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SARS-CoV-2 saliva testing using RT-PCR: a systematic review

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A B S T R A C T

Objectives: There remain challenges in using SARS-CoV-2 RNA diagnostic assays in the respiratory tract in a pandemic. More so certain countries such as Hong Kong have already included saliva as part of their mass-testing protocol. The aim of this study was to conduct a systematic review on the alternate use of saliva as a SARS-CoV-2 RNA testing specimen in the context of mass screening with reverse transcription polymerase chain reaction.

Methods: Our search methodology was modeled after the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) checklist, and the risk of bias of the selected studies was qualitatively assessed. The percentage individual positive and percentage agreement of both index (saliva) and reference (nasopharyngeal swab), in preference to specificity and sensitivity, were estimated using Kappa statistics.

Results: A total of 44 studies met the inclusion criteria. The average percentage positive saliva cases was 72.7% (95% confidence interval), which was lower but not substantially different from the percentage positive NPS of 78.7% (95% confidence interval), and there was an average overall agreement of 89.7% (95% confidence interval).

Conclusion: Although the literature supports nasopharyngeal swab as a superior testing specimen, an alternative clinical specimen in saliva may offer potential benefits such that a potentially reduced accuracy may be tolerated, especially in low socioeconomic regions.

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Introduction

Coronavirus (CoV) is a nonsegmented, enveloped, positive-sense RNA virus belonging to the Coronaviridae family and is generally found in humans and other mammals (Rothan et al., 2020). Coronavirus can be detected in the respiratory system and previous outbreaks of coronaviruses, such as the Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV), have been characterized as agents of great threat to public health (Rothan et al., 2020; Guo et al., 2020). In December 2019, a group of pneumonia cases occurring in Wuhan, China was confirmed to be caused by a newly identified β-coronavirus, and in January 2020; it was named the SARS-CoV-2 virus by the International Committee on Taxonomy of Viruses and the disease was named COVID-19 (Guo et al., 2020). Thus far, over 204 million confirmed cases of COVID-19 with >4.3 million deaths across over 210 countries have been reported worldwide as of August 11, 2021 (https://covid19.who.int/table). The disease has since been called a pandemic, with several confinement measures and comprehensive vaccination programs put in place in many different countries to curb its further spread. The COVID-19 infection typically appears after an incubation period of approximately 5.2 days and the symptoms range from completely asymptomatic to symptomatic (Wang et al., 2020). The period from the onset of COVID-19 symptoms to death is generally dependent on patient's age and immune status and this ranges from 6–41 days, with a median of 14 days (Huang et al., 2020). The most frequently seen symptoms are cough, fever, and fatigue, whereas other symptoms such as headache, diarrhea, sputum production, hemoptysis, and dyspnea may also occur (Wang et al., 2020; Carlos et al., 2020; Huang et al., 2020; Ren et al., 2020). Screening by RT-qPCR for targets of SARS-CoV-2 genome from respiratory (nasopharyngeal) specimen remains the gold standard for detection, and in a pandemic-testing context, it is the first crucial step toward surveillance and effective control (Pan et al., 2020). Although there is need to increase the

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capacity for diagnostic testing, there remains diagnostic assay challenges with detecting SARS-CoV-2 RNA from different specimens. A clinical study on RT-PCR detection rates of SARS-CoV-2 RNA from several specimens comprising nasopharyngeal, oropharyngeal, sputum, feces, urine, ocular fluid, and blood highlighted the discordance in detection of viral material because sputum and fecal samples returned the most positive tests (HIQA, 2020); this informed the current guidance of collection of a combined nasopharyngeal and oropharyngeal swabs for routine SARS-CoV-2 testing (Wong et al., 2020). Additional challenges associated with nasopharyngeal swabbing include its relative invasiveness and discomfort, particularly among children, patients with reduced cognitive function, and those undergoing serial testing for surveillance. In addition, it requires a degree of clinical skill, close contact, and substantial PPE due to risk of transmission (Cheng et al., 2020). Alternatively, the use of saliva has less adverse risk because it is less invasive and requires no transport media, no PPE, and a reduced number of healthcare personnel because it can be self-collected. Furthermore, its diagnostic assay can be a simple QE (QuickExtract DNA) buffer-based preparation compared with the column-based nucleic acid purification that is currently used for nasopharyngeal swab specimen. In the context of mass testing, such as in a pandemic, certain countries such as Hong Kong have already included saliva as part of their mass-testing protocol (Wong et al., 2020). The pathophysiology behind the use of saliva for testing lies in the high salivary gland expression of host angiotensin-converting enzyme, which regulates the host receptor-cellular entry of SARS-CoV-2, compared with the lungs (Hoffmann et al., 2020; Xu et al., 2020).

