Oral saliva swab reverse transcription PCR for Covid-19 in the paediatric population

Cinta Moraleda ,[1,2] Sara Domínguez-Rodríguez,[2] Juan Miguel Mesa,[3] Paula García-Sánchez,[4] María de la Serna,[3] José Antonio Alonso-Cadenas ,[5] Amanda Bermejo,[6] Gema Sabrido,[7] Leticia Martínez-Campos,[8] Aránzazu Flavia González-Posada,[9] Marta Illán-Ramos,[10] Elena Cobos-Carrascosa,[2] Álvaro Ballesteros,[2] Juan Carlos Galán,[11] Francisco Llorente,[12] David Aguilera-Alonso ,[13] Ana Belén Jiménez,[14] Pilar Catalán,[15] Elias Dahdouh,[16] Ignacio Navarro,[3] Bárbara Fernández-Garoz,[17] Pablo Mendoza,[18] Concepción Pérez-Jorge,[19] Teresa Cabezas-Fernández,[20] Daniel Blázquez-Gamero ,[1,2,21] Gonzalo Rivas,[22] Patricia Gonzalez-Donapetry,[16] Elena Sáez,[23] Jovita Fernández-Pinero,[12] Jesús Lucas-Fernández,[11] Elisa Pérez-Ramírez,[12] Paloma Merino,[24] Sandra Miragaya,[17] Jorge Lorente,[25] Irene Iglesias,[12] Alfredo Tagarro ,[2,3] the EPICO working group

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/archdischild-2021-323712).

For numbered affiliations see end of article.

Correspondence to
Dr Cinta Moraleda, Department of Pediatrics, Hospital Universitario 12 de Octubre, Madrid 28041, Spain; cintamoraledaredecilla@gmail.com

Received 13 December 2021
Accepted 12 May 2022

To cite: Moraleda C, Domínguez-Rodríguez S, Mesa JM, *et al*. *Arch Dis Child* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/archdischild-2021-323712

ABSTRACT

Objectives To evaluate the performance of oral saliva swab (OSS) reverse transcription PCR (RT-PCR) compared with RT-PCR and antigen rapid diagnostic test (Ag-RDT) on nasopharyngeal swabs (NPS) for SARS-CoV-2 in children.

Design Cross-sectional multicentre diagnostic study.

Setting Study nested in a prospective, observational cohort (EPICO-AEP) performed between February and March 2021 including 10 hospitals in Spain.

Patients Children from 0 to 18 years with symptoms compatible with Covid-19 of ≤5 days of duration were included. Two NPS samples (Ag-RDT and RT-PCR) and one OSS sample for RT-PCR were collected.

Main outcome Performance of Ag-RDT and RT-PCR on NPS and RT-PCR on OSS sample for SARS-CoV-2.

Results 1174 children were included, aged 3.8 years (IQR 1.7–9.0); 73/1174 (6.2%) patients tested positive by at least one of the techniques. Sensitivity and specificity of OSS RT-PCR were 72.1% (95% CI 59.7 to 81.9) and 99.6% (95% CI 99 to 99.9), respectively, versus 61.8% (95% CI 49.1 to 73) and 99.9% (95% CI 99.4 to 100) for the Ag-RDT. Kappa index was 0.79 (95% CI 0.72 to 0.88) for OSS RT-PCR and 0.74 (95% CI 0.65 to 0.84) for Ag-RDT versus NPS RT-PCR.

Conclusions RT-PCR on the OSS sample is an accurate option for SARS-CoV-2 testing in children. A less intrusive technique for younger patients, who usually are tested frequently, might increase the number of patients tested.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ SARS-CoV-2 saliva tests have not been widely implemented in children.
⇒ The accuracy of reverse transcription PCR (RT-PCR) on saliva and nasopharyngeal swab is similar.
⇒ Studies in this population are scarce, particularly in preschoolers.

WHAT THIS STUDY ADDS

⇒ RT-PCR on oral saliva swab is an accurate option for SARS-CoV-2 testing in children.
⇒ RT-PCR on oral saliva swab shows higher sensitivity than the antigen rapid diagnostic test on nasopharyngeal swab.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Saliva swab may be more acceptable for younger patients than nasopharyngeal swab, possibly increasing the capacity of testing in this age group.
⇒ Saliva swab could be added to the SARS-CoV-2 diagnostic protocols as an alternative specimen to nasopharyngeal swab in children.

INTRODUCTION

Nucleic acid amplification testing in nasopharyngeal swab (NPS) samples is considered as the gold standard technique for the diagnosis of SARS-CoV-2 infection[1]; however, this technique requires trained staff, is associated with an increased risk of complications (epistaxis, retained swabs, cerebrospinal fluid leak),[2] is unpleasant and generates anxiety mainly in children. Indeed, patients with mild symptoms are often put off accessing the test.[2 3]

Saliva swab reverse transcription PCR (RT-PCR) is an alternative and minimally invasive test for the diagnosis of SARS-CoV-2 infection. Data from a recent systematic review showed that given a proper RT-PCR kit is chosen, the accuracy of RT-PCR on

saliva and NPS is similar, particularly in ambulatory patients,[4] yet saliva tests have not been widely implemented in children. Indeed, studies in this population are scarce, particularly in preschoolers.[5–10]

We performed this study with the hypothesis that oral saliva swab (OSS) RT-PCR is as sensitive as RT-PCR on NPS, which is considered the gold standard. Our objective was to analyse the performance of OSS RT-PCR in comparison to (1) the gold standard (RT-PCR on NPS) and (2) the Panbio SARS-CoV-2 antigen rapid test (Ag-RDT) on NPS, a technique widely implemented, in a symptomatic paediatric population evaluated at the emergency departments of 10 Spanish hospitals.

METHODS

Study design

Cross-sectional multicentre diagnostic study nested in a prospective, observational cohort, the Epidemiological Study of Covid-19 in Children of the Spanish Society of Pediatrics (EPICO-AEP). Participants were children from 0 to 18 years old with symptoms compatible with SARS-CoV-2 infection of ≤5 days of duration, seen in the emergency departments of 10 secondary and tertiary hospitals, in Madrid and Almería, Spain.

The study was performed between February and March 2021. At that time, the peak incidence of Covid-19 in Spain was 559/100 000 individuals.[11] Standards for Reporting Diagnostic Accuracy (STARD) 2015 guidelines for reporting diagnostic accuracy studies were followed throughout.[12]

SARS-CoV-2 testing

Trained nurses collected three samples consecutively from each patient following a standardised procedure: two NPS samples (for Ag-RDT and RT-PCR) and one OSS sample for RT-PCR. The Panbio SARS-CoV-2 Ag-RDT was purchased from Abbott Rapid Diagnostics (Jena, Germany). OSS samples were collected using the identical brand of the swab for the NPS samples. The procedure included smearing the swab under the tongue, between the gums and lips and in the inner part of both cheeks, but not the pharynx. Older patients were invited to cough with their mouths closed before sampling. A minimum amount of saliva was not necessary for the oral smear. Clinicians recommended patients not eat or drink at least 30 min before sampling. OSS and NPS to be used for RT-PCR were placed in standard viral transport media (Delta Labs, Barcelona, Spain).

The nurses in charge of collecting samples performed Ag-RDT on-site and results were interpreted following the manufacturer's instructions, whereas OSS and NPS for RT-PCR were immediately transported to each local microbiology laboratory of each participating centre after specimen collection.

RT-PCR testing in OSS and NPS was performed immediately after specimen collection, targeting at least two viral genomic fragments following the manufacturer's recommendations in each laboratory. RNA was extracted from samples by using the automated systems in place in each of the participating centres. OSS were processed using the same protocol as NPS in each centre. It was considered indeterminate those samples in which only one of the genes was amplified with a high cycle threshold (Ct). Turnaround time was less than 12 hours at all the centres. Nurses who interpreted the Ag-RDT did not have access to the RT-PCR result. Lab technicians had access to the Ag-RDT result. Clinicians attending each patient had the information of the three tests once available. Clinical information was available for all of them.

