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REVIEW ARTICLE

Upper respiratory tract sampling in COVID-19

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Abstract

Introduction: To review the present literature on upper respiratory tract sampling in COVID-19 and provide recommendations to improve healthcare practices and directions in future studies. *Methods:* Twelve relevant manuscripts were sourced from a total of 7288 search results obtained using PubMed, Medline and Google Scholar. The search keywords used were COVID-19, nasopharyngeal, oropharyngeal, swabs, SARS and CoV2. Original manuscripts were obtained and analysed by all authors. The review included manuscripts which have not undergone rigorous peer-review process in view of the magnitude of the topic discussed. *Results:* The viral load of SARS-CoV-2 RNA in the upper respiratory tract was significantly higher during the first week and peaked at 4-6 days after onset of symptoms, during which it can be potentially sampled. Nasopharyngeal swab has demonstrated higher viral load than oropharyngeal swab, where the difference in paired samples is best seen at 0-9 days after the onset of illness. Sensitivity of nasopharyngeal swab was higher than oropharyngeal swabs in COVID-19 patients. Patient self-collected throat washing has been shown to contain higher viral load than nasopharyngeal or oropharyngeal swab, with significantly higher sensitivity when compared with paired nasopharyngeal swab. *Recommendations:* Routine nasopharyngeal swab of suspected COVID-19 infection should take anatomy of the nasal cavity into consideration to increase patient comfort and diagnostic yield. Routine oropharyngeal swab should be replaced by throat washing which has demonstrated better diagnostic accuracy, and it is safe towards others.

Keywords: SARS-CoV-2; swab; nasopharyngeal; oropharyngeal; viral; analysis; coronavirus

INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or more aptly known as Coronavirus Disease 2019 (COVID-19) was an outbreak in Wuhan, Hubei province China. The World Health Organisation classified COVID-19 as a global health emergency on 30th January 2020. By 11th March 2020 it was declared as a global pandemic. The world has not seen a global pandemic for a century, the last being the Spanish Flu in 1918 to 1920 with similar or even less virulence to COVID-19.[1]

COVID-19 first arrived in Malaysia on the 25th January 2020 by travelers from China via Singapore.[2] The cases have been rapidly increased in numbers with new clusters leading to the introduction of the Movement Control Order (MCO) on the 13th March 2020. At the same time, healthcare facilities under the Ministry of Health (MOH) and university hospitals geared up to manage the overwhelming crisis.

COVID-19 spreads via airborne droplets. Upon entering the human body, the virus first break will be the upper respiratory tract. Replication begins and a myriad of symptoms such as high fever, sore throat, myalgia and fatigue may set in. COVID-19 may be asymptomatic for 5.2 to 12.5 days, thus acclaiming its reputation as a 'stealth virus'.[3] During this period, viral samples may be positive but the patient may be an asymptomatic carrier.

COVID-19 unlike SARS replicates in the upper respiratory tract which peaks at day five of infection.[4] Viral uptake in the upper respiratory tract is believed to be facilitated by the cleavage in S1-S2 regions of the viral protein.[5] In the lower respiratory tract, Angiotensin Converting Enzyme II (ACEII) receptors act as a binding side

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for the viral capsid antigen and allow entry into alveoli cells. These ACEII receptors are absent in the upper respiratory tract but are also present in the gastrointestinal system, heart, kidneys and alveolar cells.[6]

Viral samples for diagnostic tests can either be taken from the upper (nasopharyngeal/ oropharyngeal swabs or saliva) or lower respiratory tract (sputum or tracheal aspirate or bronchoalveolar lavage- BAL).[7] The lower respiratory tract specimen commonly sampled in symptomatic or severe cases. Sampling procedure is an important factor in ensuring fast and accurate results from laboratory testing. There are still ongoing issues on false negative results from specimens taken in the upper respiratory tract in asymptomatic individuals or mild cases, and the need to repeat sampling and tests. This may worsen the issues of the backlog of specimens that need to be tested and causes spread of disease from the false negative individuals as well as the risk of transmitting the infection to the healthcare workers during the sampling procedure. Although Nasopharyngeal (NP) and Oropharyngeal (OP) swabs are often used, there has yet to be any local data in relation to viral loads and the efficacy of NP and OP swabs as a procedure in obtaining diagnosis for asymptomatic and mild to moderate disease.

