COVID – 19 Diagnosis: How Reliable is Saliva Test?

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Abstract

Collection of appropriate specimens is key to a prompt, precise and reliable diagnosis of COVID-19. Saliva has been studied extensively as a potential diagnostic specimen for many diseases over the years and many breakthroughs have been reported. Saliva as a diagnostic fluid offers many advantages over other specimens. Saliva collection is a simple non-invasive procedure that requires less skill with minimal risk. Several surveys/cross-sectional hospital-based studies using proteomics and microbial analysis have varying sensitivities of saliva test for COVID-19 diagnosis. Saliva test is thus a promising reliable diagnostic test for COVID-19 despite the identified limitations. More studies are however needed to validate the diagnostic use of saliva as well as to develop cheap, rapid, point of care saliva-based tests for COVID-19.

Keywords: COVID-19, Saliva, Diagnosis, Test

Introduction

Towards the end of 2019, a novel coronavirus was discovered as the cause of a remarkable respiratory tract infection in Wuhan, China which became an epidemic after it rapidly spread to other parts of China. Since the emergence, the virus has also spread to many countries throughout the world becoming a pandemic as declared by the World Health Organization (WHO). In February 2020, the WHO termed the disease COVID-19 (Coronavirus Disease 2019)[1] and the viral agent was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is presently a global health burden with more than 26 million confirmed cases and 800 thousand deaths reported as at September 5, 2020[2]. COVID-19 can affect any age group but commoner in adults and males[3]. It has also been reported that individuals at most risk are immunocompromised individuals such as older people and those with underlying systemic diseases (e.g. chronic kidney disease, diabetes mellitus, hypertension, coronary heart diseases, malignancies, liver diseases etc.)[4,5].

Whilst the respiratory system is the primary site of infection for COVID-19, studies have shown the involvement of other body systems including the gastrointestinal system among others[3,6]. However, the clinical manifestation is still not completely clear, as the reported symptoms range from mild to severe flu-like symptoms, with some cases even resulting in death[5,7]. Oral conditions such as dysgeusia/ageusia, xerostomia and mucosal lesions have also been reported in patients with COVID-19[8,9] and these may be early symptoms of the infection even before the appearance of distinctive clinical symptoms. Importantly, it has been reported that self-diagnosed loss of taste and smell is a significant predictor of a positive COVID-19 diagnosis[10,11]. Laboratory diagnosis of COVID-19 is however pertinent for adequate surveillance as well as for appropriate treatment. Different diagnostic techniques have been reported out of which the manual laboratory based real time polymerase chain reaction (RT-PCR) was most commonly used[12,13]. Even though SARS-CoV-2 has been isolated in many biologic samples (such as oropharyngeal swab/lavage, sputum, urine, feces etc.)[14-16] the nasopharyngeal swab is the 'reference standard' sample for the diagnosis of COVID-19 at present. In the same way, saliva was implicated as a potential specimen for the diagnosis of COVID-19 by earlier studies[17-20] due to the vulnerability of the oral cavity to the infection as well as its close proximity to the respiratory tract. The reliability of saliva as a diagnostic fluid for COVID-19 is discussed in this review.

Diagnosis of COVID-19

Collection of appropriate specimens at the right time from the right anatomic site is key to a prompt and precise diagnosis of COVID-19. Similarly, adequate and proper measures are obligatory to keep the medical personnel safe while taking samples as well as performing laboratory analysis of the samples to produce reliable test results. At the early onset of COVID-19 pandemic, laboratory diagnoses required the collection of respiratory specimens such as nasopharyngeal or oropharyngeal aspirates or washes, oropharyngeal or nasopharyngeal swabs, sputum, bronchoalveolar lavage and tracheal aspirates etc., which were used for viral culture or serological tests, electron microscopy for viral particles, conventional and real-time reverse transcriptase polymerase chain reaction (RT-PCR)[21-24]. Subsequently, the widely acceptable method for diagnosis of COVID-19 in patients with suspected infection was the real-time fluorescence polymerase chain reaction (RT-PCR) which detects as well as quantifies positive nucleic acid of SARS-CoV-2 in the specimens[23]. Thus, a confirmed case is one which is positive for the SARS-CoV-2 by the real-time PCR test. The main benefit of real-time RT-PCR technique is that amplification and analysis are carried out concurrently in a closed system to reduce false-positive results accompanying amplification sample contamination. Some of the demerits of RT-PCR however, include long processing time and need for expensive apparatus and equipment. Thus, RT-PCR test is not suitable for point-of-care diagnosis which is a much-desired diagnostic method for many disease conditions such as COVID-19. Point-of-care diagnostic tests have many advantages over the laboratory-based tests because they can be performed by trained non-laboratory personnel in patient care facilities, such as doctors' offices, clinics, emergency units and healthcare centers making the diagnostic test of COVID-19 closer to the patient[25]. Some respiratory samples based commercial point-of-care tests for COVID-19 are available and more are still being developed. The current methods for the diagnosis of COVID-19 are shown in **Table 1**.

