Received: 20 April 2020 | Accepted: 22 April 2020

DOI: 10.1002/hed.26232

SPECIAL ISSUE

WILEY

Respiratory sampling for severe acute respiratory syndrome coronavirus 2: An Overview

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Abstract

The novel coronavirus disease 2019 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was declared a pandemic in March 2020. A plethora of respiratory sampling methods for SARS-CoV-2 viral detection has been used and in the current evolving situation, there is no international consensus on the recommended method of respiratory sampling for diagnosis. Otolaryngologists deal intimately with the upper respiratory tract and a clear understanding of the respiratory sampling methods is of paramount importance. This article aims to provide an overview of the various methods and their evidence till date.

KEYWORDS

coronavirus, COVID-19, respiratory sampling, RT-PCR, SARS-CoV-2

1 | INTRODUCTION

The novel coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an evolving pandemic.[1] Various detection methods have been described. Presently, respiratory sampling is the commonest method used for viral detection. Reverse transcription real-time polymerase chain reaction (RT-PCR), targeted at the E gene of SARS-CoV-2, was developed based on the protocol by Corman et al and this assay was officially released by the World Health Organization.[2] Positive results can be confirmed by a subsequent real-time RT-PCR assay targeted at the ORF1b-NSP-14 gene, based on the protocol by Chu et al.[3] In this article, we provide an overview of the plethora of respiratory sampling methods for SARS-CoV-2 that have been published to date. These methods may be divided into two main categories: upper respiratory sampling (nasal, nasopharyngeal, oropharyngeal, lingual, and gargle lavage) and lower respiratory sampling (sputum, tracheal, and bronchoalveolar).

2 | UPPER RESPIRATORY SAMPLING

2.1 | Nasal and nasopharyngeal specimens

The nasal swab (NS) refers to flocked swab stick sampling of the anterior nasal cavity, which is bounded anatomically by the nares anteriorly and the hard-soft palate transition posteriorly. In contrast, the nasopharyngeal swab (NPS) involves the introduction of a flocked swab stick deep into the nasopharynx (beyond the hard-soft palate transition) to achieve direct contact with the posterior nasopharyngeal mucosal wall. It is not possible to obtain a pure nasopharyngeal specimen with a flocked swab stick as its introduction mandates contact with the nasal cavity on the way in. It is, however, perfectly possible to obtain a pure NS, by avoiding deep insertion of the flocked swab stick. The average length of the anterior nasal cavity in a healthy adult is 5 to 7 cm.[4] A review of the English literature on PubMed confirmed that there is no published comparison study examining the SARS-CoV-2

viral load yield between nasal and nasopharyngeal flocked swab specimens. A study of such nature is also not clinically significant and would not have been logically undertaken.

In the 2003 SARS outbreak, an alternative method of nasopharyngeal sampling known as "nasopharyngeal aspirate" (NPA) was described by Chan et al.[5] This method allows for pure nasopharyngeal sampling without anterior nasal cavity contamination. Instead of the flocked swab stick, a suction catheter is threaded into the nasopharynx and suction is then activated, aspirating nasopharyngeal mucus into a trap. Chan et al compared NPA specimens with "nasal and throat swab" specimens and found a marginally better performance of NPA in detecting SARS viral RNA in confirmed SARS patients (NPA 29.6% vs NS/OPS 28.3%, no *P*-value provided).[5] It should be noted, however, that no details of the "nasal and throat swab" procedure were available. It is unclear if the "nasal" component referred to an anterior nasal cavity sampling (NS) or a combined NS/NPS sampling. Contrary to Chan et al's results, another study conducted in Hong Kong during the 2003 SARS outbreak reported that "pooled throat and nasal swabs" provided a higher diagnostic yield compared to NPA specimens. It was further highlighted that the former has the additional benefit of less risk of generating aerosols since no suction is involved.[6]

In the current COVID-19 pandemic, literature surrounding NPA as a form of respiratory sampling for SARS-CoV-2 viral shedding is lacking. Most studies appear to utilize swabbing as the main method of upper respiratory sampling. Comparisons of the combined NS/NPS vs oropharyngeal swab (OPS) have been published. In a study by Zou et al, 72 NS/NPS specimens sampled from the middle turbinate and nasopharynx, across various days of illness, were analyzed in 18 COVID-19 patients. Higher viral loads (cycle threshold [C_t] values) were detected in these specimens, compared to 72 OPS specimens in the same group of patients.[7] In the authors' view, compared to the NS, NPS and OPS, the NPA technique is time-consuming, resource intensive and unsuitable for mass testing in a pandemic situation.

