Saliva sample for the massive screening of SARS-CoV-2 infection: a systematic review

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Objective. This systematic review aims to describe the value of saliva as a noninvasive sample for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in comparison with the current method for sample collection, the nasopharyngeal swab.

Study Design. We conducted a systematic review of the literature following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations. We searched in 5 databases (PubMed, Cochrane, EBSCO, Elsevier, and MEDLINE) and included articles published between December 2019 and July 2020.

Results. This review included 22 publications that met inclusion criteria, 17 of which were case series, 2 of which were case reports, and 3 of which were massive screenings. All articles compared saliva with nasopharyngeal swabs. The detection rate of SARS-CoV-2 in saliva was similar to that for nasopharyngeal swabs. The sensitivity ranged between 20% and 97%, and specificity ranged between 66% and 100%.

Conclusions. This systematic review found that saliva might be an appropriate, fast, painless, simple, and noninvasive sample for SARS-CoV-2 detection, making it ideal for massive screening of SARS-CoV-2 infection. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:540−548)

Since the first outbreak was reported in Wuhan, China, in December 2019, more than 62 million cases of coronavirus disease 2019 (COVID-19) had been reported through the end of November 2020,[1] and more than 1.4 million people had died worldwide, according to World Health Organization (WHO) statistics.[1] The global pandemic was declared on March 11, 2020, and, since then, government regulations have been adopted to reduce the transmissibility. In addition, many countries declared quarantine, closing their borders.[2,3] Since many regions have not controlled the outbreak to date, regulations are heavily affecting the economy, and incomes have been drastically reduced. These measures have been enforced and maintained in some countries up until now mainly because mass screening of the infected population is not yet available. Thus, traceability remains one of the biggest challenges in controlling the pandemic while newly available COVID-19 vaccines are deployed globally.[4-7] Mass screening for SARS-CoV-2 is defined as a high number of tests performed in a population, regardless of their status (symptomatic or asymptomatic), in order to identify and quarantine positive cases.[6]

According to WHO recommendations, case tracing and boosting testing capacity should be regarded as the main policy of disease surveillance, especially when cases begin to drop or when facing a second wave of infection, in order to rapidly detect and isolate positive cases. These measures, along with social distancing, are the foundations for a public health−based COVID-19 response.[4,5,7]

Determining the exact number of asymptomatic or mild cases, which until now seem to be underreported, is the utmost important factor to ensure case tracing, given that these cases are the ones driving the pandemic. Massive screening or early testing of suspicious asymptomatic cases may help in reducing the transmission—limiting the spread of the disease. For this purpose, widely available rapid diagnostic tests are necessary.[8-10]

COVID-19 diagnosis currently requires obtaining a sample by using a nasopharyngeal swab (NPS), which must be performed by medical or trained personnel. Afterward, a real-time reverse transcription−polymerase chain reaction (rRT-PCR) test is performed. The rRT-PCR test has high specificity for the detection of SARS-CoV-2, being the gold standard for SARS-CoV-2 infection diagnosis. However, the NPS is not the ideal sample collection method regarding patient compliance. Moreover, use of the NPS is an invasive technique that can expose health care personnel to aerosols due to patients' coughing, and associated complications include epistaxis (which can be severe in anticoagulated patients).[11] In this context, other

Statement of Clinical Relevance

The results of this systematic review highlight the advantages of saliva as a sample for severe acute respiratory syndrome coronavirus 2 detection. As opposed to nasopharyngeal swabs, saliva is a painless, noninvasive, and fast test that can be used for diagnosis and massive screening.

sources of sample collection have been proposed, including saliva, which offers some advantages over NPSs.