Patients with discordant diagnostic test results from any of the three different tests were invited to a second visit and a blood sample was drawn for SARS-CoV-2 IgG detection. Samples were performed in less than 24 hours after the researchers were aware of the results and less than 48 hours from the initial clinical visit. IgG antibodies directed against the SARS-CoV-2 surface S1 domain of the spike protein or the internal nucleocapsid protein were measured in the serum samples using commercial enzyme-linked immunosorbent assay (ELISA) or chemiluminescent immunoassay kits, depending on availability in each centre following manufacturer's instructions. Besides, in these patients, a study of viral viability was performed whenever possible. All the information related to this additional analysis is included in the online supplemental material.

Data collection

Clinical and laboratory data were collected using RedCap electronic data capture tools[13 14] hosted at the 12 de Octubre Hospital.

Statistical methods

The intended sample size was 1500 patients to estimate a 70% sensitivity for OSS and 90% of sensitivity for Ag-RDT to reach a statistical power of 80% with acceptable precision.

The study population was described using counts and percentages for categorical variables and medians and IQRs for continuous variables. Categorical variables were compared with χ^2 or Fisher's test, and continuous variables with Mann-Whitney U test. The performance of the test was evaluated estimating the sensitivity, specificity, negative and positive predictive values and the kappa index. Additionally, we provided the sensitivity and specificity of the different diagnostic techniques stratified by age (≤3 and >3 years old). The performance of the test was evaluated by estimating the sensitivity, specificity, negative and positive predictive values and the kappa index taking RT-PCR on NPS as the gold standard. A confusion matrix was assessed to calculate the diagnostic accuracy. CIs for sensitivity and specificity are exact Clopper-Pearson CIs. Besides, the performance of the three tests was also estimated by Bayesian Latent Class Models (BLCA) using extensions of the three tests in one population model implemented in a simplified interface application.[15] The BLCA[16] was used to approximate the prevalence and the sensitivities and specificities of all tests. This model does not assume that any test is perfect but considers that each test could be imperfect in diagnosing the true disease status. The true disease status of the patient population was then defined based on overall prevalence (the probability that a patient with suspected SARS-CoV-2 infection is truly infected with SARS-CoV-2). BLCA estimates the prevalence and accuracy of each test based on the observed frequency of the possible combinations of test results.

All the analyses were performed using R software V.4.1.2,[17] including caret R package V.6.0-90,[18] MKmisc R package V.1.8[19] and compareGroups Package v.4.0.[17]

RESULTS

Overall, 1186 patients were included in the study. Eight children did not provide the three samples, and four children presented indeterminate RT-PCR results (two for OSS and two for NPS) and were excluded. Thus, 1174 children were included in the final analysis (figure 1). Median age was 3.8 years (IQR 1.7–9.0), and 516/1174 (44.0%) were ≤3 years old. A total of 647/1174 (55.1%) children were males. The median duration of symptoms

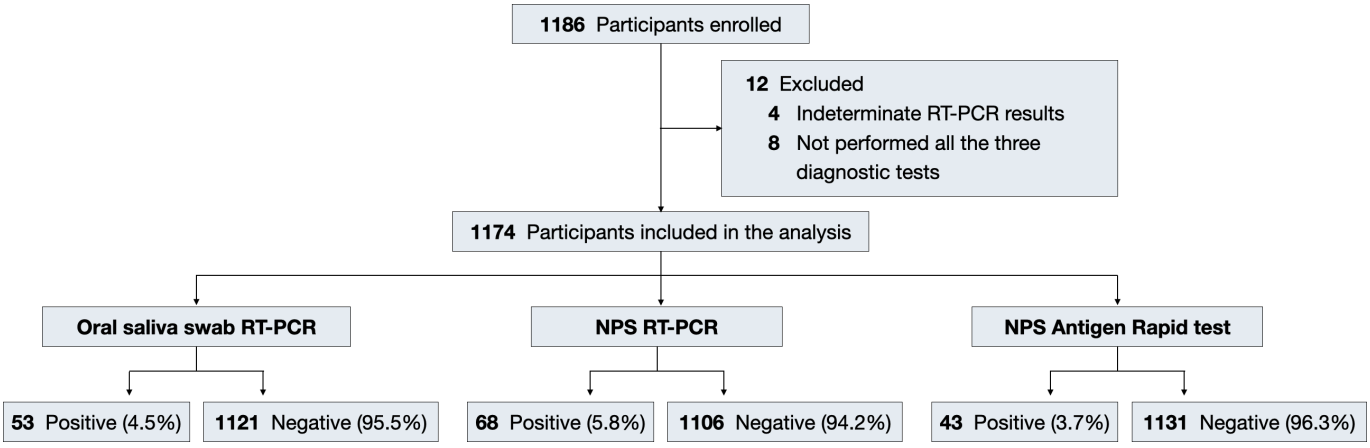

Figure 1 Flow chart of the study and positive results in each of the three evaluated techniques. One NPS antigen rapid test result missing and seven oral saliva swabs collected but not processed due to laboratory workflow problems. NPS, nasopharyngeal swab; RT-PCR, reverse transcription PCR.

before emergency department admission was 1.0 days (IQR 1.0–2.0) (table 1).

In total, 73/1174 (6.2%) patients tested positive by at least one of the diagnostic techniques. Out of those 73 patients, 68 tested positive by NPS RT-PCR (93.2%; 5.8% of the total), 53 by OSS RT-PCR (72.6%; 4.5% of the total) and 43 were positive by NPS Ag-RDT (58.9%; 3.7% of the total) (figure 1).

The comparison of OSS RT-PCR and the Ag-RDT in NPS samples versus NPS RT-PCR (gold standard) is shown in table 2. The overall sensitivity was 72.1% (95% CI 59.7% to 81.9%) for OSS RT-PCR and 61.8% (95% CI 49.1% to 73.0%) for the Ag-RDT. The specificity was 99.6% (95% CI 99.0% to 99.9%) for OSS RT-PCR and 99.9% (95% CI 99.4% to 100%) for the Ag-RDT. The kappa index for the OSS RT-PCR and NPS RT-PCR was 0.79 (95% CI 0.72 to 0.88) versus 0.74 (95% CI 0.65 to 0.84) for the Ag-RDT. OSS RT-PCR showed similar performance in children stratified by age. By contrast, the Ag-RDT showed lower sensitivity in children younger than 3 years (table 3).

The estimated medians with 95% credible intervals (Crs) in the whole cohort and in the BLCA model are shown in table 2. Sensitivity for OSS RT-PCR was 84.8% (95% Cr 71.5%–93.6%) and 72.5% (95% Cr 58.8%–83.6%) for the Ag-RDT. Specificity for OSS RT-PCR was 99.7% (95% Cr 99.2%–99.9%) and 99.9% (95% Cr 99.6%–100%) for the Ag-RDT.

The median Ct values for those patients with positive RT-PCR results were 21.1 (IQR 17.8–30.0) on NPS samples and 32.9 (IQR 28.8–35.6) on OSS samples. The Cts were higher in OSS than in NPS samples (Ct (NPS)=0.5×(Ct saliva)+4.5), p=0.027).

In total, 36/1174 patients (3.1%) tested positive with the three diagnostic tests, 13 cases (1.1%) tested positive only by NPS RT-PCR, 4 cases (0.3%) only by OSS RT-PCR and 1 case (0.1%) only by Ag-RDT (table 4). Ct values for patients with discordant results are shown in online supplemental table S1.

A total of 37 patients had a discordant result in at least one of the three tests (table 3 and online supplemental material). All were invited to provide a blood sample for SARS-CoV-2 serology, and it was available for 22/37 (59.5%) patients.