The aim of this study was to contribute and clarify the alternate use of saliva as a testing specimen for mass SARS-CoV-2 screening by systematically appraising the detection accuracy of nucleic acid assay between saliva and nasopharyngeal samples.

Methods

Our search methods was modeled from the checklist of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Page et al., 2021). A Cochrane style MeSH terms and keywords comprising COVID-19, SARS-CoV-2, real-time RT-PCR, RNA, Saliva (SA), and nasopharyngeal swabs (NPS) were used for the initial search with the search tools: Pubmed, Ovid Medline, and Web of science from January 1, 2020–August 1, 2021. English publications of COVID-19 testing assays with paired specimens, i.e., saliva with NPS as the comparator, were selected. Additional publications were retrieved from the reference list of selected articles from the initial search, and the full text of these retrieved publications were reviewed to identify those suitable for inclusion. Studies considered were:

- Cross-sectional, case-controlled studies, and controlled clinical trials;
- SARS-CoV-2 saliva and NPS RNA RT-PCR-based studies were also included.

To qualitatively assess the risk of participant selection bias of the selected studies, a modified quality assessment of diagnostic accuracy studies 2 (modified QUADAS-2) (Whiting et al., 2011) was used and two factors were appraised: the experimental design and assay accuracy for index (SA) and reference (NPS) samples. As part of appraisal of the experimental design, the respective RT-PCR kit targets with minimum C_t (cycle threshold) for positive results were collated. In addition, a result comparison on the basis of SA collection protocol, such as deep throat collection (coughing) versus spitting/drooling, was also appraised. A box plot for index and reference positive detection values was also generated using MS excel (Microsoft Excel for Mac version 16.59). SPSS 27

software package (IBM Company, Armonk, NY, USA) was used for statistical analysis. Median of individuals with paired specimens in addition to sample size, sex ratio, and sample collection method were captured for descriptive statistical information. On the basis of the assumption that no testing specimen type is superior to the other, individual positive and negative cases (% of individual positive and negative for both index and reference specimens) and reports of percentage agreement of index specimen (% of positive and negative agreements with confirmed samples), preferred to specificity and sensitivity, were estimated using Kappa statistics (Table 1) (Obermeier et al., 2016). Purely clinical or pathological articles, conference articles, and abstract-only articles were excluded.

Patient & participant involvement

No patient involvement was required because this is a review paper study.

Results

Characteristics of studies included

The electronic search yielded 1907 entries, of which 1817 publications were removed for having no correlation to SARS-CoV-2 saliva-based test, absence of comparators, and for study duplicity, resulting in 90 publications being eligible for full-text review. Of these, 44 studies (Azzi et al., 2020; Wong et al., 2020; Griesemer et al., 2021; Iwasaki et al., 2020; Kojima et al., 2020; Leung et al., 2021; McCormick-Baw et al., 2020; Pasomsub et al., 2021; Wyllie et al., 2020; Williams et al., 2020; Barat et al., 2021; McMillen et al., 2021; Nacher et al., 2021; Migueres et al., 2020; Otto et al., 2021; Hanson et al., 2020; Rao et al., 2021; Byrne et al., 2020; Skolimowska et al., 2020; Dogan et al., 2021; Landry et al., 2020; SoRelle et al., 2020; Jamal et al., 2021; Bhattacharya et al., 2021; Rutgers, 2020; Hitzenbichler et al., 2021; Aita et al., 2020; Babady et al., 2021; Braz-Silva et al., 2020; Chen et al., 2020; Güçlü et al., 2020; Hasanoglu et al., 2021; Kandel et al., 2020; Kim et al., 2020; Lai et al., 2020; Li et al., 2020; Lin et al., 2020; Moreno-Contreras et al., 2020; Perchetti et al., 2020; Procop et al., 2020; Senok et al., 2020; Sohn et al., 2020; Vaz et al., 2020; Yokota et al., 2021) met the eligibility criteria and were included in the study, whereas 46 articles were removed for not using PCR assay and the absence of NPS as a specific a comparator (Table 2). All selected studies comprised studies on the basis of clinical surveys among patients with confirmed SARS-CoV-2 infection in the United States, the United Kingdom, China, Hong Kong, Turkey, India, Italy, Japan, France, Republic of Thailand, Malaysia, Australia, and Canada.