Considering that massive screening will probably be adopted in more countries to control the pandemic, we propose that saliva could be a better sample than NPS because saliva collection can be performed by the patient himself, avoiding the need to attend a health care facility, thus diminishing crowding and health care personnel exposure.[12] It has been reported that saliva sample collection is easy, fast, widely accepted by patients, and cost-effective—all conducive to massive testing.[13,14] In addition, saliva is a feasible specimen type for respiratory virus diagnosis.[15] To et al.[16] reported a high overall agreement (93.3%) between saliva and nasopharyngeal aspirate specimens when other respiratory viruses were tested.

To date, several studies comparing the efficacy of rRT-PCR detection of SARS-CoV-2 between NPS and saliva samples have been published. Hence, the purpose of this review is to describe the value of saliva as a noninvasive sample for the detection of SARS-CoV-2 compared with the current method for sample collection (NPS), assessing if saliva offers adequate sensitivity for SARS-CoV-2 detection and providing a critical review of the selected articles.

METHODS

We conducted a systematic review of the literature following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations:

1. **Focused question:** We formulated a focused question following the patient, intervention, comparison, outcome (PICO) strategy: Is saliva a reliable source for SARS-CoV-2 quantitative RT-PCR (RT-qPCR) detection compared with NPS in the general population?
2. **Inclusion criteria:** We included case reports and case series that evaluated saliva as a sample for SARS-CoV-2 detection. We included articles published between December 2019 and October 2020 that were published in English and available in full text.
3. **Databases:** We searched 5 databases: PubMed, Cochrane, EBSCO, Elsevier, and MEDLINE.
4. **Search strategy:** A search strategy was developed according to the PICO strategy. Thus, we combined the following search terms: (saliva) AND (COVID-19), (saliva) AND (SARS-CoV-2), (saliva) AND (COVID-19) AND (diagnostic), (saliva) AND (COVID-19) AND (diagnosis), (saliva) AND (SARS-CoV-2) AND (diagnostic), (saliva) AND (SARS-CoV-2) AND (diagnosis).

We also screened the references of the selected articles to identify any relevant articles that were not included through the search strategy.

1. **Screening of selected articles and data extraction:** The literature search was performed independently by 2 authors, and the results were then compared. First, the title and abstract were screened to evaluate if the inclusion criteria were met. Afterward, both authors compared the selected articles and discussed their inclusion for full-text review and final inclusion. All duplicate articles were excluded. All disagreements were settled by discussion between the 2 authors. All articles that did not answer the focused question were excluded.

Both authors extracted the relevant data from the selected articles, including first author, year of publication, country, number of subjects, sex and age of the included patients, sample collection method (methods, storage, and processing), and diagnostic method for SARS-CoV-2 detection.

RESULTS
Selected studies

In total, 48 articles were found with the search strategies combining the 5 databases. Forty of them were selected by screening titles and abstracts. After a discussion between the 2 authors, 34 articles that met the inclusion criteria were included for full-text review. Manual screening was performed for the 34 articles; no new articles were included this way. After full-text review, an agreement was reached by the 2 reviewers to include 22 articles in the present review. The process of article selection is depicted in a flowchart (Figure 1).

Main characteristics of included articles

The included articles are case series, case reports, and massive screenings published during 2020 in Australia, Belgium, Canada, China, Italy, France, Hong Kong, Japan, South Korea, Thailand, and the United States. The main characteristics of the included articles are summarized in Table I.