Table 1: Available methods for the diagnosis of COVID-19.

Method	Available technique
Viral culture	Growing of virus in cell culture
Manual laboratory-based nucleic acid test	Real-time quantification (RT-PCR)
	Nested RT-PCR
	Droplet digital PCR
Point-of-care nucleic acid test	Loop-mediated isothermal amplification
	Nanoparticles-based amplification
	Portable benchtop-sized analyzers
Manual laboratory-based antibody test	Enzyme-linked immunosorbent assay (ELISA)
	Immuno-fluorescence assay
	Chemiluminescence immunoassay
Point-of-care antibody test	Lateral flow assay
	Microarray
	Microfluidic chip

Concerning the diagnostic test of any disease, the biologic sample for the analysis is key to reliability of the test regardless of the laboratory or diagnostic method employed. The reliability of any diagnostic test is a function of the accuracy and reproducibility of the test which are usually determined by the sensitivity, specificity, positive predictive value as well as negative predictive value of the test[26]. For COVID-19 diagnosis, studies have reported varying levels of sensitivity using different samples[21-24]. A study reported that a single nasopharyngeal swab taken in the early course of disease showed 70% sensitivity[27] thus, a single nasopharyngeal swab may not be completely reliable. Also, a study documented that negative nasopharyngeal or oropharyngeal swabs do not rule out COVID-19 infection[28]. Subsequently, studies have reported detection of SARS-CoV-2 in many other biologic samples including urine[29], stool[30], tear[31] and conjunctival swab[32] using different techniques including serological and PCR tests. Serological tests can serve as indicators of the spread of SARS-CoV-2 in the body system, while RT-PCR tests can show current infection. Thus, a combination of molecular and serological tests is needed to improve the diagnostic accuracy of COVID-19.

Saliva as a diagnostic tool

Saliva is a unique fluid that maintains the normal functioning of the oral tissues by means of its peculiar properties and numerous constituents. Saliva has been described as the "mirror of oral health and a reservoir of analytes" from various diseases (oral and systemic). Thus, saliva has emerged as a diagnostic specimen for many diseases of which viral infections take a significant part. Saliva has been studied extensively as a potential diagnostic tool for many diseases over the years and many breakthroughs have been reported[33-38]. Several clinical conditions can be assessed using saliva as a diagnostic biofluid. For example, numerous salivary markers of oral diseases such as caries, periodontal disease, oral cancers, Sjogren syndrome, lichen planus, aphthous ulcer, etc. have been reported[33,34]. Similarly, studies have shown correlation between blood and salivary markers of several systemic diseases such as diabetes, chronic kidney disease, head and neck cancers, breast cancer, cardiovascular diseases etc[35,36]. Saliva samples have been used as sources of DNA for a variety of forensic applications including gene expression profiling on saliva stains from crime scenes[37]. Saliva-based tests are also available for the diagnosis of numerous infectious diseases such as HIV, parvovirus, acute hepatitis, dengue fever and malaria, along with detection of substance use, drugs, alcohol and hormone levels[38], even though many more saliva-based tests are still being developed.

Saliva as a diagnostic fluid offers many advantages over other specimens. Saliva collection is a simple non-invasive procedure that requires less skill with minimal risk. Saliva sampling is appropriate for all age groups and can be repeated more frequently. It is of particular acceptability in children because of its pain free collection. It also offers a cost-effective method for the screening of large populations. Other key acknowledged advantages of saliva as a diagnostic tool is that it avoids the discomfort as well as the inconveniences associated with collecting other samples such as blood and urine. We recently assessed the awareness and preference of patients[39] and health care providers[40] on the diagnostic use of saliva. Majority (80.4%) of the patients were aware of its usefulness/usage, 85% would prefer it to other body fluid but 10.6 % had been previously sampled. In similar manner, 95.7% of the health care workers knew the various uses and advantages of saliva as a diagnostic specimen but few (26%) indicated previous use. Thus, use of saliva as a diagnostic tool is highly desirable among patients as well as health care professionals. However, further education and appropriate practical approaches are essential to widespread use of saliva as a diagnostic fluid because low patronage of known "saliva tests" renders it useless. Hence, it is essential to increase advocacy to promote its use especially in the area of disease susceptibility or screening[33].

