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BRIEF REPORT

Saliva is inferior to nose and throat swabs for SARS-CoV-2 detection in children

It is important to identify children and adolescents infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as they are often asymptomatic and may unintentionally spread the virus. However, information on the best sampling methods is limited. Most sampling tests analyse nose and throat swabs with real-time polymerase chain reaction (RT-PCR), but these tests are uncomfortable, and young children may not co-operate, increasing suboptimal sample collection and false-negative results.

Detecting SARS-CoV-2 in adult saliva, using RT-PCR,[1,2] has shown promise. However, young children struggle to produce saliva spontaneously, and there have been conflicting results about using this method for children.[3,4] In contrast, oral swabs cause minimal discomfort, do not generate aerosols, collect adequate viral material[5] and can be used by parents or day-care staff without personal protective equipment.

This prospective cohort study evaluated using saliva to detect SARS-CoV-2 in nonhospitalised children. We recruited 20 children aged 0–17 years with an RT-PCR-positive SARS-CoV-2 test from a nose or throat swab, taken at the Hvidovre University Hospital or Rigshospitalet, Copenhagen, Denmark, from 10 May to 4 December 2020. The parents brought their children to the hospital, where a project nurse collected nasal and throat swabs and saliva samples. The children were followed up weekly for four weeks, and the parents were given oral and written instructions and asked to perform home saliva sampling between hospital visits. The families were called weekly to monitor symptoms and provide sampling reminders.

Saliva samples were taken using an Oracol device (Malvern Medical Developments, Worcester, UK), which is a cylindrical polystyrene sponge attached to a plastic stick. Designed to be used as a toothbrush, it was rubbed against the gums for one minute. Two devices were used for infants younger than six months. The samples were stored at 4–8°C until they were brought to the hospital or mailed to the laboratory. Nose and throat samples were collected using flocked nylon swabs placed in inactivated transport media (NEST Biotechnology, Jiangsu, China). All samples were stored at −80°C until analysed by the hospital's laboratory.

Total nucleic acids were extracted using an in-house silica-based procedure on Beckman i7 robotic platforms (Beckman Coulter). SARS-CoV-2 ribonucleic acid was detected using a multiplexed version of the CDC N-gene one-step RT-PCR (Pentabase Ltd) that targeted two N-gene segments, with the RNase P ribozyme as the inhibition control. Samples with a cycle threshold (Ct) of <36 for at least one of the virus specific N-gene targets were considered positive. Samples with no N-gene targets Ct <36 and a valid inhibition control (Ct <23) were considered negative. The results were inconclusive if the inhibition control Ct was ≥23.

The study protocol was approved by the National Danish Ethics Committee (H-20028631) and Danish Data Protection Agency (P-2019–29) and registered at ClinicalTrials.gov (NCT04666207). Informed consent was obtained from the parents and adolescents.

We studied 13 boys and seven girls, with a median age of five (7 weeks - 16 years) years. All had symptoms for at least one day, including fatigue (n = 16), cough (n = 14) and coryza, fever and reduced appetite (n=12). Diagnostic RT-PCR tests were performed 0–17 days after symptoms started.

During the first and second weeks after the PCR-RT tests, SARS-CoV-2 was detected in 29% and 11% of saliva samples, 86% and 50% of nasal swabs and 58% and 40% of throat swabs, respectively (Table 1). The median Ct in week one was 25 in saliva, 22 in nasal swabs and 23 in throat swabs, with no correlation between the test results and symptoms, age or gender.

Our findings agreed with those of other studies that saliva was less sensitive than nasopharyngeal swabs for diagnosing SARS-CoV-2 in children. Two studies showed this in 53% of 18 children[3] and 8/11 children, respectively.[4]

Adult studies have shown that collection methods, collection devices and processing methods are critical when testing saliva.[2] The reason for the numerous negative saliva samples in our study was not clear. Six children had their first saliva sample collected more than one week after the first diagnostic test and early sampling may have resulted in more positive saliva samples. However, all the children had a positive nose or throat swab in the first two weeks, indicating that these secretions are more useful than saliva for detecting SARS-CoV-2. Saliva was not added to a viral transport media and arrived at the laboratory a few days after testing, which may have degraded the viral ribonucleic acid. Despite the small sample size, our data indicate that collecting saliva using an oral swab without virus preservation media was inferior to nose or throat swabs and should not be used to screen for SARS-CoV-2 in children. Further studies should explore whether other saliva collection methods could detect SARS-CoV-2 in children.

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, real-time polymerase chain reaction; Ct, cycle threshold.

TABLE 1 Positivity and viral load of SARS-CoV-2 in saliva, nasal swabs and throat swabs in 20 children with mild COVID-19

	Saliva ($n = 20$)		Nasal swab ($n = 19$)		Throat swab ($n = 20$)	
Days after positive test	No positive/total (%)*	Ct value (interval)	No positive/total (%)	Ct value (interval)	No positive/total (%)	Ct value (interval)
Total	5/20 (25)	24–30	15/19 (79)	7–35	12/20 (60)	17–32
1–7	4/14 (29)	24–29	12/14 (86)	7–30	7/12 (58)	17–30
8–14	2/18 (11)	26–30	8/16 (50)	16–35	6/15 (40)	19–31
15–21	1/18 (6)	24	3/12 (25)	25–33	2/12 (17)	22–27
22–36	0/12 (0)	-	1/10 (10)	31	1/11 (9)	32

*SARS-CoV-2 positivity calculated from number of patients with known test results.

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CONFLICT OF INTEREST

None.

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