A total of 19/68 (27.9%) patients were positive for NPS RT-PCR and negative for OSS RT-PCR. Of these 19 patients, 4 were serology positive and could be considered not contagious.[20] Four patients had OSS RT-PCR as the unique positive test, but three of them with serology available and positive, suggesting old infections.

DISCUSSION

This study evaluates the performance of three SARS-CoV-2 diagnostic tests in a large paediatric population with Covid-19 symptoms showing that the RT-PCR on OSS is a valid diagnostic option in the paediatric population, even better than the widely used Ag-RDT on NPS. If the turnaround time is sufficiently rapid, OSS RT-PCR would seem to be the better choice for children. The performance of the test improved when NPS RT-PCR was considered as an imperfect gold standard.[21 22]

NPS is the most widely used sample for SARS-CoV-2 testing, although it is not always well accepted by patients.[1–3 23] Several studies have shown that different sample types of saliva (dribble pots, suck swabs, oral and oropharynx swabs) are a valid alternative for SARS-CoV-2 testing, but most of the studies included only adults.[4] To our knowledge, this study is the largest paediatric study comparing OSS with the established gold standard. The results are in concordance with the few studies performed on children.[5 7 9 10 24] A recent study performed in Dubai with 476 children demonstrated a sensitivity even higher (87.7%) than found here; however, the authors used a saliva sample rather than saliva swabs, and the children were older (mean 10.8 years old).[10] Obtaining direct saliva samples in children younger than 3 years of age and children with special needs, who could be at a higher risk of severe Covid-19 (ie, neurological diseases) is challenging. However, unlike direct saliva, oral swabs can be obtained with practically no collaboration from patients. For this reason, we used oral swabs, which is a sample easily feasible and painless for all ages including those children with special needs. We had a high proportion of children younger than 3 years old, which we believe fills the gap of knowledge on the performance of saliva samples at this age. Moreover, analysis by age showed that the accuracy is similar for both groups (≤3 years vs >3 years), unlike the Ag-RDT, which performed poorly in children under 3 years. Children with special needs were not specifically included in this study so the results cannot be directly extrapolated to them.

We found that OSS RT-PCR could detect some cases that were missed by the NPS RT-PCR; four cases were positive for OSS RT-PCR but negative for NPS RT-PCR, as has been described previously.[7–9] Also, three of those four cases had positive serology but were negative for the Ag-RDT. These data suggest that the positivity on saliva RT-PCR could remain even longer than in NPS RT-PCR, but a SARS-CoV-2 reinfection cannot be fully discarded.

Table 1 Clinical characteristics of the children included in the study

	Overall	Negative	Positive*	
	N=1174	n=1101	n=73	P value†
Sex (male; n, %)	647/1165 (55.1%)	609/1093 (55.7%)	38/72 (52.8%)	0.716
Age (median, IQR)	3.79 (1.67–9.00)	3.68 (1.65–8.65)	8.12 (3.06–12.2)	<0.001
Groups of age				0.001
≤3 years	516 (44.0%)	498 (45.2%)	18 (24.7%)	
>3 years	658 (56.0%)	603 (54.8%)	55 (75.3%)	
Fever				0.429
No	414 (35.3%)	392 (35.6%)	22 (30.1%)	
Yes	760 (64.7%)	709 (64.4%)	51 (69.9%)	
Cough				0.321
No	625 (53.2%)	581 (52.8%)	44 (60.3%)	
Yes	547 (46.6%)	518 (47.0%)	29 (39.7%)	
Unknown	2 (0.17%)	2 (0.18%)	0 (0.00%)	
Sore throat				0.143
No	872 (74.5%)	824 (75.0%)	48 (65.8%)	
Yes	286 (24.4%)	262 (23.9%)	24 (32.9%)	
Unknown	13 (1.11%)	12 (1.09%)	1 (1.37%)	
Runny nose				0.602
No	566 (48.3%)	528 (48.1%)	38 (52.1%)	
Yes	603 (51.5%)	568 (51.7%)	35 (47.9%)	
Unknown	2 (0.17%)	2 (0.18%)	0 (0.00%)	
Wheezing				0.229
No	1071 (91.4%)	1001 (91.1%)	70 (95.9%)	
Yes	101 (8.62%)	98 (8.92%)	3 (4.11%)	
Myalgia				<0.001
No	1042 (88.8%)	987 (89.7%)	55 (75.3%)	
Yes	84 (7.16%)	69 (6.27%)	15 (20.5%)	
Unknown	47 (4.01%)	44 (4.00%)	3 (4.11%)	
Arthralgia				0.064
No	1101 (93.8%)	1036 (94.1%)	65 (89.0%)	
Yes	23 (1.96%)	19 (1.73%)	4 (5.48%)	
Unknown	50 (4.26%)	46 (4.18%)	4 (5.48%)	
Fatigue				0.096
No	1008 (86.0%)	951 (86.5%)	57 (78.1%)	
Yes	139 (11.9%)	125 (11.4%)	14 (19.2%)	
Unknown	25 (2.13%)	23 (2.09%)	2 (2.74%)	
Dyspnoea				0.110
No	1066 (91.4%)	996 (91.0%)	70 (97.2%)	
Yes	100 (8.58%)	98 (8.96%)	2 (2.78%)	
Unknown	8 (0.7%)	7 (0.6%)	1 (1.37%)	
Chest indrawing				0.764
No	1116 (95.9%)	1045 (95.8%)	71 (97.3%)	
Yes	48 (4.12%)	46 (4.22%)	2 (2.74%)	
Unknown	6 (0.51%)	6 (0.55%)	0 (0.00%)	
Headache				<0.001
No	915 (81.1%)	873 (82.4%)	42 (61.8%)	
Yes	213 (18.9%)	187 (17.6%)	26 (38.2%)	
Unknown	45 (3.84%)	40 (3.64%)	5 (6.85%)	
Abdominal pain				0.058
No	879 (77.2%)	818 (76.6%)	61 (87.1%)	
Yes	259 (22.8%)	250 (23.4%)	9 (12.9%)	
Unknown	33 (2.82%)	31 (2.82%)	2 (2.78%)	
Vomiting/nausea				0.012
No	855 (72.9%)	792 (72.0%)	63 (86.3%)	
Yes	318 (27.1%)	308 (28.0%)	10 (13.7%)	
Diarrhoea				<0.001
No	948 (80.8%)	877 (79.7%)	71 (97.3%)	
Yes	225 (19.2%)	223 (20.3%)	2 (2.74%)	

Continued

Table 1 Continued

	Overall	Negative	Positive*	
	N=1174	n=1101	n=73	P value†
Conjunctivitis				0.622
No	1152 (98.5%)	1079 (98.4%)	73 (100%)	
Yes	18 (1.54%)	18 (1.64%)	0 (0.00%)	
Unknown	2 (0.17%)	2 (0.18%)	0 (0.00%)	
Oral inflammation				0.619
No	1098 (93.6%)	1028 (93.5%)	70 (95.9%)	
Yes	75 (6.39%)	72 (6.55%)	3 (4.11%)	
Rash				0.722
No	1137 (96.9%)	1065 (96.8%)	72 (98.6%)	
Yes	36 (3.07%)	35 (3.18%)	1 (1.37%)	
Alteration taste				0.202
No	917 (97.6%)	856 (97.7%)	61 (95.3%)	
Yes	23 (2.45%)	20 (2.28%)	3 (4.69%)	
Unknown	230 (19.7%)	221 (20.1%)	9 (12.3%)	
Others				0.461
No	1098 (94.1%)	1030 (93.9%)	68 (97.1%)	
Yes	68 (5.83%)	66 (6.02%)	2 (2.86%)	
Unknown	1 (0.09%)	1 (0.09%)	0 (0.00%)	
Days of symptoms (median, IQR)	1.00 (1.00–2.00)	1.00 (1.00–2.00)	1.00 (1.00–2.00)	0.714
Days of fever (median, IQR)	1.00 (0.00–2.00)	1.00 (0.00–2.00)	1.00 (1.00–1.50)	0.464

P values were calculated excluding unknown.
*Positive by any technique.
†P value for the comparison test (continuous, Mann-Whitney U test or categorical χ^2 test).

