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# Methods to evaluate the impact of SARS-CoV-2 nucleocapsid mutations on antigen detection by rapid diagnostic tests

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## ABSTRACT

Mutations in the nucleocapsid of SARS-CoV-2 may interfere with antigen detection by diagnostic tests. We used several methods to evaluate the effect of various SARS-CoV-2 nucleocapsid mutations on the performance of the Panbio™ and BinaxNOW™ lateral flow rapid antigen tests and a prototype high-throughput immunoassay that utilizes Panbio antibodies. Variant detection was also evaluated by immunoblot and BIAcore™ assay. A panel of 23 recombinant nucleocapsid antigens (rAgs) were produced that included mutations found in circulating SARS-CoV-2 variants, including variants of concern. All mutant rAgs were detected by all assays, at a sensitivity equivalent to wild-type control (Wuhan strain). Thus, using a rAg approach, we found that the SARS-CoV-2 nucleocapsid mutations examined do not directly impact antigen detection or antigen assay performance.

## METHOD SUMMARY

Expression, purification and testing of wild-type and mutant recombinant SARS-CoV-2 nucleocapsid antigens by the lateral flow SARS-CoV-2 antigen tests BinaxNOW™ and Panbio™, an ARCHITECT® SARS-CoV-2 antigen prototype automated immunoassay, immunoblot and BIAcore™ (surface plasmon resonance) activity assays.

**KEYWORDS:**

antigen ● BinaxNOW ● immunoassay ● lateral flow assay ● nucleocapsid ● Panbio ● recombinant protein ● SARS-CoV-2 ● variant

SARS-CoV-2 has continued to evolve during the COVID-19 pandemic, with new lineages containing a variety of mutations that could alter virus transmissibility and infectivity. While variants of concern (VOCs) are primarily defined by mutations in the spike protein that affect receptor binding and host cell entry, the effects of mutations in the nucleocapsid are less well understood. Most SARS-CoV-2 antigen assays target the nucleocapsid protein, raising the possibility that continued evolution of the virus may impact antigen assay performance. Antigen tests provide an important frontline defense against the SARS-CoV-2 pandemic; however, only a few studies have examined the effect of nucleocapsid protein variants on assay performance. A recent study by Bourassa *et al.* reported that variants containing the D399N mutation could not be detected by the Quidel Sofia SARS Antigen fluorescent immunoassay (Quidel, CA, USA), but were detected by the BinaxNOW™ COVID-19 Ag card (Abbott Laboratories, IL, USA) [1]. Another group recently described in a preprint study that the Abbott Panbio™ COVID-19 Ag Rapid Test Device was unable to detect SARS-CoV-2 variants containing both A376T and M234I mutations in the nucleocapsid region [2]. Here we used several methods to evaluate the effect of nucleocapsid mutations found in 23 circulating SARS-CoV-2 variants, including VOCs, on the performance of the Panbio and BinaxNOW lateral flow rapid antigen tests as well as a prototype SARS-CoV-2 immunoassay run on the automated, high-throughput Abbott ARCHITECT® system.

## Materials & methods

### Expression & purification of wild-type & mutant recombinant SARS-CoV-2 nucleocapsid antigens

A total of 23 recombinant nucleocapsid antigens (rAgs) were generated. This panel included rAgs based on the sequence of the wild-type (WT) nucleocapsid (strain: Wuhan-Hu-1; Genbank Accession: NC_045512) and nucleocapsid amino acid mutation patterns commonly found in several circulating SARS-CoV-2 variants as well as strains reported by Del Vecchio *et al.* [2]; details are provided in Supplementary Table 1. Based on the WT nucleocapsid reference sequence described above, a WT rAg was synthesized as well as 22 mutant rAgs containing mutation patterns of 25 amino acid substitutions or deletions (-) made at 24 unique positions in the WT sequence: P13L,

E31-, R32-, S33-, D63G, P80R, R203K, R203M, G204R, T205I, R209I, G214C, G215C, A220V, Q229H, M234I, S235F, S327L, D348Y, P365S, E367Q, A376T, D377Y, Q384H and S413R (Table 1). The rAgs in Table 1 are sorted chronologically, in the order in which each variant was identified and rAg was expressed, purified and tested; the three most recently tested Omicron variants are listed last.

