Journal of Iranian Medical Council, Volume 6, Issue no. 1
http://dx.doi.org/10.18502/jimc.v6i1.11839

Review Article

Check for updates

Common Coronavirus Disease 2019 Upper Respiratory Tract Sampling Techniques in Children

Reza Shervin Badv[1], Maryam Sotoudeh Anvari[2], Farhoud Toutounchian[3], Moones Toosang[4], Maryam Monajemzadeh[4], MohammadTaghi Haghi Ashtiani[4], Setareh Mamishi[5], Keyvan Mirnia[6], Rohola Shirzadi[7] and Abbas Mohammadi[8]*

1. Pediatrics Centre of Excellence, Department of Pediatric Neurology, Children's Medical Centre, Tehran University of Medical Sciences, Tehran, Iran
2. Molecular Pathology and Cytogenetics Division, Pathology Department, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
3. Pediatrics Centre of Excellence, Department of Forensic Medicine and Clinical Toxicology, Children's Medical Centre, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Pathology, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
5. Pediatric Gastroenterology and Hepatology Research Center, Children's Medical Center, Children Centre of Excellence, Tehran University of Medical Sciences, Tehran, Iran
6. Pediatrics Centre of Excellence, Department of Neonatology, Children's Medical Centre, Tehran University of Medical Sciences, Tehran, Iran
7. Pediatric Respiratory and Sleep Medicine Research Center, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
8. Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: During COVID-19 pandemic, most studies have focused on sampling technique in adults. Considering the need to be aware of the effectiveness and evaluation of sampling methods in children, we have motivated a search for introducing and performing sampling techniques, especially upper respiratory tract sampling in children. We systematically reviewed the literature to understand the performance of different sampling methods in children in COVID-19.

Methods: We systematically reviewed PubMed, Google Scholar, medRxiv, and bioRxiv (last retrieval August 1st, 2021) for comparative studies of deferent sampling techniques by using the search keywords including: children, pediatric sampling, nasopharyngeal, COVID-19, oropharyngeal, swabs, SARS, CoV2. 8 relevant manuscripts were sourced from a total of 4852 search results.

Results: Nasopharyngeal (NP) swabs testing significantly had higher positivity rate over oropharyngeal swab in detecting SARS-CoV-2. Nasal swab has a low sensitivity in detecting SARSCoV-2 in children when referred to the Nasopharyngeal Aspiration (NPA), whereas its specificity is high. Therefore, NPA can be as the gold standard for detection of SARS-CoV-2.

Conclusion: Saliva is not a useful for diagnosing COVID-19 in children. Negative nasopharyngeal and oropharyngeal swabs do not rule out COVID-19 and in patients with strong clinical suspicion, and Bronchoalveolar lavage (BAL) can be helpful.

Keywords: Bronchoalveolar lavage, Children, COVID-19

*** Corresponding author**

Abbas Mohammadi, MD
Eye Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 939 060 2177
Email: abbas19941373@gmail.com

Received: Jan 29 2022
Accepted: May 14 2022

Citation to this article:
Shervin Badv R, Sotoudeh Anvari M, Toutounchian F, Toosang M, Monajemzadeh M, Haghi Ashtiani MT, et al.Common Coronavirus Disease 2019 Upper Respiratory Tract Sampling Techniques in Children. *J Iran Med Counc.* 2023;6(1):29-37.

Introduction

New type of coronavirus was reported in Wuhan, Hubei Province, China, like Severe Acute Respiratory Syndrome (SARS) in Guangzhou, and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causes severe respiratory disease (1). The outbreak of the disease began in China, has brought a burden on whole world and stated as a global pandemic (2). Most reports mainly concern adults and the mortality rate is higher in the elderly than the children. Despite the large number of articles on adults, data regarding children infected with coronavirus are so limited (3). Wu *et al* in a review on 44,672 laboratory-confirmed cases of COVID-19, reported 1% of cases in children from 10 to 19 years of old and 1% in children of 9 years or younger, with no deaths in the latter group (4). Overall, children younger than 18 years appear to be less vulnerable to the infection and have less severe disease course compared to adults. In a Chinese observational study, of the 1391 children younger than 16 years tested for SARS-CoV-2, only 171 (8.1%) resulted positive test and among these, 15.8% were asymptomatic while the rest showed only mild symptoms (5), but unfortunately, recently rate of infection with the Delta variant of COVID-19 (because of high transmissibility) in children has also increased (6).

