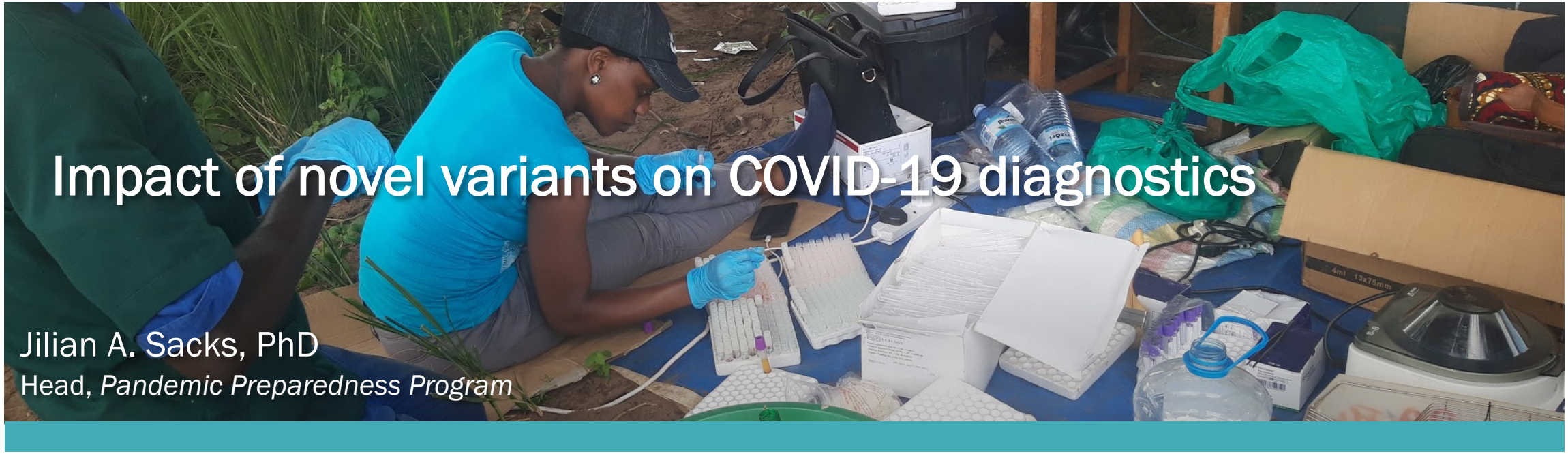
FIND

Because diagnosis matters



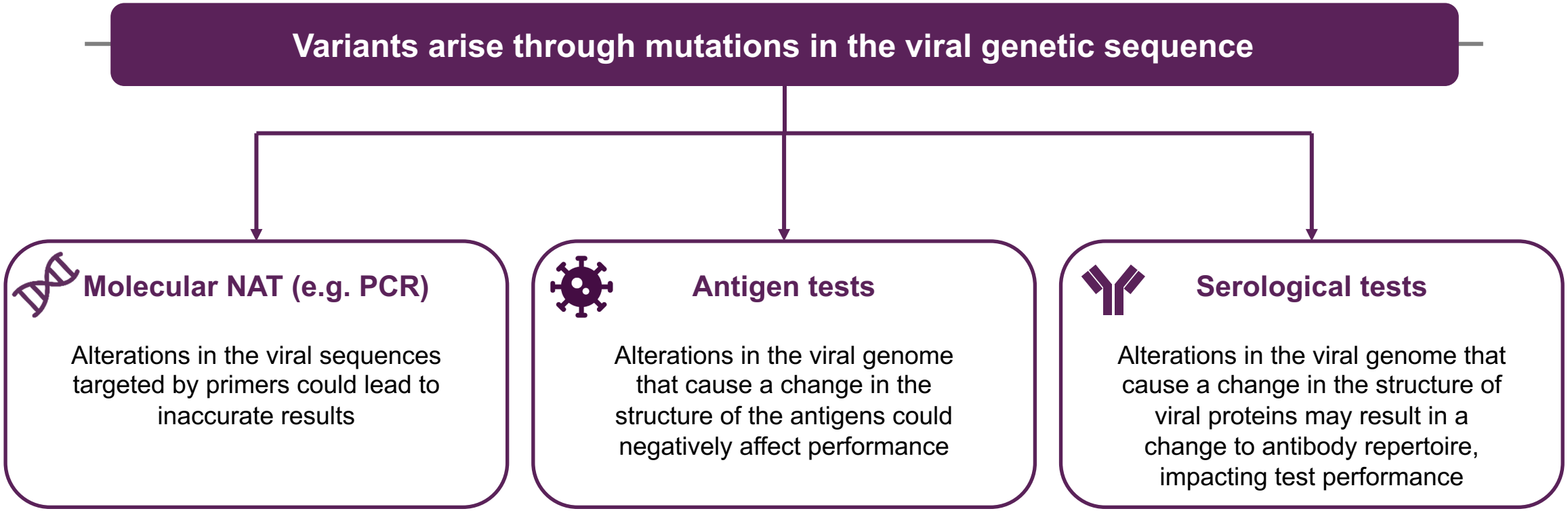
Impact of novel variants on COVID-19 diagnostics