Therefore, the present review was performed on available studies to explore the efficacy of upper respiratory tract sampling in detecting SARS-CoV-2 in COVID-19 patients. Related published manuscripts were sourced using PubMed, Medline and Google Scholar. Keywords used were COVID-19, nasopharyngeal, oropharyngeal, swabs, SARS and CoV2. The search yielded 7288 results which were then browsed through, and narrowed down to 26 relevant manuscripts. Twelve original full manuscripts were obtained and reviewed by the authors. The relevant data related to the site of sampling from the upper airway, viral load, diagnostic tests accuracy and recommendation on sampling procedure were tabulated in Table 1.

Anatomic considerations in sampling of the upper respiratory tract for diagnosis of COVID-19

The nostril and the mouth are external openings that can be used to reach the nasopharynx and the oropharynx, which are suggested sites to obtain respiratory secretions for SARS-CoV-2 testing. A brief knowledge of the relevant anatomy is worth revisiting to increase the yield and reduce discomfort to the patients, since most of the tests are performed without an aid of an endoscope for precise localisation. The nose is a sensate organ. It does not only contain olfactory receptors to identify odour but tactile and thermal receptors supplied by the ophthalmic and maxillary division (CN V1) of the trigeminal nerve (CN V2). The location of these tactile receptors is debatable as in the empty nose phenomenon. The nasal vestibule followed by the inferior meatus is more sensitive to airflow compared to the middle meatus.[8] The nasopharynx sits behind the nasal cavities and estimation of the distance from the nares to the nasopharynx is more than 10mm shorter than the distance from the nares to the tragus.[9] The nasopharynx is rectangular in shape and is connected from the two nasal cavities via posterior choanae (FIG. 1).

Mucociliary action directs respiratory secretions from the nasal cavity to the posterior choanae and nasopharynx in both physiological and pathological conditions. The mucosa of the nasal cavities is highly susceptible to nociception and there are mechanical receptors in the naso and oropharynx which stimulates the cough reflex in both physiological and pathological conditions.[10] Stimulation of cough reflex during sampling is an unwanted sequela that causes aerosolization of the virus. The medial wall of the nasal cavity is constituted by the nasal septum, which is more rigid but the lateral wall is constituted by nasal turbinates that have compressible submucous structures (Figure 2a). There are confluences of blood vessels at the anterior part of the septum (Kiesselbach plexus-KP) and posterior part of the septum (Woodruff plexus-WP) which can potentially bleed following trauma.

The nasal cavity assumes a pyramidal shape where its inferior portion near the floor of the nose is much roomier compared to the superior portion (FIG. 2a). Nasal diseases such as deviated nasal septum (FIG. 2b), benign and malignant tumours of the paranasal sinuses may potentially cause problems during sampling, which can be confirmed with the help of an endoscope. Scope guided swab are often ideal for better visualisation and comfort for the patient. However, in view of potential transmission via airborne droplets and upper respiratory tract endoscopy procedures, many international Ear, Nose and Throat (ENT) organisations have advised against endoscopic procedure and anaesthesia in the form of aerosol droplets and if necessity dictates otherwise,

TABLE 1: Analysed manuscripts with region, specimen numbers, analysing technique, relevant findings and recommendations

Author (Year)	Region, Country	Studied specimens/ Confirmed PUI	Timing	Laboratory Technique(s) Findings	Recommendation(s)
Yang *et al*. (2020)	Shenzen, China	213 patients 866 samples nasal swb(n=490), throat swb (n=205), sputum(n=142), BAL (n=29)	From the day of admission among severe/mild patients 0 to 7 ds; 8 to 14 ds; >15 ds	RT-PCR 0 to 7 ds (severe/mild) Nasal (73.3%, 72.1%) Throat (60%, 61.3%) Sputum (88.9%, 82.2%) BAL (0,0) 8 to 14 ds (severe/mild) Nasal (72.3%, 53.6%) Throat (50%, 29.6%) Sputum (83.3%, 74.4%) BAL (100%, 0) >15 ds (severe/mild) Nasal (50%, 54.5%) Thorat (36.8%, 11.1%) Sputum (61.1%, 42.9%) BAL (29.8%, 0%)	Sputum is most accurate, followed by nasal swbs Throat swbs were not recommended in diagnosing Covid-19. Viral RNAs in BAL is necessary for diagnosis and monitoring in severe cases
Liu *et al*. (2020)	Wuhan, China	4880 samples Nasal swb Pharyngeal swb Sputum BAL	Not Specified	RT-PCR Total +ve Swbs: 1875 (38.42%) NP & OP (38.25% +ve) Sputum (19.12% +ve) BAL (100% +ve)	Combined clinical, imaging and RT-PCR for diagnosis confirmation Repeat RT-PCR for false negatives