Saliva as a diagnostic tool for COVID-19

Various uses of saliva for the diagnosis of COVID-19 have been reported and while some studies were narratives[41-43], others were cross-sectional studies/surveys[44-54]. These studies reported varying detection rates (ranging from 13% to 100%) of SARS-CoV-2 using saliva. An earlier study reported the detection of SARS-CoV-2 in the self-collected saliva of 91.7 % (11/12) of patients and the median viral load was 3.3 × 106 copies/mL (range = 9.9 × 102 to 1.2 × 108 copies/mL)[20]. Interestingly, saliva samples also tested negative in 33 patients whose nasopharyngeal samples tested negative. Viral culture using saliva samples were also positive in 2 out of 3 patients tested[20]. In another cross sectional study, SARS-CoV-2 was detected in saliva from all 14 (100 %) available specimens with a median value of 9.92 x 104 copies/mL (range = 7.08 x 103 to 6.38 x 108 copies per mL) which was higher than the detection in throat wash (median value of 3.56 x 103 copies/mL; range = 9.58 x 102 to 5.93 x 106 copies per mL)[20]. Also, every patient showed higher copies of SARS-CoV-2 in their saliva samples than throat wash samples although this may be explained by dilution effect of the throat wash. A linear relationship was also observed between the copies of SARS-CoV-2 in the saliva and throat wash, suggesting that the samples could have originated from a common source in the respiratory tract[20]. Similarly, a cohort study of 23 patients showed that the viral load in their early morning saliva samples from the posterior oropharynx (i.e. coughed up by clearing the throat) could be determined from 87% of the patients[45]. It was also reported that the viral load in the samples was highest during the first week of symptomatic infection which then gradually declined. Thus, in spite of the body immune response to SARS-CoV-2, viral RNA could still be detected in the saliva samples from majority of the patients for as long as 20 days or more[45]. In addition, a survey of 25 COVID-19 patients, reported that SARS-CoV-2 was detected in all (100%) patients[43]. Remarkably, positive saliva results were recorded in two patients, who had negative results from pharyngeal or bronchoalveolar swabs[46]. Thus, this finding emphasizes the better diagnostic value of saliva over nasopharyngeal swab in some cases. Furthermore, Pasomsub et al.[47], in a cross-sectional study, compared the COVID-19

diagnostic ability of saliva samples and standard nasopharyngeal/throat swabs using two-hundred pairs of samples. Their results showed that the sensitivity and specificity of the saliva sample RT-PCR were 84.2% and 98.9 %, respectively. An analysis of the agreement between the two specimens also demonstrated 97.5% observed agreement. In addition, positive predictive value and negative predictive values were 88.9% and 98.4% respectively. Also, RNase P gene was detectable in all (100%) saliva specimens[47]. Also, Williams et al.[48] in a cross-sectional study reported that 33 out of 39 patients (84.6%) with positive nasopharyngeal tests for COVID-19 also had positive saliva tests, while 1 (2%) out of 50 patients who had negative nasopharyngeal tests also showed positive saliva test for COVID-19[48]. Importantly, it was observed that there were correlations between median cycle threshold (CT) values of saliva samples and days of symptoms. One animal study also reported detection of SARS-CoV-2 in saliva specimens of ferrets using RT-PCR technique[52]. A recent study[53] reported detection of antibodies against SARS-CoV-2 in saliva samples at a sensitivity of 84.2% and a specificity of 100% in a population of asymptomatic COVID-19 patients using SARS-CoV-2 IgG and IgA ELISAs for serum as gold standard. The characteristics of some studies on the diagnostic use of saliva for COVID-19 test are shown in **table 2**.

Table 2: Characteristics of reports on the use of saliva test for COVID-19 diagnosis

Authors	Study type	Sample size	Saliva sampling method	Laboratory technique	Findings
Han et al.[18]	Case report	2	NS	RT-PCR	50 % positive
To et al. [20]	Survey	12	Self-collected 'coughed-out' saliva	RT-qPCR Viral culture	91.7 % positive for PCR 75 % positive for viral culture
To et al. [45]	Survey	23	'Coughed out' saliva	RT-PCR	87 % positive
Azzi et al.[46]	Survey	25	Drooling Use of pipette	RT-PCR	100 % positive
Pasumsob et al.[47]	Cross sectional	200	Spitting (devoid of cough)	RT-PCR	84.2% positive
Williams et al.[48]	Survey	39	Spitting	RT-PCR	84.6 % positive
Chen et al.[49]	Survey	31	Saliva collection from opening of submandibular duct	RT-PCR	13 % positive
Zheng et al.[50]	Survey	42	'Coughed out' saliva	RT-PCR	88.7 % positive
Zheng et al.[51]	Retros-pective cohort	96	'Coughed out' saliva	RT-PCR	100 % positive
Mac Mullan et al.[54]	Cross sectional	149	Orasure saliva sample collection Mouthwash saliva samples	ELISA	84.2 % sensitivity 100 % specificity

