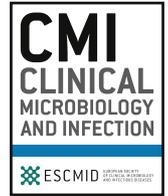




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## Original article

## Saliva for molecular detection of SARS-CoV-2 in school-age children

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## ABSTRACT

**Objectives:** The high diagnostic accuracy indices for saliva severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reverse transcriptase PCR (RT-PCR) reported in adults has not been demonstrated in children, and adequately powered studies focused on the paediatric population are lacking. This study was carried out to determine the diagnostic accuracy of saliva for SARS-CoV-2 RT-PCR in ambulatory children.

**Methods:** During 1 to 23 October 2020, we recruited a population-based sample of children presenting for coronavirus disease 2019 (COVID-19) screening in Dubai, United Arab Emirates. Each child provided paired nasopharyngeal (NP) swab and saliva for SARS-CoV-2 RT-PCR N, E and RdRp gene detection.

**Results:** Paired NP swab and saliva samples were obtained from 476 children with mean  $\pm$  standard deviation age of  $10.8 \pm 3.9$  years, and 58.2% were male (277/476). Nine participants were sampled twice, so 485 pairs of NP swab/saliva were tested. Virus detection in at least one specimen type was reported in 17.9% (87/485), with similar detection in NP swab (16.7%, 81/485) and saliva (15.9%, 77/485). Sensitivity and specificity of saliva RT-PCR was 87.7% (95% confidence interval (CI) 78.5–93.9) and 98.5% (95% CI 96.8–99.5). The positive and negative predictive values were 92.2% (95% CI 84.2–96.3) and 97.6% (95% CI 95.7–98.6), with a kappa coefficient of 0.879 (95% CI 0.821–0.937). Concordance of findings between NP swab and saliva did not differ by age ( $p$  0.67) or gender ( $p$  0.29). Cycle threshold (Ct) values were significantly higher in NP swab/saliva pairs with discordant findings compared to those with both specimens positive.

**Conclusions:** In light of these findings, we recommend saliva as a diagnostic specimen for COVID-19 screening in children. **Hanan Al Suwaidi, Clin Microbiol Infect 2021;27:1330**

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## Introduction

With the reopening of schools, repeated screening for coronavirus disease 2019 (COVID-19) is vital to identify new clusters of infection in children and to prevent outbreaks in educational settings. The collection of nasopharyngeal (NP) swabs for COVID-19

screening is difficult in children because of the invasive nature of the sampling process. The ease of collecting noninvasive drooling saliva makes it an attractive specimen for COVID-19 screening in children, particularly when repeated testing is required. On the basis of data from various studies, saliva is increasingly recognized as an alternative specimen for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using reverse transcriptase PCR (RT-PCR) [1–3].

Depending on the studied population, the reported diagnostic accuracy of saliva-based SARS-CoV-2 RT-PCR has been shown to be higher or comparable to NP swab-based testing [1–7]. Furthermore, recent reports have demonstrated the utility of saliva alone,

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without transport media, for SARS-CoV-2 RT-PCR in ambulatory symptomatic and asymptomatic adults at community-based screening centres [2]. Studies confirming the usefulness of saliva as an alternative specimen for COVID-19 screening have largely been in adults, with a paucity of data in children. Furthermore, a recent report from a small clinical case series suggested poor diagnostic accuracy for saliva specimens in hospitalized children with COVID-19 [8]. In the context of this inconsistency in diagnostic accuracy for saliva SARS-CoV-2 RT-PCR in children, further investigation with adequately powered studies using representative samples of children is warranted.

Therefore, this study was carried out to determine the diagnostic accuracy of saliva for SARS-CoV-2 RT-PCR in ambulatory children presenting for COVID-19 screening.