In pandemic situations, the first step in the preventive strategy, is exhaustive case finding. Nevertheless, the best type of clinical specimen for the initial diagnostic test of COVID-19 in children remains controversial. As of October 2020, interim guidance from the Centers for Disease Control and Prevention (CDC) recommends upper respiratory tract specimen for COVID-19 testing with any of the following specimens without focus on specific age categorization: Nasopharyngeal (NP) swab, NP wash/aspirate, nasal wash/aspirate, Oropharyngeal (OP) swab, nasal Mid-Turbinate (MT) swab using a flocked tapered swab, an Anterior Nares (AN) nasal swab using a flocked or spun polyester swab, or a saliva specimen obtained by supervised self-collection (7). Nasopharyngeal specimen collection is usually recommended in children, but its sensitivity has been questioned if compared with other clinical specimens, and it is not always feasible in young children (8-10).

There is no sufficient information on different sampling methods and efficacy of upper respiratory tract sampling in children, as a diagnostic procedure in COVID-19.

Here, we aim to clarify the implementation of variable specimen types for diagnosis of SARS-CoV-2 in children by systematically reviewing the literature on this topic available through August 2021. A review of the literature was obtained by searching in PubMed, Medline and Google Scholar. Keywords used were children, sampling, nasopharyngeal, COVID-19, oropharyngeal, swabs, SARS, and CoV2. The search yielded 4852 results which were then browsed through, and narrowed down to eight relevant manuscripts. Eight original full articles were reviewed by authors. The relevant data related to the site of sampling, diagnostic tests accuracy and recommendation on sampling procedure were tabulated in table 1.

Saliva

saliva sampling can circumvent the shortage of collection supplies and be a sufficient noninvasive and more cost-effective alternative for SARS-CoV-2 testing. Less-invasive and cost-effective collection methods are indispensable in a pandemic scenario as large-scale tests to understand the actual evolution of contagion in different populations (11-13).

Salivary droplets are the main source of human-to-human transmission of the virus. It is a good alternative sample for diagnosis as easily and quickly collected, without specific devices and cause less discomfort during collection, as an important factor for specimen collection in children (14).

Saliva specimens have been used in detection of Zika virus in children. The sensitivity of saliva for detection of SARS-CoV-2 in adults has been shown to be less than that of NPS in different studies, ranging from 72-86% (15). Pasomsub *et al* prospectively compared the detection rate of SARS-CoV-between saliva and nasopharyngeal specimens in 76 adult patients by RT-PCR. Both samples were simultaneously collected from patients who were either suspected to have COVID-19 or had a positive diagnosis of COVID-19. The results revealed that the concordance rate of the virus detection between the two samples was as high as 97.4% (16).

Yee *et al* in 2020 prospectively collected paired

Table 1. Manuscript with region, specimen numbers, technique, relevant findings and recommendations