1. **Study design:** This review included 17 case series, 2 case reports, and 3 massive screenings. Two articles were not peer reviewed.[11-14,17-34]
2. **Patients:** A total of 7630 patients were tested and included in this review. Of these, 862 patients had positive test results for SARS-CoV-2. Only 16 articles reported the sex of the patients; in total, 1390 men and 1205 women were included in this review. Their mean age was 55.6 years (range, 18[25] to 106 years[27]; Williams et al.,[13] Becker et al.,[23] Iwasaki et al.,[24] Caulley et al.,[31] Sakanashi et al.,[33] and Mestdagh et al.[34] did not report the sex of the patients, whereas Williams et al.,[13] Becker et al.,[23] Nagura-Ikeda et al.,[28] Caulley et al.,[31] Sakanashi
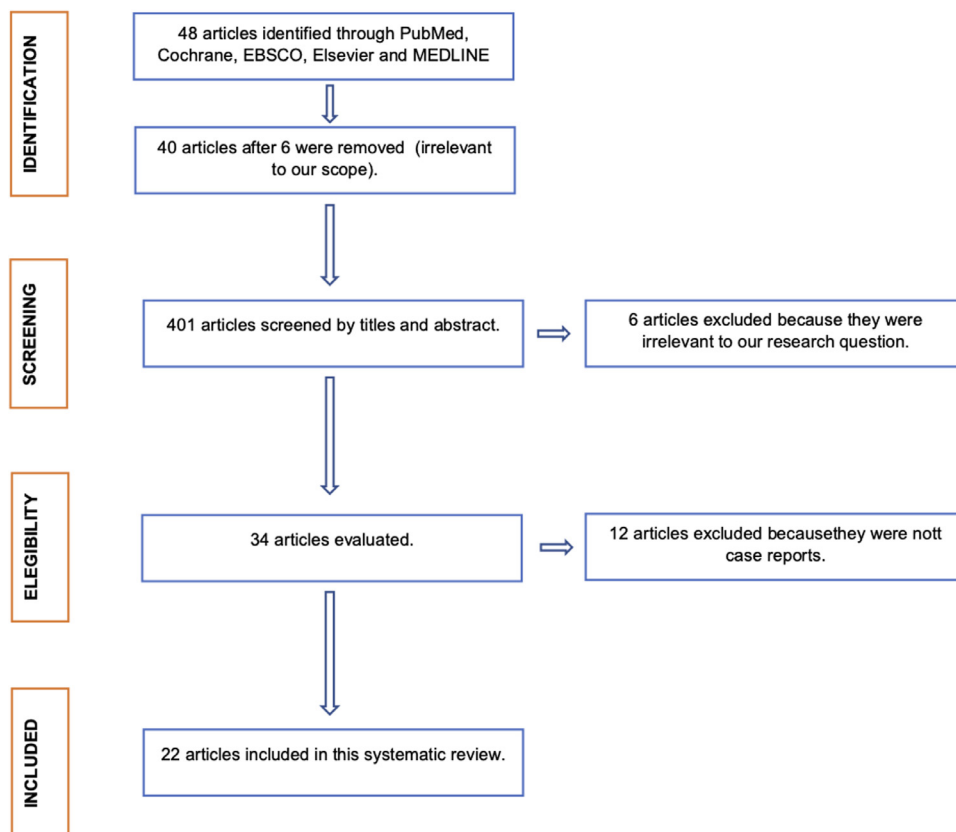
Figure 1. Flow chart of study selection process.

et al.,[33] and Mestdagh et al.[34] did not report the age of the patients).

3. **Saliva sample collection:** All included articles compared saliva with NPS for SARS-CoV-2 diagnosis. Saliva samples were self-collected by drooling (n = 1), spitting (n = 10), coughing up (n = 2), drooling or pipetting (n = 2), coughing up and spitting (n = 2), and spitting and saliva swabs (n = 1). Yoon et al.,[14] Pasomsub et al.,[11] McCormick-Baw et al.,[22] and Yokota et al.[32] did not report the saliva collection method.

4. **Obtention and preservation of the sample:** Six articles documented the procedures and conditions necessary for the sample collection. To et al.[18] obtained an early morning saliva sample, and the procedure was supervised by nurses. Zheng et al.[17] requested patients not to drink water, eat, or gargle in the 30 minutes preceding the sample collection. In addition, all patients were asked to wear a mask and cough 3 to 5 times before spitting out saliva into a sterile container. Wyllie et al.[21] instructed patients to avoid eating, brushing their teeth, and drinking water upon awakening and until samples were obtained. Nagura-Ikeda et al.[28] collected saliva without restriction on timing or food intake. McCormick-Baw et al.[22] and Mestdagh et al.[34]

recommended to patients that they not have any food, drink, tobacco, and gum at least 30 minutes before sample collection.