Jilian A. Sacks, PhD

Head, *Pandemic Preparedness Program*

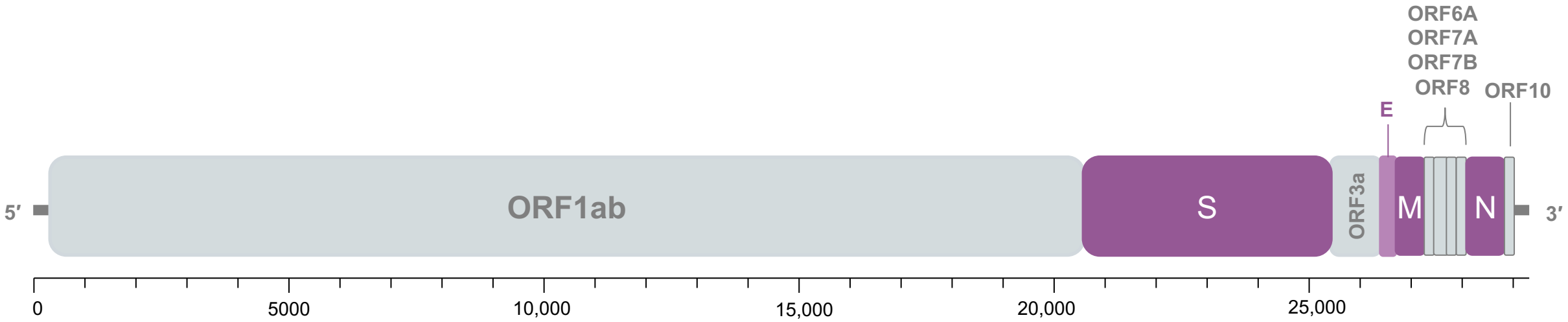
05 March 2021, ALSM Webinar

How might novel variants affect diagnostic 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

