

# Partial Sanger Sequence Analysis of SARS-CoV-2 Spike (S) Gene Detects the Delta SARS-CoV-2 Variant of Concern In Bungoma, Nakuru and Nyamira Counties

*This is a preliminary report prepared by CDC Kenya's Diagnostic Laboratory Systems Program and the KEMRI Centre for Global Health Research on June 25, 2021.*

## Summary of Findings

- The Sanger partial sequencing method provides a quick, low-cost pre-screening approach to rapidly identify variants of concern (VOC) or interest (VOI).
- Nineteen (19) SARS-CoV-2 RT-PCR positive samples from Bungoma, Nakuru, and Nyamira counties were sequenced including: Bungoma (4), Nakuru (12) and Nyamira (3). These samples were collected between June 15 - 21, 2021 from patients aged 19-82 years; eleven (57.9%) were female and eight (42.1%) were male. Eighteen were Kenyan nationals and one Ugandan. Two cases did not indicate their age on case investigation form (CIF) and none of the 19 cases indicated a recent travel history on their CIF (i.e., travel within the past 14 days).
- **Fourteen samples (73.7%) aligned with the Delta SARS-CoV-2 VOC (B.1.617.2, first identified in India).** This is the first time we have detected Delta SARS-CoV-2 VOCs in the counties of Bungoma and Nyamira.
- Five samples (26.3%) aligned with Alpha SARS-CoV-2 VOC (B.1.1.7, first identified in the United Kingdom).

## Background

There is increasing need for genomic surveillance of SARS-CoV-2 to determine new introductions and current circulation of variants of concern (VOC) and variants of interest (VOI) in the local population. Timely release of sequencing data is important for initiating mitigation measures and linking variants to clinical manifestation and vaccine effectiveness in the population.

## Methods

Here we describe findings from partial Spike (S) gene sequencing at the CDC-funded KEMRI laboratory in Kisumu of 19 SARS-CoV-2 samples collected from Bungoma, Nakuru and Nyamira counties. These samples were collected between June 15 -21, 2021.

Portions of S gene at nucleotide positions 21358 to 23847 of SARS-CoV-2 reference sequence (NC\_045512) genome were amplified using Qiagen Onestep RT-PCR kit, and nested PCR was performed using New England BioLabs Taq DNA polymerase. The PCR products were purified using ExoSAP-IT (ThermoFisher) and cycle sequencing was performed using BigDye sequencing kit v3.1. Excess Bigdye removal was done using DyeEx 96 kit (Qiagen) and resultant purified products read-out in ABI 3730xl genetic sequence analyser. Multiple sequence alignment (MSA) was generated using BioEdit vr 7.2. Maximum likelihood (ML) tree was constructed in MEGA vr 7.

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## Results - Sequence and Phylogenetic Analysis

Fourteen samples (73.7%) that aligned with the sub-lineage B.1.617.2 (Delta SARS-CoV-2), contained amino acid changes at L452R, T478K and D614G. All the samples in this sub-lineage also had amino acid changes at G142D and deletions at amino acid positions 156 and 157.

Five samples (26.3%) that aligned with B.1.1.7 (Alpha SARS-CoV-2), contained amino acid changes F490S, N501Y, A570D, D614G and amino acid deletions at 69/70 and 144. Two samples (40%) contained amino acid changes D138H.

Information including age, gender, county, testing facility and cluster are shown in the table below.

## Conclusions

- This report provides preliminary sequencing results identifying the Delta VOC (B.1.617.2, first identified in India) in Bungoma and Nyamira for the first time. These findings may inform public health action in these counties.
- Using Sanger partial sequencing of the SARS-CoV-2 S gene, 14 cases (73.7%) of specimens submitted for sequencing were identified as the Delta VOC (B.1.617.2) in Bungoma, Nakuru and Nyamira counties from samples collected between June 14 - 21, 2021.
- We also detected Alpha VOC (B.1.1.7) from five cases (26.3%) in Bungoma (2) and Nakuru (3) counties.
- The cases ranged from 19-82 years old and eleven (57.9%) were female. Two cases did not include their age on the CIF.
- Although one case of the Delta VOC was detected in a foreign national, none of the cases indicated a history of travel reported on their CIF within the last 14 days prior to collection of specimens.
- All results from this report require further confirmation with whole genome sequencing to confirm the lineages identified by partial sequencing.

## Acknowledgement

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**Table. Case information and sequencing results from Bungoma, Nakuru and Nyamira counties**

Age (Years)	Gender	Nationality	County	Facility	Symptoms	Date of sample collection	Pango lineage	WHO lineage
NA	F	Kenya	Bungoma	Bungoma County Referral Hospital	Yes	6/15/2021	B.1.1.7	Alpha
48	F				No	6/15/2021	B.1.1.7	Alpha
78	M		Nakuru	Nakuru PGH	No	6/18/2021	B.1.1.7	Alpha
82	M				No	6/18/2021	B.1.1.7	Alpha
60	F				No	6/18/2021	B.1.1.7	Alpha
66	F		Bungoma	Bungoma County Referral Hospital	Yes	6/15/2021	B.1.617.2	Delta
61	F				Yes	6/15/2021	B.1.617.2	Delta
51	F		Nakuru	Nakuru PGH	No	6/18/2021	B.1.617.2	Delta
64	F				No	6/18/2021	B.1.617.2	Delta
29	F				No	6/18/2021	B.1.617.2	Delta
43	M				No	6/18/2021	B.1.617.2	Delta
60	M				No	6/18/2021	B.1.617.2	Delta
43	F				Yes	6/18/2021	B.1.617.2	Delta
39	M				No	6/18/2021	B.1.617.2	Delta
52	M				No	6/18/2021	B.1.617.2	Delta
49	M		Uganda		Yes	6/18/2021	B.1.617.2	Delta
22	M		Kenya	Nyamira	Nyamira County Referral Hospital	No	6/20/2021	B.1.617.2
NA	F	Yes				6/20/2021	B.1.617.2	Delta
19	F	Yes				6/21/2021	B.1.617.2	Delta

Note: No cases reported a travel history within the last 14 days from the date of sample collection; NA = not available