Two articles documented the storage conditions and handling of the samples after obtention.[21,27] Wyllie et al.[21] stored the samples at room temperature and transported them to the laboratory for processing within 5 hours. Yokota et al.[32] transported saliva at 4°C without transport media. Jamal et al.[27] froze the samples at −80°C and transported them to the laboratory within 8 hours. Authors of 3 articles used commercial kits to collect and preserve nucleic acids: Oragene·Dx Kit (DNA Genotek, Ottawa, ON, Canada)[23]; OMNIgene·ORAL, OM-505 (DNA Genotek)[31]; and the Saliva RNA Collection and Preservation Device (Norgen Biotek, Thorold, ON, Canada).[34] Most articles specified the handling for transportation of the sample. After collection, 2 to 2.5 mL of viral transport medium (VTM), phosphate-buffered saline (PBS), or universal transport medium (UTM) were added to the sample.

1. **SARS-CoV-2 detection method:** Various RT-qPCR kits for SARS-CoV-2 detection in saliva samples were used in the included articles. Similarly, viral RNA extraction was performed using different

Table I. Main characteristics of included articles

	Reference	Country, year	Study design	No. of patients	Sex	Mean age (years)	Saliva collection method	Saliva sample preservation	SARS-CoV-2 detection method	Sensitivity (%) for saliva	Specificity (%) for saliva	Viral load in saliva (copies per mL or Ct)	Viral load in NPS (copies per mL or Ct)
1	To et al.[12]	Hong Kong, China, February 2020	CS	12	7 M 5 W	62.5	Spit	VTM	RT-qPCR	–	–	3.3×10^6 copies/mL (range, 9.9×10^2 to 1.2×10^8 copies/mL)	–
2	Zheng et al.[17]	China, February 2020	CS	65	40 M 25 W	54	Coughing up and spitting	–	RT-qPCR	–	–	–	–
3	To et al.[18]	Hong Kong, China, March 2020	CS	23	13 M 10 W	62	Coughed up	VTM	RT-qPCR	–	–	2 $\log_{10}$ copies per mL to 6 $\log_{10}$ copies per mL	5 × 2 $\log_{10}$ copies per mL (IQR, 4.1-7.0)
4	Azzi et al.[19]	Italy, April 2020	CS	25	17 M 8 W	61.5	Drooling and pipetting	PBS (2 mL)	RT-qPCR	–	–	Ct 27.16 ± 3.07[†]	–
5	Azzi et al.[20]	Italy, April 2020	CR	2	2 M	67.5	Drooling and pipetting	PBS (2 mL)	RT-qPCR	–	–	–	–
6	Williams et al.[13]	Australia, April 2020	CS	522	–	–	Spit	Liquid Amies media	RT-qPCR	84.62%	98%	–	–
7	Wyllie et al.[21]	USA, April 2020	CS	44	23 M 21 W	61	Self-collected saliva and spit	–	RT-qPCR	–	–	–	–
8	Pasomsub et al.[11]	Thailand, May 2020	CS	19	9 M 10 W	33	–	UTM	RT-qPCR	84.21%	98.9%	ORF1 ab gene Ct 32.7 (28-35.0) N gen Ct 31.8 (28.4-33.7)*	ORF1 ab gene Ct 32.0 (27.434.3) N gen Ct 30.5 (26.1-32.3)*
9	Yoon et al.[14]	South Korea, May 2020	CR	2	2 W	55.5	–	VTM (2 mL)	RT-qPCR	–	–	Patient 1 = 6.63 $\log_{10}$ copies/mL Patient 2 = 7.10 $\log_{10}$ copies/mL	Patient 1: 8.41 $\log_{10}$ copies/mL Patient 2 = 7.49 $\log_{10}$ copies/mL
10	McCor-mick-Baw et al.[22]	USA, May 2020	CS	156	90 M 66 W	47.8	–	Unpreserved	RT-qPCR	95.92%	99.06%	E gene Ct 26.10 ± 11.20; N2 gene Ct 30.40 ± 9.67[†]	E gene Ct 23.83 ± 7.78; N2 gene Ct 26.70 ± 7.61[†]
11	Becker et al.[23]	USA, May 2020	CS	112	–	–	Self-collected and spit	Oragene·Dx Kit	RT-qPCR	40%-60% 20%-50%	97%-100% 75%-94%	–	–
12	Iwasaki. et al.[24]	Japan, June 2020	CS	76	–	69	Self-collected saliva and spit	PBS (600 μL)	RT-qPCR	88.89%	98.51%	4.1 ± 1.4 $\log_{10}$ gene copies/mL Ct 30.6 ± 4.6[†]	5.4 ± 2.4 $\log_{10}$ gene copies/mL Ct 26.5 ± 8.1[†]
13	Hung et al.[25]	Hong Kong, China, June 2020	CS	18	8 M 10 W	39.1	Coughed up	VTM (2 mL)	RT-qPCR	–	–	Early morning Ct 34.5 (32.5-41); before lunch Ct 38.2 (33.9-41); before teatime Ct 36.3 (34.5-41); before dinner Ct	Ct on admission 28.09 (17.71-36.6)*