A similar finding was observed in the majority of the 19 cases with a negative result on the OSS but positive on the NPS. Of these 19, 4 had positive serology. An observational study showed that the concordance between paired samples (NPS and saliva) decreased with time, with saliva false negatives increasing in older infections.[25] We observed a lower sensitivity of the Ag-RDT in children younger than 3 years old. Moreover, among the four cases with negative Ag-RDT and positive OSS, three were younger than 3 years. Thus, younger children might benefit from the OSS RT-PCR in testing guidelines possibly before Ag-RDT.

Table 2 Diagnostic accuracy and agreement of oral saliva swab RT-PCR and rapid antigen test compared with nasopharyngeal swab RT-PCR

Parameters	NPS RT-PCR (assumed as a perfect gold standard (%))*	Bayesian latent class model (%)†
Prevalence	5.8 (4.6–7.3)	5.0 (3.8–6.4)
RT-PCR NPS		
Sensitivity	100	99.3 (92.1–100)
Specificity	100	99.1 (98.3–99.7)
Positive predictive value	100	84.4 (73.3–94.6)
Negative predictive value	100	100 (99.6–100)
RT-PCR oral saliva swab		
Sensitivity	72.1 (59.7–81.0)	84.8 (71.5–93.6)
Specificity	99.6 (99.0–99.9)	99.7 (99.2–99.9)
Positive predictive value	92.5 (80.9–97.6)	92.6 (83.3–98.3)
Negative pedictive value	98.3 (97.3–98.9)	99.2 (98.4–99.7)
Antigen rapid test		
Sensitivity	61.8 (49.1–73.0)	72.5 (58.8–83.6)
Specificity	99.9 (99.4–100)	99.9 (99.6–100)
Positive predictive value	97.7 (86.2–99.9)	97.6 (89.9–100)
Negative predictive value	97.7 (96.6–98.5)	98.6 (97.6–99.2)

*Gold standard model assumed that RT-PCR NPS test is perfect (100% sensitivity and 100% specificity; all patients with positive gold standard test are diseased and all patients with negative gold standard test are non-diseased). Values shown are estimated means with 95% CI.
†Bayesian latent class model assumed that all tests evaluated are imperfect. Values shown are estimated median with 95% credible interval.
NPS, nasopharyngeal swab; RT-PCR, reverse transcription PCR.

At the time of performing the study, the cost of SARS-CoV-2 Ag-RDT was US$2–US$5, which was around 10 times lower than the average price of NPS RT-PCR including the swab and reagents. OSS RT-PCR uses the same diagnostic approach as NPS RT-PCR, but in a different sample, so the cost in our study was the same. Additionally, RT-PCR tests require the prior acquisition of expensive devices to perform the analysis and specialised staff. Considering only the cost, Ag-RDT is a better choice than RT-PCR tests. Furthermore, the turnaround is lower (15 min vs 2–12 hours). However, compared with OSS RT-PCR, it has some disadvantages, such as a lower sensitivity and NPS being a more uncomfortable sample than OSS. So, OSS RT-PCR could be a good alternative to NPS RT-PCR in case of aiming a high sensitivity test and comfortable for patients. Besides, a new approach for SARS-CoV-2 RT-PCR (SalivaDirect) that simplifies RNA extraction has been published.[26] In this study, the standard RNA extraction and the SalivaDirect procedure were performed followed by the same RT-PCR with similar results. The potential implementation of the simplified procedure could greatly reduce the cost and the time of the OSS RT-PCR testing, making this method even more attractive.

No systematic or standardised information of the acceptance of the patients, families and operators were collected in this study, but several publications support that OSS is the preferred technique by patients.[27 28] The acceptability of participating in the study (>99%, data not shown) shows how families acknowledge that finding a more accepted technique to test children is needed. Besides, the collection and processing of samples

Table 3 Performance of the studied tests compared with the gold standard test stratified by age

(%, 95% CI)	All N=1174	≤3 years old n=516	>3 years old n=658
RT-PCR on oral saliva swab			
Sensitivity	72.1% (59.8% to 82.27%)	75% (47.6% to 92.73%)	71.1% (56.9% to 82.87%)
Specificity	99.6% (99.1% to 99.9%)	99.6% (98.6% to 99.9%)	99.7% (98.8% to 99.9%)
Positive predictive value	92.4% (82.0% to 97.1%)	85.7% (59.4% to 96.1%)	94.9% (82.1% to 98.7%)
Negative predictive value	98.3% (97.5% to 98.8%)	99.2% (98.1% to 99.7%)	97.6% (96.3% to 98.4%)
Kappa index	0.79 (0.72 to 0.88)	0.79 (0.63 to 0.95)	0.8 (0.71 to 0.89)
Positive likelihood ratio	199.24 (74.1 to 535.9)	187.5 (45.7 to 769.4)	215.6 (53.4 to 869.4)
Negative likelihood ratio	0.28 (0.19 to 0.41)	0.25 (0.11 to 0.59)	0.29 (0.19 to 0.44)
Ag-RT on nasopharyngeal swab			
Sensitivity	61.8% (49.2% to 73.3%)	43.7% (19.8% to 70.1%)	67.3% (52.9% to 79.7%)
Specificity	99.9% (99.5% to 100%)	100% (99.2% to 100%)	99.8% (99.1% to 100%)
Positive predictive value	97.67% (85.4% to 99.7%)	100%	97.2% (83.0% to 99.6%)
Negative predictive value	97.7% (96.9% to 98.3%)	98.2% (97.3% to 98.4%)	97.3% (96.0% to 98.1%)
Kappa index	0.74 (0.65 to 0.84)	0.6 (0.36 to 0.83)	0.78 (0.68 to 0.87)
Positive likelihood ratio	97.7 (85.4 to 99.7)	–	407.9 (57.0 to 2917.6)
Negative likelihood ratio	0.38 (0.3 to 0.5)	0.56 (0.37 to 0.87)	0.33 (0.22 to 0.48)

Ag-RDT, antigen rapid test; RT-PCR, reverse transcription PCR.

showed scarce difficulties as only eight children did not provide the three samples, and four children presented indeterminate RT-PCR results.

This study has several limitations. RT-PCR tests and serology were not centralised, which may have introduced some heterogeneity in the results and difficulties in reproducibility. It accounted for only one wave, including the alpha variant as dominant (>70% of isolations), and it may not be fully extrapolated to Omicron or future variants. The possibility of reinfections was not addressed, and not all discordant patients had serology available. Finally, this study included only symptomatic patients (≤5 days of duration), so the results cannot be extrapolated to asymptomatic patients and new studies will be necessary to evaluate this test in SARS-CoV-2 contacts and as a screening test in the general paediatric population. However, as a strength, we evaluate a novel, comfortable and easy-to-collect sample in children, which has not been previously thoroughly evaluated in the paediatric population, mainly in the younger children.

In conclusion, OSS RT-PCR seems a more suitable and friendly technique for younger patients who must be tested very frequently and might help to maximise the number of patients tested, playing an important role in the control of the disease.

Table 4 Detection of SARS-CoV-2 by RT-PCR on NPS or OSS and Ag-RDT in NPS in all 1174 tested children

RT-PCR NPS	RT-PCR OSS	NPS Ag-RDT	Total
Positive	Positive	Positive	36 (3.1%)
Positive	Positive	Negative	13 (1.1%)
Positive	Negative	Negative	13 (1.1%)
Positive	Negative	Positive	6 (0.5%)
Negative	Positive	Negative	4 (0.3)
Negative	Negative	Positive	1 (0.1%)
Negative	Positive	Positive	0 (0.0%)
Negative	Negative	Negative	1101 (93.8%)

Ag-RDT, antigen rapid test; NPS, nasopharyngeal swab; OSS, oral saliva swab; RT-PCR, reverse transcription PCR.

