FLUIDIGM® | Technical Note

Detection of Delta+ Variant and Inferred Identification of Delta Variant Using Advanta SARS-CoV-2 Mutation Assay Panel for Research and Community Surveillance

Increased Prevalence of Delta and Decline of Epsilon in Global Epidemiology

The spike protein mutation L452R, historically linked to the Epsilon variant of SARS-CoV-2, more recently is identified as a key mutation in the Delta and Delta+ variants. While the Epsilon variant has declined below 1% of cases in the United States, its origin and primary location of existence, the Delta variant has increased beyond 20% and is on the verge of becoming the dominant variant globally.[1] Based on these epidemiological statistics, it is clear that detection of the L452R mutation can be used to infer the presence of the Delta variant for surveillance purposes.

The Advanta™ SARS-CoV-2 Mutation Assay Panel (Table 1) can be used to detect the L452R mutation associated with the Delta variant and the K417N mutation associated with the Delta+ variant. Combined with prevailing epidemiology, this assay (when run with additional reagents not provided) can be a quick, cost-effective tool for community surveillance of the fast-growing Delta and Delta+ variants.

Assay Design for L452R

The detection of L452R is conducted in a two-step process (Figure 1): 1. One-step RT-preamplification with forward and reverse primers that produces a 99 bp amplicon product, and 2. qPCR-based detection of L452 wild-type (CUG) or L452R mutant (CGG) that produces a 90 bp amplicon product. The qPCR assay uses either a mutant or wild-type-specific forward detection primer, a common detection probe with FAM-quencher, and a common reverse primer. The specificity of the assay relies on two target-specific primers and the probe.

Specific Detection of L452R

In order to demonstrate specific detection of L452R, Twist Synthetic SARS-CoV-2 RNA Controls, Wuhan-Hu-1 (wild type; Control 2; PN 102024), and India/CT-ILSGS00361/2021 Kappa variant (mutant to isolate for L452R; Control 18 (B.1.617.1); PN 104338) were tested using the Advanta SARS-CoV-2 Mutation Assay Panel with the 192.24 GE IFC (integrated fluidic circuit) with 20 replicates each. The test also included assays for N1, N2, and RNase P in quadruplicate for COVID-19. The heat map of the Ct value is shown in Figure 2. The N1, N2, and RNase P were positive for both wild-type and Kappa variant for all the assay replicates. The no template control (NTC), negative control (NC), and positive control were tested in duplicate, and controls met the expected results. The results from Biomark™ Pathogen Detection Software are compiled in Table 1 and aligned with expected detection outcome based on the current assays in the panel.

Mutation (where first reported)	B.1.1.7 (UK)	B.1.351 (S. Africa)	P.1 (Brazil)	B.1.427 B.1.429 (US–CA)	B.1.526 (US–NY)	B.1.617.2 (India)	B.1.617.2.1 (India)
WHO identification	Alpha	Beta	Gamma	Epsilon	Iota	Delta	Delta+
K417N		✓				Not detected	✓
K417T			✓			Not detected	Not detected
L452R				✓		✓	✓
E484K		✓	✓		✓	Not detected	Not detected
N501Y	✓	✓	✓			Not detected	Not detected
Δ69/70	✓					Not detected	Not detected

Table 1. Mutations detected by the Advanta SARS-CoV-2 Mutation Assay Panel

L452R assay design strategy

One preamplification assay for both wild type and mutant
Two detection assays: one for wild type and one for mutants

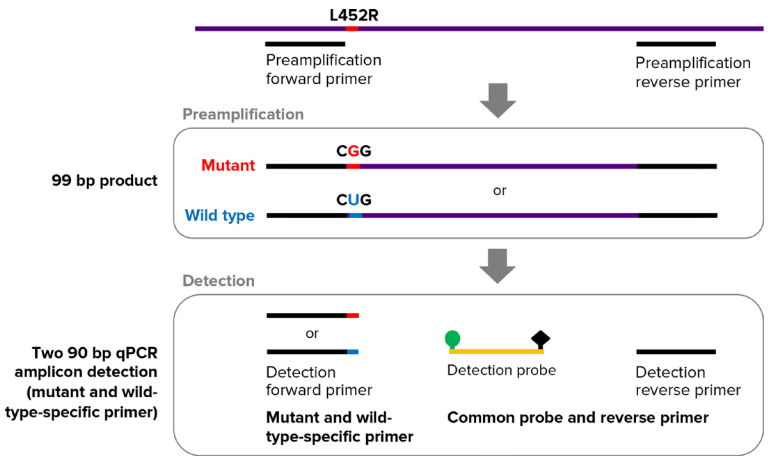

Figure 1. Schematic of assay design for L452 mutation

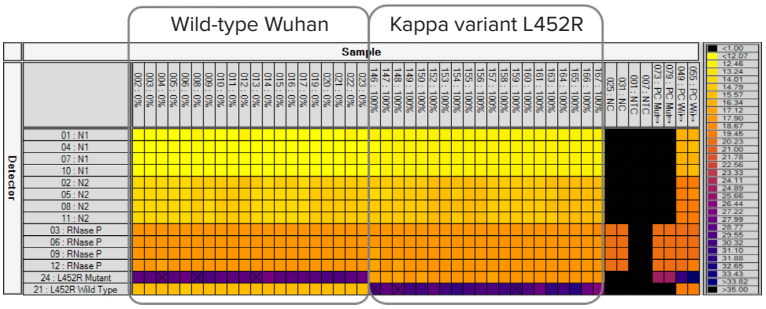

Figure 2. Heat map of threshold cycle (Ct) for Wuhan wild-type and Kappa variant testing

Sample Name	Interpretation COVID-19	Interpretation T22917G (L452R)
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Wild Type Wuhan-Hu-1	Detected	Absent
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present
Variant Kappa (B.1.617.1)	Detected	Present

Table 2. Results of Wuhan wild-type and Kappa variant testing using Advanta SARS-CoV-2 Mutation Assay Panel

Advanta SARS-CoV-2 Mutation Detection Assay

The Fluidigm RT-PCR-based Advanta SARS-CoV-2 Mutation Assay Panel is a fast, cost-effective, saliva and extracted RNA-based assay that leverages our proprietary microfluidics technology along with the Biomark™ HD platform, an IFC controller, and additional reagents acquired separately to detect mutations associated with Variants of Concern identified by CDC and WHO for research or surveillance. As the virus continues to evolve, the assay's open design enables this solution to evolve along with it. To stay up to date on our mutation detection solutions, visit our web page, **fluidigm.com/singlearticles/sars-cov-2-mutation-detection**

References

1. covid.cdc.gov/covid-data-tracker/ #variant-proportions

Learn more at **fluidigm.com**

Or contact: **tech.support@fluidigm.com**

For Research Use Only. Not for use in diagnostic procedures.

Information in this publication is subject to change without notice. **Patent and License Information:** fluidigm.com/legal/notices. **Trademarks:** Fluidigm, the Fluidigm logo, Advanta, and Biomark are trademarks and/or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of their respective owners. © 2021 Fluidigm Corporation. All rights reserved. 07/2021

FLDM-00618 Rev 01