Author (year)	Country	Studied specimens	Laboratory technique(s), findings	Recommendation(s)
L. Robinson *et al* (2008)	Alberta, Canada	-137 children and adolescents aged <17 years -137 NP, 105 throat swabs, 104 saliva specimens	-DFA testing of the NP: respiratory syncytial virus, influenza A and B viruses, parainfluenza virus -If NP positive: NAT performed: for the same virus in throat swab and saliva specimens -105 of the 137 NP specimens -87 (83%) of 105 throat swabs -77 (74%) of 104 saliva specimens	Throat swab and saliva specimens, inferior to NP specimens for the detection of respiratory viruses
B. Lambert *et al* (2008)	Queensland, Australia	-303 sets of paired NPA/NTS specimens (295 children)	-PCR for: influenza A virus, respiratory syncytial virus -270 (89%) paired specimens were concordant -NTS: sensitivity of 91.9% for influenza A, 93.1% for respiratory syncytial virus	NTS specimen is a less invasive diagnostic respiratory specimen with adequate sensitivity for use in the clinic and hospital outpatient.
Palmas *et al* (2020)	Florence, Italy	-11 SARS-CoV-2 +ve patients (age 0–18)-52 paired clinical specimens (26 NS, 26 OS)	-qRT-PCR -24 of 26 NS+ -20 of 26 OS+ -4 NS positive, but paired to a negative OS -higher positivity rate of NS: Fisher exact test 0.046, Cohen K 0.43, confidence interval 95%, 0.014–0.855	NS preferred choice for swab-based SARS-CoV-2 testing in children
Di Pietro *et al* (2020)	Milan, Italy	- 134 children (most<6 years) -600 samples: 300 paired NS and NPA specimens	- RT-PCR-43 positive NPA, 31 positive NS, 257 negative NPA, and 269 negative NS -276 were concordant; 24 were discordant, naïve concordance: 92.0% (95% CI: 88.3–94.6%) -NS: specificity of 97.7% sensitivity of 58.1% -NS has a low sensitivity in detecting SARSCoV- 2 in children when referred to the NPA	in children under 6 years of age, NS should be preferred whenever possible
Yin Chong *et al* (2020)	Singapore, Republic of Singapore	-18 children (mean age 6.6 years), SARS-CoV-2 +ve detected by NP -paired NP and saliva specimens	-RT-PCR -Saliva PCR sensitivity was highest 52.9% on day 4-7 -Saliva PCR had higher Ct compared to NP swabs	saliva is not a useful specimen for diagnosing COVID-19 in children

Cont Table 2

Yee *et al* (2020)	California, USA	-300 SARS-CoV-2 +ve, adult and pediatric -paired NPS and saliva	-qRT-PCR -concordances for saliva and NPS were 91.0% (273/300) and 94.7% (284/300) -PPA for saliva and NPS were 81.4% (79/97) and 89.7% (87/97)	saliva can be an appropriate sample choice alternative to NPS for detection of SARS-CoV-2 in children and adults
Kam *et al* (2020)	Singapore, Republic of Singapore	-11 SARS-CoV-2 +ve children (median ages of asymptomatic and symptomatic were 8.4 years and 3.8 years) -paired NPS and buccal S	-rRT-PCR -detected from at least 1 buccal specimen in 9 of 11 children (81.8%) -the mean difference of Ct values between buccal NP was 10.7 (range, 6.1–16.1), statistically significant (P<.001).	-Buccal swabs are not good as COVID-19 screening specimens in children
Li *et al* (2020)	Sichuan, China	Case report	-SARS-CoV-2 infection in a baby with non-productive cough and normal chest computed tomography, only anal swabs tested positive by r RT-PCR	Infants with a history of SARS-CoV-2 exposure and mild symptomsshould be tested using anal swabs

NPS and saliva samples from a total of 300 adult and pediatric patients confirmed their SARS-CoV-2 RNA by RT-PCR. The overall concordances for saliva and NPS were 91.0% (273/300) and 94.7% (284/300) and values for positive percent agreement (PPA) for saliva and NPS were 81.4 and 89.7%, respectively and finally they concluded that saliva is a reliable diagnostic specimen particularly for both symptomatic and asymptomatic children and symptomatic adults (17).

Chong *et al* in 2020 analyzed NP and saliva specimens using a real-time reverse transcription (rRT)-PCR assay for the E gene of SARS-CoV-2. They recorded Cycle threshold (Ct) values of specimens according to the day of illness (onset of symptoms) for symptomatic patients or day of diagnosis for asymptomatic patients. A sample of 18 children that confirmed their SARS-CoV-2 RNA by RT-PCR in from the first day to the 11th day was evaluated. In 5 (27.8%), saliva PCR was persistently negative, including 1 asymptomatic child who only had samples tested on day 6 of admission (NP Ct 37.9, saliva negative). In other 5 (27.8%) patients, saliva that was initially negative on day 1-3 turned positive on day 4-7. Saliva PCR had higher Ct compared to NP swabs. The Ct differences were statistically significant for all time periods except days 11-15. They found that saliva is not a useful specimen for COVID-19 determination in children and maximum saliva sensitivity was only 52.9% compared to NP swab (18).