## Methods

### Setting and participants

This prospective observational diagnostic study is reported according to the 2015 Standards for the Reporting of Diagnostic Accuracy Studies (STARD) guidelines [9]. From 1 to 23 October 2020, we recruited a population-based convenience sample of school-children presenting for COVID-19 screening at Dubai Health Authority (DHA) community-based screening centres in Dubai, United Arab Emirates. Indications for testing included contact with confirmed COVID-19 patients, presence of presumptive symptoms or testing for return to school. All children presenting for COVID-19 screening were eligible for participation. Ethical approval for the study was obtained from the DHA research and ethics committee (approval DSREC-06/2020\_15).

### Sample collection

Informed consent was obtained from parents or guardians, and each child provided paired concurrent NP swab and saliva samples. Using sterile containers without transport medium, self-collected saliva samples (1–3 mL) were obtained at least 30 minutes after abstinence from food or drink as previously described [2]. Participants were asked to close their mouths, allow saliva to pool in the mouth for 1 or 2 minutes and then gently spit into the provided sterile container. NP swab specimens were obtained by trained healthcare personnel using standardized DHA NP swab collection protocol for COVID-19 screening. The NP swabs were placed in Greiner Bio-One universal transport system (Greiner Bio-One, Kremsmünster, Austria). Both NP swab and saliva specimens were transported in cool boxes with ice packs to the DHA virology laboratory for processing.

### SARS-CoV-2 detection

Samples were processed using validated RNA extraction and SARS-CoV-2 RT-PCR protocols used in the DHA virology laboratory. Viral RNA was extracted from 200  $\mu$ L of each sample using the EZ1 DSP Virus Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. No additional buffer was added to the saliva to reduce its viscosity before processing it for RNA extraction.

The internal control (10  $\mu$ L), which was composed of a MS2 phage genome for validation of the RNA extraction and reverse transcription, was added before extraction. SARS-CoV-2 RT-PCR for the detection of three gene targets (N, E and RdRp genes) was carried out using the Allplex 2019-nCoV assay (Seegene, Seoul, South Korea) in accordance with manufacturer-provided instructions [10]. The Allplex 2019-nCoV RT-PCR assay detects the SARS-CoV-2 N and RdRp genes as well as the E gene shared by the

Sarbecovirus [10]. Purified nucleic acid was reverse transcribed using 5  $\times$  Real-time One-step Buffer/Real-time One-step Enzyme into complementary DNA, which was then subsequently amplified using the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). A cycle threshold (Ct) of  $\leq 40$  was taken as the cutoff for a positive result for the target genes as per the manufacturer-provided protocol. Seegene Viewer 3.20 software was used for analysis and interpretation of results. The NP swab and saliva samples were processed separately with blinding in place until all results were available. A presumptively positive result was rendered if only the E gene target was detected, and a positive result was based on the detection of any two target genes. A negative result was reported if no gene targets were amplified and the internal controls were validated.

### Statistical methods

As previously reported, on the basis of an estimated infection prevalence of  $\sim 5.0\%$ , a sample size of 400 participants (including 20 positive cases) was required to detect a sensitivity of 80% and a specificity of 95% [2,11]. Descriptive statistics for categorical variables are presented as number (percentage) and for continuous variables as mean  $\pm$  standard deviation (SD) or median (interquartile range, IQR). Comparison of means was carried out by Student *t* tests. Using swab RT-PCR as the reference standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for saliva RT-PCR were calculated, along with their associated 95% confidence limits. The kappa coefficient was used to estimate the agreement between NP swab and saliva RT-PCR. All analyses were performed by SPSS 24 statistical software (IBM, Armonk, NY, USA), and statistical significance was set at  $p \leq 0.05$ .

## Results

The 476 children who participated in the study had a mean  $\pm$  SD (min–max) age of  $10.8 \pm 3.9$  years (3–18 years); 58.2% of the subjects were male (Table 1). All children provided a single set of paired NP swab and saliva samples, except for nine participants who were sampled on two occasions (Table 1); hence, a total of 485 pairs of NP swab/saliva RT-PCR were tested. The second sampling was carried out for those who had discordant results or who were being seen for clinical reassessment. Of the nine children with repeated sampling, three had discordant results at their first sampling (NP swab negative/presumptively positive saliva,  $n = 1$ ; NP swab negative/saliva positive,  $n = 2$ ), but NP swab positive/saliva positive RT-PCR was found at the second sampling. There were 11 paired NP swab/saliva samples which had a presumptively positive result in one or both specimens (Fig. 1). The presumptively positive results were considered as positive for this analysis [12].

