Public Health Ontario | Santé publique Ontario

COVID-19 Laboratory Testing Q&As

Swabs, Kits and Media

1. Q. What is the difference between a swab and transport media?

A. A swab is a brush-like device used to collect the patient's specimen. The swab must collect cells from a suspected infected area. The laboratory will lyse the cells to recover either intact virus or viral nucleic acid and identify the virus through various laboratory methods.

The transport media is a solution that keeps the specimen stable until it can be tested by the laboratory. The laboratory method performed depends on the type of media that the swab is placed in, e.g. molecular testing, virus culture, or rapid flu testing.

Media containing molecular preservatives destroy the viruses, while keeping only their nucleic acid intact. Swabs submitted in molecular preservative media are only suitable for testing for viruses by molecular test methods.

2. Q. On your website, why do you indicate that some media are also suitable for rapid influenza testing while others are not?

A. It is important to note that public health units who are investigating respiratory outbreaks may require rapid testing for influenza virus in addition to COVID-19. Rapid influenza testing uses a non-molecular method that detects viral proteins (antigens) Universal transport media, also referred to as 'Multitest' or 'Virus Transport' media contain components that will preserve the intact virus rendering the sample suitable for rapid influenza testing as well as molecular testing for any virus including COVID-19.Therefore in these circumstances, it is important to have the correct media.

3. Q. What if I need rapid influenza testing in addition to COVID-19 and I received a kit that is not suitable for influenza?

A. Rapid influenza testing is only performed at the request of health units and is only performed on four specimens per outbreak. If you are concerned that your institution is having an influenza outbreak, and you do not have virus transport or universal transport media (UTM) please contact the PHOL Customer Service Centre

4. Q. Why are some swabs stated as suitable for nasopharyngeal collection (NPS), while others for deep nasal, and/or throat?

A. The physical characteristics of the swab are designed for specific purposes. Thin flexible swabs are used to collect NPS, while larger, sturdier swabs are used for deep nasal, nasal, and throat swabs. The swabs are recommended for specific specimen collection depending on the physical characteristics that will be comfortable for the patient.

5. Q. Are the swabs I received suitable to detect COVID-19?

A. The Infectious Diseases Society of America (IDSA) suggests collecting nasopharyngeal, or mid-turbinate or nasal swabs for SARS-CoV-2 RNA testing in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19.

While NP swab collection is widely used and the primary specimen type for commercial direct SARS-CoV-2 test platforms, based on current available evidence, clinical practice, and availability of testing resources, the IDSA believes there are comparable alternative methods for sampling the nasal passages.

References:

- https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/ (Recommendation #3)
- https://www.idsociety.org/globalassets/idsa/practice-guidelines/covid-19/diagnostics/table-3.png

6. Q. When I order collection kits, I do not always receive the kit that I ordered.

A. Due to global shortages, our laboratory leaders are working diligently to secure suitable swabs and collection kits so that testing is not interrupted. You may receive various combinations of swabs and transport media depending on what is available to fulfill orders. You will receive kit instructions with links to visual diagrams to illustrate how to collect specimens. The instructions will also indicate if the collection media is suitable for molecular and other types of test methods.

PHOL performs sterility evaluations on all swabs to ensure they are safe to use for the patient. We also perform evaluations to determine if the swabs are able to recover human cells. Only swabs that pass sterility and recovery evaluations are approved for distribution.

7. Q. Is there a minimum age limit on the swabs, or can they be used for any age person?

A. We do not have age limits on any swab. If the swab fits in the nose, it can be used as directed.

8. Q. Is the pediatric swab (Copan CA56750CS01) supposed to be used only on pediatric patients? If so, what would be the age recommendations for this?

A. The Copan CA56750CS01 swab can be used for pediatric patients, but so can the other swabs. There is no age recommendation, but it is expected to be used on pediatric patients. However is not appropriate for use on neonates as it is too wide for this age group. There are limited numbers of Copan CA56750CS01 available.

9. Q. If a pediatric swab is not available, can the practitioner choose an alternative site for a pediatric client? For example, choose deep nasal when the swab is an NPS.

A. Yes, an alternative site such as nasal or deep nasal can be used, as long as the swab tip fits in the patient's nose. Alternatively a throat collection can be done.

10. Q. Can adult patients choose an alternative site even though the swab is an NPS?

A. For best sensitivity an NPS swab should be used for NPS collection, however nasal or deep nasal collection is also acceptable if NPS can't be performed.