SARS-CoV-2 nucleocapsid protein expression vectors were constructed by cloning DNA encoding full-length WT and mutant nucleocapsid proteins with a C-terminal 6×His tag into the pD451-SR backbone. DNA synthesis, cloning and sequence verification was performed by ATUM (CA, USA). Each vector was transformed into *Escherichia coli* BL21(DE3) cells (New England Biolabs, MA, USA). Preinoculums were grown at 37°C and 80% humidity, with shaking (250 rpm), overnight in Super Broth medium (Teknova, CA, USA) supplemented with 0.5% glucose (Sigma-Aldrich, MO, USA) and 50 μg/ml kanamycin (Teknova). Cells were inoculated into Terrific Broth medium (Teknova) supplemented with 50 μg/ml kanamycin (Teknova) at an initial optical density ($OD_{600}$) of 0.05 and shaken (250 rpm) at 37°C and 80% humidity until an $OD_{600}$ of 0.8 was reached. Protein expression was induced by the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (Teknova), and the cultures were further incubated with shaking (250 rpm) for ~18 h at 25°C.

Cells were harvested at 7741 × *g* for 10 min, resuspended in 50 mM sodium phosphate buffer (pH 8; Sigma-Aldrich), 500 mM NaCl (Invitrogen, MA, USA) plus 1.5 mM $MgCl_2$, 2 mM imidazole, 6 M urea, 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 U/ml benzonase, a protease inhibitor cocktail tablet and 1.5 mg/ml lysozyme (all from Millipore Sigma, MA, USA) and lysed by sonication. The resulting cell extracts were centrifuged at 17,700 × *g* for 20 min to remove cellular debris, and supernatants were filtered using a 0.45-μm vacuum filtration system (VWR, PA, USA).

The proteins were purified with 1 ml cOmplete™ His-Tag Purification Column (Roche, Basel, Switzerland) using an ÄKTA™ Pure protein purification system (Cytiva, MA, USA). The columns were equilibrated with 50 mM sodium phosphate buffer (pH 8), 1 M NaCl and 2 M urea before loading the sample. The resin was washed first with 50 mM sodium phosphate buffer (pH 8), 1 M NaCl and 6 M urea to remove nonspecifically bound proteins, followed by the same buffer with 2 M urea. Proteins were step-eluted with 25 mM sodium phosphate buffer (pH 7.2), 50 mM NaCl, 2 M urea and 500 mM imidazole. Peak fractions were pooled, concentrated with a 10,000-Da (MW cutoff) Amicon Ultra centrifugal filter unit (Millipore Sigma), desalted with a PD-10 column into 50 mM sodium phosphate buffer (pH 7), 350 mM NaCl, 5 mM EDTA (Millipore Sigma) and 1 M urea, and stored at -80°C. The protein concentration was determined using Pierce 660 nm protein assay reagent (Thermo Fisher Scientific, MA, USA) with bovine serum albumin (BSA) as a standard. Purity of each protein sample was observed by SDS-PAGE analysis.

### Panbio COVID-19 Ag Rapid Test Device

To avoid potential variability introduced by spiking swabs with rAg, rAg dilutions were prepared in assay elution buffer or storage buffer and then directly pipetted onto the device (20–50 μl to normalize the concentration of each rAg stock to 200, 20 or 2 pg/device), followed immediately by five drops of elution buffer. Elution buffer was used as a negative control. All subsequent steps were followed according to the manufacturer's instructions (Abbott) [3]. Note that the antibodies used in the Panbio device are referred to as Ab1 and Ab2.

### BinaxNOW COVID-19 Ag Card

All rAgs were tested using the BinaxNOW lateral flow sandwich immunoassay, following the manufacturer's instructions (Abbott) [4]. As with the Panbio assay, to avoid introducing variability by spiking swabs, mutant rAg and WT control samples were diluted and applied directly to the card at 200, 20 and 2 pg/device to demonstrate specificity. The diluent was tested as a negative control prior to sample testing. For each test, 20 μl of diluted sample or negative control was pipetted into the top hole of the BinaxNOW swab well. This was immediately followed by pipetting 140 μl of the BinaxNOW COVID-19 Ag Reagent provided in the kit into the top hole of the swab well. All subsequent steps were followed according to the manufacturer's instructions. Diluted samples and negative controls were evaluated at 15 min per the manufacturer's instructions; for the research application of the device in this study, we confirmed all negative control results at 20 min. Results were interpreted as positive or negative by experienced users. Note that the antibodies used in the BinaxNOW assay are designated Ab3 and Ab4.