Table I. Continued

	Reference	Country, year	Study design	No. of patients	Sex	Mean age (years)	Saliva collection method	Saliva sample preservation	SARS-CoV-2 detection method	Sensitivity (%) for saliva	Specificity (%) for saliva	Viral load in saliva (copies per mL or Ct)	Viral load in NPS (copies per mL or Ct)
14	Chen et al.[26]	Hong Kong, China, June 2020	CS	58	28 M 30 W	38	Coughing up followed by spit.	VTM (2 mL)	RT-qPCR	89%	66%	41 (34.7-41); before bedtime Ct 41 (34.7-41)* E gene Ct 29.7 (27.2-37.2); N2 gene Ct 32.3 (29.9-38.6)*	E gene Ct 26.8 (20.7-33.5); N2 gene Ct 29.3 (23.3-36.5)*
15	Jamal et al.[27]	Canada, June 2020	CS	91	52 M 39 W	66	Spit	PBS (2.5 mL)	RT-qPCR	68.75%	70.37%	Ct 34.0 (31-37)*	Ct 30.0 (26-35)*
16	Nagura-Ikeda et al.[28]	Japan, July 2020	CS	103	66 M 37 W	–	Self-collected and spit	–	RT-qPCR RT-LAMP	81.6%	–	–	–
17	Migueres et al.[29]	France, August 2020	CS	123	49 M 74 W	43	Spit	–	RT-qPCR	82.93%	96.34%	RDrP gene Ct 29.5 (27.6-33.3)*	RDrP gene Ct 24.9 (21.9-30.9)*
18	Kim et al.[30]	South Korea, August 2020	CS	15	5 M 10 W	59	Spit	UTM	RT-qPCR	64%	–	Ct 32.0 (28-38)*	Ct 33.0 (27-35)*
19	Caulley et al.[31]	Canada, August 2020	Mass screening	1939	–	–	Self-collected and spit	OMNIgene·ORAL, OM-505 (DNA Genotek)	RT-qPCR	60.71%	99.26%	–	–
20	Yokota et al.[32]	Japan, September 2020	Mass screening study	1924	981 M 858 W 95 unknown	Cohort 1: 44.9 Cohort 2: 33.5	–	None	RT-qPCR RT-LAMP	92%	>99.9%	–	–
21	Sakanashi et al.[33]	Japan, September 2020	CS	12	–	–	Self-collected (drooling)	VTM (3 mL)	RT-qPCR	79%	69%	–	–
22	Mestdagh et al.[34]	Belgium, October 2020	Mass screening	2289	–	–	Spit and saliva swab	Norgen Biotek's Saliva RNA Collection and Preservation Device Dx 53800	RT-qPCR	30.8% overall (spit) 22.4% Overall (swab) 97% in high viral load samples (spit) 76.7% in high viral load samples (swab)	–	–	–

