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Commentary

Saliva testing for severe acute respiratory syndrome coronavirus 2 in children

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Children are less likely than adults to suffer symptomatic or severe disease from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) but they have nevertheless been significantly impacted by the coronavirus disease 2019 (COVID-19) pandemic [1]. In particular, they have suffered from the physical, emotional and mental health consequences of the various public health restrictions needed to manage the pandemic, including lengthy lockdowns and school closures. In high transmission settings, it has been suggested that frequent surveillance testing within school settings may allow children and their teachers to safely return to their classrooms.

If found to have adequate sensitivity and specificity, saliva would be an ideal sample type for identifying SARS-CoV-2 and other respiratory viruses in children, because its collection is less invasive than other standard upper respiratory tract sample types such as nasopharyngeal swab, deep nasal/oropharyngeal swab or nasopharyngeal aspirate. Collection of nasopharyngeal swabs or aspirates involves both discomfort for the child and potential risk to the health-care personnel collecting the sample, who may be exposed to SARS-CoV-2 through aerosol-generating procedures and behaviours. The use of saliva could also reduce time and cost for sample collection [2].

Before the COVID-19 pandemic, studies in adults reported that saliva might be an adequately sensitive sample type for detection of respiratory viruses (e.g. influenza and respiratory syncytial virus), either on its own [2,3] or as an adjunctive sample type to complement nasopharyngeal swab testing [4]. However, there have been surprisingly few studies in children. At present, the majority of commercial SARS-CoV-2 assays do not list saliva as an acceptable sample type for nucleic acid testing, so validation studies assessing the use of this sample type are welcome.

In this edition of *Clinical Microbiology and Infection*, Al Suwaidi et al. report on a prospective evaluation of saliva as an alternative upper respiratory tract sample type for the diagnosis of SARS-CoV-2 infection in children [5]. In a study of 476 children attending a screening clinic, 485 paired samples were collected, comprising saliva collected in a dribble pot and a nasopharyngeal swab. These were tested for SARS-CoV-2 by RT-PCR using a commercially available extraction and diagnostic testing platform. The median age of children in the study was 10 years; the youngest was 3 years old. A total of 87 samples were positive from either saliva or nasopharyngeal swab, with an overall sensitivity of saliva compared with nasopharyngeal swab of 88%. Paired samples were concordantly positive in 71 cases (82%) and discordant in 16 samples: in six children only saliva was positive and in ten children only nasopharyngeal swab was positive.

Although there have been several recent large studies in adults comparing saliva with other upper respiratory tract sample types for the diagnosis of SARS-CoV-2 [6], this is the largest published study evaluating this in children. Studies in adults have found variable concordance between saliva and other upper respiratory tract sample types, with saliva usually reported as slightly less sensitive than other samples types [7]. The relatively few studies carried out specifically in children [8–10], or that included children within a larger cohort [10,11] are summarized in Table 1.

Although small, these studies suggest that the sensitivity of saliva as a paediatric diagnostic sample type is acceptable compared with nasopharyngeal swabs, but not perfect. Interestingly, although missing

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Table 1
Summary of recent studies comparing SARS-CoV-2 detection in saliva and nasopharyngeal swabs in children

Author Country	No. of participants (No. positive[a])	Age	Severity of disease (Setting)	Saliva collection method (Volume)	Platform	Saliva sensitivity compared with NPS	Saliva sensitivity compared with all positive tests	Positive			Comments
								Total	NPS only	Saliva only	
Al Suwaidi et al. 2021 [5] UAE	476 (87 positive)	Mean 10.8 y Range 3–18 y	39/87 mild symptoms (Screening clinic)	Dribble pot (1–3 mL) 200 μL undiluted saliva	EZ1 extraction (Qiagen) Allplex 2019-nCoV Assay kit (Seegene) (N, E and RdRp genes) Positive = more than two genes detected; 'presumptive positive' = E gene detected	71/81 (88%)	77/87 (89%)	87	10	6	Ct values significantly higher in saliva
Borghi et al. 2020 [11] Italy	109 (27 positive)	Range 0-17y	Not stated (Attending hospital)	Sterile dental role	SalivaDirect™ process [19] using N1, RdRp primers/probes (CDC) Applied Biosystem 7500 Fast platform Positive = N1-gene detected at Ct < 40	20/21 (95%)	26/27 (96%)	27	1	6	
Yee et al. 2021 [15] USA	43 cases <18 y positive Part of larger study	Median 12 y Range 4–18 y	Variable (Inpatients, outpatients and household contacts)	Dribble pot (3 mL) 250 μL undiluted saliva	Applied Biosystems MagMAX extraction kit (ThermoFisher) TaqPath COVID-19 Combo kit (ThermoFisher) Positive = one or more of N, S and ORF-1 genes	29/38 (76%)	34/43 (79%)	43	9	5	Performance superior for saliva in asymptomatic compared with symptomatic children
Han et al. 2020 [8] Korea	(11 positive)	Median 6.5 y Range 27 d, 16 y	9 mild symptoms; 3 asymptomatic	Not stated	Allplex 2019-nCoV Assay kit (Seegene) (N, E and RdRp genes)	8/11 (73%)	8/11 (73%)	11	N/A	N/A	Saliva positivity declined rapidly after first week
Chong et al. 2021 [9] Singapore	(18 positive)	Mean 6.6 y IQR 1.8–11 y	6 symptomatic; 12 asymptomatic	Dribble pot (minimum 0.5 mL)	Extraction method not stated RT-PCR E gene	52.9% (day 4–7 of illness)	N/A	18	N/A	N/A	Ct values higher in saliva. Saliva positivity declined rapidly over first week
Guzman-Ortiz et al. 2021 [12] Mexico	156 (23 positive)	Range 5–18 y	22 symptomatic 1 asymptomatic	Spit five times into sterile tube	QIAmp Viral RNA mini kit (Qiagen) extraction GeneFinder COVID-19 PLUS RealAmp Kit (Elitech)	14/17 (82%)	20/23 (87%)	23	3	6	

