

LABSTRACT – March 2021

SARS-COV-2 (COVID-19 virus) Variant of Concern (VOC) Testing

Audience

Health care providers, including laboratory and public health staff

Overview

Effective Monday March 22, 2021

- All COVID-19 PCR-positive specimens with adequate viral load (Ct value ≤ 35) in Ontario will be tested for the N501Y and E484K mutations using a multiplex real-time PCR assay
- Only specimens positive for the E484K mutation (with or without the N501Y mutation) will be routinely sequenced and reported for surveillance purposes
- Specimens that are N501Y positive and E484K negative are presumed to be Variants of Concern (VOCs) (likely B.1.1.7) based on current epidemiology, and will not require further sequencing

Background information

Since the detection of the first confirmed case of B.1.1.7 on December 26, 2020, Ontario has been actively testing for the presence of VOCs.

The approach initially targeted high priority specimens, followed by universal VOC mutation testing of all SARS-CoV-2 positive specimens in Ontario for the presence of the N501Y mutation present in all three most commonly described VOCs (B.1.1.7, P.1, and B.1.351) since February 3, 2021. When the N501Y mutation is detected, appropriate specimens are tested by whole genome sequencing (WGS) for VOC identification and classification.

To date, this approach has revealed that B.1.1.7 now represents a common variant in Ontario at > 40% of all specimens tested. Therefore, PHO Laboratory is modifying the VOC testing algorithm to ensure ongoing monitoring of B.1.1.7 as well as more rapid and comprehensive detection and characterization of B.1.351, P.1, and novel VOCs. To enable this change PHO is introducing a new multiplex real-time PCR assay on March 22, 2021.

What has changed?

VOC testing algorithm to start March 22, 2021

Multiplex real-time PCR assay for mutations associated with VOCs

All COVID-19 PCR-positive specimens that meet assay testing specifications in Ontario will be tested for the N501Y and E484K mutations by the multiplex real-time PCR assay. This test replaces the N501Y single target test that was implemented February 3, 2021. Due to the real-time PCR assay specifications, only specimens with adequate viral load (CT value ≤ 35) will be sent for testing.

Whole genome sequencing for VOC confirmation and lineage designation

Only specimens that are positive for the E484K mutation (with or without the N501Y mutation) will be routinely sequenced and reported for surveillance purposes. Specimens that are N501Y positive and E484K negative are presumed to be VOCs (likely B.1.1.7) based on current epidemiology, and will no longer be sequenced to report the lineage. Specimens not undergoing whole genome sequencing will be reported out as final upon completion of the multiplex real-time PCR assay. Due to whole genome assay specifications, specimens testing CT ≤ 30 with the real-time PCR assay that detects mutations associated with VOCs will be sequenced to ensure adequate quality of sequencing data generated.

Table 1 outlines the new testing algorithm based on the results for the multiplex real-time PCR assay for mutations associated with VOCs.

Table 1: Testing algorithm for whole genome sequencing based on multiplex real-time PCR assay for VOC mutation results

N501Y Mutation	E484K Mutation	Whole Genome Sequencing	Notes
Not Detected	Not Detected	Not performed	A subset may be tested for surveillance purposes Likely B.1.1.7 based on current epidemiology
Detected	Not Detected	Not performed	A subset may be tested for surveillance purposes
Detected	Detected	Performed*	Likely P.1 or B.1.351 based on current epidemiology
Not Detected	Detected	Performed*	
Unable/Invalid	Unable/Invalid	Not performed	

* Dependent on Ct value ≤ 30 on real-time PCR assay for mutations associated with VOCs

For further information

- Please see the PHO Test Information Sheet on SARS-CoV-2 (COVID-19 Virus) Variant of Concern (VoC) Surveillance at <https://www.publichealthontario.ca/en/laboratory-services/test-information-index/covid-19-voc>
- Contact the PHO's Laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567 (toll-free), or by email at customerservicecentre@oahpp.ca
- For PHO's Laboratory specimen collection information and previous Lababstracts, refer to [publichealthontario.ca/test directory](https://publichealthontario.ca/test-directory)
- The current versions of the PHO Test Requisition and other forms are available at publichealthontario.ca/Requisitions
- To subscribe to receive future Lababstracts, [register on our website](#)
- To register for Autofax and receive laboratory reports by fax directly from our laboratory information system as soon as they are released, contact the PHO's Laboratory Customer Service Centre.

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