REVIEW

Salivary Diagnosis in Covid-19 Infection-A Brief Review

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Abstract

Introduction: SARS-CoV-2 viral infection and the consequent COVID-19 disease rolled over the globe sweeping human lives and national health systems. Early diagnosis plays an important role in stopping its further escalation.

Saliva as a Diagnostic Tool: Reverse transcription polymerase chain reaction (RT-PCR) remains the gold standard in the diagnosis of COVID-19 disease. Nasopharyngeal/oropharyngeal swabs are the recommended specimen types for identification of viral RNA. However, false negative results may occur due to inadequate or improper oropharyngeal sampling. Saliva, as a promising alternative, circumvents the limitations associated with the use of nasopharyngeal/oropharyngeal swabs and lessens the exposure risk of health care professionals.

Salivaomics or salivary diagnostics includes the study of salivary proteins, salivary RNAs, salivary metabolites, salivary microRNAs and salivary microbiota. Saliva sample collection is easy, non-invasive and more acceptable for repeat testing and can be performed by non-healthcare professionals or even be self-sampled. Recent studies suggest that the sensitivity of saliva-based SARS-CoV-2 RNA detection methods seem to be comparable to or better than that of nasopharyngeal swabs.

Conclusion: This paper reviews the role of saliva in the diagnosis of covid-19 infection, with special emphasis on its advantages, limitations and clinical implications.

Key words: COVID-19 disease, Salivaomics, salivary diagnostics,

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Introduction

A major coronavirus outbreak reported, with the spread of the 2019 novel Coronavirus (2019-nCoV, or SARS-CoV-2), which is known to cause the Coronavirus Disease-2019 (COVID-19). On 11 March 2020, the WHO declared COVID-19 as a pandemic and around 20 Cr cases and 42.5 deaths has been reported globally till July 30 2021[1]. The pandemic is still spreading, early diagnosis and social distancing are the most effective ways to protect public from the disease. COVID19 laboratory tests either detect virus or antibodies that are produced by the body in response to infection. At present, the real-time quantitative reverse transcription PCR (rRT-PCR) on specimens such as nasopharyngeal and oropharyngeal swabs (NP/OP) or wash in ambulatory patients from the upper respiratory tract is the 'gold standard' for the diagnosis of COVID-19. NP/OP swab sampling is technically challenging and it requires healthcare professional's direct interaction with patients. It is also invasive and painful and poses difficulty in serial sampling[2]. These drawbacks necessitate the adaption of newer diagnostic approaches.

Study of genome, the epigenome, the transcriptome, the proteome, the microbiome, and the metabolome of human saliva also known as Salivaomics, it can be applied in the detection of SARS-CoV-2 virus as salivary glands are its significant reservoirs[3]. Studies show that epithelial cells of salivary gland has elevated ACE-2 expression in those who are infected, as virus invades human cells by its spike protein binding to the cell membrane protein receptor (angiotensin-converting enzyme 2, ACE2. The ACE-2 expression in minor salivary glands was high in some studies, indicating that a target for COVID-19 may possibly be salivary glands. Furthermore, before lung lesions emerge, SARS-CoV RNA has been found in saliva. This could be the reason for asymptomatic infections. The positive rate of COVID-19 in patient's saliva can exceed upto 92%, and the live virus can also be cultivated through saliva samples. This suggest that asymptomatic COVID-19 infection may be from contaminated saliva and that the source of asymptomatic infection could be salivary glands. Hence saliva may be a promising non-invasive sample specimen for the diagnosis of COVID-19[4].

Salivary sampling characteristics

Early morning saliva before brushing and breakfast has been preferred as the test sample, as during night in the supine posi-

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tion, the nasopharyngeal and bronchopulmonary secretions get collected in the posterior oropharyngeal area. The secretions can be collected by deep cough, spitting, or gargling saline [5].Different studies included in this review used different saliva collection techniques and has not differentiated between the collection techniques or samples. Different saliva collection techniques may have an impact on the sensitivity of the method[6]. Two studies used the drooling technique to collect saliva while one study collected salivary swabs from the opening of the salivary gland duct. Tajima et al. compared early morning saliva samples (EMSSs) with daytime saliva samples (DSSs) and found 66.7% sensitivity in EMSSs (4/6) compared to 25.0% (2/8) in DSSs, both EMSS and DSS had a similar specificity (100%), though the sensitivity of EMSS was much better than DSS[7]. Avoidance of food, drink, tobacco, or gum for 30 min before saliva collection has been followed in one study[8]. Room conditions of airborne isolation has also been considered in another study[8]. For clinical applications needing a strong positive rate of virus identification, saliva from deep throat provides the strongest positive rate, which could account for early-diagnosis of COVID-19[8]. Saliva extracted from salivary gland ducts is consistent with acute COVID-19 which may likely be a reliable and non-invasive test

Various diagnostic methods using saliva:

RT-PCR

Saliva sample testing using RT-PCR shows a lower sensitivity when compared to nasopharyngeal swab sample. The diagnostic sensitivity of RT-PCR on saliva samples is variable considering the different salivary collection techniques. A meta-analysis of salivary sampling studies shows 91% sensitivity for saliva tests and 98% sensitivity for nasopharyngeal swab tests in previously confirmed COVID-19 patients, with moderate heterogeneity among the studies. However, most studies do not specify the sample collection technique used. Higher sensitivity were observed in the early morning posterior oropharyngeal spitting (95% CI -42.9 to 73.7), the lowest sensitivity was observed in the general spitting (95% CI -15.3 to -0.9). Furthermore, sensitivity decreases after the first five days from symptom onset[9]. However extraction of RNA from salivary sample is cumbersome.