Testing Results and Performance

1. Q. What is the test performance of the PCR assay in use at PHO Laboratory?

A. PHO Laboratory validated the PCR assay currently in use in close collaboration with the National Microbiology Laboratory (NML), Canada's reference microbiology laboratory. We have excellent concordance with NML from the parallel testing done with them at set up, which included a large number of negatives (over 100) and positives (over 20). The sensitivity and specificity of the assay, comparing to NML as the gold standard, is close to 100%.

However, there are many commercial and laboratory developed assays being released and used in Canada, and it is not possible to compare assay performance with every one of them. It is expected there will be some variance in performance if multiple assays are compared to each other, especially around the limit of detection of the individual assays. Parallel testing with the commercial kits in use so far shows similar performance with the assay in use at PHO Laboratory.

More information on the testing done at PHO Laboratory from our COVID-19 Test Information Sheet: https://www.publichealthontario.ca/en/laboratory-services/test-information-index/wuhan-novel-coronavirus

2. Q. What is the positive predictive value of COVID-19 PCR assays?

A. In general, the positive predictive value of COVID-19 PCR assays is excellent, and approaches 100%. At PHO Laboratory, we know this, as we are able to generate viral sequence from samples that are positive provided the viral copy number is not near the limit of detection of the assay.

3. Q. To what degree are the reverse transcriptase polymerase chain reaction techniques used for COVID-19 testing standardized in laboratories across Canada?

A. There is some variability in assays used across Canada. PHO Laboratory currently uses the same assay as the National Microbiology Laboratory, Canada. There are many commercial assays that have been released and are now being used in hospital and community laboratories, and have been introduced at PHO Laboratory to increase testing capacity. Overall, early information suggests that the performance of the different assays is similar. The Canadian Public Health Laboratory Network (CPHLN) is has conducted an evaluation of several of the different assays being used and has found that the assays in use are of similar analytical sensitivity (they have similar lower limit of detection). This information has been summarized and will be submitted for publication.

4. Q. What is the sensitivity, and how often is the COVID-19 test false negative?

A. It is hard to answer this question objectively on how many false negatives there are, as the only way to know is to retest patients who are initially negative, or retest a large number of the same samples with a different assay.

Several studies with small sample sizes have been published, and have estimated that the first test done has a sensitivity of 70% to 90% for detecting SARS-CoV-2.

When PHO Laboratory set up the assay, close to 200 patients were retested at The National Microbiology Laboratory (NML) and got almost identical results, giving a specificity of over >99%. False positive results were not observed (see Q2). However, NML use the same assay for testing as PHO Laboratory, and ideally, it would be compared to a different assay, which tests for a different gene target to more thoroughly evaluate for false negative results.

A preliminary review of PHO Laboratory data for patients tested by nasopharyngeal swab (NP) and/or throat swab between January 11 and April 14 has been conducted. Other specimen types were not included in the analysis (bronchoalveolar lavage, endotracheal aspirate, lung tissue).

a) Based on 569 positive patients (defined as having any 1 test positive over multiple testing episodes) who had more than 1 swab collected and were positive on at least one sample, the first PCR test has a sensitivity of 85% for detecting COVID-19 - 484/569 were positive on first testing episode, while 85 were negative on the first testing episode, and became positive on a second or subsequent testing episode.

b) Among 4220 patients who were negative on their first testing episode, and subsequently retested, only 85/4220 (2.5%) flipped from negative to positive. This gives a negative predictive value (NPV) of the first test of 97.5% (3335/3420).

Note that the subgroup of patients that were retested may not be the same as those patients that only get one test and never get retested. A high proportion of these may have been tested in suboptimal conditions initially e.g. if they were asymptomatic.

It is presumed that the large majority of patients included in this analysis were symptomatic. The sensitivity in patients with milder forms of illness, including asymptomatic patients, is likely very different, and has not been well elucidated. Available data suggests that patients with more severe illness have higher viral loads than those with milder illness.

When testing asymptomatic patients, a negative test does not rule out infection, as it could be early in the incubation period before the virus is actively replicating at a level that can be detected by PCR assays. At this time, there is not enough data to know the sensitivity, specificity, and predictive values of testing in asymptomatic persons. This would require testing a large cohort of patients, and adjusting these values for the day within the incubation period that the person is tested at. This would also require a large cohort of patients tested in a controlled fashion, with clinical follow up and repeat retesting at intervals.