Virus detection in at least one specimen type was reported in 17.9% (87/485) of specimen pairs. The prevalence of COVID-19 diagnosis by NP swab RT-PCR was 16.7% (81/485) and 15.9% (77/485) by saliva RT-PCR. Both NP swab and saliva were positive in 71 paired samples; there were 16 discordant NP swab/saliva RT-PCR findings (Table 2). Among the 87 children with a positive test by either specimen, 39 (44.8%) had self-reported symptoms, predominantly fever ( $n = 25$ ), cough ( $n = 16$ ) and sore throat ( $n = 15$ ). All samples arrived in the laboratory within 3 hours of collection, and the laboratory processing time (from sample receipt to test result) was comparable between the two specimens ( $9.62 \pm 4.34$  vs.  $10.19 \pm 4.74$  hours in NP swab vs. saliva respectively,  $p = 0.06$ ).

Using the NP swab RT-PCR as the reference standard, the sensitivity and specificity of saliva RT-PCR was 87.7% (95% CI 78.5–93.9) and 98.5% (95% CI 96.8–99.5) respectively. The PPV and

**Table 1**  
Profile of study participants

Characteristic	Value
Overall no. of participants	476
Age (years), mean $\pm$ SD	10.8 $\pm$ 3.9
Male gender	277 (58.2)
No. of participants with two samplings of paired NP swab and saliva	9
Interval between first and second sampling (days), median (IQR)	2 (1–9)
Total no. children with positive result from at least one sample type	87
Age (years), mean $\pm$ SD	10.0 $\pm$ 4.0
Self-reported symptoms	
Children with symptoms	39 (44.8)
Age (years), mean $\pm$ SD, of symptomatic children	9.3 $\pm$ 3.8
Reported symptoms	
Fever (self-reported)	25 (64.1)
Cough	16 (41.0)
Sore throat	15 (38.5)
Nasal congestion	8 (20.5)
Muscle pain	6 (15.4)
Abdominal pain	2 (5.1)
Shortness of breath	2 (5.1)
Diarrhoea	1 (2.6)
Anosmia	0
Loss of taste	0

Data are presented as *n* (%) unless otherwise indicated. Abbreviations: IQR, interquartile range; NP, nasopharyngeal; SD, standard deviation.