There are three protocols for collecting saliva (17,18):

1) asking patients to cough or clear their throat before submission of sample (likely mixed sputum and saliva specimen or deep throat saliva specimen)

2) requesting collection by spitting or drooling

3) Saliva specimen should be collected more than one hour after breastfeeding with inserting a sterile swab into the baby's mouth between the gum and cheek and swirl for several seconds, then remove the swab and place into buffer formulated for PCR diagnostic testing (https://www.cdc.gov/cmv/clinical/lab-tests.html).

Although the first two is user- friendly in adults, based on authors' unpublished experience, it seems that accurate saliva collection in children younger than five is less feasible.

Therefore, for younger children, who seem to be

one of the target audiences in the context of saliva sampling instead of respiratory secretions, a saliva test is much more attractive.

The nasopharyngeal swab and nasopharyngeal wash/aspiration

The nasopharyngeal epithelium is an entrance and an important transmission point of SARS-CoV-2, as in other viral upper tract respiratory infections. SARS-CoV-2 uses Angiotensin-Converting Enzyme 2 (ACE2) as a receptor for cellular influx and activity. Binding affinity of the Spike (S) protein and ACE2 is the main determinant of viral replication and disease severity. Since the ACE 2 receptors expression is higher in nasopharyngeal areas, it has been considered that sampling from this region provides better yield of the virus (19,20).

Previously, nasopharyngeal swab was routinely used for diagnosis of viral upper respiratory tract infections in adults and children, and performed by experienced caregivers (21). If sample is not collected correctly, it may lead to false positive/negative results and have dire consequences specially in outbreak (22).

Specimen collection has to be performed in special place with strict sterilization of the entire environment to avoid the spread of the virus. Personnel are required to wear protective equipment including FFP2 (N95) mask (or higher level), disposable cap, goggles, gown, apron, latex gloves and shoe covers (8).

With focus on nasopharyngeal sampling technique in children, parents are necessary to reassure to improve collaboration during the test. Patients and parents should be informed that the procedure is uncomfortable and induce discomfort and retching (8,23).

Past medical history should be obtained to identify any contraindications (any pathology or medication with high risk of epistaxis) or information to facilitate the sample (better air flow on one side for example indicating a wider nasal cavity). Technique is similar to an adult; other than how deep the swab should go and pattern of supporting the infant/child. The approximate external depth is calculated from the patient's entrance of the ear canal to the tip of their nose (8,24).

In child positioning, first, it needs holding forehead with one hand, and arm and leg with another hand, with the assistance of parents or other trained nurses, laboratory staff or physicians. Then with slightly chin elevation, access to the nostril will be better. Next swab, held like a pen is moved perpendicular to the plane of the face, along the floor of the nose. Progression of the swab continue until a resistance is encountered indicating posterior wall of the nasopharynx. The distance between the nostril and posterior wall of the nasopharynx is between 6 and 7 *cm* in children, defined as depth of swab movement (8,23,24).

In infant positioning, developmentally swaddle infant, parents can hold to ensure they are not able to move their head, and/or offer a pacifier, and it will be better performed in supine position. The sampling instruction is similar to older children. If not able to pass, pull back slightly and then rotate swab and advance in downward arc. If it will not pass, it should be stopped and reevaluated (23,24).

In the usual proposed technique in nasopharyngeal sampling, we should gently rub and roll the swab and then leave the swab in place for several seconds to absorb secretions and then slowly remove the swab while continuing to rotate it (25,26).

Kinloch *et al* in 2020 compared two nasopharyngeal sampling methods, ''with and without swab rotation" in 69 adults, and found that swab rotation following nasopharyngeal contact did not recover additional nucleic acid. Rotation was also less tolerable for participants. Finally, they suggest that it is unnecessary to rotate the swab in place following the contact with the nasopharynx (25). Therefore, given that this technique is more problematic in children, it could be recommended that rotating the swab is not necessary, although more studies are needed.