Author (Year)	Region, Country	Studied specimens/ Confirmed PUI	Timing	Laboratory Technique(s) Findings	Recommendation(s)
Roxby *et al*. (2020)	Washington, USA	142 people at nursing home (80 residents, 62 staff) NP swb	d1 and d7 (7d apart)	RT-PCR d1 Res: 3 +ve, 1 -ve Staff: 2 +ve, d7 Res: 3+ve, 1 -ve Staff: not tested	Symptom based screening may not identify COVID-19
To *et al*. (2020)	Hong Kong, China	Self collected saliva 12 patients	Median 2d (0-7 d)	RT-PCR/Viral culture 2019-nCoV detected in 11/12 (91.7%) 3 cultures +ve ; 2 cultures -ve; Rest (7) pending	Saliva is preferred over NP for more comfort, non-invasive, less HCW exposure Suggest saliva for diagnosis and viral load monitoring
Wang *et al*. (2020)	Beijing, China	205 COVID-19 +ve patients 1070 specimens BAL (n=15) Bronchoscopic brush biopsy(n=13) Sputum (n=104) Nasal swb (n=8) Pharyngeal swb (n=398) Feces (n=153) Blood (n=305) Urine (n=72)	Pharyngeal swb: d1-d3 of hospital admission Others throughout admission	RT-PCR Described positive rates in all specimen sites as cycle thresholds less than 40 to indicate higher viral copy number; Extracted sensitivity, mean cycle thresholds BAL (93%, n=31.1), Bronchoscopic brush biopsy (46%, n=33.8), Sputum (72%, n=31.1), Nasal swb (63%, n=24.3), Pharyngeal swb (32%, n= 32.1) Feces (29%, n=31.4) Blood (1%, n=34.6), Urine (0%, ND)	Testing from multiple sites increases sensitivity and reduces false negative results.

Author (Year)	Region, Country	Studied specimens/ Confirmed PUI	Timing	Laboratory Technique(s) Findings	Recommendation(s)
Wolfel *et al*. (2020)	Munich, Germany	9 subjects NP swb (9) OP swb (9) sputum (n=7)	Between 2-4 ds post onset	RT-PCR No difference in detection rates and viral loads between NP swb and OP swb. Compare virus concentration: NP/OP swb > sputum (n=2) sputum > NP/OP swb (n=2) NP/OP swb = sputum (n=5)	Throat swb will provide sufficient sensitivity at early stage of infection (viral load peak before day 5)
Guo *et al*. (2020)	Guangzhou, China	11 subjects. 24 pairs of throat washing & NP swbs	Samples taken 48-57 ds after symptom onset	RT-PCR Commercial kits (unsure of processing methods) Sensitivity of throat washing (29.17%) and NP swbs (4.17%) Chi-square (p=0.031)	Throat wash is safe and reliable, better sensitivity compared to NP swb
Lo *et al*. (2020)	Macau, China	10 subjects. 213 NP swb, sputum, urine & stool specimens	Varying interval d1-d24 of admission, some paired and some not	SARS-CoV-2 viral RNA RT-PCR Sensitivity, viral RNA conversion time. 90% subjects diagnosed via NP swb 1 patient via sputum specimen, stool specimen NP swb (n=57/84; 68%; 18.2 days) Sputum (n=1/1; 100%; NA) Urine (n=0/49; 0%; NA) Stool (n=46/79; 58%; 19.3 days)	Universal combined NP swb and stool sampling in hospitalised patients to improve sensitivity

Author (Year)	Region, Country	Studied specimens/ Confirmed PUI	Timing	Laboratory Technique(s) Findings	Recommendation(s)
Yu *et al*. (2020)	Beijing, China	76 subjects. 323 nasal and throat swbs, sputum, blood and urine specimens	Samples are not paired, timing not mentioned.	droplet digital PCR (ddPCR) and RT-PCR based on two target genes (ORF1ab and N) High correlation between ddPCR and RT-PCR ORF1ab and N (ORF1ab R2 = 0.83; N gene, R2 = 0.87). Based on ddPCR, 16.4% (9/55) nasal swbs, 37.3% (50/134) throat swbs, and 66.4% (77/116) sputum samples were positive. Viral load: Sputum: (17429 ± 6920 copies/test) Throat swbs (2552 ±1965 copies/test, $p < 0.001$) Nasal swbs (651 ± 501 copies/test, $p < 0.001$).	Quantitative monitoring of viral load in lower respiratory tract samples helps to evaluate disease progression both RT-PCR and ddPCR were accurate and reliable in high-viral-load samples and negative samples, but ddPCR was better at detecting samples with low viral load.
Zou *et al*. (2020)	Zhuhai, Guangdong, China	18 subjects. 72 pairs of nasals (mid-turbinate, nasopharynx) and throat swbs	Consecutive paired sampling up to 9 times since day of admission	RT-PCR with primers and probes targeting the N and Orf1b genes of SARS-CoV-2 Higher viral loads (lower Ct values) detected in the nasal than in the throat swb	Viral load in asymptomatic patients was similar with symptomatic patients.