The studies cumulatively included 8555 samples of paired saliva and NPS, although nine studies used both nasopharyngeal and oropharyngeal samples as comparators (Pasomsub et al., 2021; Byrne et al., 2020; Chen et al., 2020; Hasanoglu et al., 2021; Hitzenbichler et al., 2021; Kim et al., 2020; Lai et al., 2020; Moreno-Contreras et al., 2020; Vaz et al., 2020). The median number of participants included in the studies was 91.5. All studies used PCR assay, amplifying five different SARS-CoV-2 targets (E, N, ORF1, RdRp, and S) and compared NPS and oropharyngeal samples with SA samples. A total of 21 studies used 2–3 RT-PCR SARS-CoV-2 targets for test detection, 16 studies used one target, whereas seven studies did not provide details of the control used. (Hanson et al., 2020; Landry et al., 2020; Aita et al., 2020; Chen et al., 2020; Jamal et al., 2021; Güçlü et al., 2020; Hasanoglu et al., 2021). A total of 27 studies used C_t values 35–45 as their positive test indicator (Table 2). After removing outliers, the mean percentage positive SA cases (72.7%) (95% confidence interval [CI] 49%–100%) was lower than the mean percentage positive NPS cases (78.7%) (95% CI 47%–99%), and there was

Table 1
Formulas for % positive agreement (PPA); % negative agreement (PNA); and Overall Agreement (OA).

	Index (SA) +ve	Index (SA) -ve	
Reference (NPS) +ve	A	B	$PPA* = 100\% \times \dfrac{A}{(A+B)}$
Reference (NPS) -ve	C	D	$PNA* = 100\% \times \dfrac{D}{(C+D)}$
Kappa			$OA* = 100\% \times \dfrac{(A+D)}{(A+B+C+D)}$

Key: * = index case, SA = Saliva, NPS = Nasopharyngeal swab

Table 2
List of selected publications with data.