Author affiliations
[1]Pediatric Infectious Diseases Unit. Department of Pediatrics, Hospital Universitario 12 de Octubre. Pediatric Research and Clinical Trials Unit (UPIC). RITIP (Translational Research Network in Pediatric Infectious Diseases), Madrid, Spain
[2]Instituto de Investigación 12 de Octubre (imas12), Fundación para la Investigación Biomédica del Hospital 12 de Octubre, Madrid, Spain
[3]Paediatrics Department. Pediatrics Research Group, Hospital Universitario Infanta Sofia, San Sebastian de los Reyes, Madrid, Spain
[4]Emergency Pediatric Department. Instituto Investigación Hospital La Paz (IDIPaz), Hospital Universitario La Paz, Madrid, Spain
[5]Emergency Pediatric Department. Instituto Investigación La Princesa, Hospital Infantil Universitario Niño Jesús, Madrid, Spain
[6]Pediatric Department, Hospital Universitario de Móstoles, Móstoles, Madrid, Spain
[7]Pediatric Department, Hospital Universitario Rey Juan Carlos, Móstoles, Madrid, Spain
[8]Pediatric Infectious Diseases, Materno Infantil. Hospital Universitario Torrecárdenas, Almeria, Spain
[9]Emergency Pediatric Department, Hospital Universitario 12 de Octubre, Madrid, Spain
[10]Pediatrics Department, Hospital Universitario Clínico San Carlos, Madrid, Spain
[11]Microbiology Department, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS). Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[12]Centro de Investigacion en Sanidad Animal INIA-CSIC, Valdeolmos, Madrid, Spain
[13]Pediatric Infectious Diseases Unit. Department of Pediatrics, Hospital Universitario Gregorio Marañón. Unidad de Investigación Maternoinfantil Fundación Familia Alonso (UDIMIFFA), Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM). CIBER en Enfermedades Infecciosas (CIBERINFEC), Madrid, Spain
[14]Pediatrics Department, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain
[15]Servicio de Microbiologia Clinica y Enfermedades Infecciosas, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[16]Clinical Microbiology and Parasitology Department, Hospital Universitario La Paz. IdiPAZ, Madrid, Spain
[17]Clinic Analysis Department, Hospital Infantil Universitario Niño Jesús, Madrid, Spain
[18]Microbiology Department, Hospital Universitario de Móstoles, Mostoles, Madrid, Spain
[19]Microbiology Department, Hospital Universitario Rey Juan Carlos, Mostoles, Madrid, Spain
[20]Microbiology Department, Hospital Universitario Torrecárdenas, Almería, Spain
[21]Universidad Complutense, Madrid, Spain
[22]Microbiology Department, Hospital Universitario 12 de Octubre, Madrid, Spain
[23]Microbiology Department, UR Salud. Hospital Universitario Infanta Sofía. Paediatrics Research Group, San Sebastian de los Reyes, Madrid, Spain
[24]Microbiology Department, Hospital Universitario Clínico San Carlos, Madrid, Spain
[25]Emergency Pediatric Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain

Twitter David Aguilera-Alonso @DavidAguileraAl

Acknowledgements We thank all the patients and families for their participation in the study, and the laboratory staff and clinical staff members who cared for them and the EPICO working group. We also thank Kenneth McCreath for the meaningful comments and suggestions on English writing. K.M. is a medical English consultant funded by Universidad Europea de Madrid.