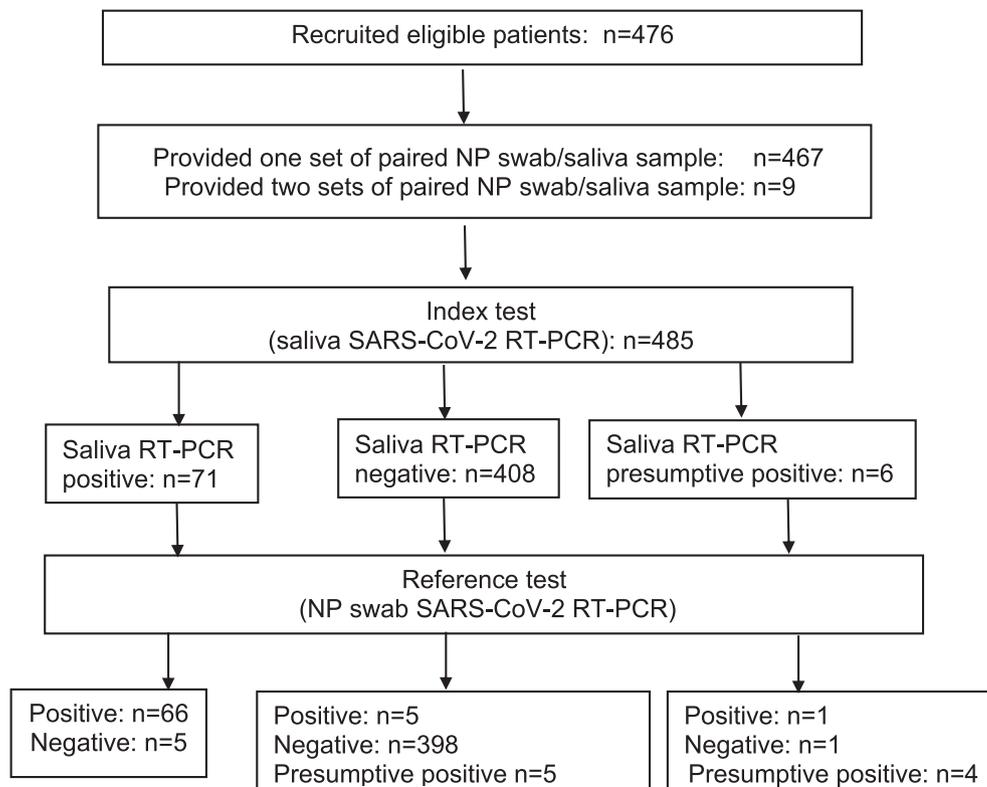
NPV were 92.2% (95% CI 84.2–96.3) and 97.6% (95% CI 95.7–98.6) respectively. The accuracy was 96.7% (95% CI 94.7–98.1), and the agreement by kappa coefficient was 0.879 (95% CI 0.821–0.937). With the exclusion of the duplicate samples for nine patients as shown in Table 3, similar sensitivity (86.5%) (95% CI 76.6–93.3) and specificity (98.5%) (95% CI 96.8–99.5) were observed. The PPV

(91.4%) (95% CI 82.8–95.9), NPV (97.5%) (95% CI 95.7–98.6), accuracy (96.6%) (95% CI 94.6–98.1) and kappa coefficient (0.869) (95% CI 0.806–0.932) were also comparable. Furthermore, in sensitivity analyses excluding the specimens with presumptively positive results, the corresponding sensitivity and specificity were 92.9% (95% CI 84.3–97.7) and 98.8% (95% CI 97.1–99.6) respectively, while the PPV and NPV were 92.9% (95% CI 84.6–96.9) and 98.8% (95% CI 97.2–99.5) respectively, with an accuracy of 97.9% (95% CI 96.2–98.9) and a kappa coefficient of 0.917 (95% CI 0.866–0.968).

The median Ct values for the E, RdRp and N gene targets in NP swab were 23.9 (IQR 17.3–31.9), 27.8 (IQR 19.8–35.5) and 26.6 (IQR 21.5–31.1) respectively. In saliva, median Ct values were E gene 27.2 (IQR 19.9–32.0), RdRp gene 29.5 (IQR 23.6–34.1) and N gene 28.2 (IQR 19.4–32.0). Fig. 2 shows the Ct values of E, RdRp and N gene targets in paired saliva and NP swab specimens. Notably, mean Ct values were higher in the pairs where only one sample was positive (discordant findings) compared to samples where both specimens were positive (29.3 vs. 24.7 for E gene, 35.9 vs. 26.9 for RdRp gene, 34.3 vs. 25.8 for N gene,  $p < 0.05$  for all comparisons). The concordance of findings between NP swab and saliva samples did not differ by age ( $p = 0.67$ ) or gender ( $p = 0.29$ ). However, for patients with self-reported symptoms compared to those without symptoms, we found statistically significant differences in the mean Ct values for the E gene in NP swab and saliva as well as the N gene in saliva (Fig. 3).