No.	Publications	Sample size	Mean/ Median ages	M: F	No of test targets	Ct value (Mean)	+ve percent SA agreement (%)	-ve percent SA agreement (%)	Overall agreement (%)	Risk of bias
1.	Azzi et. al.	114	54	1:2	NS	<30(27.2)	90.9	100.0	94.1	U
2.	Wong et al.	229	39 / 36*	2:1	NS	≤ 40	85.3	65.4	76.0	U
3.	Griesemer et al.	463	NS	1:4	N	<45	82.5	99.4	95.7	H
4.	Iwasaki et al.	76	69*	NS	NS	(30.6)	88.9	98.5	97.4	M
5.	Kojima et al.	177	42	NS	N	(34.1)	90.0	NS	NS	H
6.	Leung et al.	62	42	1:2	NS	<37.9	NS	NS	79.8	U
7.	McCormick-Baw et al.	156	48	1.5:1	E & N2	<41 (30.4)	97.9	99.0	98.7	H
8.	Pasomsub et al.	200	36	1:3	ORF1 & N	≤ 38	84.2	98.9	97.5	M
9.	Wyllie et al.	70	NS	NS	N1	<24.4	NS	NS	NS	M
10.	Williams et al.	522	NS	NS	NS	<17	84.6	NS	NS	U
11.	Barat et al.	918	42*	1:1.5	N	<40 (<31)	81.1	99.8	98.3	M
12.	McMillen et al.	20	NS	NS	2 targets -NS	<40	100.0	NS	NS	H
13.	Nacher et al.	776	40	1:1.6	N & RdRp	<35	50.0	98.4	88.9	H
14.	Otto et al.	92	NS	NS	RdRp	NS	100.0	91.5	95.7	U
15.	Migueres et al.	606	33	1:1	ORF1 RdRp	<40	79.2	99.2	95.7	H
16.	Hanson et al.	1104	35	1:1	ORF1	≤ 42	93.8	97.8	96.9	U
17.	Rao et al.	160	27	NS	E & RdRp	<38	86.9	0.0	45.6	M
18.	Byrne et al.	110	NS	1:1	NS	NS	NS	NS	NS	U
19.	Skolimowska et al.	132	39*	1:1.5	ORF1	<34	83.3	99.1	96.9	H
20.	Dogan et al.	200	NS	NS	ORF & N	≤ 29	54.5	88.4	69.4	U
21.	Jamal et al.	91	66*	2:1	N, RdRp, E	<34	68.8	70.4	69.2	H
22.	Landry et al.	124	NS	NS	N	<40	84.8	97.8	94.4	H
23.	SoRelle et al.	83	NS	NS	E & N2	<40	82.0	100.0	91.6	U
24.	Rutgers Lab.	53	NS	NS	N, S, ORF1	<37	100.0	100.0	100	H
25.	Bhattacharya et al.	53	NS	NS	ORF1 & E	(29.1)	90.6	NS	NS	H
26.	Hitzenbicher et al	34	57	3:1	E	NS	81.3	40	71.4	H
27.	Aita et al.	43	62	2:1	E	NS	100	97.2	97.7	H
28.	Babady et al.	87	NS	NS	N, E, ORF1	<40	94.1	98.6	97.7	H
29.	Braz-Silva et al.	201	40	1:1.5	E & S	<40	71.1	87.9	83.6	M
30.	Chen et. al.	58	38	1:1	ORF1 & N	<35	89.1	0.0	84.5	H
31.	Guclu et al.	64	51	1:1	NS	<45 (32.9)	85.2	89.2	87.5	H
32.	Hasanoglu et al.	60	34	1:1	RdRp	<40	56.3	75	60	M
33.	Kandel et al.	215	42	1:1.5	ORF1 & E	<37	90.7	99.2	98.4	M
34.	Kim et al.	15	59	1:2	E & RdRp	≤ 35	NS	NS	NS	U
35.	Lai et al.	50	NS	1:1	N	≤ 39.9	NS	NS	NS	M
36.	Li et al.	13	52.8	1:1	RdRp,E,N	NS	NS	NS	NS	H
37.	Lin et al.	52	57.3	1:1	ORF1, N, E	≤ 30	82.6	27.6	51.9	M
38.	Moreno-Contreras et al.	71	41	1:1	E	≤ 38	67.9	86.1	78.9	U
39.	Perchetti et al.	20	NS	NS	N	(35.4)	NA	NS	NS	M
40.	Procop et al.	216	44	NS	N	(24.2)	100	99.4	NS	H
41.	Senok et al.	401	35.5	4:1	RdRp,N	<40	73.1	97.6	96.0	M
42.	Sohn et al.	48	32.6	3:1	RdRp,E,N	<40	100	97.6	97.9	H
43.	Vaz et al.	155	40	1:3	E,RdRp	≤ 40	94.4	97.6	96.1	H
44.	Yokota et al	161	44.9	1:1.5	N	<37.3	92.7	95.0	94.4	H

Key: * = Median age; NS = Not Stated, SA = Saliva, C_t = Cycle threshold, N=N gene, E=E gene, ORF1=Open read frame 1 gene, RdRp = RNA dependent RNA polymerase gene, M = Medium, H = High, U = Unknown

