Impact of novel variants on COVID-19 diagnostics

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How might novel variants affect diagnostic performance?

Variants arise through mutations in the viral genetic sequence

- **Molecular NAT (e.g. PCR)**
  Alterations in the viral sequences targeted by primers could lead to inaccurate results

- **Antigen tests**
  Alterations in the viral genome that cause a change in the structure of the antigens could negatively affect performance

- **Serological tests**
  Alterations in the viral genome that cause a change in the structure of viral proteins may result in a change to antibody repertoire, impacting test performance

NAT, nucleic acid amplification test
PCR, polymerase chain reaction
The SARS-CoV-2 genome

- S gene encodes the **spike glycoprotein**
- E gene encodes the **envelope protein**
- M gene encodes the **membrane glycoprotein**
- N gene encodes the **nucleocapsid protein**
- ORFs encode **non-structural proteins and polypeptides**

The majority of mutations in novel SARS-CoV-2 variants occur in the S gene, but other genes are also affected.

Adapted from Khailany RA et al. Gene Rep 2020;19:100682
ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2
Three SARS-CoV-2 variants have been designated ‘variants of concern’

<table>
<thead>
<tr>
<th></th>
<th>B.1.1.7</th>
<th>B.1.351</th>
<th>P.1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alternative nomenclature</strong></td>
<td>VOC-202012/01 501Y.V1</td>
<td>501Y.V2</td>
<td>501Y.V3</td>
</tr>
<tr>
<td><strong>Location first identified</strong></td>
<td>United Kingdom</td>
<td>South Africa</td>
<td>Brazil</td>
</tr>
<tr>
<td><strong>Number of mutations</strong></td>
<td>23*</td>
<td>21†</td>
<td>21*</td>
</tr>
<tr>
<td><strong>Genes with mutations</strong></td>
<td>ORF1ab, S, ORF8, N</td>
<td>ORF1ab, S, ORF3a, E, N</td>
<td>ORF1ab, S, ORF8, N</td>
</tr>
<tr>
<td><strong>Number of mutations in S gene</strong></td>
<td>8</td>
<td>10†</td>
<td>10</td>
</tr>
</tbody>
</table>

*Includes 6 synonymous mutations for B.1.1.7 and 4 for P.1. Synonymous mutations do not change the amino acid sequence of the resulting protein
†Includes one mutation and one deletion at the same site
Prevalence of variants of concern in Africa

**B.1.1.7**
Countries with >10 cases:
- Ghana
- Nigeria

Cases also reported in Gambia, DRC, Kenya, Rwanda, Mayotte, Senegal, South Africa

**B.1.351**
Countries with >10 cases:
- Botswana
- Mayotte
- Mozambique
- South Africa
- Zambia

Cases also reported in Kenya, Ghana, Rwanda, DRC

Data from [www.cov-lineages.org/global_report.html](http://www.cov-lineages.org/global_report.html), last checked: 4 March 2021

DRC, Democratic Republic of Congo
B.1.525 – a new variant under investigation

First detected in November 2020 in Nigeria and the United Kingdom

Nigeria is currently the only African country with >10 cases
Cases also reported in Ghana and Mayotte

As of 04 March 2021, detected in 23 countries across Europe, North America, Oceania, Asia and Africa

Mutations in ORF1ab, S, E and N genes

Not yet considered a variant of concern, but shares mutations with B.1.1.7, B.1.351 and P.1

Data from www.cov-lineages.org/global_report.html, last checked: 4 March 2021
Impact of SARS-CoV-2 variants on molecular test performance

- Impact **expected to be minimal**, as molecular tests in widespread use generally target ≥1 gene
- No reports of molecular tests being substantially affected by new variants except for S gene dropout with certain tests\(^1\)
- Laboratories should routinely review testing data for unusual/unexpected results, including **monitor for target gene dropout**

<table>
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<tr>
<th>Variant</th>
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<th>B.1.351</th>
<th>P.1</th>
<th>B.1.525</th>
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<td>Deletion at position 69–70 can cause S gene dropout(^1)</td>
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FIND. [https://www.finddx.org/covid-19/novel-variants/](https://www.finddx.org/covid-19/novel-variants/)
## Impact of SARS-CoV-2 variants on antigen test performance

- **Impact expected to be minimal**, as most antigen detection tests (including rapid lateral flow devices) target the C-terminus of the viral nucleocapsid protein (N gene)
- A few antigen tests target the spike protein; performance of these tests may be impacted

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<tr>
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<th>Impact</th>
<th>Details</th>
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<td>B.1.1.7</td>
<td>Minimal impact</td>
<td>N gene mutations are located at the N-terminal</td>
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Impact of SARS-CoV-2 variants on serological test performance

There is potential for the performance of assays detecting antibodies nucleocapsid and more likely the spike protein to be affected, but to date, no systematic evaluations have been performed.

As these assays do not directly detect the virus, but rather the immune response generated in response to infection, it will take longer to understand how performance of individual assays may be impacted.

Initial data characterising the immune response to the variants, shows differing impact on host antibody response, so more data is needed.