Limited studies have been published on nasopharyngeal sampling in children. Robinson *et al* in 2008 firstly determined the sensitivity of respiratory viral detection in throat swab and saliva specimens in comparison to NP specimens as the reference method. The yield of throat swab and saliva specimens were 83 and 74%, respectively which were less than NP specimens. The viral yield of Direct Fluorescent Antigen detection (DFA) of NP specimens and Nucleic acid Amplification Tests (NAT) in DFA–negative NP specimens were compared with the viral yield of NAT of throat swab and saliva specimens for detection of respiratory viruses in children. Children under the age

of 17 underwent (DFA testing of the NP specimen for respiratory syncytial virus, influenza A and B viruses, and parainfluenza virus. If the virus is not detected, NAT was performed for respiratory syncytial virus, influenza A and B viruses, and parainfluenza virus, adenovirus, and human metapneumovirus. In case the virus was detected by both methods, they performed NAT for the same virus for the corresponding saliva specimens and throat swab. Finally, in 105 of the 137 NP specimens, at least one virus was detected. In 77 of 104(74%) saliva specimens and 87 of 105(83%) oropharyngeal swab specimens, the same virus was detected by NAT. Finally, they concluded that although saliva specimens and oropharyngeal swab are inferior to NP specimens for detecting respiratory viruses, in cases where obtaining an NP sample is impractical, it might be acceptable for screening (27).

Lambert *et al*, firstly by using 303 sets of paired Nose-Throat Swabs (NTS) and nasopharyngeal aspirates (collected from 295 children), calculated sensitivity values for the detection of major respiratory viruses of childhood. 270 (89%) paired specimens were concordant, with the same result in the NPA and NTS specimens. It was finally concluded that nose-throat swab specimens, in combination with sensitive molecular testing, are a less invasive diagnostic respiratory specimen with adequate sensitivity for being used in the clinic and hospital outpatient settings and large-scale community studies through parent collection. For children who present to a hospital in which an avian or pandemic strain of influenza virus is reasonably part of the differential diagnosis, nasopharyngeal aspirates or a similar collection technique (*e.g*., nasal washes) should continue to be used (28).

Di Pietro *et al* in 2020 answered the question that is concerning whether nasopharyngeal swabs are comparable with nasopharyngeal aspirate to detect SARS-CoV-2 in children. They collected 300 paired specimens (NS/NPA) from 136 patients (134 hospitalized and 2 outpatients) were tested for SARS-CoV-2. Of the 300 paired specimens evaluated: 276 were concordant, 24 were discordant, thus the naive concordance was 92.0% (95% CI 88.3–94.6%) with Cohen's kappa (K) 0.63. Among the paired specimens whose NPA resulted positive, 41.9% (95% CI 28.2–56.9%) had NS negative while among the paired specimens whose NPA resulted negative, 2.3% (95% CI 1.1– 5.1%) had NS positive. They finally concluded that the NS has, in any case, a low sensitivity in detecting SARS-CoV-2 in children when referred to NPA. They prefer the collection of NPA whenever possible for the detection of SARS-CoV-2 in children (29).

Since nasopharyngeal sampling is uncomfortable, researchers evaluated nasal sampling rather than nasopharyngeal sampling. Palmas *et al* in first pediatric study focused on application of nasal (mid-turbinate) and oropharyngeal swabs for severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) detection. 11 patients with laboratory-confirmed SARS-CoV-2 infection were selected for further evaluation. A total of 52 paired clinical specimens (26 nasal swabs and 26 oropharyngeal swabs) were collected. Given comparison of Cycle Threshold (CT) values, they observed higher positivity rate of nasal (mid-turbinate) swab over oropharyngeal swab in detecting COVID-19 (Fisher exact test 0.046, Cohen K 0.43, 95% CI, 0.014–0.855%), and suggested that a diagnostic approach based on only oropharyngeal samples may cause SARS-CoV-2 infected patients to be missed (30). Management of children in the COVID-19 era follows specific guidelines, and the preferred testing technique depends on who performs the test. Nurses are comfortable with nasal aspiration because this technique is used routinely in pediatric departments to diagnose viral upper respiratory tract infection. However, we still do not know whether this technique is as reliable as a nasopharyngeal swab. If a trained caregiver is performing the test, a nasopharyngeal swab may be done even in young children and age limit may be determined by the collaboration of the child and parents. Fixed 50% nitrous oxide oxygen mixture might help and a local anesthetic spray may also be used if the child is 6 years old or over.

It is interesting to notice that in Chinese publications, the nasopharyngeal swab has been the gold standard method for diagnosis even for children (5).

