Is saliva a reliable biofluid for the detection of COVID-19?

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Abstract

This review aimed to assess the current evidence on the diagnostic potential of saliva regarding the detection of coronavirus disease 2019 (COVID-19). The literature published until May 24, 2020 was searched in the Web of Science, PubMed and Google Scholar databases with the keywords “COVID-19”, “SARS-CoV-2”, “2019-nCoV”, “oral fluid”, “saliva”, and “diagnosis”, individually and in combination, and 11 studies that explored the efficacy of saliva in the diagnosis of COVID-19 in different patient groups were found. Together, these studies suggest that saliva is a safe and reliable tool for the diagnosis of COVID-19. Further, saliva offers enhanced safety as well as logistical and economic benefits as compared to the current methods used to diagnose COVID-19. However, there is still limited evidence in the literature to make a definitive, clinically appropriate decision. The ideal specimen for the detection of acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still an issue under investigation. Thus, new studies with large sample sets for the validation of easy, safe and reliable methods applicable for large-scale testing are immediately required.

Keywords: saliva, 2019-nCoV, SARS-CoV-2, COVID-19

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Introduction

In late December 2019, the outbreak of a disease of unknown biological origin occurred in Wuhan, China. The disease gave pneumonia-like symptoms and rapidly spread to nearly all continents.^{1,2} The pathogen responsible for this epidemic was referred to as the 2019 novel coronavirus (2019-nCoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the World Health Organization (WHO) and the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV), respectively. SARS-CoV-2 is a new type of coronavirus that can infect mammals and humans in a similar manner as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV).^{1,3} As of March 11, 2020 WHO announced a global emergency and officially declared coronavirus disease 2019 (COVID-19) a pandemic.⁴ Because of the novelty of 2019-nCoV, the information regarding the biological characteristics of the virus was largely unknown, and researchers are still trying to find innovative ways to prevent the spread of the infection. One of these preventative strategies is the development and implementation of large-scale rapid diagnosis protocols and technologies.⁵

In the last 2 decades, saliva has gained enormous attention in biomedical sciences. Furthermore, the development and improvement of highly sensitive diagnostic technologies have overcome the barriers preventing the broad implementation of saliva diagnostics.^{6,7} Saliva research has advanced rapidly due to its use in metabolomics, genomics, proteomics, and bioinformatics approaches for the evaluation of overall health and disease.^{8–10} These data has shown that saliva is an ideal, non-invasive material for the diagnosis of several diseases as well as for use in pharmacokinetic studies and therapeutic drug monitoring.^{10,11}

The fast testing and isolation of the infected individuals, particularly the asymptomatic cases, which account for approx. 79% of the spread of COVID-19, are considered as the key issues in controlling the pandemic.^{12,13} Currently, respiratory tract specimens are used for detecting the virus; however, the acquisition of such specimens has numerous shortcomings in terms of collection, exposure of the healthcare workers (HCW), patient discomfort, problems related to self-collection, shortage of testing materials, and equipment.^{13–15} To overcome these shortcomings, new efficient methods that can be easily rolled out for large-scale virus scanning are required. From this point of view, the use of saliva seems a reliable alternative for the diagnosis of the 2019-nCoV presence, as the collection of saliva is minimally invasive, does not cause discomfort, can be easily performed by the patient, prevents the contamination of the healthcare staff, and decreases the use of swabs and personal protective equipment (PPE).^{3,10,16,17} Moreover, saliva has been reported to exhibit sensitivity comparable to respiratory tract swabs in the detection of other respiratory pathogens.^{15,18,19}

This review aimed to present a concise update on the diagnostic role of saliva and to discuss the recent data regarding its diagnostic potential for COVID-19. To achieve this goal, we performed a literature search in the Web of Science, PubMed and Google Scholar databases with the keywords “COVID-19”, “SARS-CoV-2”, “2019-nCoV”, “oral fluid”, “saliva”, and “diagnosis”, individually and in combination, taking into consideration the research published until May 24, 2020. Clinical studies that used saliva as a diagnostic fluid for the detection of COVID-19 were included in this review, while case reports without enough clinical data and in vitro studies were excluded.

What is the entrance mechanism and transmission route of 2019-nCoV?