Author (Year)	Region, Country	Studied specimens/ Confirmed PUI	Timing	Laboratory Technique(s) Findings	Recommendation(s)
Pan *et al*. (2020)	Ghuang Zou, China	Nasal swb (n=1) Throat swb (n=67) Sputum (n=42)	2 patients, serial collection 3 to 15 ds after onset of symptoms	RT-PCR Viral loads ranged from 641 copies per mL to $1.34x10^{11}$ copies per mL **Throat swb**: median of $7.99x10^{4}$ copies per mL **Sputum**: median of $7.52x10^{5}$ copies per mL **Nasal swb**: $1.69x10^{5}$ copies per mL (day 3 post onset) **Sputum**: $1·34x10^{11}$ copies per mL (day 8 post-onset) **30 pairs of throat swb and sputum** showed significant correlation of viral load. d1-3 (r=0.50, p=0.02) d4-7 (r=0.93, p<0.001) d7-14 (r=0.95, p=0.028)	No recommendation
To *et al*. (2020)	Hong Kong, China	23 subjects. 173 respiratory samples	Serial self-collected posterior OP sample during admission	RT-qPCR Mean viral load at presentation = 5.2 log_{10} copies per mL. Viral load in oropharyngeal saliva samples was highest during the first week after onset, subsequently declined with time.	High viral load on presentation suggests that SARS-CoV-2 can be transmitted easily at early phase.

Abbreviations: swb: swabs, +ve: positive; -ve: negative; d: day, n/a: not applicable, n/d: not detected; p<0.05 is considered significant

adequate personal protective equipment should be donned.[11]

Based on these anatomic considerations, the authors would like to suggest several steps to reduce complications associated with NP swab. Firstly, using the distance between the nares to the tragus, estimate the required depth of insertion to sample the nasopharynx (FIG. 3). Secondly, direct the swab posteriorly along the floor of the nose, aiming laterally rather than medially to reduce the risk of pain and bleeding. Tilting the head by 70 degrees can be performed to ensure that the swab remains on the floor of the nose[12]. Thirdly, do not use excessive force to perform the procedure as the patient may have associated pathologies causing an obstruction not known to the healthcare practitioner. In addition to the nasopharynx, mid nasal turbinate and anterior nasal swabs (around the vestibule or nares) can be used to detect SARS-CoV-2. The use of a flexible silicone swab as recommended by the CDC can reduce patient discomfort. Both oropharyngeal and nasopharyngeal swabs need to be left for several seconds to allow absorption of respiratory secretion at the respective sites.[12] Upon removal, the operator may opt to rotate the shaft to overcome resistance.

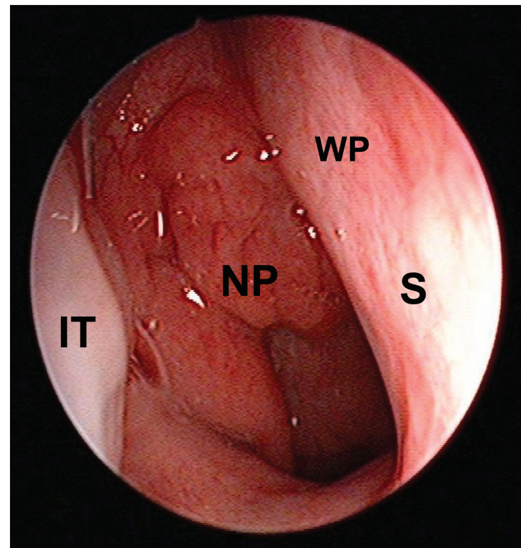

FIG. 1: Endoscopic picture of the nose showing the posterior part of the septum (S) with the Woodruff plexus (WP) and posterior part of the inferior turbinate (IT) opening into the nasopharynx (NP) via the posterior choana.

