

FOCUS ON

The Use of Saliva as an Alternate Specimen for SARS-CoV-2 (COVID-19) PCR Testing

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Introduction

- Nasopharyngeal (NP) swabs are considered the gold standard specimen type for SARS-CoV-2 PCR testing. Saliva is an alternative specimen collection type for SARS-CoV-2 (COVID-19) Polymerase Chain Reaction (PCR) testing. Saliva can be collected in several ways including direct pooled saliva or as a swish and gargle approach, and complements other non-NP swab collection methods including throat and nasal swabs for SARS-CoV-2 PCR testing.
- While there is some heterogeneity of performance data of saliva as a specimen type for SARS-CoV-2, and a higher rate of invalid samples when compared to NP swabs, more recent publications have shown better performance, making saliva a reasonable specimen type to consider for use in the ambulatory, non-hospitalized setting.
- The use of saliva as a collection method in Ontario may ensure more accessible testing, particularly for children. Implementation of this collection method will require securing a stable supply of an acceptable specimen collection device that can be incorporated into laboratory workflows, and validation on a sufficient number of assays and laboratories to provide testing across the provincial diagnostic network.

Background

Current specimen collection has mainly relied on trained healthcare providers wearing full personal protective equipment (PPE), with collection of nasopharyngeal (NP), deep or anterior nasal, or throat (oropharyngeal) swabs, with NP swab considered the gold standard specimen type.

One challenge with NP, nasal or throat collection is that many patients do not tolerate having these collected, due to discomfort, or anxiety around having the procedure done, especially among children. Other patients can't have these done for medical reasons (e.g. coagulopathy or anticoagulant therapy, significant nasal septum deviation, or local oropharyngeal disease).

There have been several studies published in recent weeks that have shown acceptable performance of saliva collection when compared to NP collection. Although saliva takes more time to collect, and may not be suitable for all patients, it does provide an option for patients for whom NP, nasal or throat swab collection are not feasible. In particular, it provides an alternative collection method for testing children that are able to produce saliva and are not likely to tolerate NP, nasal or throat collection, especially if having repeat testing done as part of surveillance activities.

Using saliva as an alternate specimen would also provide the option of specimen self-collection, potentially reducing the amount of close contact required between the healthcare provider (HCP) and the patient, and reducing associated PPE use. It would also provide more flexibility around testing - although some supervision may be required, the HCP will not need to be trained in respiratory specimen collection, which is considered a controlled act.

Performance characteristics of saliva specimens for the detection of SARS-CoV-2 (COVID-19)

This document complements and updates the information provided on saliva testing provided by PHO on September 15, 2020 in the document titled, [EVIDENCE BRIEF: The Use of Alternate Sample Collection Methods for COVID-19 PCR Testing](#).

Several studies have evaluated the performance characteristics of saliva for detection of SARS-CoV-2, and new publications are being released at a rapid pace. Some key recent studies and their findings are as follows:

- Saliva as a possible alternative to NP samples has been reported in several studies for COVID-19 testing. The studies showed that sensitivity in saliva samples varied from 69.2% to 97.1% when compared to NP samples.¹⁻³
- A recent August 2020 meta-analysis including studies published between January 1 and April 25, 2020 calculated sensitivity of the saliva testing to be 91% [95% CI: 80%—99%] compared to 98% [95%CI: 89%—100%] in NP for previously confirmed COVID-19 patients.⁴ The authors also found that viral loads were higher in NP tests compared to saliva, and concluded that saliva-based testing is promising, though more data is needed from diagnostic accuracy studies. The authors also noted that there were 18 registered ongoing clinical trials of saliva-based tests for detection of SARS-CoV-2.
- An Ontario hospital-based study involving hospitalized patients showed a significant difference in the sensitivity between NP swabs versus saliva (89% versus 72%).⁵ In addition, they and other Ontario researchers found that there were a higher number of invalid tests observed in saliva-based testing compared to NP testing and that high volumes of saliva were required, making collection cumbersome. This problem has been overcome with centrifugation at one Ontario laboratory, which has completed a full validation of saliva as an acceptable specimen for SARS-CoV-2 PCR testing on a commercial PCR platform (cobas® SARS-CoV-2, Roche Diagnostics), already in use at that laboratory (2020 email from L. Goneau; unreferenced).
- In April 2020 the Food and Drug Administration (FDA) granted emergency use authorization (EUA) to Rutgers' RUCDR Infinite Biologics and its collaborators for a collection approach that utilizes saliva as the primary test biomaterial for the SARS-CoV-2 coronavirus.⁶ Another accelerated EUA for the "Curative-Korva SARS-Cov-2 Assay," which was specifically designed for use with saliva, was also approved.⁷
- A recent publication from investigators at Yale has presented a detailed validation of using saliva as a specimen for testing using the N1 gene target of Centers for Disease Control and Prevention (CDC's) real-time PCR assay without nucleic acid extraction.⁸⁻⁹ This protocol (SalivaDirect) provides an easy saliva self-collected method, into a dry, sterile container. Agreement of >94%

with NP swab testing was documented by the investigators. The testing approach has received a lot of attention in the media, and a trial is ongoing including NBA players. It received Emergency Use Authorization from the FDA on August 15, 2020.¹⁰