CR, case report; *CS*, Case-series; *IQR*, interquartile range; *M*, men; *n*, number of included patients, considers patients with PCR SARS-CoV-2 diagnosis; *PBS*, phosphate-buffered saline; *RT-LAMP*, reverse transcription loop-mediated isothermal amplification; *RT-PCR*, reverse transcription polymerase chain reaction; *SARS-CoV-2*, severe acute respiratory syndrome coronavirus 2; *UTM*, universal transport medium; *VTM*, viral transport medium; *W*, women.
*Median (IQR).
†Mean ± standard deviation.

commercial kits (only 3 articles did not report the RNA extraction kit used).

The amplification targets were different according to the authors. Within the reported amplified sequences are genetic regions belonging to genes E, S, N, N2, RDrP, ORFI ab, and 5′-untranslated region.

6. **Salivary SARS-CoV2 detection:** This review includes reports with small cohorts of patients with positive COVID-19 test results and large cohorts of asymptomatic close contacts and high-risk individuals. Moreover, some selected articles lacked information regarding SARS-CoV-2 viral load as well as sensitivity and specificity. Of the 22 included articles, 12 (54.5%) reported viral load as the number of copies and/or cycle threshold (Ct) values, and 14 (63.6%) of the included articles reported sensitivity and specificity.

   a. **SARS-CoV-2 viral load:** To et al.[18], Yoon et al.,[14] Pasomsub et al.,[11] and Iwasaki et al.[24] reported the number of viral particles and found that the viral load was greater in NPS than in saliva samples. Also, Azzi et al.,[19] Pasomsub et al.,[11] McCormick-Baw et al.,[22] Iwasaki et al.,[24] Hung et al.,[25] Chen et al.,[26] Jamal et al.,[27] Migueres et al.,[29] and Kim et al.[30] found that saliva samples showed higher Ct values than the NPS samples, indicating that the viral load was higher in NPS.

   b. **SARS-CoV-2 sensitivity and specificity:** Eleven studies reported the sensitivity and specificity of saliva for SARS-CoV-2 detection. Three studies only reported sensitivity. Sensitivity ranged between 20%[23] and 97%,[34] with a mean of 79.47% for all included studies. Specificity ranged between 66%[26] and 100%,[23] with a mean of 90.48% for all included studies.

Additionally, Iwasaki et al.[24] reported that the concordance index between saliva and NPS was 97.4% with a kappa of 0.87%.

DISCUSSION

Salivary SARS-CoV-2 detection

According to To et al.,[12] saliva is an alternative to NPS for SARS-CoV-2 detection and even other respiratory viruses. Xu et al.[35] stated that saliva may be easily obtained from the patient, coincident with reports from Azzi et al.[19] and Yoon et al.,[14] who reported that the sample may be self-collected by the patient.

Viral load in saliva

Some studies have shown that epithelial cells from the oral mucosal lining, including those from the tongue dorsum, express a high amount of angiotensin-converting enzyme 2

(ACE2) receptors, critical for COVID-19 pathogenesis.[36,37] Baghizadeh Fini[38] proposed that SARS-CoV-2 can attach to ACE2 receptors on the epithelium of salivary glands, allowing the virus to enter the cell and replicate, consequently leading to their lysis and triggering an acute or chronic sialadenitis. This may explain the high concentrations of viral load detected in saliva.[37] Interestingly, Zhu et al.[39] reported a viral load peak in saliva (10^4 to 10^8 copies per mL) during the first week of symptoms that gradually declines over time. Moreover, no significant difference in salivary viral load between mild and severe cases of COVID-19 was found. On the other hand, To et al.[12] proposed that SARS-CoV-2 detection in saliva may be due to nasopharyngeal epithelial debris accumulating in the oral cavity. However, studies in animals demonstrate that SARS-CoV-2 is capable of infecting ductal epithelial cells from salivary glands, which may explain the viral presence in saliva.[40] It is important to point out that epithelial cells of the salivary glands may engulf viral particles or virus-containing exosomes on their basolateral portion, which may then be secreted to the acinar lumen, as suggested by Dawes et al.[41]