Viral load in different anatomical sites and sampling methods

Most of the coronaviruses are known to replicate in the epithelial cells of the respiratory tract. Viral loads on different time courses of COVID-19

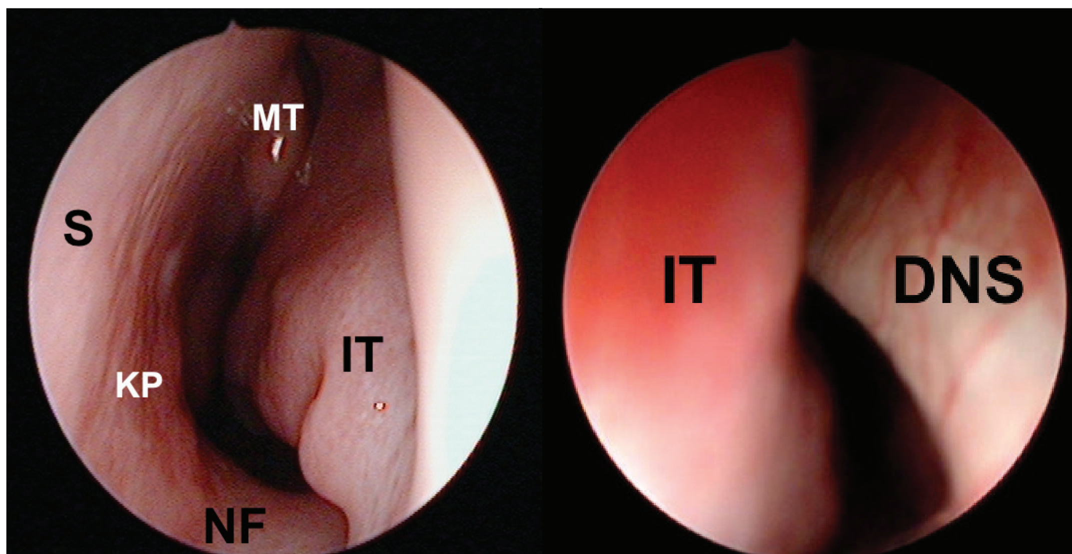

FIG. 2A (left): Endoscopic picture of the left nasal cavity showing the rigid septum (S), compressible inferior turbinate (IT) and roomier cavity at its inferior portion above the nasal floor (NF). The superior portion is less roomy at the level of the middle turbinate (MT). The Kiesselbach plexus is located at the anterior part of the septum (KP). **B (right):** Endoscopic picture of the right nasal cavity showing a deviated nasal septum (DNS) touching the inferior turbinate (IT) which can potentially cause problems during nasopharyngeal sampling.

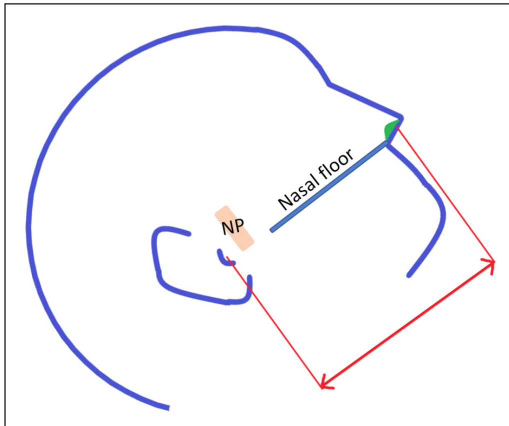

FIG. 3: Schematic diagram of the recommended head position. The nares (green) to nasopharynx (NP) distance is shorter than the distance between the nares and tragus (posterior to the NP) marked with red line.

indicate the dynamic changes in disease stages. High level of replication indicates progressive nature of the disease. Wang *et al*. in 2004 reported high SARS viral load in throat gargle and saliva samples, with median SARS-CoV RNA amount of 3.56 x 10^6copies per mL and 9.92 x 10^8 copies per mL respectively.[13] Although the genomic sequence of SARS-CoV-2 has close similarity to that of SARS-CoV, the viral shedding pattern in patients with SARS-CoV-2 resembles more to that of influenza virus.[14] It was reported that viral load was high in the early and progressive phase of the disease and gradually decreased in the recovery phase.[15,16] The amount of SARS-CoV-2 RNA in the upper respiratory tract was significantly higher during the first week, peaked at 4 to 6 days after onset of symptoms, ranging 10^4 to 10^7copies per mL.[4,17] This pattern is in part contradictory to SARS, which the viral load usually peaked at 10 days after onset. The high viral load at early phase of disease also proved that the SARS-CoV-2 virus replicates actively in the upper respiratory tract of the infected patients, rather than passive shedding from lungs. There was no documentation that viral RNA detected in urine sample.[17] However, low viral load was reported in stool samples, ranging 550 copies to 1.21 x 10^5copies per mL.[18]