RT-PCR = Real time polymerase chain transcriptase, NS= not specified, ELISA = Enzyme linked immunosorbent assay

Regarding review papers on the use of saliva for the diagnosis of COVID-19, most papers emphasized the importance of COVID-19 saliva test and made general submissions as well as recommendations. In a systematic review[41], it was concluded that saliva could be considered as a non-invasive specimen for screening SARSCoV-2 suspected patients owing to the fact that saliva sampling has good accuracy and reliability concerning viral load assessment using RT-PCR technique. They also recommended saliva sampling after deep cough, as a suitable alternative to oropharyngeal samples which may cause

uneasiness to patients. It was also suggested that more research is necessary to provide additional evidence for the diagnostic use of saliva in patients with COVID-19. Similarly, Kurshid et al.[42], in their review recommended further researches on the diagnostic competence of human saliva for various viral infections including COVID-19, SARS-COV, MERS, ZIKV, and others at different locations such as homes, cities, airports, and clinics within few seconds using the cost-effective point-of-care (POC) technology. More importantly, it was recommended that all research centers, health agencies, and health care providers should explore the diagnostic capability of saliva using "rapid automated molecular point-of-care assays" during pandemic situation like we have presently. Likewise, Ceron et al.,[43] indicated four recommendations for an ideal use of saliva as diagnostic sample for COVID-19:

1) use of a standardized method (with minimal potential risk of transmission via contact with saliva droplets or aerosol) for saliva collection;
2) use of appropriate conditions for sample preservation;
3) use of appropriate assays, validated and with sufficient sensitivity for application in saliva and
4) increase in the knowledge base on clinical applications, allowing for a more accurate interpretation of the results. They also concluded that use of saliva for COVID-19 diagnosis could be explored for various application such as direct detection of the virus, quantification of specific immunoglobulins against the virus, and assessment of the non-specific immune response to the virus. Thus, it was suggested that saliva proteomics might offer more diagnostic biomarkers as well as mechanisms underlying COVID-19.

One of the breakthroughs for the use of saliva for the diagnosis of COVID-19 is the recent emergency use authorization (EUA) of a new method of saliva test (SalivaDirect) for COVID-19 infection by the U.S. Food and Drug Administration (FDA). The SalivaDirect test is a rapid detection of SARS-CoV-2 in saliva samples developed by a group of researchers from Yale School of Public Health. The test is cheap (costs about 5 USD per test), highly sensitive (average, 93%; range, 88-94%) and can detect as low as 6 to 12 copies per mL. Another remarkable feature of the test is that saliva sample can be collected in any sterile container. In addition, the test does not need any special type of swab or collection device and a separate nucleic acid extraction step is not required. The SalivaDirect test has been validated and approved for use with diverse combinations of commonly used reagents and instruments in most laboratories[54].

Generally, most studies have documented high sensitivity of saliva test for COVID-19 although there are still methodological issues that require improvements to enhance the reliability of saliva test for the diagnosis of COVID-19. For example, whereas randomized control studies are generally better in evaluating the diagnostic value of any test[55], most of the studies on saliva and COVID-19 detection were surveys/cross sectional hospital-based. Also, the studies used different methods of saliva collection while some did not indicate the method used for saliva collection and this could have influence on the sensitivity of the test. For example, Chen et al.[51], collected saliva samples from the submandibular duct and reported 13% sensitivity while others that used 'coughed out' whole saliva samples reported 87% to 100% sensitivity. Similarly, methods of storage and handling of saliva samples differ among the studies and this could also account for the disparities in their results[33]. In addition, majority of the available studies employed laboratory-based analysis of saliva samples whereas the rapid point of care test is more desirable for screening of diseases such as COVID-19[17]. Furthermore, most studies used nasopharyngeal sample as the reference standard (for the saliva test) which however showed false negative results in some cases and lower detection rate in some reports[27,28]. Thus, there is need for well-designed studies that will include appropriate sample size, standard methods of saliva collection and handling as well as standard laboratory procedures. In addition, most of the studies were hospital based including patients with moderate to severe clinical conditions, whereas there is need for epidemiological studies on the evaluation of COVID-19 saliva screening test in the general population.

In conclusion, saliva test for COVID-19 is sensitive and user-friendly, thus it is a promising reliable diagnostic test despite the observed limitations in the available data supporting this assertion. More studies are needed however, to validate the diagnostic use of saliva for COVID-19 as well as to develop cheap, rapid, point of care saliva-based diagnostic tests.

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