Accuracy of saliva for SARS-CoV-2 detection

Saliva has been proposed as a reliable sample for SARS-CoV-2 detection; thus, specificity and sensitivity must be greater than or equal to the those reported for NPS. So far, this seems to be the case, according to Pasomsub et al.[11] and Williams et al.,[13] although contingency tables are lacking. According to Zhu et al.,[39] the sensitivity and specificity of saliva were 86.4% (95% confidence interval [CI], 82.8%-89.4%) and 97.0% (95% CI, 95.0%-98.3%), respectively, in 944 patients from 12 independent cohorts. Moreover, concordance analysis revealed a 92.1% observed virus detection accuracy between the respiratory tract and saliva samples (Cohen's kappa coefficient, 0.840; 95% CI, 0.805-0.874).[39]

Interestingly, the studies by Becker et al.,[23] Caulley et al.,[31] and Mestdagh et al.,[34] who obtained the lowest sensitivities and specificities, used commercial kits to obtain and preserve saliva samples. On the one hand, it is possible to hypothesize that transfer of the sample in VTM, UTM, or PBS is preferable because the results have been better. Further studies are required to confirm this hypothesis. On the other hand, Mestdagh et al.[34] mentioned that sensitivity improves dramatically in individuals with high viral load, which is greater at the onset of symptoms[12,18,23,27] and which would allow the virus to be identified in asymptomatic individuals, with a consequent high viral load.

It has recently been reported that NPS has a significantly higher detection rate for SARS-CoV-2 than oropharyngeal swabs (OPSs),[42] mainly due to the low viral load found in OPS. According to the results of this review, the viral load in saliva is high in symptomatic patients, as stated above[37]; thus, the detection rate is high and comparable to NPS in

these patients. Even when the viral load in saliva is lower in asymptomatic patients, it may be detected with a salivary test, as reported by Chen et al.,[26] Williams et al.,[13] Pasomsub et al.,[11] McCormick et al.,[22] Iwasaki et al.,[24] Nagura-Ikeda et al.,[28] Migueres et al.,[29] and Yokota et al.[32] According to the results of Wyllie et al.,[21] a salivary test detected all positive cases, including health care professionals who were screened.

Use of saliva for diagnostic and screening of SARS-CoV-2 infection

Countries such as China, Japan, Australia, Spain, and Germany have made some progress toward lifting COVID-19 containment measures, and these have unavoidably led to an increase in new positive cases.[1] Results reported by López et al.,[43] showed that early quarantine, social distancing measures, and self-care measures are the recommended interventions for the initial containment of the epidemic outbreaks.[44,45] However, to safely lift containment and prevent the much-feared "second wave," population-wide screening is needed to rapidly detect and isolate positive cases. The available tests for SARS-CoV-2 detection include serologic testing and rRT-PCR.[11,43] Regarding rRT-PCR, SARS-CoV-2 RNA is usually detectable in nasopharyngeal samples, and, more recently, saliva sampling has been proposed as an alternative.

Saliva offers advantages over NPS regarding population-wide screening for SARS-CoV-2 and may also be helpful for regular screening for asymptomatic health care workers in health care facilities. Saliva is a noninvasive sample that can either be self-collected by the patient or collected by medical personal when the patient is unwilling or unable to cooperate. Most important, saliva reduces the risk of transmission between health care workers and shortens both the sample collection time and diagnosis time. Pasomsub et al.[11] pointed out that, given the current complex economic situation in many countries, saliva is a viable alternative for reducing the costs of SARS-CoV-2 detection. Still, new investigations should focus on assessing the analytical and clinical sensitivity and specificity of saliva for SARS-CoV-2 detection; we were unable to retrieve these data from the included publications for this review.