an average overall agreement of 89.7% (95% CI 60%–100%). A boxplot showed that the interquartile range and the median percentage positive NPS was greater than that of SA cases, suggesting a higher percentage positive NPS (wFig. 1). Also, the boxplot was skewed to the left (above median) for NPS, suggesting the higher percentage positive NPS values are closer to the mean percentage positive NPS than that of SA cases, which was skewed to the right (below the mean), suggesting that the lower percentage positive are closer to the mean percentage positive SA cases (Fig. 1). Saliva collection protocol for studies were also assessed with respect to deep throat collection through coughing and spitting/drooling. No difference in percentage positive SA was found between the deep throat group (Rao et al., 2021; Leung et al., 2021; Kojima et al., 2020; Wong et al., 2020; Otto et al., 2021) and the
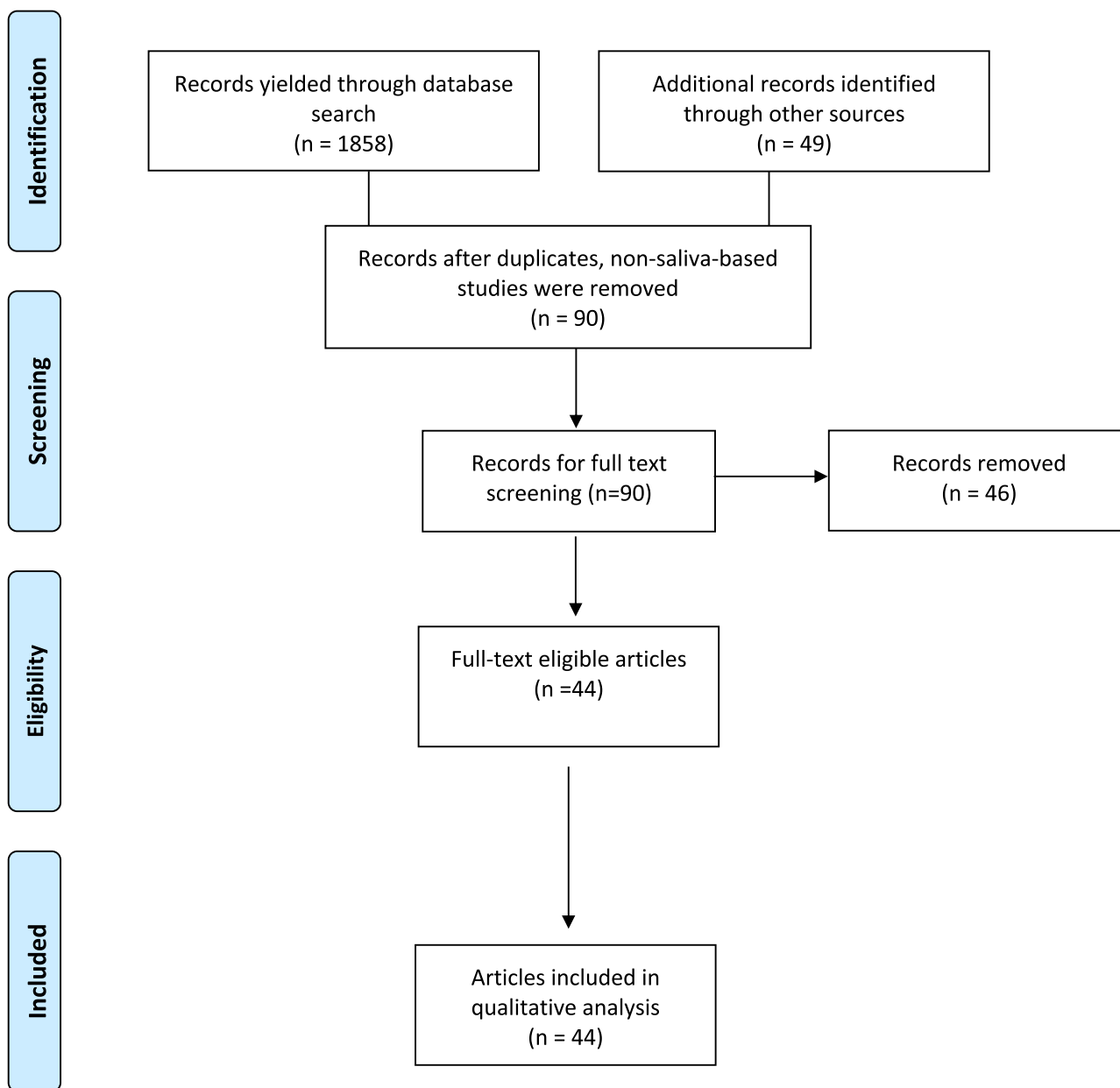
Fig. 1. Boxplot for % of positive sample for index and reference

spitting group, this is despite saliva dilution or pretreatment due to saliva viscosity, which was done in three studies (Jamal et al., 2021; Landry et al., 2020; Hanson et al., 2020). Differences in diagnostic performance at different points of presentation between SA and NPS samples were also reviewed. Further analysis of these studies showed that percentage positive SA was lower at <7 days (before or at early symptom onset) than >7 days (after symptom onset), and although this was similar for NPS, the SA test positivity was lower (Landry et al., 2020; Jamal et al., 2021; Dogan et al., 2021; Migueres et al., 2020; Byrne et al., 2020). All but 12 studies (Iwasaki et al., 2020; Wyllie et al., 2020; Pasomsub et al., 2021; Braz-Silva et al., 2020; Barat et al., 2021; Hasanoglu et al., 2021; Rao et al., 2021; Kandel et al., 2020; Lai et al., 2020; Lin et al., 2020; Perchetti et al., 2020; Senok et al., 2020) had a high patient selection bias and all assays had low biases (Table 2), (Fig. 2).