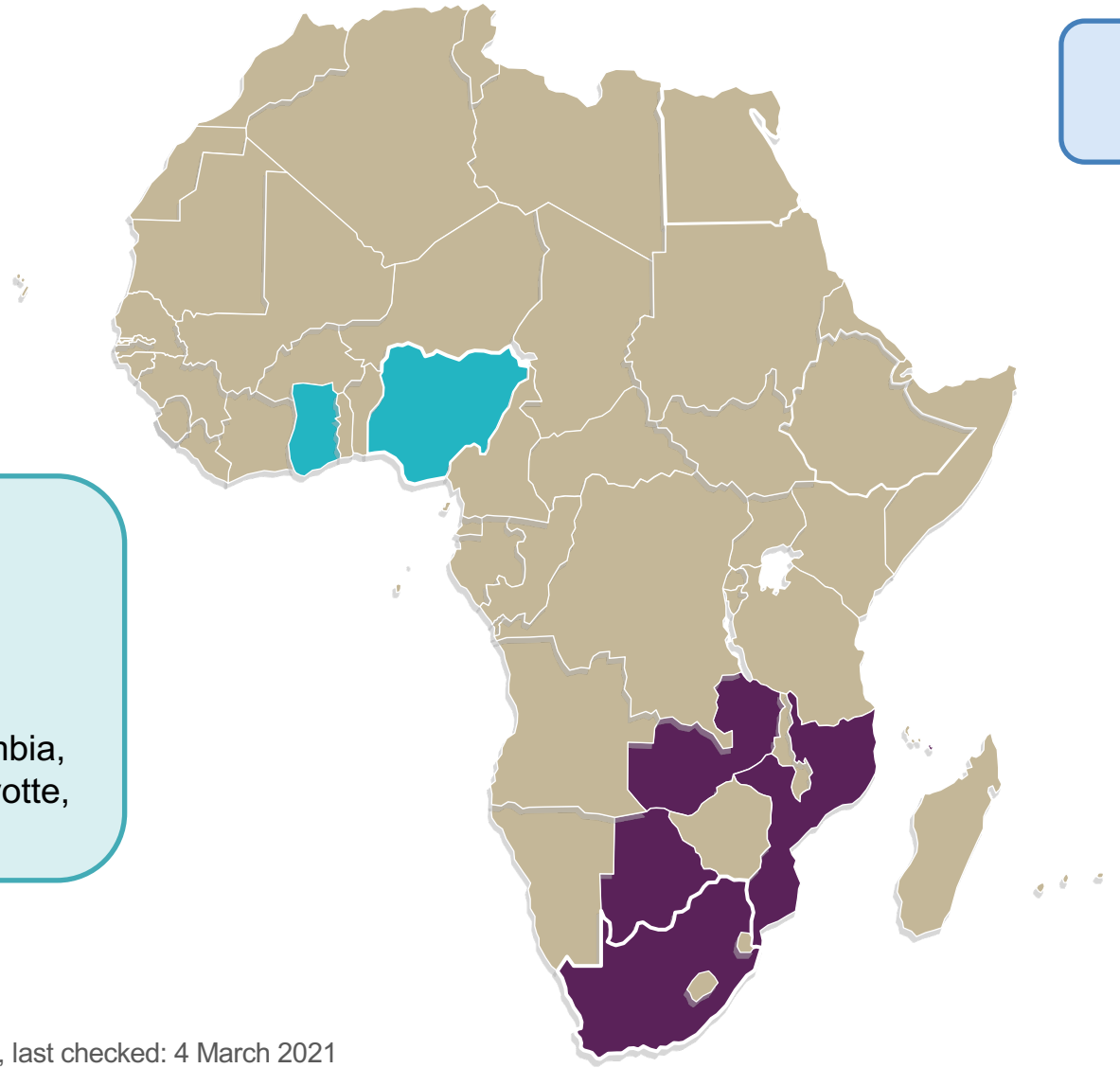
SARS-CoV-2 variants of concern

Three SARS-CoV-2 variants have been designated ‘variants of concern’

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

*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
Rambaut A et al. Virological.org 2021; Tegally H et al. medRxiv 2020; doi.org/10.1101/2020.12.21.20248640; Faria NR et al. Virological.org 2021

Prevalence of variants of concern in Africa



P.1
No cases in Africa reported to date

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

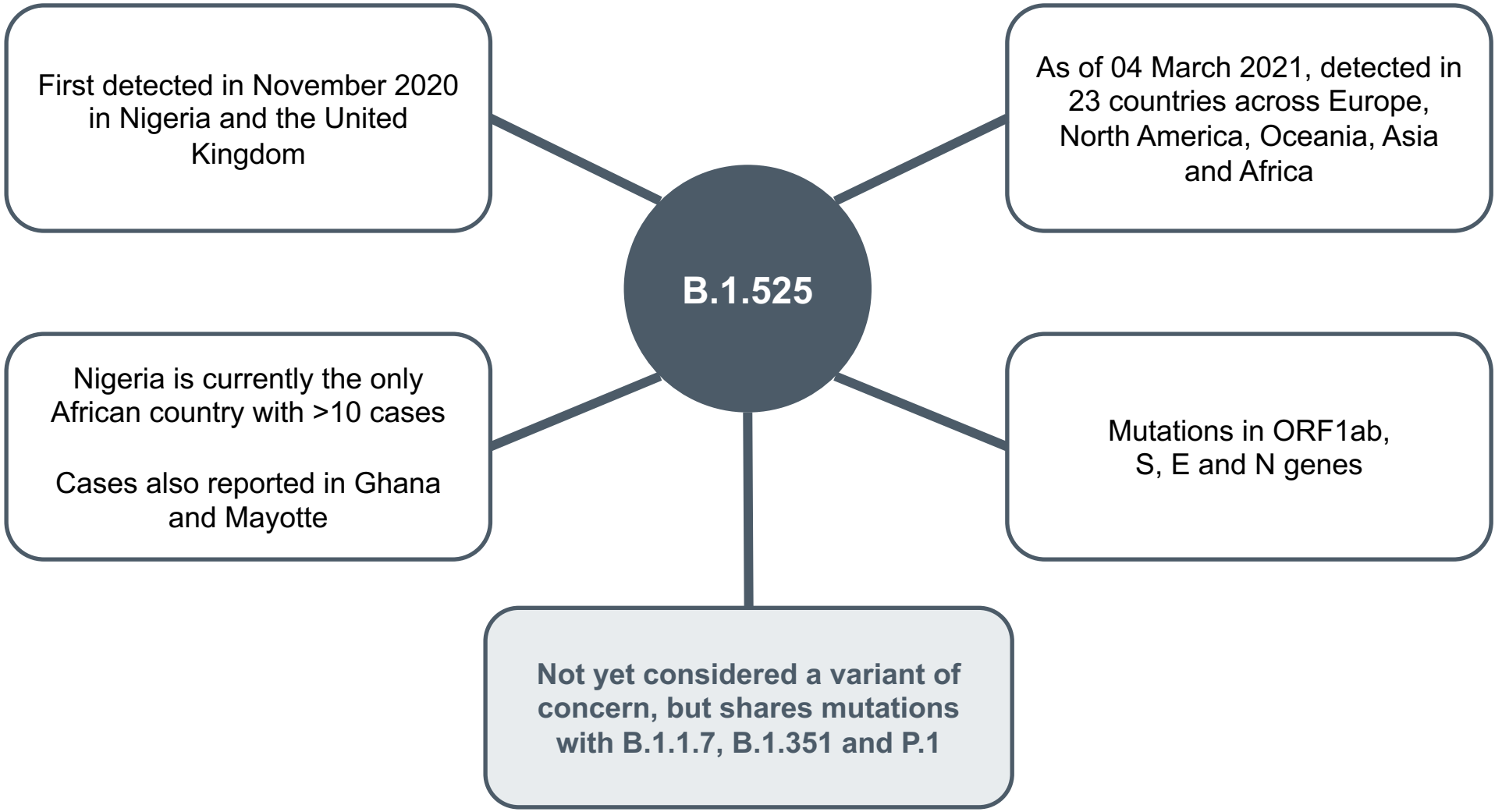
Cases also reported in Gambia, DRC, Kenya, Rwanda, Mayotte, Senegal, South Africa