During the recovery phase, patients may still carry the virus for a long duration before clinical cure. The viral RNA remained detectable at low level in OP swab samples for 9 days after full resolution of symptoms before turning negative.[4,15] To *et al*. reported that viral RNA was persistently detected for 20 days or longer after symptom onset in 33% of patients, up to 25 days in one patient[19]. Sputum sample remained positive even longer despite clinical improvement. Apart from this, SARS-CoV-2 was detected in asymptomatic patients and the viral load was similar to that in symptomatic patients.[14] Therefore, patients with no symptom or minimal symptoms carry the potential to transmit the disease.

Quantitative monitoring of the virus in respiratory tract samples is important to evaluate the disease progression, efficacy of antiviral drugs and prognosis.[15] It was generally found that the viral load in sputum samples is consistently higher than that in NP swabs, OP swabs or throat gargle.[4,15,17] Comparing the upper respiratory samples, NP swab has higher viral load than OP swab, where the difference in paired samples is best seen at 0-9 days after onset.[14]

Several authors had proposed the use of throat wash or gargle sampling technique that showed to contain higher viral load than NP swab and OP swab.[18,20] The authors postulate that more respiratory secretions and cells are removed from the upper respiratory tract via throat gargle than via NP swab and OP swab, thus generating higher yields. A study in Hong Kong reported the use of a self-collected early morning saliva sample from posterior oropharynx by asking patients to cough up and clear the throat.[19] The authors explained the high viral load in oropharynx because nasopharyngeal and bronchopulmonary secretions that move by mucociliary activity towards the oropharyngeal area while the patients are in a supine position during sleep. Besides, there was evidence that coronavirus is present in cell free specimens, which suggest that the virus is released and accumulates in the oropharynx and oral cavity.

Sampling of OP swab requires healthcare workers to inspect the patient's oropharynx directly. While NP swab is a relatively blind and invasive procedure, which can cause bleeding if it is not done properly. Both of these sampling methods can induce coughing and sneezing, which generates aerosol and carries risk of transmission of infection to healthcare workers. Throat gargle and saliva samples are non-invasive and easier to collect, compared to nasal or NP swabs. The samples are self-collected without close contact with healthcare workers, thus reducing the risk of virus transmission. Tu *et al*. demonstrated high correlation between patient-collected nasal swab (in anterior nares

and mid-turbinate) and healthcare worker-collected NP swab, with correlation coefficients of 0.78 and 0.86 respectively.[21]

Diagnostic accuracy of upper respiratory sampling in laboratory diagnosis of COVID-19

The 2019 novel coronavirus (SARS-CoV-2) pandemic has caused a global state of health emergency and demands for laboratory testing for the SARS-CoV-2, has increased exponentially. Many countries, including Malaysia are facing challenges in ramping up testing capacity to keep up with the rapid spread of the disease within the community. The rapid spread leading to a pandemic outbreak is largely attributed to high viral load in asymptomatic individuals infected with SARS-CoV-2 and the incubation period prior to disease manifestation where human to human transmission is still possible.

Standardising laboratory testing of SARS-CoV-2

The Centers for Disease Control and Prevention has suggested that polymerase chain reaction is used to target independent regions of the SARS-CoV-2 nucleocapsid gene in the specimens tested. This recommendation followed the data that emerged from Wuhan, China where the pandemic first started.[12] From February 29, 2020 many centers and referral laboratories receive guidance from the Food and Drug Administration to develop their own tests for the detection of SARS-CoV-2. These laboratories design, manufacture or obtain reagents that may still be labelled as research use to develop in-house testing.[22]

At the heart of the pandemic in Wuhan, China, Liu *et al*. in his report of 4880 patients that required COVID-19 screening, the real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) based viral nucleic acid test (NAT) from respiratory secretion was used because of its efficiency to confirm the virus infection within only 2 hours.[23] Real-time RT-PCR amplification of SARS-CoV-2 open reading frame 1ab (ORF1ab) and nucleocapsid protein (NP) genes fragments were performed using commercial kits provided by their local company in specific temperature conditions. A laboratory confirmed case was described when both targets (ORF1ab, NP) are tested positive. Additionally, a cycle threshold value (Ct-value) less than 37 was defined as a positive test, and a Ct-value of 40 or more was deemed a negative test[23]. Literatures on laboratory diagnosis of COVID-19 have, since then, detailed their sample preparation and result interpretation to mirror this landmark paper.[3,4,17-29,24,25]