RT-LAMP testing

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a technique that allows rapid and sensitive detection of SARS-CoV-2. Studies on saliva testing using RT-LAMP is inconclusive. A direct colorimetric saliva-based RT-LAMP has a sensitivity of 72.7% when compared with nasopharyngeal laboratory RT-PCR, and when measured on the healthcare worker population, the specificity was 95.7%. Further studies are needed to validate the available data[10].

Table 1: Advantages and Disadvantages of using saliva in diagnosis

Advantages	Disadvantages
Non-invasive approach	Not always reliable for measurement of certain markers
Painless (no patient discomfort and anxiety for sampling).	Contents of saliva can be influenced by the method of collection,
Easy collection	Serum markers can reach whole saliva in an unpredictable way
Safer collection for health professionals	Lack of standardisation of sample collection and viral detection protocols

Rapid antigen testing

Theoretically, saliva can serve as sample material for rapid antigen tests based on a lateral flow principle, as been shown by a few academic groups. The nature of the samples, however, can cause difficulties in the processing of the tests, and sensitivity compared to RT-PCR may be reduced with this sample type.[11,12]

Antibody testing

Saliva is an appropriate specimen for the detecting IgA antibodies early on during onset of disease, i.e. as early as two days after the onset of symptoms as their concentration appears to be higher in the mucosal secretions, compared with blood[13]. Varadhachary et al. reported a positive predictive value (PPV) of 92% and a negative predictive value (NPV) of 97% for a test protocol developed specifically to measure IgA detection in saliva[14]

Salivary/serum antibody response: The seropositivity was detected after 10 days of symptom onset with immunoglobulin G (IgG) values greater than IgM both for anti-nucleoprotein (NP) and anti-receptor-binding domain (RBD), which correlated with the virus neutralisation titre, also used for retrospective diagnosis[15]. The SARS- CoV-2 virus in samples (salivary/respiratory) varies depending upon the duration of onset of disease, sample quality (pure saliva or mixed with sputum/bronchopulmonary secretions), and the severity of disease.[16] The salivary sensitivity gradually decreases from 95 to 54% from the first week to the fourth week and the decrease is significant in severe diseases when compared to mild disease[17]. Studies with paired saliva and NPS samples showed a high positive percent agreement of 84.5%(32) and 96%(25) in both the samples. The overall positivity of nasopharyngeal and salivary sample combined was found to be 32.1% in probable SARS-CoV-2 patients (50 out of 156). Two studies reports that the longevity of the virus in salivary/respiratory sample of mild and severe disease is about 18–20 days.[18]

Point-of-care tests and rapid tests

Recent trend in the fight against COVID-19 is the POC (point-of-care) tests and the Rapid tests[19]. Point-of-care tests are simple medical tests which are performed near the patients' point of care. The advantage is that it is faster and cheaper than the time-consuming molecular tests. Abbott Diagnostics (Abbott ID NOW COVID-19), Cepheid (Cepheid Xpert SARS-CoV-2) and Credo (Singapore) report high sensitivity that reaches 100%.[20]

Advantages and Disadvantages of Salivary Sampling

Saliva is likely to become an alternate for serum or urine in the field of diagnosis. Compared with other investigative fluids, saliva sampling has both advantages and disadvantage in use for the diagnosis of COVID-19. Table 1.

Considerations to use Saliva as a Diagnostic Fluid

There is an ultimate need for standardizing the approach and protocol for collection of saliva sample. Deciding the appropriate RNA sequence to be targeted in qRT-PCR is crucial because diagnostic pitfalls of PCR can be avoided. The Charité protocol, using the RdRP_SARSr-P1 probe (Pan Sarbeco-Probe) that detects all corona viruses under the subgenus Sarbecovirusis is considered as a secure option[20]. The Institute Pasteur protocol with two RdRP targets and the E gene as a confirmatory assay is another preferable choice. Viral transport medium (VTM) is desirable to be added in

the specimen in order to retain viral integrity and consists of Earle's Balanced Salt Solution (BioSource International, Camarillo, CA), 4.4% bicarbonate, 5% bovine serum albumin, vancomycin (100 µg/mL), amikacin (30 µg/mL), and nystatin (40 U/mL)[21]. The need of a cell lysis buffer is yet under question and has to be clarified. The frequency of sampling is also another factor to be considered[22]. Salivary viral loads are reported to be elevated in the initial days of infection and then decline as the infection progresses into the lungs. The advantage of using saliva samples are that large samples are assembled (2-5ml) and if the first result is negative; diagnostic test can be repeated[23].

Conclusion

As salivary diagnostics provide a reliable and cost-effective non-invasive method for the fast and early detection of COVID-19 infection, active research involving large cohorts of patients, comparing the results using saliva samples with nasal and oropharyngeal swabs, defining the optimal RNA extraction protocol from the saliva and sample processing, detecting the levels of salivary immunoglobulin and the quality of salivary anti-SARS-CoV-2 antibodies are to be evaluated.

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