## Discussion

Although there is a growing number of studies evaluating the utility of saliva for COVID-19 screening in adults, there is a paucity of data for the paediatric population. In this study of a large community-based paediatric cohort, we demonstrated that the diagnostic accuracy of saliva RT-PCR is comparable to NP swab RT-



**Fig. 1.** Study flowchart. Abbreviations: NP, nasopharyngeal; RT-PCR, reverse transcriptase PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

**Table 2**

Detection of virus via SARS-CoV-2 RT-PCR in all 485 tested paired saliva and NP swab specimens

	NP swab result	
	Positive	Negative
Saliva result		
Positive	71	6
Negative	10	398

Presumptively positive results were considered positive. We processed paired NP swab and saliva samples from 476 participants (including nine with two paired sets). Abbreviations: NP, nasopharyngeal; RT-PCR, reverse transcriptase PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

**Table 3**

Detection of virus via SARS-CoV-2 RT-PCR in 476 paired saliva and NP swab specimens (excluding duplicate samples)

	NP swab result	
	Positive	Negative
Saliva result		
Positive	64	6
Negative	10	396

Presumptively positive results were considered positive. We processed only one set of paired NP and saliva samples from 476 participants, with duplicate samples of nine participants with two paired sets excluded. Abbreviations: NP, nasopharyngeal; RT-PCR, reverse transcriptase PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

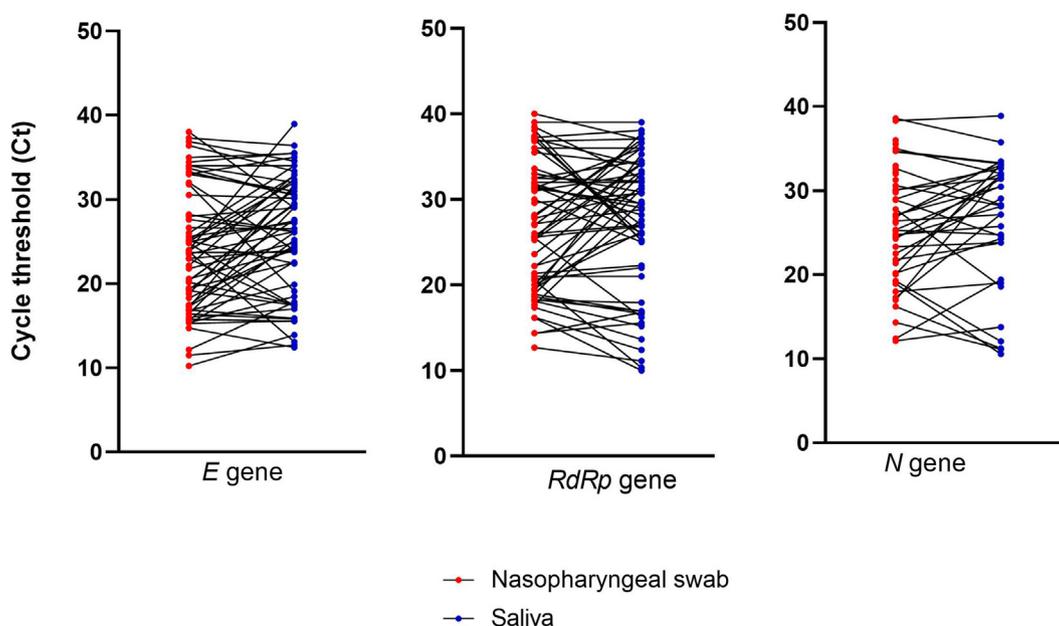
PCR. This finding confirms the utility of saliva as a noninvasive diagnostic specimen for COVID-19 screening in ambulatory school-age children. This finding is aligned with the published literature investigating the utility of saliva SARS-CoV-2 RT-PCR in ambulatory COVID-19 adults [1,2,13,14], which have been instrumental in driving the call for the use of saliva as an alternative noninvasive specimen for COVID-19 screening [15,16].