Samples from respiratory secretions can be obtained from either upper or lower respiratory tracts for SARS-CoV-2 detection. Examples of upper respiratory tract samples suggested include NP and OP swabs, as well as NP wash or aspirate. Meanwhile, examples of lower respiratory tract samples include bronchial-alveolar lavage, bronchoscopic brushing and sputum.[1] Although the diagnostic yields of these samples are different, there are obvious advantages of sampling the upper respiratory tract compared to the lower respiratory tract. The guidelines by WHO states that upper respiratory tract sampling is preferable in ambulant, asymptomatic or mild cases since it is less invasive and carries less risk of aerosolization translating to reduced transmission to healthcare workers.[1]

Nasopharyngeal swab has better diagnostic accuracy compared to oropharyngeal swab

In a pandemic situation, many countries have set up national guidelines to allow optimal yield from testing so that high-risk patients can be isolated. Preanalytical variabilities including type of sample, collection, handling and transportation of samples are integral in ensuring a reliable result.[26] At the time of writing, the best level of evidence to prioritise NP over OP swab was a comparative analysis of 72 pairs of nasal (middle turbinate and nasopharynx) and oropharyngeal swabs for RT-PCR targeting SARS-CoV-2 ORF1ab and NP genes. In this study, higher viral loads (lower Ct- values) were detected in the nasal than in the oropharyngeal swabs where differences were best seen at 0 to 9 days after onset of symptoms. Although this study demonstrated a significantly lower Ct values by 2.8 (95% confidence interval [CI], –2.4 to 8.0) in swabs taken from severe patients compared to patients with mild-moderate cases, 2.5 (95% CI, –0.8 to 5.7), further statistical analysis comparing the performance of NP (excluding middle turbinate samples) compared to OP swab was lacking.[14]

Two other high volume, non-comparative cross-sectional studies recorded higher sensitivity in swabs taken from the nasal cavity (73.3% and 63%) compared to the oropharynx (60% and 32%) in samples taken from patients from periods before the illness to up to 7 days after the onset of illness. Both studies utilised RT-PCR detection of SARS-CoV-2 RNA in their

samples.[18,24] Yang *et al*. demonstrated a temporal relationship between sensitivity of the nasal swabs with the onset of illness in their study of 213 patients.[24] Nasal swabs taken from periods before the onset of illness to 7 days after onset of illness showed higher sensitivity at 73.3% compared to swabs taken more than 15 days after the onset of illness at 50%. A similar trend was seen with sensitivity values of OP swab which declined at two weeks after the onset of illness.[24] Findings of this study demonstrated that it is important to consider the timing after the onset of illness in interpretation of both NP and OP swabs. At more than 15 days after the onset of illness, high false negative rates are seen, such that in patients who demonstrate progression in symptoms, it is important to repeat and combine samples from the lower respiratory tract, feces or even serological testing to monitor disease progression. Wang *et al*. pointed that during the COVID-19 outbreak in China, the OP swabs were used more frequently (n=398) compared to nasal or NP swabs (n=8). However, the SARS-CoV-2 RNA was detected in a significantly lower proportion of OP swabs (32%) compared to NP swabs (63%), affirming the evidence favouring NP swabs.

Wolfel *et al*. in a report of 9 patients with COVID-19 in Germany conversely reported no significant difference in detection rates and viral loads between 9 paired NP and OP swabs.[4] Although we are not able to refute the possibility of geographical differences in the diagnostic accuracy of NP swabs, the number of samples described in this study is small in comparison with the experiences reported in China.

At the time of review, earlier literature have described sampling of the nose as nasal swab with limited data describing the actual site of sampling in the methodology sections[24,18] Therefore, it is difficult to confirm whether the nasal swab would sample the nasopharynx as recommended by the WHO. However, throat swabs can be interchangeably described as oropharyngeal swabs. In conclusion, the higher diagnostic accuracy of NP swabs compared to OP swabs should prime healthcare workers to prioritise NP swabs over OP swabs. Sampling the oropharynx is of lower priority, but, if collected, should be combined in the same tube as the NP swab. To improve diagnostic accuracy, the samples should be taken within 7 days after the onset of the illness.