As antibody tests are generally not used for primary diagnosis, the overall impact on testing programmes is likely to be low.
US FDA guidance for molecular test developers

1. Design test to minimize impact of viral mutations on test performance
   - Consider performance across all known variants at time of validation
   - Describe in EUA how test design mitigates risk of future variants impacting performance
   - Including a highly-conserved pan-SARS-CoV-2 target may improve performance, but number of targets in the test should still be appropriate to provide resilience

2. Routinely monitor for viral mutations that may impact test performance
   - Periodically conduct sequence alignment of primer/probe sequences with available SARS-CoV-2 genomes (in silico analysis)
   - If mutations are identified that may reduce test performance by ≥5% or to below EUA recommendations, perform wet testing with clinical sample
   - If difference of ≥3-fold in LOD is detected, prepare risk analysis and notify FDA

3. Clearly convey any test limitations in the test labelling
   - If a potential impact on performance of an authorized test is identified, FDA will work with the test developer to address issues, e.g. by updating their labelling to reflect potential changes in performance
   - FDA may also take additional actions, such as revocation of an EUA, as appropriate

FDA. https://www.fda.gov/media/146171/download

EUA, emergency use authorization; FDA, United States Food and Drug Administration; LOD, lower limit of detection
US FDA guidance for antigen and serological test developers

1. Engage in discussion with FDA early in test development
   - FDA is still considering how best to evaluate the impact of novel variants on performance of antigen and serology tests
   - Developers should engage in discussion with FDA to ensure that they are apprised of the most recent developments

2. Consider potential impact of variants on test performance
   - Consider potential impact of variants already in circulation
   - Routinely monitor for new genetic mutations and viral variants and assess impact on performance
   - Consider potential for aggregate of mutations to reduce test performance by ≥5% or to below EUA recommendations

FDA. [https://www.fda.gov/media/146171/download](https://www.fda.gov/media/146171/download)
EUA, emergency use authorization; FDA, United States Food and Drug Administration
WHO guidance to end-users

Routinely review testing data to look for changes

- Positivity rate
- Invalid rate
- Drop out or discrepant detection of different gene targets for multi-target assays

Notify Suppliers of any unusual results

- Increased discrepancies in Ct values across gene targets
- Failure to detect specific targets
- Misdiagnosis

WHO Information Notice for Users: https://www.who.int/news/item/19-01-2021-who-information-notice-for-ivd-users-2021-01
Company responses to mutations in SARS-CoV-2 and VOCs

- Most companies have conducted *in silico* analysis and issued letters to customers or posted information through press releases/on their website.

**Abbott**

Bioinformatics analysis confirmed that the target regions used in the Abbott Alinity m SARS-CoV-2, RealTime SARS-CoV-2 and Alinity m Resp-4-Plex assays would not be impacted by the following new variant strains.

**SD BIOSENSOR**

According to our investigations, several site mutations have occurred on the N-terminal side of the nucleocapsid protein at positions of 3, 203, 204, 235 for U.K(VUI-202012/01) and of 205 for S.A(501.V2), and there were no mutations on the C-terminal side. Since the recognition site of the raw materials used in our antigen test are the C-terminal side different from mutation sites, we expect our products are theoretically able to detect variants including U.K(VUI-202012/01) and S.A(501.V2).

**Cepheid**

The Cepheid Xpert Xpress SARS-CoV-2 and Xpert Xpress SARS-CoV-2/Flu/RSV tests detect the nucleocapsid (N2) and envelope (E) genes of SARS-CoV-2. Cepheid is monitoring strain surveillance data and has performed routine in silico analysis of SARS-CoV-2 sequences (over 500,000 from GISAID database as of February 2021 https://www.gisaid.org/) since the launch of our Xpert Xpress SARS-CoV-2 test. These include the spike protein variant strains listed above. Coverage is currently at 100% for the E target and greater than 99% for the N2 target based on in silico analysis of our two-target design. Data from field reports are consistent with this analysis. The implications of these findings are that for the Xpert Xpress SARS-CoV-2 test a PRESUMPTIVE POSITIVE callout may occur for strains with point mutations in the N2 target, whereas the results from the Xpert Xpress SARS-CoV-2/Flu/RSV test are not impacted. Modifications in test design to accommodate known N2 variants are currently underway.

**NOTE:** this is meant for illustrative purposes and is not exhaustive. FIND is working to compile official company responses to have move comprehensive information on our website: https://www.finddx.org/covid-19/novel-variants/
Analysis of the specific mutations carried by the novel variants suggests that the majority of tests currently used in primary detection of SARS-CoV-2 are not affected.

However, testing programmes and laboratories must be aware of potential effects on certain diagnostic tests, in order to be able to rapidly take any necessary precautionary measures.

As part of routine QA/QC, end-users should actively monitor test result trends.

The rapid emergence of novel variants demonstrates the need for:

- Robust and widespread genomic surveillance, to ensure that other novel mutations are detected promptly.
- Continued monitoring of diagnostic test results to identify any changes in test performance and determine whether amendments to testing practices are required.
Thank you

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