Collaborators EPICO working group: Francisco José Sanz MD, María Isabel Iglesias-Bouzas MD, Jose Antonio Alonso Cadenas MD, Blanca Herrero (Hospital Infantil Universitario Niño Jesús, Madrid, Spain), Teresa del Rosal MD, Ana Méndez-Echevarría MD, Talía Sainz MD, Clara Udaondo MD, Fernando Baquero MD, Cristina Calvo MD, Carlos Grasa MD, Paula R Molino MD, María José Mellado MD, María Ceano MD, Victor Galán MD, Paula Garcia Sanchez MD, Sonsoles San Roman (Hospital Universitario La Paz Madrid, Spain), Alicia Hernanz Lobo MD, Mar Santos MD, Marisa Navarro MD, Elena Rincón MD, David Aguilera-Alonso MD, Begoña Santiago MD, Jesús Saavedra, Jorge Huerta MD, Eduardo Bardón MD, Jorge Lorente MD, Pilar Catalán MD (Hospital Universitario Gregorio Marañón, Spain), Pablo Rojo MD, Daniel Blázquez MD, Luis Prieto MD, Elisa Fernández-Cooke MD, David Torres-Fernández MD, Ángela Manzanares MD, Jaime Carrasco MD, Cristina Epalza MD, Jesús Contreras MD, Sara Domínguez MD, Sara Villanueva MD, Arantxa Gonzalez (Hospital Universitario 12 de Octubre, Madrid, Spain), Cinta Moraleda MD, Alfredo Tagarro MD, Elena Cobos MD, Álvaro Ballesteros MD, Sara Domínguez-Rodriguez (Instituto de Investigación 12 de Octubre, Madrid, Spain), Gemma Pons MD, Silvia Simó MD, Miguel Lanaspa MD, Victoria Fumadó MD, Rosa María Pino MD, María Melé (Hospital Sant Joan de Déu, Barcelon, Spain), María Espiau MD, Jacques G. Rivière MD, Pere Soler-Palacín MD, Antonio Soriano Arandes MD, Natalia Mendoza (Hospital Universitari Vall d'Hebron, Barcelona, Spain), Mercedes Herranz MD, María Urretavizcaya Martínez (Complejo Hospitalario de Navarra, Navarra, Spain), Fernando Cabañas MD, Fátima Ara MD, Marta Baragaño (Hospital Universitario Quirón salud Madrid, Spain), Rut del Valle MD, Ana González-de-Zárate MD, Mónica Pacheco MD, María Luisa Herreros MD, Julia Yebra MD, Beatriz Pérez-Seoane MD, María Fernández MD, Teresa Raga MD, María de la Serna MD, Ane Plazaola MD, Juan Miguel Mesa MD, Rosa Batista MD, Ana Barrios MD, Ignacio Navarro MD, Jana Rizo MD, Teresa Reinoso MD, Alfonso Cañete (Hospital Universitario Infanta Sofía, Madrid, Spain), María Dolores Martín MD, Elena Sáez MD, Olga Nerea Coya MD, Fernando Cava (BR Salud, Madrid, Spain), Enrique Otheo MD, Juan Carlos Galán MD, José Luis Vázquez MD, Carmen Vázquez MD, Victor Quintero (Hospital Universitario Ramón y Cajal, Madrid, Spain), Lola Falcón MD, Olaf Neth MD, Peter Olbrich MD, Walter Goicoechea MD, Cati Márquez MD, Marisol Camacho MD, Inés Marín Cruz (Hospital Universitario Virgen del Rocío, Sevilla, Spain), Laura Martín (Hospital Universitario Regional de Málaga, Málaga, Spain), Lucía Figueroa (Hospital de Villalba, Madrid, Spain), María Llorente (Hospital Universitario del Sureste, Madrid, Spain), María Penin MD, Claudia García MD, María García MD, Teresa Alvaredo (Hospital Universitario Príncipe de Asturias, Madrid, Spain), Mª Inmaculada Olmedo MD, Agustín López MD, María Jose Pérez (Hospital Universitario Puerta de Hierro, Madrid, Spain), Elvira Cobo (Hospital Fundación Alcorcón, Madrid, Spain), Mariann Tovizi (Hospital del Tajo, Madrid, Spain), Pilar Galán (Hospital Universitario de Fuenlabrada, Madrid Spain), Beatriz Soto MD, Sara Guillén (Hospital de Getafe, Madrid, Spain), Adriana Navas (Hospital Universitario Infanta Leonor, Madrid, Spain) M. Luz García (Hospital de Leganés, Madrid, Spain), Sara Pérez (Hospital de Torrejó, Madrid, Spain), Amanda Bermejo MD, Pablo Mendoza (Hospital de Móstoles, Spain), Gema Sabrido (Hospital Rey Juan Carlos, Madrid, Spain), María José Hernández (Hospital Central de la Defensa, Madrid, Spain), Ana Belén Jiménez (Fundación Jiménez Díaz, Madrid, Spain), Arantxa Berzosa MD, José Tomás Ramos MD, Marta Illán (Hospital Clínico San Carlos, Madrid, Spain), Ana López MD, Nerea Gallego (Hospital Universitari Son Espases, Mallorca, Spain), Beatriz Ruiz (Hospital Universitario Reina Sofía, Granada, Spain), Santiago Alfayate MD, Ana Menasalvas MD, Eloísa Cervantes (Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain), María Méndez (Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Barcelona, Spain), Ángela Hurtado (Instituto Hispalense de Pediatría, Sevilla, Spain), Cristina García MD, Inés Amich MD, Yolanda Ruiz (Hospital San Pedro, Logroño, Spain), Manuel Oltra MD, Álvaro Villarroya-Villalba MD, Adela Cañete MD, Bienvenida Argiles (Hospital Universitari i Politècnic La Fe, Valencia, Spain), Angustias Ocaña (Hospital La Moraleja, Madrid, Spain), Isabel Romero MD, María Fernanda Guzmán (Hospitales Madrid, Madrid, Spain), M.J. Pascual (Hospital Nisa, Madrid, Spain), Francisco Llorente de Gracia, Jovita Fernández-Pinero, Miguel Ángel Jiménez-Clavero, Pilar Aguilera-Sepúlveda, Cristina Cano-Gómez (Centro de Investigación en Sanidad Animal INIA-CSIC, Valdeolmos, Madrid, Spain), María Sánchez-Códez (Hospital Universitario Puerta del Mar, Cádiz, Spain), Elena Montesinos (Consorci Hospital General Universitari de València, Valencia, Spain), Julia Jensen MD, María Rodríguez (Hospital Universitario Infanta Cristina, Madrid, Spain), Gloria Caro (Hospital Universitario Infanta Elena, Madrid, Spain), Neus Rius MD, Alba Gómez (Hospital Universitari Sant Joan de Reus, Reus, Spain), Rafael Bretón (Hospital Clínico Universitario de Valencia, Valencia, Spain), Margarita Rodríguez MD, Julio Romero MD, Juan Francisco Pascual Gazquez (Hospital Universitario Virgen de las Nieves, Granada, Spain), Ana Campos (Hospital Universitario Sanitas La Zarzuela, Madrid, Spain), Mercedes García (Hospital de Mérida, Mérida, Spain), Rosa María Velasco (Complejo Hospitalario de Toledo, Toledo, Spain), Zulema Lobato Althaia MD, (Xarxa Assistencial Universitària de Manresa, Manresa, Spain), Fernando Centeno MD, Elena Pérez MD, Alfredo Cano (Hospital Universitario Río Hortega, Valladolid, Spain), Paula Vidal (Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain), Corsino Rey MD, Ana Vivanco MD, Maruchi Alonso (Hospital Universitario Central de Asturias, Oviedo, Spain), Pedro Alcalá MD, Javier González de Dios MD, Laura Ureña Horno (Hospital General Universitario de Alicante, Alicante, Spain), Eduard Solé MD, Laura Minguell (Hospital Universitari Arnau de Vilanova, Catalunya, Spain), Itziar Astigarraga MD, Olatz Villate MD, Susana García Obregón (Hospital Universitario de Cruces, Bilbao, Spain), Mª Ángeles Vázquez MD, Miguel Sánchez MD, Leticia Martínez Campos (Hospital Universitario Torrecárdenas, Almería, Spain), Elena Díaz (Hospital Virgen de la Luz, Spain), Eduardo Consuegra MD, Susana Riesco MD, Almudena González MD, Maika Mendoza (Hospital Universitario de Salamanca, Salamanca, Spain), María Cabanillas (Complejo Asistencial Universitario de Palencia, Palencia, Spain), Yeray Novoa-Medina MD, Elena Colino-Gil MD, Ana Reyes-Domínguez MD, Luis Peña-Quintana (Hospital Universitario Materno Infantil de las Palmas, Las Palmas, Spain), Elisa Garrote MD, Maite Goicoechea (Hospital Universitario de Basurto, Basurto, Spain), Irene Centelles (Hospital General Universitari de Castelló, Castelló, Spain), Santiago Lapeña MD, Sara Gutiérrez MD, Soraya Gutiérrez (Complejo Asistencial Universitario de León, León, Spain), Amparo Cavalle (PIUS Hospital de Valls, Valls, Spain), José María Olmos (Hospital Mare de Déu dels Lliris, Catalunya, Spain), Alejandro Cobo MD, Sara Díaz MD, Cristina Martinez Faci MD, Macarena Gonzalez Cruz MD, Beatriz Castro (Hospital Universitario de Canarias, Tenerife, Spain), Beatriz Jiménez MD, Cristina Alvarez Alvarez (Hospital Universitario Marqués de Valdecilla, Santander, Spain), Raúl González (Hospital Sant Joan d'Alacant, Alicante, Spain), Miguel Lafuente MD, Matilde Bustillo (Hospital Infantil de Zaragoza, Zaragoza, Spain), Natividad Pons MD, Julia Morata (Hospital Lluís Alcanyis, Catalunya, Spain), Elsa Segura (Hospital Universitario Son Llatzer de Palma de Mallorca, Spain), María Bernardino (Universidad Europea de Madrid, Spain), Marta Pareja León (Complejo Hospitalario Universitario de Albacete, Albacete, Spain), Ana Domingo Ruiz (Hospital de Manacor, Mallorca, Spain), Eider Oñate MD, Nagore García de Andoin Baran (Hospital Universitario Donostia, Donostia, Spain), Nerea Domínguez-Pinilla (Hospital Virgen de la Salud, Spain), María Teresa Coll Sibina (Hospital General de Granollers, Granollers, Spain), María Jesús García García (Hospital Universitario de Cáceres, Cáceres, Spain), Marta Osuna (Hospital HM Montepríncipe, Madrid, Spain), Raquel Portugal (Hospital Universitario de Burgos, Burgos, Spain), Leonor García Maset (Hospital de Sagunto, Valencia, Spain), Belén Sevilla (Hospital Universitario San Cecilio, Granada, Spain), Noelia Berciano Jiménez (Hospital Virgen Macarena, Sevilla, Spain).

Contributors AT, CM, EC-C and JMM designed the study, contributed to the collection and interpretation of the data, drafted the initial manuscript and reviewed the final version. SD-R and AB designed the study, made the statistical analysis and drafted the initial manuscript, and reviewed the final version. FL, JF-P, EP-R and II carried out the viral cultures, and JCG and JL-F carried out the sgRNA analysis. PGS, MdlS, JAA-C, AB, GS, LM-C, AFG-P, MI-R, DA-A, ABJ, PC, ED, IN, BF-G, PM, CP-J, TC-F, DB-G, GR, PG-D, PM, SM, and JL contributed to the collection and interpretation of the data, the creation of the manuscript and reviewed the final version. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work. AT and CM accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

Funding The study was funded by: Project PI20/00095, from the Instituto de Salud Carlos III (Ministry of Economy, Industry and Competitiveness) and cofounded by the European Regional Development Fund, by Infanta Sofia University Hospital and Henares University Hospital Foundation for Biomedical Research and Innovation (FIIB HUIS HHEN), and by SERMAS-Fundación para la Investigación Biomédica del Hospital 12 de Octubre. EC-C is supported by the Spanish Society of Paediatrics (Asociación Española de Pediatría); Grant COVID-19 EPICO-AEP 2020. JMM is funded by SERMAS-Fundación para la Investigación Biomédica del Hospital Infanta Sofía y del Henares and by Universidad Europea de Madrid, Spain. MdlS is funded by Grant Cantera de Investigación Santander, Fundación Universidad Europea de Madrid, Spain. ED is funded by the Juan de la Cierva–Incorporación granted by the Spanish Ministry of Science and Innovation. DB-G is funded by the Spanish Ministry of Science and Innovation—Instituto de Salud Carlos III and Fondos FEDER by 'Contratos para la intensificación de la actividad investigadora en el Sistema Nacional de Salud, 2020 (INT20/00086)'.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by Ethics Committee of the "Fundación de Investigación Biomédica del Hospital 12 de Octubre" (code 20/101) and by the Ethics Committees of the other participating hospitals. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Not applicable.