2.2 | Oropharyngeal swab

The OPS is also known as the "throat swab." It refers to the sampling of one or more of the four oropharyngeal subsites (tonsils, soft palate, base of tongue, and posterior pharyngeal wall). Just as the nasopharynx is paired posterior to the nasal cavity, the oropharynx is situated behind the oral cavity and commences at the hard-soft palate transition. The commonest target site of an OPS is the posterior pharyngeal wall as it considered the anatomical continuum of the nasopharynx. Throat swabs or OPS are commonly used for respiratory sampling in the current COVID-19 outbreak. An early landmark study detailing the clinical characteristics of Wuhan COVID-19 patients used OPS as the sole respiratory sampling method.[7] In the largest study to date of a Chinese non-Wuhan COVID-19 cohort in Zhejiang, OPS was similarly used as the sole sampling method.[8] However, low negative predictive value of OPS has been reported. Xie et al reported that only 9 out of 19 (47%) OPS from ultimately seropositive COVID-19 patients were positive, calling to attention the importance of repeated sampling from multiple sites, including the lower respiratory system, to increase diagnostic yield.[9]

The NS, NPS, NPA and OPS methods were compared in a Chinese study by Ye et al, which reviewed SARS, MERS, and H1N1 respiratory sampling literature and concluded that among all the upper respiratory sampling methods, NPA had a higher positive rate within 2 weeks of symptom onset, while combined NS + OPS were the least harmful to medical staff during sampling.[10] It remains to be evaluated if the above findings can be extrapolated to SARS-CoV-2.

2.3 | Lingual swab

The lingual swab (LS) or oral cavity swab (OCS) for detection of SARS-CoV-2 involves swabbing the anterior two-thirds of the tongue or the oral tongue. A Wuhan study compared LS with OPS and found that in 91 patients, the positive rate of OPS was higher than that of LS. However, the authors noted that this difference may have been attributable to a single experienced nurse collecting all of the samples.[11]

Azzi et al reported the use of OCS and "oral saliva pipette collection" for viral detection. In a cohort of 25 severe to very severe COVID-19 patients (severity not otherwise specified but all patients were mechanically ventilated in intensive care unit), it was reported that SARS-CoV-2 was detected in all 25 patients' salivary swabs, with different C_t values (range 18.12-32.23, mean value 27.16 $\pm$ 3.07). All patients had prior positive NPSs at point of diagnosis.[12] The authors thus concluded that the oral cavity saliva collection was a reliable method of viral detection. It should be noted, however, that these results were from critically ill patients whose viral shedding patterns may be different from suspect cases. Hence, the utility of oral cavity salivary swabs or LS may not be reproducible in screening scenarios. Although the LS or OCS is simple and presumably causes less discomfort, its use in the current pandemic is unlikely to be prevalent due to the need to maximize sites sampling and secretions collection to accurately diagnose infection.

2.4 | Gargle lavage

Exposure risk to healthcare workers are inherent while obtaining flocked swab samples as these methods may provoke sneezing, coughing and retching. Saito et al reported the use of gargle lavage as a safe and sensitive alternative to swab sampling. Gargle lavages using 10 mL of normal saline and OPS were compared in a COVID-19 patient on Days 8 and 9 of illness. Notably, higher amounts of viral genome were seen in the gargle lavage samples.[13] This was, however, a single case report. Notwithstanding the above, it had been previously reported that for respiratory pathogens such as influenza A, influenza B, and respiratory syncytial virus, gargle lavage is more sensitive than throat swabs.[14] In the current outbreak, there is scant evidence of gargle lavage's sensitivity and specificity for viral detection.

