Saliva in SARS-COV-2 Detection

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In the wake of the COVID-19 pandemic, it is crucial to assess the application of a multitude of effective diagnostic specimens for conducting mass testing, for accurate diagnosis and to formulate strategies for its prevention and control. As one of the most versatile and amenable specimen options, saliva offers great advantages for widespread screening strategies due to its non-invasive properties, cost-effectiveness, excellent stability and minimal risk of cross-infection.

Keywords: saliva ; COVID-19 ; SARS-CoV-2 ; infection ; diagnosis ; polymerase chain reaction

1. Introduction

SARS-CoV-2 can be transmitted via direct or indirect contact. One of the primary sources of transmission of coronavirus is through salivary aerosols emitted from coughing, breathing, and even during speaking [1]. Figure 1, below, illustrates three potential trajectories for the presence of the virus in saliva as explained by Sabino-Silva et al. [2]. Contemporary studies have gathered evidence demonstrating molecular strategies adopted by the SARS-CoV-2 virus, enabling it to enter the host cell, causing a high rate of infectivity. Possible activation of the SARS-CoV-2 virus by gene expression of furin in salivary glands is also a noteworthy finding explained by Shang et al. [3]. Furin is typically expressed by salivary glands and its components are responsible for the regulation of different specific proteins while the gene itself is believed to cleave different viral toxins including coronaviruses. As a result, the severity of COVID-19 disease is increased if salivary infection is withdrawn from the salivary glands, while the presence of furin in saliva leads to a rapid progression of the disease through salivary droplets [4].

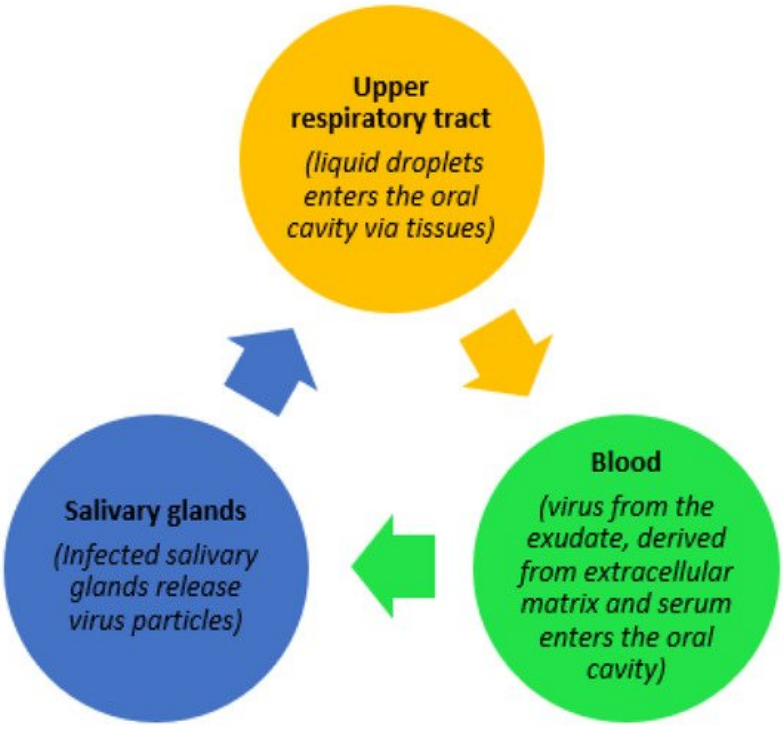

Figure 1. Possible trajectories for the presence of SARS-CoV-2 in saliva.

2. Salivary Diagnostics

Table 1 describes assays that have received FDA EUA approval containing more recent data. Different approaches and collection techniques were used in the trials included here, such as collection of saliva by cough, passive collection from posterior oro-pharynx, simple swab or a whole saliva collection technique. As of 22 July 2020, RT-PCR using

Oropharyngeal respiratory specimens is very much the gold standard for the qualitative detection of the SARS-COV-2 virus.

Table 1. Comparison of studies using saliva-based testing versus conventional swab-based testing for the detection of SARS-COV-2.

Study	Ref	Saliva Collection Method	Swabs and Lavage for Comparison	Diagnostic Test	N	TP	FP	FN	TN	Sensitivity	Specificity	PPV	NPV
Azzi, L et al., 2020 (Italy)	[5]	Drooling	NPS	RT-PCR	25	25	0	0	0	1	uc	1	uc
Azzi, L et al., 2020 (Italy)	[6]	Drooling	BAL	RT-PCR	2	0	2	0	0	uc	0	0	uc
Chen, Lili et al., 2020 (China)	[7]	Cotton Swabs—Saliva from orifices	OPS	RT-qPCR	31	4	0	9	18	0.31	1	1	0.66
Han, Mi Seon et al., 2020 (Korea)	[8]	Saliva	NPS, OPS	qPCR	2	1	0	1	0	0.50	uc	1	0
Wang, To et al., 2020 (Hong Kong, China)	[9]	Sputum/Coughed-out Saliva (self-collected)	NPS	RT-qPCR	12	11	0	1	0	0.92	uc	1	0
Wang, To et al., 2020 (Hong Kong, China)	[10]	Coughed-up Saliva—Posterior OroPharynx	NPS, Sputum	RT-qPCR	23	20	0	3	0	0.87	uc	1	0
Wyllie Anne et al., 2020 (USA)	[11]	Saliva (spitting)	NPS	rRT-PCR	46	38	1	7	0	0.84	0	0.97	0
Zheng Shufa et al., 2020 (China)	[12]	Sputum (hospitalized patients)	Stool, Serum, Urine	RT-qPCR	96	96	0	0	0	1	uc	1	uc
Zhang Wei et al., 2020 (China)	[13]	Oral Swabs (hospitalized patients—baseline)	Blood, Anal	RT-qPCR	16	8	0	8	0	0.50	uc	1	0
Pasomsub, E et al., 2020 (Thailand)	[14]	Saliva	NPS, TS	RT-PCR	200	16	2	3	179	0.84	0.98	0.88	0.98
Somrak et al., 2021	[15]	Self-collected	NPS	RT-PCR	32	12	0	20	0	0.37	1	1	0.91
Basso et al., 2021	[16]	Self-collected	NPS	RT-PCR	84	67	0	17	0	0.78	uc	1	0