5. Q. What is the incidence of false positive COVID-19 tests? What is the cause of this and what is done to minimize this from occurring?

A. Based on PHO Laboratory data, we are aware that the incidence of false positive tests that are later corrected is extremely low. The exact incidence for the province is not known, as individual reports that are corrected do not require notification.

As of May 23, 2020, PHO Laboratory has detected false positive SARS-CoV-2 results on approximately 20 occasions among over 228,000 specimens tested to date for COVID-19, with ~11,000 specimens testing positive. This represents a false positivity rate of less than 0.01% (specificity of >99.99%), which is well beyond performance targets for a laboratory test, even acknowledging there may be some false positive tests that are not detected.

In general, the positive predictive value of COVID-19 PCR assays is excellent, and approaches 100%. At PHO Laboratory, we know this as we are able to generate viral sequence from samples that are positive provided the viral copy number is not near the lower limit of detection of the assay.

False positive results can occur at various stages of laboratory testing, which can be grouped into the following categories:

> **1. Pre-analytical errors.** These are errors that occur prior to the actual testing being done. These could include mislabelling of samples that result in incorrect results being reported. They may also result from specimen contamination in transport or during aliquoting in the laboratory.
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> **2. Analytical errors.** These occur during the actual laboratory testing. These could occur for various reasons such as reagent contamination. Reagents can arrive contaminated from the supplier. To mitigate this, each new batch undergoes a quality assurance check before being put into use. False positive results can also arise from contamination due to pipetting errors, which can be due to human error or defects in automated equipment. Such analytic errors are controlled by having negative and positive controls on each run of the assay, which are reviewed prior to releasing results.
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> **3. Post-analytical errors.** This involves result interpretation by the technologist and reporting of results. Incorrect interpretation could lead to a false positive result. Transcription errors could also result in false positive results being generated. Such errors are controlled by having a second technologist review results prior to them being reported out.

6. Q. What are the difference targets that are detected in the COVID-19 PCR assays?

A. The majority of COVID-19 PCR assays target one or more regions in the viral genome, including the envelope (E), nucleocapsid (N), open reading frame (Orf) 1a/b, spike (S) protein, and RNA-dependent RNA polymerase (RdRp) genes. The sensitivity and specificity of each target depends on the design of the primers and probes and the testing conditions of the PCR assay. In general, the various PCR assays used in Ontario are comparable in test performance.

PHO Laboratory uses two commercial assays, Roche (Orf1a/b and E genes) and Abbott (N and RdRp genes), as well as a laboratory-developed test (LDT), which detects the E and RdRp genes. The E gene assay was found to be more sensitive compared to the RdRp gene assay and therefore was chosen as the primary target when testing with the LDT. The LDT E gene assay can detect other sarbecoviruses, including SARS-CoV and bat-related coronaviruses; however, this is not a concern since these viruses are not currently circulating. The LDT E gene assay will not detect the human seasonal coronaviruses (OC43, HKU1, 229E, NL63).

7. Q. How do nasopharyngeal swabs (NPS) and throat swabs compare in their test performance?

A. Upper respiratory tract specimens include a nasopharyngeal swab (NPS), deep nasal swab, anterior nasal swab **OR** viral throat swab. **NPS is the preferred specimen when swabs are available, followed by a deep nasal swab.** An analysis of a subset of specimens tested at PHO Laboratory shows that throat swabs are less sensitive than NPS.

The analysis looked at approximately 2400 episodes of parallel NPS and throat swab collections (separate throat swabs and NPS collected at the same time). Sixteen (18.4%) of 87 parallel collections that were positive by NPS were negative by throat swab. In addition, only 7 (0.3%) of 2307 parallel collections episodes with a negative NPS were positive by throat swab collected at the same time. Similar analysis of the performance of nasal swabs, or different types of nasal swabs (deep or anterior) could not be performed at PHO Laboratory, as this information was not consistently provided on the laboratory requisition.

Although a NPS or nasal swab is preferred over a throat swab due to increased sensitivity, PHO Laboratory continues to accept and test throat swabs. Throat swabs may be the only testing option for specific reasons such as limited supplies of NPS and nasal swabs, or patient factors e.g. nose bleeds with nasal/NP swabbing.