Diagnostic accuracy of other samples from the upper respiratory tract

The potential risk of virus transmission during sampling of the nasopharynx and oropharynx has led to reports of novel techniques in sampling the respiratory secretions of the upper respiratory tract. Sampling techniques that allow self-collection by the patient can potentially reduce the risk of transmission to the healthcare provider. However, these techniques should be valid and reliable when compared to the more established NP swabs. In the setting of a pandemic, it is almost impossible to carry out reliability and validity studies of large scales to prove such hypotheses. A more practical approach is to infer findings of previous research on viruses that behave similar to the SARS-CoV-2. Viral load studies have suggested that the nucleic acid shedding patterns in SARS-CoV-2 resembles that of patients with influenza[27] and is different from SARS-CoV.[28] Viral loads in asymptomatic patients were found to be similar to that in symptomatic patients, with higher loads in the nose compared to the throat.[14]

To *et al*. in 2018 studied paired samples of NP aspirate and saliva, then performed influenza and Respiratory Syncitial Virus (RSV) assays in 214 patients. The sensitivity and specificity of viral detection in saliva specimens were 90.8% and 100% respectively with overall agreement of 93.3% with NP aspirate.[29] The high diagnostic accuracy of salivary testing opened new avenues as it potentially reduces healthcare cost, increases patient comfort and reduces risk to others. In a study of 12 patients with COVID-19, To *et al*. in 2020, later tested patient self-collected saliva specimens where 91.7% (11/12) of patients demonstrated the novel coronavirus 2019 (2019 n-CoV). Viral culture testing detected live viruses in 3 specimens, showing the potential application of self-collected salivary testing as a non-invasive specimen for diagnosis and monitoring of the disease.[19] A follow-up study by To *et al*. involving 23 patients with COVID-19 later described the salivary samples as posterior oropharyngeal saliva, which were self-collected by a throat clearing manoeuvre and was serially measured using RT-PCR. The viral load studied in the specimens was highest during the first week and it correlated with clinical disease progression.[16] It is unclear whether this sampling method sampled the oropharynx or the nasopharynx. Regardless, the diagnostic yield obtained seemed promising.

Guo *et al*. in 2020 reported a novel technique of throat washing where 11 COVID-19 patients

were instructed to oscillate 20 mls of sterile water at the posterior pharynx for 5 to 10 secs and spit into sterile containers. In this sampling technique, patients will need to extend their neck to direct the sterile water to the posterior pharynx, potentially sampling the respiratory secretions from the NP region. Nucleic acid extraction and real-time RT-PCR of paired throat washing and NP swabs were compared using locally approved commercial kits. The patients were sampled 48 to 57 days after the onset of the symptoms when viral shedding was low. Sensitivity of throat washing was higher than NP swab at 29.17% and 4.17% comparatively (p=0.031).[30] Similarly, a case report by Saito *et al*. showed higher viral genome detection using real time RT-PCR in four gargle lavages of a COVID-19 patient compared to OP swabs[20]. These preliminary results seem promising since it allows sampling of the nasopharynx, where viral loads have proven to be higher than the oral cavity.

RECOMMENDATIONS AND CONCLUSION

Based on review of the papers performed in this study, the authors support recommendations from WHO for prioritising NP swab over OP swab in testing ambulant, asymptomatic and mild cases suspected of SARS-CoV-2 infection within 7 days from the onset of illness. In the light of more NP swabs needing to be undertaken, healthcare practitioners should adhere to strict infection prevention protocols and respect the anatomy of the nasal cavities to prevent untoward complications.

Review of the current literature has shown that OP swabs have poor sensitivity, which later declines after more than 15 days from the onset of illness. Therefore, the authors would like to make strong recommendations for routine concurrent NP swab and throat wash or oropharyngeal saliva instead of OP swab so that a better understanding of its diagnostic accuracy can be obtained. Although a recent study by Guo *et al*. has demonstrated the superiority of throat wash over NP swabs, the laboratory processing of the samples was not detailed in his writing, such that it is difficult to ascertain whether it was processed using standards outlined by the FDA. Routine concurrent NP swab and throat wash or oropharyngeal saliva may be a potential solution for false negative results and help reduce the spread of this pandemic.

In the event of a negative NP and OP swab in a highly suspicious patient, consider repeat samples from multiple sites other than the upper respiratory tract. Lastly, the authors would like to suggest a quantitative viral load study in our local population to address possible geographical differences in the diagnostic accuracy of multiple sampling sites of the upper respiratory tract.

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Conflict of Interest

The authors declare that they have no competing interests and have not received any financial support for this research manuscript. We do not believe that there is a conflict of interest that could potentially be construed to affect the material contained in the manuscript that is being submitted to the Journal.

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