Sample size (N); true positive (TP); false positive (FP); true negative (TN); false negative (FN); positive predictive value (PPV); negative predictive value (NPV); nasopharyngeal swab (NPS); broncho-alveolar lavage (BAL); oropharyngeal swab (OPS); throat swab (TS); real-time reverse transcription polymerase chain reaction (rRT-PCR); reverse transcription polymerase chain reaction (RT-PCR); unable to calculate (uc). Sensitivity and specificity calculations were performed through an online tool (http://vassarstats.net/clin1.html) (accessed on 29 April 2021).

A study in Hong Kong is the earliest available reported study during the course of pandemic, which investigated the presence of SARS-CoV-2 in saliva in 11 COVID-19-positive patients. The patients were tested at various phases including during their recovery phase and, at that time, a decline in salivary SARS-CoV-2 RNA was observed [17]. Early evidence from Wuhan (China), revealed that in a cohort of 16 COVID-19 patients, the SARS-CoV-2 viral titers were discovered

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3. Sensitivity and Specificity of Salivary Diagnostics for SARS-CoV-2 Testing

Limited studies have demonstrated the comparability or superiority of saliva sampling, relative to conventional swab-based sampling; however, the results for saliva are compelling. As mentioned earlier, we were able to compile the results from current and previously reported studies and have been able to calculate a pooled sensitivity and specificity for saliva specimen collection in the detection of SARS-CoV-2. The table below (see Table 1) demonstrates a pooled sensitivity of approximately 87% and a specificity of 98%. From the combined results, the probability of a positive test result being a true positive (PPV/True-positive) is 98%, and the probability of a negative test result being a true negative result (NPV/False-positive) is 86%. These findings indicate that salivary-based detection of SARS-CoV-2 using the RT-PCR method has a high diagnostic accuracy for positive cases but may lack accuracy for the detection of false-positive cases. However, these performance data mirror that of data obtained using nasopharyngeal swabs and represents a solid alternative for diagnostic purposes.

3.1. Strengths and Limitations of Salivary Diagnostics

The inclusion of saliva samples for the detection of SARS-CoV-2 coronavirus is a major step forward in the fight to identify patients suffering from the disease. Due to its easily accessible nature, it can be readily obtained from patients in a non-invasive fashion thereby reducing the risk of nosocomial infections among healthcare workers [32][33]. Equally importantly, saliva samples possess high sensitivity and specificity when compared to nasopharyngeal swabs for the detection of SARS-CoV-2 coronavirus (refer to Table 1). Intriguingly, there are also a few studies that report that SARS-CoV-2 is detectable in saliva samples but not in nasopharyngeal swabs [6][11]. It is documented that saliva collection is beneficial in cases where screening of infected individuals is required on a larger scale such as in the community, in a drive-through setting, in a hospital setup or in locations with access to limited medical resources. In addition, it has also been previously documented that certain viral strains may survive in saliva for 29 days post-infection, enhancing the possibility of disease detection even at later stages of the disease [2].

3.2. Emerging Technologies in Salivary Diagnostics for COVID-19

Besides the CDC-approved RT-PCR test for the detection of SARS-COV-2, many other inexpensive, fast detection methods for mass screening purposes have been recently approved by the FDA under the EUA (Emergency Use Authorization) mechanism [34]. As illustrated in Table 2, the diagnostic specificity and sensitivity of oral fluids (saliva and sputum) are very high in controlled studies with smaller sample sizes.

4. Direction for Future Studies

Large-scale prospective studies are needed to establish the temporal trends in salivary viral titers and tie them in with the course of infection or as markers of disease severity. Although preliminary evidence indicates that salivary detection of SARS-CoV-2 is feasible in mild or asymptomatic cases [9][10], once again this finding needs to be validated in a larger

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cohort. Large-scale, epidemiological studies are needed to compare the sensitivity and specificity of swab-based methods versus methods where saliva specimens are used. Here we note that there are differences in the literature where various types of oral samples collected have contributed to variability in the detection of SARS-CoV-2 (refer to Table 1). This in turn leads us to recommend that further studies should be performed to validate different oral fluid collection protocols and to compare viral detection rates [13][35]. Moreover, it is important to extend the application of salivary diagnostics to neglected or vulnerable populations such as pediatric populations, geriatrics and pregnant females. Generally speaking, the literature is unclear on whether SARS-CoV-2 detection is dependent on ACE-2 receptor expression at oral sites, versus nasopharyngeal sites, so we believe this relationship should be investigated in future studies [36]. To avoid the frequency of false-negative results in suspected positive cases, sampling a mix of multiple specimens (including oropharyngeal, nasopharyngeal, oral, sputum and saliva specimens) is recommended [37]. For proposed future research, we recommend the application of saliva for a number of studies aimed at disease detection and looking at progression. Saliva is an excellent matrix for the evaluation of salivary antibodies, so studies on a large cohort of individuals will provide advantages for monitoring disease progression in the COVID-19 area.