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, last checked: 4 March 2021
DRC, Democratic Republic of Congo

B.1.525 – a new variant under investigation



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**

B.1.1.7	B.1.351	P.1	B.1.525
Minimal impact	**Minimal impact**	**Minimal impact**	**Minimal impact**
Deletion at position 69–70 can cause S gene dropout[1]	No data on impact of mutations on assay performance but may impact assays that target S gene sequences	No data on impact of mutations on assay performance but may impact assays that target S gene sequences	Deletion at position 69–70 can cause S gene dropout[1]

FIND. https://www.finddx.org/covid-19/novel-variants/

1. Bal A et al. medRxiv 2020. doi 10.1101/2020.11.10.20228528

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**

B.1.1.7	B.1.351	P.1	B.1.525
Minimal impact	**Minimal impact**	**Minimal impact**	**Minimal impact**
N gene mutations are located at the N-terminal A Public Health England assessment found that six rapid antigen tests targeting the nucleocapsid protein all successfully detected the variant[1]	N gene mutations are located at the N-terminal A Public Health England assessment found that six rapid antigen tests targeting the nucleocapsid protein all successfully detected the variant[1]	To date, no evaluation studies have been carried out to confirm that test performance is not affected, but no major performance deficits are anticipated for tests targeting the nucleocapsid protein	To date, no evaluation studies have been carried out to confirm that test performance is not affected, but no major performance deficits are anticipated for tests targeting the nucleocapsid protein

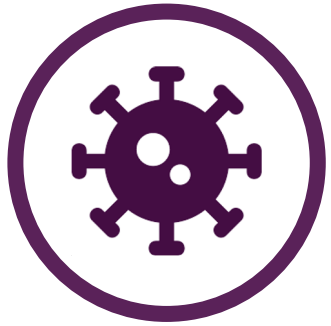
FIND. https://www.finddx.org/covid-19/novel-variants/

1. Public Health England. https://www.gov.uk/government/publications/sars-cov-2-lateral-flow-antigen-tests-evaluation-of-voc1-and-voc2/sars-cov-2-lateral-flow-antigen-tests-evaluation-of-voc1-kent-uk-and-voc2-south-africa. Tests evaluated were: Abbott Panbio, Fortress, Innova, Roche/SD Biosensor nasal swab, Surescreen and Orient Gene

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

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, RealTi*me* SARS-CoV-2 and Alinity m Resp-4-Plex assays would not be impacted by the following new variant strains.

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.

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).

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/

Summary

- 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

www.finddx.org
outbreaks@finddx.org