Public Health Ontario | Santé publique Ontario

FREQUENTLY ASKED QUESTIONS

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

03/24/2021

Introduction

This document aims to provide information about the recent changes to the Public Health Ontario's laboratory (PHO) process for detecting SARS-COV-2 variants of concern (VOCs). The previous approach tested all positive COVID-19 samples for a mutation known to be associated with VOCs called N501Y.

A second mutation associated with a subset of VOCs (E484K) is being added to PHO's VOC mutation testing by real-time PCR (hereafter referred to as VOC PCR test) starting March 22, 2021.

Additional testing called whole genome sequencing will be performed as required to understand the epidemiology of B.1.1.7 (first identified in the UK), B.1.351 (first identified in South Africa), P.1 (first associated with Brazil), and emerging variants to understand how these different types were spreading in Ontario.

About the Testing Update

Q1. What has changed?

Starting March 22, PHO's VOC PCR test will be applied to eligible SARS-CoV-2 positive samples and will detect both the N501Y and E484K mutations at the same time. Given the accuracy of PHO's VOC PCR test in Ontario, genome sequencing is no longer required to confirm that a sample is positive for the B.1.1.7 variant. As a result, instead of all N501Y positives going for whole genome sequencing, only those where the E484K mutation is detected (with or without N501Y) will be routinely sequenced. Ongoing surveillance testing will also continue for a subset of samples without the E484K mutation.

Q2. Why is this change being made?

We are making this change because over 95% of samples sequenced to date in Ontario that have N501Y in the absence of the E484K mutation can be categorized as B.1.1.7. By introducing the E484K target in our VOC PCR test, we will continue to both track the B.1.1.7 variant and be able to more closely identify potential B.1.351, P.1 or any other emerging variants associated with E484K mutations.

Q3: Why can't you continue sequencing all samples that test positive for a variant?

VOC PCR testing enables more rapid information for surveillance of VOCs. Whole genome sequencing is a complex test which takes 4-5 days to complete and is best suited for population surveillance. Using the VOC PCR test and whole genome sequencing in complementary ways ensures that labs have the capacity and resources to focus on the areas that will provide the most value in terms of detecting

spread of B.1.1.7, B.1.351 and P.1 and in identifying new and emerging variants, and to provide proactive and rapid tools to detect new and emerging variants in Ontario.

Q4. Will you still do whole genome sequencing on samples that are only N501Y positive?

Yes, to support ongoing surveillance efforts, a subset of samples that tested positive for the N501Y mutation will be forwarded for genome sequencing. Also, genome sequencing will continue to be conducted on a subset of other positive COVID-19 samples to proactively look for new and emerging variants in Ontario.

Q5: Why are there different cycle thresholds for testing and sequencing of VOCs vs. with the standard PCR diagnostic test?

VOC PCR testing and whole genome sequencing are less sensitive than the diagnostic PCR test and therefore require higher amount of virus in the sample to provide a reliable result. PHO compensates for this by selecting samples with higher viral load (lower Ct value) for the other two tests. For VOC PCR testing the Ct criteria is ≤35. Because whole genome sequencing is less sensitive than VOC PCR testing, the Ct criteria is ≤30 for this test. The CT threshold cut off for the diagnostic test has not changed.[1]

The goals of the testing are also different. The purpose of diagnostic testing is to diagnose people who are positive for the SARS-CoV-2 virus and for public health action to be taken to stop transmission of the virus. Surveillance testing, which includes VOC PCR testing and whole genome sequencing, is intended to understand trends of the virus at a population level by testing the best quality samples.

Q6. Is this updated test being shared with other laboratories in Ontario?

As with the VOC PCR test that detected the N501Y mutation, this updated test to identify the N501Y and the E484K mutations developed at PHO will be shared with other laboratories in Ontario's COVID-19 diagnostic network, as well as among labs nationally and internationally as requested.

Q7: Do you expect additional changes to VOC testing in the future?

Viruses are known to change and mutate, and the SARS-CoV-2 virus is no exception. As new and emerging variants become known, laboratory systems around the world, including PHO's, will be required to adapt and shift to proactively monitor for these variants and support surveillance efforts to identify their spread.

Questions from Health Care Providers

Q8. As a physician who orders SARS-CoV-2 testing for my patients, will I get whole genome sequence results for SARS-CoV-2 positive samples?

Whole genome sequence results identifying the VOC type will now only be reported for samples where the E484K mutation is detected and the sample meets technical criteria for sequencing. Whole genome sequencing results are for public health monitoring purposes and should not be used to inform patient care.

Q9: What does this change mean for my patient who tests positive for SARS-CoV-2?

Initial SARS-CoV-2 PCR testing has not changed and will continue to be used to identify patient cases of COVID-19, and support patient management and subsequent contact tracing. The criteria for

determining that a patient is positive or negative for SARS-CoV-2 by the initial PCR test has not changed. No further testing is required for patient management beyond the initial diagnostic SARS-CoV-2 PCR test.

Q10: My patient initially tested positive for SARS-CoV-2 but their VOC PCR test had a negative or invalid result, or their sample was not tested (did not meet the criteria for VOC testing). Does this mean the initial test result was a false positive?

The target for the diagnostic PCR test to detect SARS-CoV-2 has been validated using rigorous laboratory performance protocols. All diagnostic sample results reported positive have met the initial PCR test performance specifications.

The VOC PCR test is an additional test where all SARS-CoV-2 PCR positive samples from the initial diagnostic test are run to determine if they are positive for one of the VOCs.

The technical specification for this assay requires that only COVID-19 positives specimens from the initial test have a CT ≤35 (to ensure adequate viral load) to yield valid results for the VOC PCR test, and samples with a CT value above 35 are not tested. If the VOC PCR test is negative, your patient is still positive for SARS-CoV-2 but is not infected with one of the variants. If the VOC PCR testing is invalid, your patient is still positive for SARS-CoV-2 but the VOC assay was unable to be successfully completed, and therefore could not determine if your patient is infected with one of the variants.

References

1. Ontario Agency for Health Protection and Promotion (Public Health Ontario). SARS-CoV-2 (COVID-19 virus) variant of concern (VoC) surveillance [Internet]. Toronto, ON: Queen's Printer for Ontario; 2021 [cited 2021 Mar 23]. Available from: https://www.publichealthontario.ca/en/laboratory-services/test-information-index/covid-19-voc

Citation

Ontario Agency for Health Protection and Promotion (Public Health Ontario). SARS-COV-2 (COVID-19 virus) variant of concern testing update. Toronto, ON: Queen's Printer for Ontario; 2021.

Disclaimer

For More Information

For more information, email customerservicecentre@oahpp.ca.

Public Health Ontario

Public Health Ontario is an agency of the Government of Ontario dedicated to protecting and promoting the health of all Ontarians and reducing inequities in health. Public Health Ontario links public health practitioners, front-line health workers and researchers to the best scientific intelligence and knowledge from around the world.

For more information about PHO, visit publichealthontario.ca.

Ontario