ORCID iDs
Cinta Moraleda http://orcid.org/0000-0002-8335-903X
José Antonio Alonso-Cadenas http://orcid.org/0000-0002-7648-1336
David Aguilera-Alonso http://orcid.org/0000-0003-1017-2386
Daniel Blázquez-Gamero http://orcid.org/0000-0001-6483-8329
Alfredo Tagarro http://orcid.org/0000-0003-2860-3455

REFERENCES

1 Corman VM, Landt O, Kaiser M, *et al*. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* 2020;25.
2 Clark JH, Pang S, Naclerio RM, *et al*. Complications of nasal SARS-CoV-2 testing: a review. *J Investig Med* 2021;69:jim-2021-001962:1399–403.
3 Rigante M, Picciotti PM, Parrilla C. A nasal complication of nasopharyngeal swab for reverse transcription polymerase chain reaction (RT-PCR) detection of SARS-CoV-2 mRNA. *Cureus* 2021;13:e16183.
4 Butler-Laporte G, Lawandi A, Schiller I, *et al*. Comparison of saliva and nasopharyngeal swab nucleic acid amplification testing for detection of SARS-CoV-2: a systematic review and meta-analysis. *JAMA Intern Med* 2021;181:353.
5 Han MS, Seong M-W, Kim N, *et al*. Viral RNA load in mildly symptomatic and asymptomatic children with COVID-19, Seoul, South Korea. *Emerg Infect Dis* 2020;26:2497–9.
6 Chong CY, Kam K-Q, Li J. Saliva is not a useful diagnostic specimen in children with coronavirus disease 2019 (COVID-19). *Clin Infect Dis* 2020:ciaa1376.
7 Yee R, Truong TT, Pannaraj PS, *et al*. Saliva is a promising alternative specimen for the detection of SARS-CoV-2 in children and adults. *J Clin Microbiol* 2021;59. doi:10.1128/JCM.02686-20. [Epub ahead of print: 21 01 2021].
8 Borghi E, Massa V, Carmagnola D, *et al*. Saliva sampling for chasing SARS-CoV-2: a Game-changing strategy. *Pharmacol Res* 2021;165:105380.
9 Ana Laura G-O, Abraham Josué N-R, Briceida L-M, *et al*. Sensitivity of the molecular test in saliva for detection of COVID-19 in pediatric patients with concurrent conditions. *Front Pediatr* 2021;9:642781.
10 Al Suwaidi H, Senok A, Varghese R, *et al*. Saliva for molecular detection of SARS-CoV-2 in school-age children. *Clin Microbiol Infect* 2021;27:1330–5.
11 Ministerio de Salud, Dirección General de Salud Pública. *Centro de Coordinación de Alertas Y, Emergencias Sanitarias. Gobierno de España. Actualización no 331. Enfermedad POR El coronavirus (COVID-19)*, 2021.
12 Bossuyt PM, Reitsma JB, Bruns DE, *et al*. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ* 2015;351:h5527.
13 Harris PA, Taylor R, Thielke R, *et al*. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–81.
14 Harris PA, Taylor R, Minor BL, *et al*. The REDCap Consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
15 Lim C, Wannapinij P, White L, *et al*. Using a web-based application to define the accuracy of diagnostic tests when the gold standard is imperfect. *PLoS One* 2013;8:e79489.
16 Dendukuri N, Joseph L. Bayesian approaches to modeling the conditional dependence between multiple diagnostic tests. *Biometrics* 2001;57:158–67.
17 Subirana I, Sanz H, Vila J. Building Bivariate Tables: The compareGroups Package for *R*. *J Stat Softw* 2014;57.
18 et alKuhn M, Wing J, Weston S. Caret: classification and regression training, 2021. Available: https://CRAN.R-project.org/package=caret [Accessed 25 Feb 2022].
19 Kohl M. MKmisc: miscellaneous functions from M. Kohl, 2021. Available: https://CRAN.R-project.org/package=MKmisc [Accessed 25 Feb 2022].
20 Cohen R, Jung C, Ouldali N, *et al*. Assessment of SARS-CoV-2 infection by reverse transcription-PCR and serology in the Paris area: a cross-sectional study. *BMJ Paediatr Open* 2020;4:e000887.
21 De Virgilio A, Costantino A, Mercante G, *et al*. How to increase the SARS-CoV-2 detection rate through the nasopharyngeal swab? *Oral Oncol* 2020;106:104802.
22 Dramé M, Tabue Teguo M, Proye E, *et al*. Should RT-PCR be considered a gold standard in the diagnosis of COVID-19? *J Med Virol* 2020;92:2312–3.
23 World Health Organization (WHO). Antigen-detection in the diagnosis of SARS-CoV-2 infection: interim guidance, 2021. Available: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays
24 Fougère Y, Schwob JM, Miauton A, *et al*. Performance of RT-PCR on saliva specimens compared with nasopharyngeal swabs for the detection of SARS-CoV-2 in children: a prospective comparative clinical trial. *Pediatr Infect Dis J* 2021;40:e300–4.
25 To KK-W, Tsang OT-Y, Leung W-S, *et al*. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020;20:565–74.
26 Vogels CBF, Watkins AE, Harden CA, *et al*. SalivaDirect: a simplified and flexible platform to enhance SARS-CoV-2 testing capacity. *Med* 2021;2:263–80.
27 Siegler AJ, Hall E, Luisi N, *et al*. Willingness to seek diagnostic testing for SARS-CoV-2 with home, Drive-through, and clinic-based specimen collection locations. *Open Forum Infect Dis* 2020;7:ofaa269.
28 Plantamura J, Bousquet A, Otto M-P, *et al*. Performances, feasibility and acceptability of nasopharyngeal swab, saliva and oral-self sampling swab for the detection of severe acute respiratory syndrome coronavirus 2. *Eur J Clin Microbiol Infect Dis* 2021;40:2191–8.

Oral Saliva Swab RT-PCR for COVID-19 in the Pediatric Population

SUPPLEMENTARY DOCUMENT

Analysis of discordant patients

In order to study the infectivity of the patients with discordant diagnostic test results (37 patients) from any of the three different tests, additional analyses were performed.

The leftover original samples were sent to the Microbiology Department of the Ramón y Cajal Hospital (Madrid, Spain) for testing viral viability based on subgenomic RNA (sgRNA) determination (as a biological marker of replication viral [1]. SgRNA was determined as a surrogate marker for viral replication [1,2]. As a control for this assay, the genomic RNA (gRNA) was also simultaneously detected using in both cases the gene E as amplification target. RNA was extracted from samples by using an automated system (KingFisher Flex, ThermoFisher Scientific, Waltham, MA, USA). SgRNA was detected with an in-house RT-PCR assay, using primers sg_E_F (5'-CGATCTCTTGTAGATCTGTTCTC-3') and sg_E_R (5'-ATATTGCAGCAGTACGCACACA-3'), and the probe E_P1 (5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-3BHQ-3' following a previously described protocol)[3].