### Immunoblot

Mutant rAg, WT rAg as a positive control, and diluent as a negative control were separated by SDS-PAGE using 4–20% Tris-HCl Criterion gel (Bio-Rad, CA, USA) under reducing conditions at 1.0 μg load, and then transferred onto nitrocellulose membranes using a Trans-Blot Turbo system according to the manufacturer's protocol (Bio-Rad). After protein transfer, one membrane was stained with SYPRO™ Ruby protein blot stain according to the manufacturer's protocol (Bio-Rad). The remaining nitrocellulose membranes were treated with T20 Blocking buffer (Thermo Fisher Scientific) for 60 min, followed by three 5-min washes in 1× TRIS buffered saline (TBS). The membranes were then incubated with 1.0 μg/ml of anti-nucleocapsid antibodies (Ab1, Ab2, Ab3 or Ab4; see Supplementary Table 2) prepared in antibody buffer (1× TBS, 1% BSA, 0.05% Tween® 20) for 16–18 h at room temperature, followed by three 5-min washes (1× TBS). The membranes were then incubated with alkaline phosphatase (AP)-conjugated goat anti-mouse IgG (H&L)-AP or goat anti-human IgG (H&L)-AP antibody (Bio-Rad) at 1:2000 dilution prepared in antibody buffer (1× TBS, 1% BSA, 0.05% Tween 20) for 2 h at room temperature, followed by three 5-min washes (1× TBS). The blots were developed with colorimetric AP substrate reagent kit according to the manufacturer's protocols (Bio-Rad).

Table 1. Summary of assay results against recombinant SARS-CoV-2 nucleocapsid antigens.

| rAg | Nucleocapsid mutation | Category | Lineage mutation is present in | Panbio† | BinaxNOW† | ARCHITECT‡ | | Immunoblot | | | | BIAcore activity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | RLU @ 20 pg/ml | RLU @ 200 pg/ml | Ab1 | Ab2 | Ab3 | Ab4 | Ab1 | Ab2 |
| 1 | Wild type (WT) | WT positive | A | Detected | Detected | 2638 | 21524 | + | + | + | + | 53% | 102% |
| 2 | P13L, R203K, G204R, G214C | Lambda§ | C.37 | Detected | Detected | 2697 | 20985 | + | + | + | + | NT | NT |
| 3 | D63G, R203M, G215C, D377Y | Delta | B.1.617.2, AY.x | Detected | Detected | 2904 | 20644 | + | + | + | + | NT | NT |
| 4 | R203K, G204R, R209I | Del Vecchio§ | B.1.1.119 | Detected | Detected | 2790 | 26789 | + | + | + | + | 58% | 110% |
| 5 | A220V, P365S | Del Vecchio | B.1.177 | Detected | Detected | 1968 | 14548 | + | + | + | + | 53% | 108% |
| 6 | A220V | Del Vecchio | many | Detected | Detected | 1867 | 13359 | + | + | + | + | 51% | 106% |
| 7 | P365S | Del Vecchio | B.1.177 | Detected | Detected | 2423 | 20516 | + | + | + | + | 60% | 111% |
| 8 | M234I, A376T | Del Vecchio | B.1.160 | Detected | Detected | 2638 | 24175 | + | + | + | + | 54% | 105% |
| 9 | A220V, Q229H | Del Vecchio | B.1.177 | Detected | Detected | 1999 | 13730 | + | + | + | + | 52% | 106% |
| 10 | A220V, D348Y | Del Vecchio | B.1.177 | Detected | Detected | 1309 | 5416 | + | + | + | + | 51% | 111% |
| 11 | D63G, R203M, D377Y | Delta | B.1.617.1, AY.x | Detected | Detected | 2324 | 19420 | + | + | + | + | NT | NT |
| 12 | S235F | Alpha | B.1.1.7 | Detected | Detected | 2423 | 20516 | + | + | + | + | NT | NT |
| 13 | M234I | Eta | B.1.526 | Detected | Detected | 2258 | 18580 | + | + | + | + | NT | NT |
| 14 | D377Y | Delta | B.1.2 | Detected | Detected | 2888 | 24897 | + | + | + | + | NT | NT |
| 15 | M234I, E367Q | None | B.1.618 | Detected | Detected | 1924 | 13833 | + | + | + | + | NT | NT |
| 16 | P80R | Gamma | P.1.1 | Detected | Detected | 4213 | 18609 | + | + | + | + | NT | NT |
| 17 | P80R, R203K, G204R, | Gamma§ | P.1.2 | Detected | Detected | 3213 | 16770 | + | + | + | + | NT | NT |
| 18 | P13L, R203K, G204R, Q384H | VOI§ | C.1.2 | Detected | Detected | 2753 | 14622 | + | + | + | + | NT | NT |
| 19 | D63G, R203M, G215C, S327L, D377Y | Delta | AY.5 | Detected | Detected | 3166 | 18444 | + | + | + | + | NT | NT |
| 20 | T205I | Mu, Beta, Epsilon | Many | Detected | Detected | 2655 | 14871 | + | + | + | + | NT | NT |
| 21 | P13L, E31-, R32-, S33-, R203K, G204R | Omicron | | Detected | Detected | NT | NT | NT | NT | NT | NT | NT | NT |
| 22 | P13L, E31-, R32-, S33-, R203K, G204R, D343G | Omicron | | Detected | Detected | NT | NT | NT | NT | NT | NT | NT | NT |
| 23 | P13L, E31-, R32-, S33-, R203K, G204R, S413R | Omicron | | Detected | Detected | NT | NT | NT | NT | NT | NT | NT | NT |
| n/a | Negative control: influenza A nucleocapsid | NA | NA | NT | NT | NT | NT | NT | NT | NT | NT | -1% | -1% |