2019-nCoV is a novel enveloped, single-stranded, zoonotic, RNA virus that belongs to the β -coronavirus genus. It is reported to share a 96% identity with bat coronavirus, 91% with pangolin coronavirus and 79% with SARS-CoV.^{20–24} The 2019-nCoV virion has 4 structural proteins.^{20,24,25} Among them, the spike protein (S) facilitates the entrance of the virion into the host cell and determines the transmission ability of the virus.^{22–24} To date, the exact entry mechanism of 2019-nCoV into human cell has not been completely elucidated. However, like in the case of the SARS-CoV infection, it is hypothesized that 2019-nCoV infects humans by binding to the angiotensin-converting enzyme 2 (ACE2) receptor of the host cell. Thus, the in vivo infection dynamics of 2019-nCoV is associated with the number and distribution of ACE2 receptors.^{2,20,26–30} The expression of ACE2 has been identified in different parts of the body, i.e., the lungs, myocardial cells, esophagus, ileum, colon, kidneys, oral mucosa, and salivary glands.^{1,26,31–34} Xu et al. reported that ACE2 could be expressed and highly enriched in the epithelial cells of the oral cavity.³² Additionally, the salivary glands have been reported to express the ACE2 receptor, highlighting its potential as a target for the COVID-19 infection.^{33–35}

The transmission of 2019-nCoV takes place via the spread of the infected aerosol droplets, which may be expelled during sneezing, coughing, speaking, or singing, or via contact with the mucous membranes of the nose and eyes, and saliva. It has been reported that the incubation period during the COVID-19 infection ranges from 1 to 14 days.^{36–39} Furthermore, it has been observed that the clinical symptoms of COVID-19 differ among the infected patients and are affected by age as well as by the existing comorbidities. While some patients are asymptomatic, others present with a cough, fever and fatigue. Transmission can occur in the early period, before symptoms manifest themselves; thus, asymptomatic or mildly symptomatic patients can potentially transmit the virus

for longer periods, before they are diagnosed with the disease.^{24,40–42} It is estimated that 1 infected person can transmit the virus to nearly 3 people.^{24,43} The ability to quickly diagnose COVID-19 plays an essential role in controlling the spread of the virus through its accurate detection and the isolation of the infected persons.^{24,36}

Physiology and functions of saliva

The salivary glands in the oral mucosa are responsible for the production and secretion of saliva.¹¹ Saliva is a biofluid that is important for mastication and oral cavity homeostasis.¹¹ It is composed of water (99.5%), organic (0.3%) and inorganic (0.2%) substances.^{9,44,45} The amount of saliva produced daily by a healthy individual is approx. 1–1.5 L. Beside the standard components of saliva, whole saliva includes other substances, such as fluids from the gingival fold, desquamated epithelial cells, blood cells, mucus from the nasal cavity and pharynx, oral bacteria, and traces of medications.⁴⁵ Saliva has several functions in terms of maintaining the homeostasis of the oral cavity, namely it has a protective function for the teeth and oral mucosa, it has a buffering effect, and participates in clearing the oral cavity of residual food and deleterious bacteria. Additionally, saliva participates in digestion and taste perception. Furthermore, with a variety of components, such as lysozyme, lactoferrin, mucins, immunoglobulins, and histatins, saliva has also been shown to exhibit antimicrobial activity.^{11,44,46}

The collection of stimulated and unstimulated saliva can be performed by means of different methods, such as draining/spitting or chewing an absorbent material. Additionally, with the use of special devices or aspiration, pure glandular saliva can be collected. However, since saliva shows great variations, the standardization of saliva collection is important.^{9,11}

Saliva as a diagnostic material

Oral fluid is referred to as the “mirror of the body”, enabling the evaluation of both health and disease progression within an individual. A better understanding of the molecular profiles found within saliva and their relation to disease has raised interest in the diagnostic potential of saliva.^{7,47} Furthermore, saliva is a cost-effective and non-invasive diagnostic tool, with many advantages in terms of collection, procurement of sufficient and repeated samples, manipulation, processing, safety, longitudinal monitoring, applicability to large populations, and transportation.^{7,8,11} Notably, saliva does not coagulate like blood and is stable for 24 h at room temperature and 1 week at 4°C. These factors make saliva samples easy to transport, store and manipulate for diagnostic purposes.¹¹ Recently, lab-on-a-chip technologies/point-of-care devices

are increasingly leading to portable “labs” and real-time analyses of a variety of diseases by using saliva.^{7,47,48}