General recommendations for saliva collection and preservation

According to the results of this systematic review, saliva is a feasible option for SARS-CoV-2 detection. The collection may be performed in either asymptomatic or symptomatic patients (including those intubated). Sample collection does not require a special setting or preparation. The following are recommendations for sample collection, storage, and handling of saliva for SARS-CoV-2 detection:

1. **Time of sample collection:** It is always best to collect the sample in the morning.

2. **Before saliva collection (indications for patients):** Do not eat, drink, wash your teeth, or rinse for at least 60 minutes before the sample collection.

3. **Sample collection:** Samples may be obtained by the passive drooling method. Use stimulants only when necessary. For intubated patients, a pipette must be used to collect the sample. Label the vial with the patient's information.

4. **Sample volume:** A sample volume of 1 to 3 mL is sufficient for SARS-CoV-2 detection.

5. **After saliva collection:** If possible, freeze the sample immediately at −80°C and process it within 8 hours. Samples may be stored at room temperature for no longer than 5 hours before freezing at −80°C.

6. **Storage and handling:** Resuspend the sample in 2 to 2.5 mL of VTM or PBS.

7. **Processing and RNA extraction:** Samples are to be processed as soon as possible. Bring samples to room temperature, vortex, and then centrifuge for 15 minutes at 4°C at 20 000 × *g*. For RNA extraction, use the NucliSENS easyMAG (bioMérieux, Marcy l'Etoile, France) or the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany).

8. **SARS-CoV-2 detection method:** rRT-qPCR.

The greatest viral load has been reported in the morning; thus, the sample must be obtained during the first hours of the day to ensure good results.[25] In parallel, the patients must follow instructions before sample collection to avoid contamination.[12,17,21] The sample may be obtained by drooling technique or pipetting if the patient is intubated or is unable to cooperate, and 1 to 3 mL should be enough to perform the assays.[19] According to Sullivan et al.,[46] it is advisable to rinse the mouth with water and discard the rinse, then wait 5 minutes before collecting the sample. Thick saliva may occur in dehydrated or hospitalized patients, making the pipetting difficult and risking contamination of the workspace[47]; therefore, precautions should be taken with such patients. In addition, considering that saliva contains proteins and peptides that have exhibited antiviral effects, hyposalivation could be a risk factor for SARS-CoV-2 infection.[48]

Sample preservation is another key point. Once the sample is obtained, it must be preserved in VTM or PBS[19,27] to prevent salivary enzymes from damaging the viral RNA. The sample must be processed within the next hours. If the sample is stored at room temperature, it must be processed within the next 5 hours.[21] If the sample is stored at −80°C, it can be processed within the next 8 hours or more.[27] The subsequent RNA extraction can be performed with any available and U.S. Food and Drug Administration−approved commercial kit (such as NucliSENS easyMAG and QIAamp Viral RNA Mini Kit), and SARS-CoV-2

detection can be performed by using a validated rRT-qPCR kit. We suggest considering these key aspects of sample collection, storage, and handling of the saliva sample in order to obtain reliable results.

CONCLUSION

Saliva might be an appropriate, fast, painless, simple, and noninvasive sample for SARS-CoV-2 detection, making it ideal for massive screening of SARS-CoV-2 infection. The sample can be self-collected by the patient, thus reducing the infection risk among health care workers. Although sensitivity and specificity of saliva samples are not equivalent to NPS in all cases, values are relatively similar for symptomatic cases with high viral load and fairly acceptable for asymptomatic patients with low viral load. Thus, saliva could be considered a proper sample for massive screening or for the diagnosis of suspicious cases in health facilities where resources are scarce and NPS might not be widely available.

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