†For PanBio and BinaxNOW, each rAg was tested in triplicate at 2, 20 and 200 pg/device where at least one replicate was detected at 200 pg/device or lower for each variant, similar to WT.
‡For ARCHITECT, the calibrator A negative control (Cal A; 0 pg/ml) RLU = 1149 (background). Dilutions were tested at 20 and 200 pg/ml and all variants were positive.
§Mutations R203K and G204R are also present in the Omicron VOC.
AB: Antibody; AY.x: All AY lineages; NA: Not applicable; NT: Not tested; rAg: Recombinant nucleocapsid antigen; RLU: Relative light units; VOI: Variant of interest.

### Prototype ARCHITECT SARS-CoV-2 immunoassay

A prototype quantitative sandwich immunoassay specific for the SARS-CoV/SARS-CoV-2 nucleocapsid antigen was developed for the Abbott ARCHITECT automated immunoassay analyzer using the same anti-nucleocapsid antibodies as in the Panbio COVID-19 Ag Rapid Test Device (i.e., Ab1 and Ab2). In the prototype immunoassay, paramagnetic microparticles coated with a human monoclonal antibody serve as the solid-phase reagent for capturing the viral nucleocapsid target. The captured viral antigen is detected with a mouse monoclonal antibody conjugated with acridinium, which produces a chemiluminescent signal upon target binding, with signal quantified in relative light units (RLU). Higher RLU indicates higher binding of the antibodies to the nucleocapsid target. Mutant rAg and WT rAg control samples were diluted to 200 and 20 pg/ml, which are above the limit of detection. Diluent was tested as a negative control.

### BIAcore binding percentage activity assay

A BIAcore™ 4000 device (GE Healthcare, IL, USA) was desorbed and sanitized per the manufacturer's protocols, and a Series S CM4 Sensor Chip (Cytiva) was conditioned with short duplicate injections of 100 mM HCl (Sigma), 50 mM NaOH (Sigma), 0.1% SDS (Cytiva) and 150 mM $H_3PO_4$ (Fisher Scientific, NH, USA). BIAnormalizing solution was used for hydrodynamic addressing and normalization of the conditioned chip in 1× general purpose buffer, 0.01 M HEPES pH 7.4, 0.15 M NaCl (HBS-N) prior to successfully passing the instrument's system check with BIAtest solution (Cytiva). His capture reagent was immobilized onto the prepared CM4 chip using prepared amine coupling reagents per the manufacturer's protocols (Cytiva).