Nowadays, the general health status of the body or the presence of several diseases can be diagnosed through the detection of biomarkers in saliva. Nearly all disease markers that are found in blood can also be detected in saliva. Moreover, human saliva has been reported to be successfully used for the diagnosis of many systemic diseases, such as cancer, autoimmune diseases, infections, endocrinological diseases, psychiatric diseases, cardiovascular diseases, and gastrointestinal tract diseases.^{48–51}

Viruses frequently infect human through the mouth and eyes. Thus, the oral cavity is important in the transmission of certain viruses.⁵² Viral DNA, RNA, antigen, and antibodies are used to detect 2019-nCoV in saliva.⁵⁰ Human immunodeficiency virus, hepatitis viruses, rotavirus, norovirus, Zika virus, human papilloma virus, herpes simplex viruses, Ebola virus, SARS-CoV, Epstein-Barr virus, and influenza viruses are all common examples of viruses that can be detected in saliva.^{51,52} These viruses serve as an important precedent for the potential detection of 2019-nCoV in saliva.

Is saliva a reliable tool for the diagnosis of COVID-19?

To control the spread of the disease, the rapid and large-scale detection of 2019-nCoV is crucial. So far, respiratory tract specimens, such as throat swabs, nasal swabs, nasopharyngeal swabs, sputum, and bronchoalveolar lavage, have been used for 2019-nCoV diagnostic testing.^{36,37,53,54} However, the harvesting of oropharyngeal and/or nasopharyngeal specimens puts the healthcare staff at a high risk of accidental transmission, as obtaining these samples requires close contact with the infected patients and involves potential exposure to the virus through the patient's sneezing, gag reflex or cough.^{36,53,55} Additionally, the collection procedures cause discomfort and may result in bleeding, particularly in patients with thrombocytopenia.^{5,36} Thus, they are not suitable for the broad-scale repeated monitoring of the viral load.^{37,53} Sputum, on the other hand, is a non-invasive specimen, but not all patients with 2019-nCoV can produce sputum for diagnostic evaluation.^{5,18,36,53} However, the use of saliva has numerous advantages, such as non-invasiveness, easy self-collection, a reduced need for specific equipment and also PPE, and limited transmission to the healthcare staff. Importantly, saliva collection also facilitates taking samples from a single patient multiple times and allows for the use of point-of-care testing.^{13,48} It is understood that saliva is the meeting point of respiratory tract secretions, blood, which can access the mouth via the gingival crevicular fluid, and the secretions of the major and minor salivary glands. Thus, saliva could reflect the viral

load of the COVID-19 infection within the salivary glands and the respiratory tract.^{5,54} The findings of Liu et al. suggest that the epithelial cells of the salivary glands can be infected by the virus.³⁴ These findings imply that the salivary glands could function as an important reservoir for the virus, particularly in the early phase of the infection. This may also explain the transmission of the infection between asymptomatic cases, when the virus has not progressed to the respiratory tract.⁴¹

In the literature, we found 11 studies that explore the efficacy of saliva in the diagnosis of COVID-19 (Table 1 and Table 2). To et al. reported that the salivary viral load peaked in the 1st week after the onset of symptoms, and noted that 2019-nCoV could be easily spread by means of saliva when symptoms were mild.⁵⁴ They also reported the prolonged detection (>20 days) of 2019-nCoV in saliva and emphasized that despite the clinical recovery, the virus might be detected in saliva at low levels. Furthermore, they suggested using posterior oropharyngeal saliva, as it consists of secretions that originate from the salivary glands, the posterior nasopharynx and the tracheobronchial tree.⁵⁴ In another study by the same research group, 2019-nCoV was detected in saliva specimens from 11 of 12 patients (91.7%).⁵³ The authors reported that at least 11 days after hospitalization, 1 patient had detectable viral shedding in saliva. Based on these observations, the authors emphasized