Although children with COVID-19 are largely asymptomatic, they tend to have virus loads comparable to adults [17]. Additionally, the virus load in children under the age of 5 years has been shown to be significantly higher compared to older children and

adults [17]. Therefore, it would be expected that the diagnostic accuracy of saliva in the paediatric population should be on par with or superior to adults. Indeed, the sensitivity of saliva RT-PCR in children shown in this study is higher compared to a similar study in adults conducted by our group [2]. The observation that Ct values were higher in pairs where only one sample was positive (discordant findings) compared to samples where both specimens were positive is noteworthy. Because lower Ct values correlate with a higher likelihood of cultivable virus and infectiousness [18,19], the greater concordance between NP swab and saliva results at lower Ct values is reassuring. In other words, concordance between NP swab and saliva results is more likely when it matters the most (i.e. at lower Ct values, when children are likely to be more infectious).

Two smaller studies suggested that saliva may not be a useful specimen for diagnosing COVID-19 in children [8,20]. In a clinical case series of 18 hospitalized children with COVID-19 from a single centre in Singapore, Chong *et al.* [8] reported a peak sensitivity of 52.9% for saliva SARS-CoV-2 RT-PCR. In another study from South Korea by Han *et al.* [20], of 11 paired NP swab/saliva samples from mildly symptomatic and asymptomatic children, saliva was positive in eight. These studies had very small sample sizes and were underpowered to assess diagnostic accuracy, which limits the generalizability of their findings. Notably, the study by Chong *et al.* used an RT-PCR assay for a single gene target (E gene) to define a positive result, although detection of only the E gene target is recommended to be considered presumptively positive. Our study and that by Han *et al.* used the same SARS-CoV-2 RT-PCR kit. It is interesting that the upper 95% confidence limit for sensitivity demonstrated by Han *et al.* overlaps with the point estimate for sensitivity that we report in our larger sample size. Our analysis showed very good sensitivity and specificity, as well as NPVs and PPVs for use of saliva for COVID-19 screening, which was maintained irrespective of inclusion of presumptively positive results.

Indeed, these diagnostic accuracy indices and the kappa coefficient for the estimate of agreement between NP swab and saliva RT-PCR were improved, with the exclusion of presumptively positive results. Although obtaining NP swabs in children may be challenging and could result in poor specimen quality, the substantial agreement between both specimen types, as shown by the



**Fig. 2.** Cycle threshold (Ct) values of E, RdRp and N gene targets in paired saliva and nasopharyngeal (NP) swab specimens.

