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Review Article

Salivary Testing as a Potential and Convenient Tool for Diagnosis of COVID 19

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Abstract

RT-PCR (Reverse Transcription Polymerase chain Reaction) is the most commonly used laboratory test for detection of COVID 19 antigen. The test is done on samples obtained by using the Nasopharyngeal swab, Oropharyngeal swab, Sputum, bronchial secretions obtained via bronchial lavage or Saliva.

The nasopharyngeal or oropharyngeal are currently commonly used for the same but several studies have shown that from the point of view of diagnosis, nasopharyngeal and throat swabs are less sensitive and specific as the material obtained from these areas reportedly contain less Viral RNA than sputum or saliva. Hence a more convenient and specific sample to test would be the saliva. The advantages of testing saliva (for RT-PCR) over other samples are discussed in this article.

Keywords: COVID 19; Salivary Testing; Corona Virus; Salivary Gland; Expression of ACE 2

Introduction

The Novel Corona virus infection which began in the Wuhan province of China in the December of 2019 has taken over the whole of the world like a storm. The unpredictability of this infection, more due to lack of adequate studies have left the medical world baffled.

COVID-19 disease is caused by the SARS CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) which belongs to the genus Betacoronavirus and this is the third animal corona virus to affect humans [7], the initial two being SARS (Severe Acute Respiratory Syndrome) in 2002 - 2003 in China and MERS (Middle Eastern Respiratory Syndrome) 2012 in the Middle Eastern countries. The spread of COVID -19 is by human to human contact and the affected individual exhibits typical symptoms of fever, sore throat, non-productive cough, malaise, dyspnoea. Some have presented with anosmia and/or loss of taste and some with uncommon presentations of GI symptoms of diarrhea, nausea, vomiting and sometimes even headache.

Diagnostic testing of COVID-19 involves detection of antigen using the RT-PCR and serological test that detects antibodies produced in response to infection. RT-PCR is the molecular test of choice for diagnosis currently for COVID 19 [3].

COVID 19 patients have demonstrated high viral loads in their upper and lower respiratory tracts within 5 to 7 days of the onset of symptoms [3]. Needless to say, collecting the proper respiratory tract specimen at the right time from the right anatomic site is essential for a prompt and accurate molecular diagnosis. Currently, either a nasopharyngeal or oropharyngeal swab is collected for RT-PCR as the disease begins in the Upper respiratory tract. Nasopharyngeal swabs are more sensitive and specific than oropharyngeal swabs [3,6].

Procedure of nasopharyngeal swab collection

For proper collection of sample, the swab must reach the nasopharynx and hence has to be inserted deep into the nasal cavity. Ideally, check for any obstructions in the nose at the start of the

procedure. The patient should be asked to tilt the head back to 70 degrees and the swab is then inserted into the nostril parallel to the palate (not upwards) until a little resistance is encountered or the distance is equivalent to that from the nostrils to outer opening of the patient's ear indicating that it is in contact with the nasopharynx (CDC recommended guidelines for collection of nasopharyngeal swab specimen). The swab is believed to be in the proper position when the patient flinches a little. The swab has to be kept in place for 10 seconds while being twirled thrice. Swabs should have flocked non-toxic synthetic fibres, such as polyester, as well as synthetic nylon handles [3].

Disadvantages of nasopharyngeal swab

Since the swab has to be properly taken from the nasopharynx, the primary site of infection, the risk of transmitting SARS-CoV 2 from the patient to the health care worker is significant.

For the same reason, the Health Care Worker taking the sample should be donned in proper PPE (Personal Protective Equipment).

Also, to reach the proper site from where to collect the sample, a trained person is preferred to avoid incorrect sampling thus reducing the specificity and sensitivity of the test.

Oropharyngeal swab (OP)

Oropharyngeal swab is recommended as an alternative if Nasopharyngeal swab cannot be procured as it is relatively easier to collect since the site can be visualized. The site of sampling is the posterior pharyngeal wall and the tonsils (not from the tongue).

Disadvantages of oropharyngeal swab

Less sensitive and specific.

The patient may gag and hence swab collection may pose difficulty.

Risk to the Health Care Worker since the patient may directly cough on him/her while taking the sample and hence need to be properly protected.

Studies done on 205 patients in Wuhan show that samples taken from the Nasopharynx (the positivity rate being 63%, n = 5) have a higher sensitivity than Oropharyngeal swabs (positivity rate being 32%, n = 126) [5]. Although Bronchoalveolar fluid lavage specimen was the most sensitive with a positivity rate of 93% (n = 14), followed by Sputum samples in whom the positivity rate was 72% (n = 75). In the same study, Fibrobronchoscope brush biopsy showed a positivity rate of 46% (6/13), Faeces sample 29% (n = 44) and Blood 1% (n = 3) [5]. This shows that Saliva sample is definitely more sensitive than both Nasopharyngeal as well as Oropharyngeal specimen.