Abbreviations: N/A, not available; NPS, nasopharyngeal swab; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UAE, United Arabe Emirates.
[a] In NPS or saliva.

a few cases detected in nasopharyngeal swab samples, SARS-CoV-2 may be additionally detected in some saliva samples where the nasopharyngeal swab is negative [10–12]. Interpreting these positive nucleic acid testing results from saliva samples remains difficult in the absence of a defined reference standard comparator test.

Concordance between the two sample types is probably impacted by a variety of pre-analytical factors such as patient age, timing of onset of symptoms in relation to sample collection and severity of illness. In a small study in children hospitalized with COVID-19 in Singapore, the majority of saliva and nasopharyngeal samples were concordant in the first few days after onset of symptoms, but the sensitivity of saliva testing declined rapidly after that [13]. Test sensitivity is also impacted by the method used to collect saliva: published studies have used a variety of methods including dribble pots, suck swabs and specialized saliva collection kits [11]. Location of sampling (buccal swabs [14] versus posterior oropharynx saliva [15]) may also affect test sensitivity. Other differences that may impact test sensitivity include the volume of sample collected and use of transport medium, pre-testing storage conditions and dilution of the sample for testing (which may be required due to viscosity of some saliva samples). In the study by Al Suwaidi et al., the cycle threshold (Ct) values were higher for saliva samples (two or three cycles) than for paired nasopharyngeal swabs, which is consistent with other studies [9], suggesting a reduced analytical sensitivity of the test, possibly due to the presence of inhibitory factors in saliva. Choice of extraction and testing platforms (and variations in test interpretation based on one or more gene targets being positive) may also explain heterogeneity between study findings.

Overall, the findings of the study by Al Suwaidi et al. and other smaller paediatric studies suggest that neither nasopharyngeal swab nor saliva are perfect sample types for the diagnosis of SARS-CoV-2. In symptomatic children in the clinical setting, both sample types would ideally be collected to maximize virus detection, in addition to lower respiratory tract samples if possible. Saliva offers a stand-alone option for testing in situations where a child is significantly distressed by the collection of a standard nasopharyngeal swab, particularly early in the course of infection [16]. These include children obliged to undergo frequent testing due to exacerbations of a chronic respiratory condition, or those requiring frequent testing before repeat surgical procedures (as occurs commonly for oncology patients). It should be noted that saliva has already been adopted as an acceptable sample type in several countries [16]. There is insufficient evidence to determine the role of saliva testing in infants and pre-school age children, as very few children of this age have been included in published studies to date, although the expected high SARS-CoV-2 viral load in very young children suggests that saliva may prove to be an adequate sample type [5].

The primary use of saliva as a diagnostic sample type may be as a tool for frequent surveillance testing in high-risk asymptomatic populations. Saliva, even if a slightly less sensitive sample type than nasopharyngeal swabs, may still be an ideal option for settings where children require frequent testing, as collection of multiple sequential test samples will compensate for minor reductions in test sensitivity [17]. Modelling suggests that for successful surveillance programmes, testing frequency is more important than the sensitivity of the test [18].

Future studies should evaluate saliva for the detection of other common respiratory viruses (such as influenza and parainfluenza viruses and respiratory syncytial virus) in children. This might allow inclusion of saliva as an acceptable sample type in commercial multiplex respiratory virus nucleic acid testing assays that include SARS-CoV-2, and has the potential to both simplify and increase public health surveillance capacity in the future.

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Authors' contributions

VC drafted the commentary and NC provided oversight and revised the manuscript for critically important intellectual content.

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