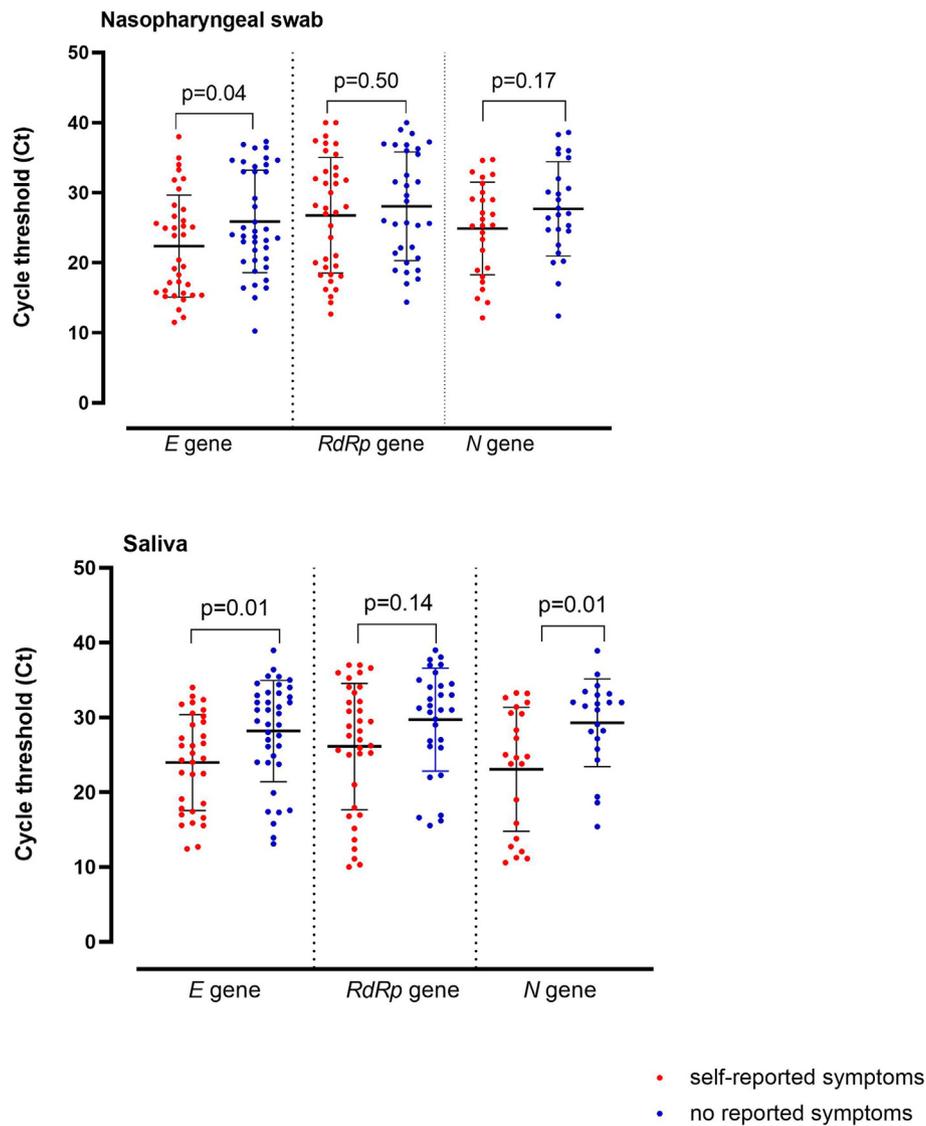


Fig. 3. Mean cycle threshold (Ct) values for E, RdRp and N genes in nasopharyngeal (NP) swab and saliva of patients with self-reported symptoms versus those without symptoms.

kappa coefficient values, suggests adequacy of the NP swabs in this study. Therefore, our adequately powered study of paediatric samples from the general population clearly shows the utility of saliva for COVID-19 screening in children. The finding of very good diagnostic accuracy has clear public health implications, as saliva is more acceptable for repeated sampling in children and will facilitate uptake of periodic screening.

To alleviate the negative impact of prolonged school closures, easing restrictions and returning to classrooms have been implemented in many countries. However, because children remain largely asymptomatic, it has been postulated that reopening schools could drive the spread of SARS-CoV-2 in the general population [21]. To mitigate against this, repeated screening for quick identification of new clusters of infection is anticipated. However, obtaining a NP swab for COVID-19 screening can be quite difficult and stressful for the child, the parent and the healthcare worker as a result of the invasive nature of the sampling process. The healthcare worker is also at higher risk of viral transmission if the child is uncooperative during the sampling process. The adoption of

saliva as a diagnostic specimen for COVID-19 screening will be useful in overcoming these challenges.

A strength of this study is the focus on the paediatric population, with an adequately powered and representative sample of school-age children drawn from the general population, thus addressing an important gap in the literature. Also, the finding of very good diagnostic accuracy for saliva despite having significantly higher Ct values (indicative of lower virus loads) for two target genes is reassuring. But for future studies where extended storage of specimen is expected, inclusion of assays to check for RNA degradation is recommended. A limitation of the study is that this was a convenience sampling of children presenting for screening, and further work on a larger population of children which includes those under the age of 3 years is recommended.

In conclusion, we demonstrate the utility of saliva as a molecular diagnostic specimen for COVID-19 screening in a large cohort of ambulatory school-age children. On the basis of our findings, saliva sampling should be routinely used for paediatric COVID-19

screening to reduce the risk of transmission to healthcare workers, limit the strain on resources and allay testing anxiety in children.

### Transparency declaration

All authors report no conflicts of interest relevant to this article.

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