Discussion

This systematic review appraised studies that compared NPS with SA specimens for SARS-CoV-2 detection using RT-PCR, with a view to accessing its suitability as an alternative to NPS during large-scale testing. We found the overall percentage of positive SA detection to be lower than that of NPS. With the increased need for worldwide testing, accurate and easy-to-use collecting methods can help in surveillance and monitoring; in this regard, several nonnucleic acid extraction protocol and new sample collecting techniques have been undertaken (Kriegova et al., 2020; Hasan et al., 2020). However, the only RNA extraction-free protocol yielded a lower rate of detection (Dogan et al., 2021). Although more than half of the study used 2–3 SARS-CoV-2 targets, which improves detection accuracy, the median C_t of 35–45 used for most of the studies, compared with a robust 25–30 value, may indicate
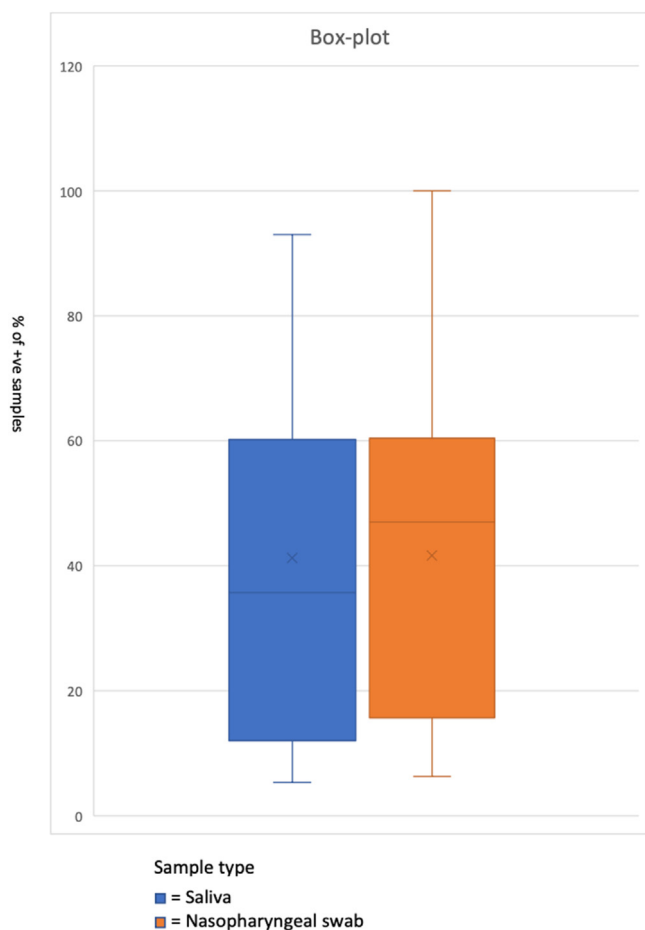
Fig. 2. Boxplot for % of positive sample for index and reference

pecially where shortage is an issue. Therefore, because of these advantages, a potentially reduced accuracy may be tolerated, especially in large-scale testing or testing in regions with lower socioeconomic or underdeveloped healthcare system.

Conflict of interest

The authors have no competing interests to declare.

Author contributions

Principal author contributed 75% of the manuscript, Co-author contributed 25% of the manuscript

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an error in the assay used or an actual low viral concentration, possibly from contamination. Increased saliva viscosity from deep throat saliva collection by coughing resulted in decreased positive saliva detection compared with self-collection through spitting, albeit not substantial. In addition, saliva viscosity is reported to result in invalid test results due to an increased automated pipetting errors and this necessitated dilution of samples (Jamal et al., 2021; Landry et al., 2020; Hanson et al., 2020). Therefore, we advocate that despite a disparity in percentage positive saliva from both sampling techniques, self-collection through spitting should be the preferred option. There are reports of SARS-CoV-2 being detected in saliva at higher titers in the early days of the onset of symptoms and the viral load decreasing over time (Comber et al., 2021). There remains no consensus on how long after symptom onset the SARS-CoV-2 RNA can be detected in saliva, with some reports suggesting within the first 13 days compared with 19 days for nasopharyngeal swabs (Comber et al., 2021). This study noted that collection of saliva 7 days after symptom onset yielded lower percentage positive detection, albeit not substantial, thus suggesting this to be a challenge in the utilization of saliva as a specimen type.

In summary, although the literature support nasopharyngeal swabs as a superior specimen for percentage positive SARS-CoV-2 detection, only surpassed by a combination with oropharyngeal swab, alternative clinical specimen such as saliva may offer potential benefits such as patient comfort, reduced invasiveness, low risk of cross infection, and large-scale testing alternative during swab shortage. In addition, its application through self-collection reduces the need for personal protective equipment, es-

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