Purified His-tagged rAgs and Flu A Perth H3N2 nucleocapsid (negative control, Abbott) were diluted into general purpose running buffer 1× 0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20 (HBS-EP) + [Cytiva], 1 M NaCl [Sigma], 0.1% BSA [Sigma] and 0.1% carboxymethyl dextran [Sigma]) and captured onto the immobilized His capture surface for 3 min. High-performance injections of the SARS-CoV-2 antibodies Ab1 and Ab2 (Abbott) at 0 and 30 μg/ml (200 nM) diluted into running buffer occurred for 3 min, immediately followed by 6 min of running buffer alone over a capture (captured nucleocapsid) and reference (no nucleocapsid) surface. The entire chip surface was regenerated with two 1-min injections of the His capture regeneration solution (Cytiva).

After removing all warm-up/conditioning cycles, the double, reference-subtracted data points were used to determine the amount (response units) of capture nucleocapsid and the amount (response units) of bound antibody. The responses were normalized by each reagent's MW and the normalized antibody response was divided by the normalized nucleocapsid response to report an observed percentage activity that correlates with the amount of antibody bound.

## Results & discussion

Both rapid lateral flow assays detected all rAgs containing mutations, alone or in combination, across several circulating lineages (Table 1). The Panbio and BinaxNOW devices detected each rAg mutant as well as the WT rAg control.

Using the same antibodies as the Panbio device, the prototype ARCHITECT immunoassay detected small quantitative differences between the various mutant rAgs. At 200 pg/ml, the RLU values for 13 of the 20 rAgs ranged from 16,770 to 26,789, but the remaining seven rAgs produced RLU less than 15,000. Among these seven rAgs, four (rAg 5, rAg 6, rAg 9 and rAg 10) shared an A220V mutation which was not found in other rAgs. The remaining three rAgs had unique mutations: E367Q (rAg 15), Q384H (rAg 18) and T205I (rAg 20). While the RLU differences suggest that certain mutations may affect the prototype immunoassay performance, it is important to note that all rAgs were detected at both 20 and 200 pg/ml, which was above the limit of detection of 2 pg/ml and in 100% concordance with the Panbio and BinaxNOW assays.

Immunoblot analysis using the same four antibodies as the lateral flow assays (Panbio, Ab1 and Ab2; BinaxNOW, Ab3 and Ab4) confirmed detection of all the electrophoresed rAgs (Supplementary Figure 1).

Both Panbio antibodies (Ab1 and Ab2) were evaluated individually for binding to rAgs using BIAcore. A range of off-rates was seen for Ab1 (Supplementary Figure 2), compared with consistent traces for Ab2 (Supplementary Figure 3). The differences observed for Ab1 can be accounted for by, first, the avidity effect of bivalent antibody binding to the target surface compared with the proper orientation for determining binding kinetics (monomeric nucleocapsid binding to antibody captured to sensor); and second, the fact that the off-rate for Ab1 is >50-fold higher than that of Ab2 when the test is in the kinetics orientation. A faster off-rate would reduce saturating monovalent interactions of antibody quickly, resulting in a greater chance of a bivalent interaction creating two off-rates (fast and slow). Nevertheless, off-rates were not considered for binding activity, and no loss of binding activity for either antibody was observed for all mutations tested, including those in VOCs.

Robust diagnostic assay design requires consideration of sequence conservation, followed by continuous evaluation of circulating strains to monitor how newly emerging mutations may impact performance. Our prior *in silico* analysis of 45 variant lineages did not identify any lineages carrying mutations in the target epitopes of Panbio or BinaxNOW antigen tests [5]; however, these *in silico* predictions must be challenged with a variety of wet-testing methods. In this study we tested the performance of the Panbio and BinaxNOW antigen assays against a comprehensive panel of mutant rAgs that reflect the variants reported in circulating SARS-CoV-2 strains, including VOCs Alpha, Beta, Gamma, Delta and Omicron. Notably, the linker region mutations R203K and G204R that are present in the Omicron variant were also included in several of the rAgs tested in this study, and the three mutant rAgs from the Omicron variants BA.1 and BA.2 were detected by the Panbio and BinaxNOW assays. Our findings provide functional confirmation of the *in silico* analysis of

assay performance in detecting SARS-CoV-2 variants [5] and are consistent with variant clinical specimen testing on BinaxNOW and Panbio for VOCs [6–8].