- Another publication by the same group of Yale investigators conducted nucleic acid extraction with real-time PCR testing among hospitalized confirmed COVID-19 cases. At one to five days after diagnosis, they reported a saliva sensitivity of 81% [95% CI: 71%—96%] and NP swab sensitivity of 71% [95% CI: 67%—94%], suggesting at least similar sensitivity during hospitalization.⁹ They also tested 495 asymptomatic healthcare workers – SARS-CoV-2 RNA was detected in 13, and all were confirmed on a follow up NP swab; 9/13 had parallel self-collected NPS, among which only 2/9 were positive.
- A recent large study was conducted at Eastern Ontario Regional Laboratory in Ottawa and the National Microbiology Laboratory (NML) including 1939 paired swab and saliva samples collected from patients who were asymptomatic or with mild symptoms. SARS-CoV-2 E gene was detected in 70 samples, 80% with swabs and 68.6% with saliva.¹¹ Thirty-four participants (48.6%) tested positive for SARSCoV-2 on both swab and saliva samples. Twenty-two (31.4%) participants tested positive with swab alone and 14 (20%) who tested positive with saliva alone. This study was conducted using a commercial collection kit and transport media, which may have contributed to lower sensitivity than has been reported in other studies where saliva was collected in a sterile dry container.
- A study conducted in British Columbia evaluated saline mouth rinse/gargle (swish and gargle) approach for collecting saliva sample for detection of SARS-CoV-2. In addition, they collected neat saliva and NP swabs to compare performance characteristics of each sample type.¹² They found the swish/gargle method had sensitivity of 97.5% [95 CI: 86.9%—99.9%] compared to 78.8% [95% CI: 61%—91%] for neat saliva. It is unclear why there was a significant difference in the sensitivity between swish/gargle method and saliva method. More studies are needed to confirm these findings.
- British Columbia Centre for Disease Control has introduced a gargling method for specimen collection on September 18, 2020 for school-aged children.¹³ As yet, no other provinces have implemented saliva as a specimen for SARS-CoV-2 PCR testing, though there is ongoing evaluation.
- Recently, a study from Japan used a saliva method for screening asymptomatic patients.¹⁴ The asymptomatic included 2 separate cohorts: 1. asymptomatic patient with exposure to a confirmed case; 2. asymptomatic travelers arriving at Tokyo and Kansai airports. The sensitivity of saliva was reported to be 92% [90% CI: 83% to 97%] compared to 86% [90% CI: 77% to 93%] for NP swab.

Conclusions

The literature on the use of saliva for COVID-19 testing is rapidly evolving and expanding. The amount of data on saliva testing is increasing at a fairly rapid pace. Although findings are variable across different studies, overall, saliva appears to be an acceptable specimen, though it is generally less sensitive than NP swabs, the gold standard for SARS-CoV-2 PCR testing.

- Based on these studies, it is reasonable to recommend accepting saliva specimens for SARS-CoV-2 testing in Ontario.
- Saliva collection provides an additional benefit of allowing self-collection, though direct supervision will still be required for some patients. This reduces the potential exposure of health care workers (HCWs) and has an added benefit of reducing the amount of PPE used. It may also facilitate self-collection away from a healthcare facility.
- The option of saliva as a clinical specimen will increase options for collection of clinical specimens in ambulatory children, including when done for surveillance initiatives. However, anterior nasal swabs may be as acceptable to children, and easier and faster to collect than saliva specimens which take some time to produce. Like saliva, anterior nasal swabs will also likely have reduced sensitivity when compared to NP swabs.
- Due to possible lower sensitivity compared to NP swabs, the use of saliva should be limited to patients seen in ambulatory settings, such as assessment centres. Whenever possible, it should not be used for hospitalized patients, or symptomatic patients tested as part of outbreaks, where the highest sensitivity specimen (NP) is preferred.
- PHO Laboratory is validating saliva testing and swish and gargle on the laboratory developed test (LDT) performed at PHO.
- For saliva sample to be used across the province, it is important for majority of laboratories, including the core group of laboratories to validate and implement this method in their settings. Furthermore, it is critical to identify and secure a stable supply of small calibre collection containers that can be stored and handled efficiently in laboratories.
- Future studies on the use of saliva as a clinical specimen for SARS-CoV-2 PCR testing will be evaluated by PHO as they are released, and if there are concerns about the performance characteristics (e.g. sensitivity, specificity), the approach to using saliva will be reassessed.

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