Saliva sample for COVID 19 testing

Rationale behind the use of saliva is the reason that saliva is shown to contain live COVID 19 viruses. Also, the viral load of SARS-CoV2 in saliva is highest [2], especially during the first week after symptom onset. Also, it can be detected in saliva for as long as 25 days after symptom onset [1], hence a convenient tool for disease monitoring as also for monitoring viral clearance of the disease [1]. The 2019 Novel coronavirus was detected in the self-collected saliva of 91.7% of patients [2].

Method of saliva collection

Patient coughs up saliva from the throat in a sterile container and 2 ml of viral transport medium is added to the sample and sent for testing [2,10]. Saliva can also be collected from swab or directly from the duct [10]. The diagnostic value of saliva collected from the deep throat specimen is the highest (between 86.96% to 91.67%), from oral cavity (50%) and from salivary glands (12.90%) [10].

Advantages of saliva testing [1,2,10]:

1. Less invasive
2. More convenient
3. Advantageous in multiple testing for disease monitoring
4. Patients can collect saliva themselves if they are given clear instructions
5. Minimises risk of virus transmission to a health care personnel
6. Avoids use of PPE (Personal Protective Equipment)
7. As it avoids invasive procedures and eliminates the services of health workers, it reduces the risk of nosocomial 2019-nCoV transmission
8. Eliminates the waiting time for specimen collection
9. Results available sooner
10. Should supply of flocked swab become scarce self-collected saliva samples are a good option
11. The salivary viral load could be checked before discharging the patient from the hospital.

Disadvantages

1. More data are needed to determine if they are as accurate as antibody titres in blood samples
2. It is uncertain how best to handle saliva samples after they have been collected.

3. There is still some debate as to whether we need transport media to keep the virus stable in saliva for transport prior to testing (According to Linoj P Samuel, PhD, division head of clinical microbiology at the Henry Ford Health System in Detroit)- May 6, 2020.
4. Sputum/saliva needs to be provided before toothbrushing and breakfast, since nasopharyngeal secretions move by ciliary activity to the posterior oropharyngeal area, while the patients are in supine position during sleep [4].
5. All patients cannot easily provide sputum with respiratory secretions, especially children.

Conclusion

Point to ponder over here is if the virus is so commonly present in the saliva, do the secretory activity of the salivary gland have a role to play in the pathogenesis or it just migrates from the nasopharynx into the oral cavity. Alternatively, COVID-19 virus is found in the saliva of infected persons can have its source either from the salivary glands via their ducts or from the gingiva (gingival crevicular fluid) [7].

Coronaviruses are enveloped, single stranded RNA viruses which have a high rate of mutation and recombination. The picture below shows the structure of the corona virus. The spike surface glycoprotein (S) is the structural protein, small membrane envelope protein (E), Matrix protein or membrane protein (M) and the nucleocapsid protein (N). The spike protein plays an important role in binding the virus to the host cell receptors.

Figure

Studies done on Chinese Rhesus Macaques have shown that the ACE2 epithelial cells of the salivary glands may be the initial target for SARS CoV early in the disease process [8]. Human ACE2 (angiotensin converting enzyme 2) is the main receptor for the viral entry into the host cell in case of SARS CoV-2 as well [7,8]. The activation site is on the spike protein (S), which is activated by an enzyme Furin [7,9]. Furin is an enzyme abundantly found in many of the human tissues including the salivary glands. In fact, salivary glands are found to have significantly high levels of furin [7,9,10]. Once the virus attaches to the host cell receptor, the spike protein is primed by cellular proteases, which in turn causes cleavage of the spike protein and this is followed by fusion of the viral and cellular membranes. The TMPRSS2(transmembrane protease serine 2) is required for priming of the spike protein [7]. Studies have also shown the expression of TMPRSS2 in the salivary glands [7,11]. Therefore, the salivary gland very likely has a role in the initial entry of the virus and the progression of infection [7].

More research needs to be done on larger groups of people as data available from the currently available research done so far is not adequate. The possibility that the salivary gland may act as a reservoir of infection, which may reactivate has also to be considered [7] and further studies need to be carried out to establish this. Also, further studies need to be made to determine salivary glands as the possible cause of asymptomatic infection [12]. Studies analyzing the expression of ACE2 in human organs have shown the expression of ACE2 in minor salivary glands higher than that in the lungs which suggests salivary glands to be the potential target for COVID 19 and a possible cause of asymptomatic infection [12]. It is to be noted however that there is no histopathological evidence [10] to support direct invasion of the 2019 Novel corona virus to the oral tissues and studies from biopsy specimen from the same are required to support the same [13].

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