Additionally, patients with discordant results were contacted and requested to visit the hospital again in less than 24 hours after the researchers were aware of the results and less than 48 hours from the first sample. New nasopharyngeal (NPS) (or oral saliva for those with only oral saliva swab (OSS) positive) samples were then collected and placed into a viral culture medium. These new samples were stored at -80 °C and sent to the Animal Health Research Center (INIA-CISA) (Madrid, Spain) for viral isolation after a new genomic detection of SARS-CoV-2 using an in-house developed kit.

Before culturing a second genomic detection of SARS-CoV-2 was performed. RNA was extracted from 200µl of NPS or OSS samples using the IndiSpin®Pathogen kit (Indical Bioscience, GmbH, Leipzig, Germany). An in-house triplex RT-PCR method was applied targeting the SARS-CoV-2 N gene (based on N1 protocol described by CDC) using β-actin gene and a synthetic extraction control (EGFP) as validation controls. Samples reporting a Ct ≤35 for SARS-CoV-2 either at hospitals (first sampling) or INIA-CISA (second sampling) were subjected to viral isolation [4]. Culturing for viral isolation was performed by placing 100µl of clinical specimens onto Vero E6 cells in 12-wells culture plates. Cells were incubated at 37ºC with 5% CO_2 and were observed for cytopathic effect (CPE) daily up to 7 days post-inoculation. Wells with CPE were collected and the supernatants of the remaining wells (without CPE) were subjected to two additional passages in Vero E6. RT-PCR analysis was done from the supernatant after each passage to follow the progress of virus culture and to confirm the success of viral isolation in the wells showing CPE.

Among the 37 patients with discordant results 23 performed sgRNA determination and 20 had available sample for viral isolation. Results of discordant patients are summarized in Table S1.

Patient	Age	Gender	Signs and Symptoms	Days with symptoms	First Sampling On each site			RNA subgenomic analysis			Viral isolation			Serology
					Antigen rapid test	Sample	RT-PCR, Ct	Sample	RT-PCR, Ct	SgRNA	Sample	RT-PCR, Ct	Viral isolation	
1	9y	F	Headache, Fever	1	Neg	NPS OSS	38 Neg	NPS	Neg	Neg	NPS	Neg		Neg
2	9y	M	Headache	1	Neg	NPS OSS	38 Neg	NPS	Neg	Neg	NPS	37.7		Neg
3	7y	F	Fever, Sore throat	1	Pos	NPS OSS	28 Neg	NPS	31	Neg				NA
4	5m	F	Fever, Sore throat, Runny nose, abdominal pain, Headache	1	Neg	NPS OSS	40 36	NPS	Neg	Neg	NPS	Neg		Neg
5	15y	M	Fever, Sore throat, Runny nose, Cough, Vomiting/ Nausea, Headache, Fatigue/Malaise, Myalgia	1	Neg	NPS OSS	40 Neg	NPS	Neg	Neg	NPS	Neg		Neg
6	2y	M	Fever, Vomiting/Nausea	1	Neg	NPS OSS	34 Neg	NPS	Neg	Neg	NPS	Neg	Neg	Pos
7	17y	F	Cough, Runny nose	1	Neg	NPS OSS	28 Neg	NPS	Neg	Neg	NPS	Neg	Neg	Neg
8	10y	F	Fever	1	Pos	NPS OSS	20 Neg	NPS	23	Pos 25				NA
9	10y	M	Fever, Myalgia, Abdominal pain	2	Neg	NPS OSS	37.9 Neg	NPS OSS	Neg	Neg	NPS	Neg		NA
10	3y	M	Abdominal pain, Vomiting/Nausea, Diarrhea	3	Neg	NPS OSS	37.9 Neg	NPS OSS	Neg	Neg	NPS	Neg		Pos
11	15y	M	Sore throat, Runny nose, Headache, Fatigue/Malaise, Myalgia	2	Neg	NPS OSS	32.9 34	NPS OSS	Neg Neg	Neg Neg	NPS OSS	18.01 29.97	Pos Neg	Neg
12	4y	F	Fever, Cough, Runny nose	1	Pos	NPS OSS	25.3 Neg	NPS OSS	29	Neg				NA

Patient	Age	Gender	Signs and Symptoms	Days with Symptoms	First Sampling RT-PCR			First sampling Ref center 1			Second sampling Ref center 2			Serology
					Antigen rapid test	Sample	RT-PCR, Ct	Sample	RT-PCR, Ct	SgRNA	Sample	RT-PCR, Ct	Viral isolation	
13	10y	F	Fever, Sore throat, Runny nose, Cough	2	Pos	NPS OSS	21.1 Neg	NPS OSS	22	Pos 24	NPS	30.9	Neg	NA
14	12y	F	Fever, Sore throat, Runny nose, Cough	2	Neg	NPS OSS	21.1 31.8	NPS OSS	30 26	Neg Pos 29	NPS	36.59	Neg	Neg
15	14y	F	Sore throat, Cough, Headache, Myalgia	3	Pos	NPS OSS	17.9 Neg	NPS OSS	23	Pos 26	NPS	18.32	Pos	Neg
16	6y	M	Fever	1	Neg	NPS OSS	36.2 28.1	NPS OSS	Neg 30	Neg Neg	NPS	16.54	Pos	Neg
17	2y	M	Cough	2	Neg	NPS OSS	35 neg.	NPS	Neg	Neg	NPS	37.99		Pos
18	20 m	M	Fever, Vomiting/Nausea	1	Neg	NPS OSS	neg. 30	OSS	Neg	Neg	OSS	Neg	Neg	Pos
19	14y	M	Headache	2	Neg	NPS OSS	35 neg.	NPS OSS	Neg	Neg	NPS OSS	Neg		Pos
20	12y	F	Headache	5	Neg	NPS OSS	neg. 31	OSS	Neg	Neg	OSS	Neg	Neg	Neg
21	3y	M	Fever, Wheezing, Dyspnea	5	Neg	NPS OSS	neg. 36	OSS	Neg	Neg	OSS	Neg		Pos
22	4y	M	Sore throat, Runny nose, Cough	3	Neg	NPS OSS	30 neg.	NPS	Neg	Neg	NPS	27.12	Neg	Neg
23	8y	F	Sore throat	1	Neg	NPS OSS	18 30	NPS OSS	23 Neg	Pos 25 Neg	NPS OSS	24 37.09	Pos Neg	Neg

Table S1. Characteristics and test results of discordant patients. y: years; m: months; M: male; F: female; NPS: nasopharyngeal swab; OSS: oral saliva swab; sgRNA: subgenomic RNA; RT-PCR: reverse transcription-polymerase chain reaction; Ct: cycle threshold; pos: positive; neg: negative; ND: not available.

REFERENCES

1 Wölfel R, Corman VM, Guggemos W, *et al.* Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;**581**:465–9. doi:10.1038/s41586-020-2196-x

2 Perera RAPM, Tso E, Tsang OTY, *et al.* SARS-CoV-2 Virus Culture and Subgenomic RNA for Respiratory Specimens from Patients with Mild Coronavirus Disease. *Emerg Infect Dis* 2020;**26**:2701–4. doi:10.3201/eid2611.203219

3 Liotti FM, Menchinelli G, Marchetti S, *et al.* Assessment of SARS-CoV-2 RNA Test Results Among Patients Who Recovered From COVID-19 With Prior Negative Results. *JAMA Intern Med* 2021;**181**:702. doi:10.1001/jamainternmed.2020.7570

4 Bullard J, Dust K, Funk D, *et al.* Predicting Infectious Severe Acute Respiratory Syndrome Coronavirus 2 From Diagnostic Samples. *Clinical Infectious Diseases* 2020;**71**:2663–6. doi:10.1093/cid/ciaa638