Buccal swabs

Children are often unable to produce saliva specimens spontaneously, so buccal swabs can be performed to obtain saliva for testing. Buccal

swabs are less invasive and cause less discomfort for children. It is also unlikely to trigger a sneezing or coughing response, in contrast to nasopharyngeal specimen collection. Also, collection of buccal swabs does not require negative-pressure isolation facilities as it is not aerosol-generating. Kam *et al* in 2020 firstly evaluated the presence of SARS-CoV-2 in buccal specimens in COVID-19 infected children. The result was detected from at least 1 buccal specimen in 9 of 11 COVID-19–infected children (81.8%) and viral loads in buccal specimens were significantly lower than nasopharyngeal specimens. The results of this study showed that buccal swabs are not good for COVID-19 screening in children (31).

Recommendations and conclusion

This study provides comparative sensitivity values in detection rate of SARS-CoV-2 in pediatrics.

To our knowledge, this is the first report to introduce and integrate different types of SARS-CoV-2 upper respiratory sampling methods in children. In fact, this is a comprehensive study in which samples belonging to 906 children with COVID-19 were examined. Evaluation of the results of the mentioned studies demonstrated the high sensitivity and specificity of the nasopharyngeal swab. As a result, we suppose that nasopharyngeal swab is still the sample of choice that support recommendations from WHO for prioritizing NP swab in adults. The two main drawbacks are its technicality and painfulness. Well-trained teams should help increase the sensitivity of the specimen collection and make it less unpleasant.

Our study has several limitations:

1- The number of studies has been very limited

2- The age of children and their classification were not mentioned in most of the studies or were not categorized clearly.

3- One of the studies was performed on children and adults and inevitably, this study was also examined.

4 - This study, as the first review study in children, could examine other sampling methods (such as anal sampling), but due to the large volume of data, it could not be examined.

Finally, deciding on the exact efficacy of sampling methods in children requires further study in the future.

Financial Support

None.

Conflict of Interest

All authors have no conflicts of interest to declare.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon request.

References

1. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003 May 15;348(20):1953-66.

2. World Health Organization. Coronavirus disease 2019 (COVID-19), situation report - 78. Available at:https://www.who.int/docs/defaultsource/coronaviruse/situation-reports/20200407-sitrep-78-covid-19. pdf?sfvrsn=bc43e1b_2. Accessed May 5, 2020.

3. Yu Y, Chen P. Coronavirus disease 2019 (COVID-19) in neonates and children from China: a review. Front Pediatr 2020 May 15;8:287.

4. Wu YC, Chen CS, Chan YJ. The outbreak of COVID-19: an overview. J Chin Med Assoc 2020 Mar;83(3):217-20.

5. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARS-CoV-2 infection in children. N Engl J Med https://pubmed.ncbi.nlm.nih.gov/32187458/

6. Lednicky JA, Tagliamonte MS, White SK, Elbadry MA, Alam MM, Stephenson CJ, et al. Emergence of porcine delta-coronavirus pathogenic infections among children in Haiti through independent zoonoses and convergent evolution. MedRxiv https://doi.org/10.1101/2021.03.19.21253391

7. Centers for Disease Control and Prevention. Information for laboratories about coronavirus 522 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical523 specimens. html. Accessed on October 4, 2020.

8. Centers for Disease Control and Prevention. Interim guidelines for collecting,handling, and testing clinical specimens from persons for coronavirus disease 2019 (COVID-19). Available at: https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html. Accessed May 5, 2020.

9. World Health Organization. Laboratory testing strategy recommendations for COVID-19: interim guidance. World Health Organization. Available at.

10. European Centre for Disease Prevention and Control. Coronavirus Disease 2019 (COVID-19) Pandemic: Increased Transmission in the EU/EEA and the UK – Seventh Update, 25 March 2020. Stockholm: ECDC; 2020.

11. Sueki A, Matsuda K, Yamaguchi A, Uehara M, Sugano M, Uehara T, et al. Evaluation of saliva as diagnostic materials for influenza virus infection by PCR-based assays. Clin Chim Acta 2016 Jan 30;453:71-4.

12. To KK, Yip CC, Lai CY, Wong CK, Ho DT, Pang PK, et al. Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: a diagnostic validity study. Clin Microbiol Infect 2019 Mar;25(3):372-378.