the possibility of the transmission of 2019-nCoV via saliva and recommended the use of surgical masks when dealing with potential cases.⁵³ Azzi et al. reported that saliva was a reliable material in the diagnosis of COVID-19, and that it could provide clinical information, useful for the monitoring of the disease.¹⁶ Those authors also detected 2 patients who tested positive when using salivary samples, while pharyngeal or bronchoalveolar swabs were negative. Based on these data, the authors hypothesized that the infected individuals could transmit the virus even though their pharyngeal swabs were negative. To combat this, the authors suggested that the assessment of the salivary viral load be mandatory before the patient can be discharged from hospital.¹⁶ In agreement with these findings, Wyllie et al. detected 2019-nCoV in saliva samples, while the matched nasopharyngeal specimens of 2 asymptomatic healthcare staff members were negative.¹⁵ Those authors noted that for the determination of mild or subclinical infections, saliva might be a viable option.¹⁵ In another study, Zheng et al. found that the viral detection rates for sputum and saliva were significantly higher relative to those observed for throat and nasal swabs.¹⁸ Williams et al. found that 33 out of the 39 patients whose nasopharyngeal swabs were positive, also had a positive result for 2019-nCoV in saliva.⁵⁶ In another study conducted on non-hospitalized patients, Kojima et al. reported

Table 1. Characteristics of the selected studies

Study	Country	Patients (N)	Sample	Analysis	Conclusions
Zheng et al. ¹⁸	China	65 CP	– TS – NS – S/SP	RT-PCR	– saliva sampling has high sensitivity and accuracy – it is more convenient in comparison with TS and NS
Wyllie et al. ¹⁵	USA	44 CP 98 HCW	– S – NPS	RT-PCR	– saliva is a reliable alternative to NPS for identifying mild/subclinical infections – it is accurate in large-scale use
To et al. ⁵⁴	China	23 CP	– S – B – U – RS	RT-PCR	– saliva a non-invasive tool for diagnosis – its collection is highly acceptable by patients and the healthcare staff
To et al. ⁵³	China	12 CP	– S	RT-PCR	– saliva is useful for the diagnosis, monitoring and control of the infection
Pasomsut et al. ⁵⁵	Thailand	200 NP	– NP/TS – S	RT-PCR	– saliva can be used for the detection of COVID-19 – it is non-invasive and non-aerosol-generating – it can be used especially in low-resource settings
Jamal et al. ⁵⁸	Canada	53 CP	– NPS – S	RT-PCR	– saliva may substitute for NPS, especially when NPS is in short supply or patients cannot tolerate it
Chen et al. ⁴	China	31 CP	– S – OPS	RT-PCR	– saliva is a useful diagnostic medium for critically ill patients – its collection is easy and non-invasive
Azzi et al. ¹⁶	Italy	25 CP	– S	RT-PCR	– saliva is a reliable tool for detecting COVID-19 – it is useful for the clinical monitoring of the disease
McCormick-Baw et al. ⁵⁹	USA	156 CP/NP	– NPS – S	RT-PCR	– saliva is an acceptable alternative specimen for detecting COVID-19
Williams et al. ⁵⁶	Australia	622 NP	– NPS – S	RT-PCR	– saliva may be a suitable alternative for first-line screening in low-resource settings
Kojima et al. ⁵⁷	USA	45 CP/NP	– S – NS – NPS	RT-PCR	– supervised self-collected S and NS performed similarly to clinician-collected NS

CP – laboratory-confirmed patients; NP – non-confirmed patients; HCW – healthcare worker; TS – throat swab; NS – nasal swab; S – saliva; SP – sputum; NPS – nasopharyngeal swab; B – blood; U – urine; RS – rectal swab; OPS – oropharyngeal swab; RT-PCR – real-time polymerase chain reaction.

Table 2. Patient characteristics and saliva collection in the studies testing saliva as a diagnostic fluid for coronavirus disease 2019 (COVID-19)

