more specific and almost as sensitive as an in-house nucleocapsid-based assay, when applied for detection from a large cohort of symptomatic and asymptomatic COVID-19 and non-COVID-19 patients [11]. Furthermore, the antibodies utilized in the assay can be employed in commercial, fast, point-of-care (POC) platforms, some of which proved very sensitive. In the context of the various screening paradigms where antigen-based tests might prove useful, a highly specific test will be most beneficial when applied for

diagnosis of symptomatic individuals in emergency wards setups. The higher specificity might lower false-positive rates thus preventing the possible hospitalization of non-COVID-19 patients in corona wards. We believe that our assay will be suitable for the detection of other, future variants, as the presence of at least two capture and two reporter antibodies should ensure sensitive detection.

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Declarations

Funding: This research received no external funding.

Conflicts of interest/Competing interests:

Patent application for the described antibodies was filed by the Israel Institute for Biological Research. None of the authors declared any additional competing interests.

Ethics approval: Ethical review and approval were waived, since the samples used for this study were leftovers of anonymized samples.

Consent for publication: All authors read and approved of the final manuscript.

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Figure 1: Binding profiles of anti-RBD Abs, anti-NTD Ab and ACE-2 receptor with W.T. SARS-CoV-2 and the 501Y.V1 and 501Y.V2 VOC. The interaction of A. Anti-RBD Abs B. Anti-NTD Ab and C. ACE-2 receptor with SARS-CoV-2 (black), 501Y.V1 VOC (blue) and 501Y.V2 (red) VOC are presented (antibodies are indicated on the graph). Antibodies were diluted (10-1500 ng/ml) and interacted with the captured viruses. The results are a summary of two independent experiments.

Figure 2: Dose response curves of wild-type (W.T.) SARS-CoV-2, 501Y.V1 and 501Y.V2 VOC in nasopharyngeal swab specimens. A. Schematic representation of the TRF-ELISA assay format. Wild-type (W.T) SARS-CoV-2 (black) and the 501Y.V1 (blue) and 501Y.V2 (red) VOC were diluted ($1 \times 10^6 - 6 \times 10^3$ pfu/ml) in B. nasopharyngeal swab buffer (COPAN) or C. A pool of qRT-PCR negative symptomatic and asymptomatic nasopharyngeal swabs and detected by the spike-based TRF-ELISA assay. Results are presented as W.T. genome-equivalents (W.T genome-eq). Dashed line represents the assays' limit of detection.

Patent application for the described antibodies was filed by the Israel Institute for Biological Research. None of the authors declared any additional competing interests.

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Researcher

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Figure 1

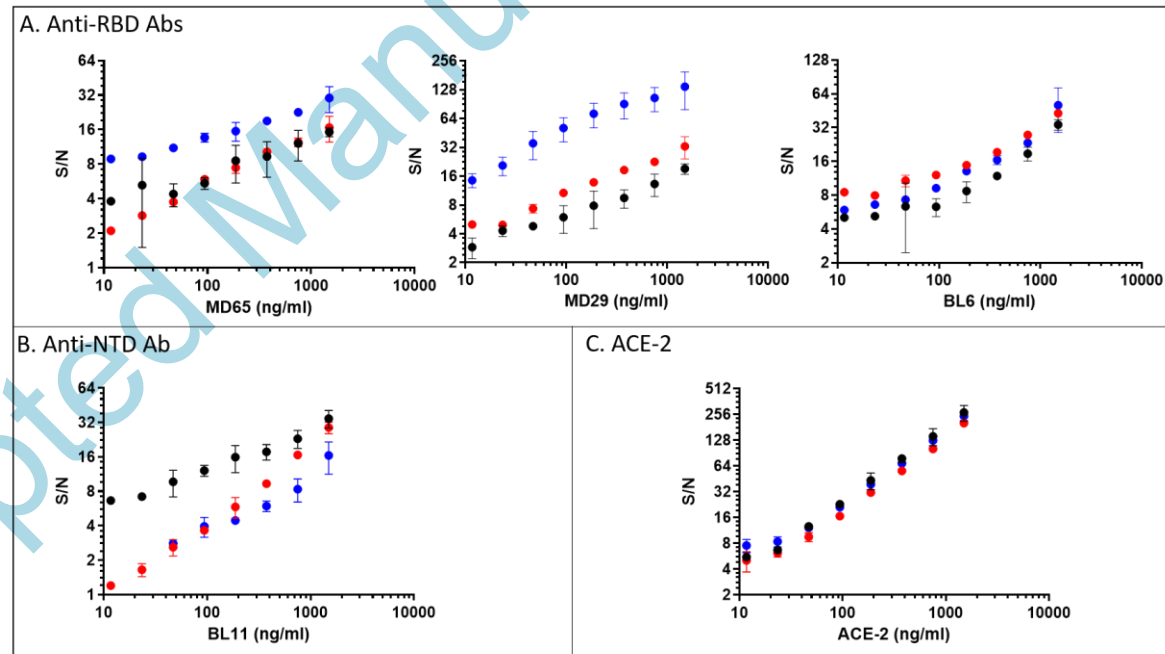


Figure 2