Our findings contradict those reported in a preprint by Del Vecchio *et al.* [2], as we found that the Abbott Panbio COVID-19 Ag Rapid Test Device and the Abbott BinaxNOW COVID-19 Ag Card could detect all variants tested, including a rAg containing both a M234I and an A376T mutation. We also confirmed that the M234I, A376T rAg was detected by immunoblotting and BIAcore binding activity assays. Furthermore, using the same antibodies as in the Panbio device, the prototype ARCHITECT immunoassay could quantitate all mutant rAgs tested. A recent study suggested that discrepancies in assay performance for detecting SARS-CoV-2 variants in patient samples versus rAgs may be related to conformational changes caused by the presence of the mutated spike antigen [9]; further studies are needed to evaluate this possibility.

Corman *et al.* independently demonstrated that the Abbott Panbio COVID-19 Ag Rapid Test Device was able to reliably detect levels as low as 4.4 SARS-CoV-2 plaque-forming units and 250 pg of recombinant SARS-CoV-2 nucleocapsid, with a 95% confidence limit to detect $3.52 \times 10^6$ copies of SARS-CoV-2 RNA per device [10]. Our study findings are consistent with that study, with detection of all mutant rAgs tested at 200 pg or less per device, and similar test results for WT rAg.

rAgs are the preferred sample for evaluating assay performance with newly emerging SARS-CoV-2 variants. Samples can be prepared far more rapidly than obtaining and isolating virus cultures or appropriately sequenced clinical samples. Furthermore, clinical samples can present a coinfection of multiple COVID-19 variants in a single sample, confounding variant specificity assessments [5]. Commercially available and characterized virus cultures of variants can take months to become available, if at all. rAgs can be used to readily demonstrate the immunological activity and sensitivity of the assay, which is the immediate interest for emerging VOCs.

rAgs may have conformational differences compared with the native virus which could affect antibody binding. However, the four antibodies tested recognize both native antigens from clinical and viral samples (data not shown) and rAgs in each assay format, as well as in orthogonal methods such as immunoblotting and BIAcore, which use denatured samples. The immunoblot and BIAcore results suggest that the antibodies bind linear epitopes (see Supplementary Figures 1–3 and [5]). The use of multiple assays and sample preparations may be needed to verify binding of assay antibodies to rAgs of emerging variants and rule out the possibility of conformational effects.

In summary, our method using rAgs provides a safe and effective approach to quickly evaluate antigen assay performance as SARS-CoV-2 variants emerge. This method could also be used to confirm assay results for patient specimens of variants with specific nucleocapsid mutations, as described by Bourassa *et al.* [1]. The use of rAgs ensures consistent sample preparation without the need for a biosafety lab, and allows rapid translation of global sequence surveillance into actionable variant testing that does not rely on procuring or shipping clinical specimens.

## Future perspective

Continued vigilance, monitoring and evaluation of new variants as they emerge will be critical to ensuring accurate diagnosis of SARS-CoV-2 infections with rapid antigen tests targeting the nucleocapsid protein.

### Executive summary

- Purified recombinant SARS-CoV-2 nucleocapsid antigens containing mutations present in various emerging variants were detected by Panbio™ and BinaxNOW™ lateral flow rapid antigen diagnostic tests.
- The antibodies from the Panbio and BinaxNOW lateral flow assays for detecting SARS-CoV-2 nucleocapsid were individually tested in immunoblot assays, BIAcore™ binding assays and a prototype ARCHITECT® immunoassay, and showed no divergence in reactivity or sensitivity for detecting wild-type (Wuhan strain) or mutant recombinant SARS-CoV-2 nucleocapsid antigens.
- The Abbott Panbio COVID-19 Ag Rapid Test Device and the Abbott BinaxNOW COVID-19 Ag Card continued to demonstrate their fitness for purpose in the diagnosis of SARS-CoV-2 during the COVID-19 pandemic.

## Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science.com/doi/suppl/10.2144/btn-2022-0020


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## Data sharing statement

Recombinant SARS-CoV-2 variant antigens can be made available under a confidential disclosure agreement and material transfer agreement or research collaboration agreement.

## Open access



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