3 | LOWER RESPIRATORY SAMPLING

3.1 | Sputum collection

Sputum collection may be performed during voluntary coughing or involuntary induction. To et al performed sputum collection by having patients cough into a sterile container, and demonstrated consistent detection of viral RNA levels in collected specimens.[15] In another limited study of 10 COVID-19 patients, Lo et al reported that SARS-CoV-2 RNA was detected in 90% of NPS specimens but falsely absent in 10% (one patient).[16] This patient was ultimately diagnosed after viral detection in collected sputum after one negative and one inconclusive result from NPSs (inconclusive result was defined as a C_t-value of 36-38). The superiority of sputum for SARS-CoV-2 viral detection was also echoed by Pan et al, who demonstrated that sputum samples generally showed higher viral loads than throat swabs.[17]

3.2 | Tracheal aspirate

Tracheal aspirates (TA) may be obtained via suction from an indwelling endotracheal tube in mechanically ventilated patients, or from direct tracheal suction of tracheotomized patients. The Chinese Society of Anesthesiology published their recommendations for tracheal intubation in critically ill COVID-19 patients, noting that this is an aerosol-producing procedure and should be avoided unless absolutely necessary.[18] In the same regard, the collection of TA for viral detection poses a significant risk to the healthcare worker. Huang et al reported viral load comparisons in upper respiratory samples vs endotracheal aspirates in a cohort of 16 intubated COVID-19 patients and found that the latter has significantly higher viral RNA values compared to nasal and OPSs.[19] However, this method of respiratory sampling is not relevant in ambulatory screening scenarios but may have a role in COVID-19 screening of patients on mechanical ventilation in intensive care units and for serial monitoring of viral load of intubated confirmed cases.

3.3 | Bronchoalveolar lavage

The earliest identification of the SARS-CoV-2 genome was performed via bronchoalveolar lavage (BAL) samples in the Wuhan Institute of Virology.[20] BAL is a form of the lower respiratory system sampling in which a bronchoscope is introduced into the trachea and bronchi, and a calculated amount of fluid is introduced and then collected for examination. Since December 2019, studies from China and Europe on the use of BAL specimens for SARS-CoV-2 viral detection have been published.[21-23] Accumulating evidence suggest that BAL may be useful in viral detection in cases of false-negative NPS and/or OPS.[24] In a study of 4880 suspect COVID-19 patients in Wuhan, Liu et al found that BAL exhibited the highest positive rate for SARS-CoV-2 detection and reported that the NS and OPS showed a poor combined positive rate of 38.25% while collected sputum exhibited a 49.12% positive rate. The case definition of a suspect COVID-19 patient in the study was based on the following: (a) typical respiratory infection symptoms such as fever, cough and hard (difficult) breathing, or (b) close contact with a SARS-CoV-2 patient.[25]

4 | CONCLUSION

Overall, aggregate studies suggest that lower respiratory tract specimens, especially BAL, tend to give a higher diagnostic yield than upper respiratory specimens in patients with pneumonia and should therefore be obtained whenever possible. However, lower respiratory sampling involves greater technical difficulty and exposure risk. TA and BAL are also unacceptable as screening tools. Hence, upper respiratory sampling remains highly relevant even though current literature increasingly highlights NS/NPS's and OPS's limitations and possible false negative results. A false negative result allows the release of an infected patient back into the community for continued viral transmission. From a public health perspective, patients who have symptomatology of SARS-CoV-2 but who tested negative initially, should be tested again

and asked to be isolated at home. It is critical for clinicians to base their diagnosis on more than one test, and to consider the patient's risk susceptibility especially in the setting of known community spread.

At present, the United States Centers for Disease Control and Prevention recommends that nasopharyngeal and oropharyngeal flocked swabs should be used for collection of specimen for SARS-CoV-2 viral detection.[26] While these methods have their limitations, both allow rapid up-scaling for mass testing and detection, as part of most countries' greater strategy to proactively test, isolate and contact-trace infected cases. Lower respiratory sampling methods lack rapid up-scaling potential.

The SARS-CoV-2 virus demonstrates affinity to angiotensin-converting enzyme 2 receptor, which is present in respiratory mucosa, heart, kidney, endothelium, and intestine tissues.[27,28] As such, numerous other biological sampling methods such as, but not limited to, blood, urine, and fecal specimen collection have been described. The in-depth discussion of each is beyond the scope of this article. Further aggregate studies may be considered to examine the diagnostic capability of each of the abovementioned methods.

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How to cite this article: See A, Toh ST. Respiratory sampling for severe acute respiratory syndrome coronavirus 2: An Overview. *Head & Neck*. 2020;42:1652–1656. https://doi.org/10.1002/hed.26232