Study	C– (n)	C+			Age [years]	Gender		Saliva collection
		AS (n)	mild (n)	severe or deceased (n)		F (n)	M (n)	
Zheng et al. ¹⁸	–	–	23 – 19 – O ₂ suppl	42 – 39 – O ₂ suppl – 2 – MV – 10 – ICU admission	54 (39.5–62)	25	40	– a deep cough with a mask – 3–5 times through spitting
Wyllie et al. ¹⁵	–	–	–	44 – 6 – ICU on admission – 19 – ICU during hospital stay – 10 – MV – 2 – deceased	61 (23–92)	21	23	– self-collected – every 3 days through spitting – throughout the clinical course
	98	–	–	–	36 (22–67)	82	16	– self-collected – every 3 days through spitting – for 2 weeks
To et al. ⁵⁴	–	–	13	10 – 10 – O ₂ suppl – 5 – ICU (3 required EI) – 2 – deceased	62 (37–75)	13	10	– posterior oropharyngeal saliva with a deep cough – endotracheal aspirate for EI
To et al. ⁵³	–	–	–	12	62.5 (37–75)	5	7	– self-collected – saliva coughed out from the throat – collected at a median of 2 days after hospitalization
Pasomsub et al. ⁵⁵	181	–	–	19	36 (28–48)	131	69	– self-collected – without coughing
Jamal et al. ⁵⁸	–	–	–	53	63 (27–106)	21	32	– through spitting – on enrollment, then 3 times at 72-hour intervals
Chen et al. ⁴	–	–	–	31 – 26 – heavy – 5 – critically ill	60.6 (18–86)	16	15	– from the salivary gland – collected with cotton swabs
Azzi et al. ¹⁶	–	–	–	25	61.5 ±11.2	8	17	– drooling technique – with a pipette for EI and MV – the 2 nd specimen after 4 days
McCormick-Baw et al. ⁵⁹	–	–	–	156	47.8	66	90	– saliva without sputum
Williams et al. ⁵⁶	–	–	–	622	NA	NA	NA	– through spitting saliva after pooling it
Kojima et al. ⁵⁷	16	–	–	29	42 (31–52)	NA	NA	– self-collected/supervised self-collected – a deep cough 3–5 times and rubbing the swab for 20 s

C–: COVID-19-negative; C+: COVID-19-positive; AS – asymptomatic; F – female; M – male; suppl – supplementation; MV – mechanical ventilation; ICU – intensive care unit; EI – endotracheal intubation; NA – not available.

Data concerning age presented as median (*Me*) (interquartile range (*IQR*)) or mean (*M*) ± standard deviation (*SD*) or *M*.

that they detected 6 cases of the 2019-nCoV infection by using saliva samples; again, 2019-nCoV was not detected in the clinician-collected nasopharyngeal swabs. Conversely, in 3 cases, they detected the 2019-nCoV infection with nasopharyngeal specimens, while it was not detected in saliva. Based on these results, the authors suggested that single-site testing might miss some cases of COVID-19.⁵⁷ Pasomsub et al. found that the detection of 2019-nCoV in saliva had a sensitivity of 84.2% and a specificity of 98.9%.⁵⁵ They also emphasized that the spectrum of the COVID-19 ranges from asymptomatic to severe. Thus, the detection of 2019-nCoV in specimens from different sample regions might also be possible.⁵⁵ Jamal et al. found that nasopharyngeal swabs were 10% more sensitive than saliva, and that the difference was more pronounced in the later stages of the disease.⁵⁸ According to their results, nasopharyngeal swabs may be preferred for 2019-nCoV

diagnostics, particularly if the patient is in the late illness period. Moreover, the authors found that none of the nasopharyngeal or saliva samples was totally sensitive for the detection of COVID-19 alone.⁵⁸ Chen et al. found that out of 13 cases, 4 tested positive in saliva and 3 of those patients were critically ill.⁴ They suggested that the salivary glands were destroyed during the infection due to a high viral load, and with the progression of the disease, the viral titer increased in saliva because of viral replication in critically ill patients with a weakened immune system.⁴ Thus, the detection of the virus in saliva may be indicative of terminal-stage disease. Studies with much larger sample sizes relative to other studies indicated that saliva was an acceptable alternative specimen for the diagnosis of COVID-19, especially for first-line screening in low-resource settings.^{15,55,56,59} However, conclusions from these studies are still contradictory.

Conclusions

The ideal medium for the detection of 2019-nCoV is still an issue under investigation. The validation of easy, safe and reliable methods that are easily applicable for large-scale testing for 2019-nCoV are immediately required. Saliva seems to be a reliable diagnostic tool for the diagnosis of COVID-19; however, there is limited evidence present in the literature to support this. Thus, new studies with large sample sizes are urgently required to accurately demonstrate the diagnostic validity of saliva in the detection of 2019-nCoV.

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