13. Kim YG, Yun SG, Kim MY, Park K, Cho CH, Yoon SY, et al. Comparison between saliva and nasopharyngeal swab specimens for detection of respiratory viruses by multiplex reverse transcription-PCR. J Clin Microbiol 2016 Dec 28;55(1):226-33.

14. Lee RA, Herigon JC, Benedetti A, Pollock NR, Denkinger CM. Performance of saliva, oropharyngeal swabs, and nasal swabs for SARS-CoV-2 molecular detection: a systematic review and meta-analysis. J Clin Microbiol 2021 Apr 20;59(5):e02881-20.

15. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. J Clin Virol 2015 Jul;68:53-5.

16. Pasomsub E, Watcharananan SP, Boonyawat K, Janchompoo P, Wongtabtim G, Suksuwan W, et al. Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study. Clin Microbiol Infect 2021 Feb;27(2):285.e1-285.e4.

17. Yee R, Truong TT, Pannaraj PS, Eubanks N, Gai E, Jumarang J, et al. Saliva is a promising alternative specimen for the detection of SARS-CoV-2 in children and adults. J Clin Microbiol 2021 Jan 21;59(2):e02686-20.

18. Chong CY, Kam KQ, Li J, Maiwald M, Loo LH, Nadua KD, et al. Saliva is not a useful diagnostic specimen in children with coronavirus disease 2019. Clin Infect Dis 2021 Nov 1;73(9):e3144-5.

19. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020 May 12;323(18):1843-4.

20. Patel AB, Verma A. COVID-19 and angiotensin-converting ezyme inhibitors and angiotensin receptor blockers: what is the evidence?. JAMA 2020 May 12;323(18):1769-1770.

21. Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J Med Virol 2020 Jul;92(7):833-40.

22. Mawaddah A, Genden HS, Lum SG, Marina MB. Upper respiratory tract sampling in COVID-19. Malays J Pathol 2020 Apr;42(1):23-35.

23. General Testing Protocol Summary Nebraska Medicine SARS-CoV-2 (COVID 19)Pediatric Nasopharyngeal Swabbing Recommendations(Updated 09/03/2020).

24. Pondaven-Letourmy S, Alvin F, Boumghit Y, Simon F. How to perform a nasopharyngeal swab in adults and children in the COVID-19 era. European Annals Otorhinolaryngol Head Neck Dis 2020 Sep 1;137(4):325-7.

25. Kinloch NN, Shahid A, Ritchie G, Dong W, Lawson T, Montaner JSG, et al. Evaluation of nasopharyngeal swab collection techniques for nucleic acid recovery and participant experience: recommendations for COVID-19 diagnostics. Open Forum Infect Dis 2020 Oct 14;7(11):ofaa488.

26. Zedtwitz-Liebenstein K. Correctly performed nasal swabs. Infection 2021 Aug;49(4):763-4.

27. Robinson JL, Lee BE, Kothapalli S, Craig WR, Fox JD. Use of throat swab or saliva specimens for detection of respiratory viruses in children. Clin Infect Dis 2008 Apr 1;46(7):e61-4.

28. Lambert SB, Whiley DM, O'Neill NT, Andrews EC, Canavan FM, Bletchly C, et al. Comparing nose-throat swabs and nasopharyngeal aspirates collected from children with symptoms for respiratory virus identification using real-time polymerase chain reaction. Pediatrics 2008 Sep;122(3):e615-20.

29. Di Pietro GM, Capecchi E, Luconi E, Lunghi G, Bosis S, Bertolozzi G, et al. Diagnosis of SARS-CoV-2 in children: accuracy of nasopharyngeal swab compared to nasopharyngeal aspirate. Eur J Clin Microbiol Infect Dis 2021 Jun;40(6):1155-60.

30. Palmas G, Moriondo M, Trapani S, Ricci S, Calistri E, Pisano L, et al. Nasal swab as preferred clinical specimen for COVID-19 testing in children. Pediatr Infect Dis J 2020 Sep 1;39(9):e267-70.

31. Kam KQ, Yung CF, Maiwald M, Chong CY, Soong HY, Loo LH, et al. Clinical utility of buccal swabs for severe acute respiratory syndrome coronavirus 2 detection in coronavirus disease 2019-infected children. J Pediatric Infect Dis Soc 2020 Jul 13;9(3):370-372.