Addition of saliva to oropharyngeal and deep nasal swabs increases PCR detection of SARS-CoV-2 in paediatric and primary care settings, Victoria, Australia July–October 2020

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The known

- Real-time polymerase chain reaction testing of oropharyngeal/bilateral deep nasal swabs (ONS) is standard-of-care for SARS CoV-2 detection in Australia. No gold standard specimen for SARS-CoV-2 detection exists.

The new

- Testing paired saliva in addition to an ONS increased case detection by 59%. Among 54 COVID-19 cases, positive test concordance was 35% but was only 11% among 19 cases under 10 years-old. Saliva was preferred to ONS by 79% of participants.

The implications

- Adding paired saliva testing to ONS testing increased case detection. Saliva might be suitable as a stand-alone test specimen for people aged 10 years and older.

Abstract

Objective

To compare the concordance and acceptability of saliva to standard-of-care oropharyngeal/bilateral deep nasal swab (ONS) testing for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) detection in paediatric and general practice.

Design

Prospective multi-centre diagnostic validation study.

Setting

Royal Children's Hospital Melbourne and two general practices (cohealth West Melbourne and Circle Health Altona North) in Melbourne, Australia from July–October 2020.

Participants

1,050 participants provided paired saliva and ONS samples; 17% were under 10 years-old.

Main outcome measures

Cases had SARS-CoV-2 detected in saliva and/or ONS using real-time polymerase chain reaction. The concordance of paired saliva and ONS testing and the positive percent agreement (PPA) values for case detection were calculated. A participant survey indicated preferences for saliva or ONS.

Results

SARS-CoV-2 was detected in 54/1,050 (5%) participants. Paired samples were concordant for 19/54 (35%) cases. PPA was 72% [95%CI 58–84%] for saliva and 63% [49–76%] for ONS (p=0.398) overall. PPA was 86% [95%CI 70–95%] and 63% [45–79%] for saliva and ONS, respectively, for cases aged 10 years and over (p=0.059). Among 19 cases aged under 10 years, 2 (11%) had concordant sample results. Adding a saliva sample to standard-of-care ONS testing increased overall case detection by 59% [95%CI 29–95%]. Saliva was preferred to ONS by 79% of participants, including 92% of children under 10 years-old.

Conclusion

Among cases aged 10 years and over, saliva may be suitable as a stand-alone test specimen. For those under 10 years-old, saliva supplemented ONS testing to increase case detection.

Introduction

Australian guidelines recommend severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) testing using real-time polymerase chain reaction (RT-PCR) with an oropharyngeal and bilateral deep nasal swab (ONS).[1] Saliva is less invasive than ONS and can easily be self-collected, reducing healthcare worker exposure to SARS-CoV-2.[2] Four meta-analyses reported the pooled sensitivity of saliva for RT-PCR SARS-CoV-2 detection as 84–86% compared to upper respiratory swabs.[3-6] One of these meta-analyses with data from 12 cohorts reported overall concordance between saliva and upper respiratory swabs of 92.1% (Cohen's kappa coefficient 0.840, 95% confidence interval [CI] 0.805–0.874).[7] Saliva and upper respiratory swab collection and processing may affect test reliability.[2] Most studies assessed self-collected saliva dribble or posterior oropharyngeal saliva. The comparator upper respiratory swab specimen was usually a nasopharyngeal swab (NPS). Other differences included variable use of transport medium; sample volumes; timing/severity of illness; different assays; and comparator swab collection technique.

Upper respiratory swab collection may cause discomfort and reduce test uptake.[8] Saliva testing presents a less invasive means of case detection. Two small studies of children with coronavirus disease 2019 (COVID-19) reported peak saliva sensitivity (compared to NPS) of 52%–80% in the first week after onset.[9,10] Another study of 43 COVID-19 cases aged 4–18 years reported the positive percent agreement (PPA) for saliva was 79.1% and for NPS was 88.4%.[11] A study with 170 children reported PPA of 93.3% for saliva and 84.4% for NPS.[12] An additional three studies reported similar SARS-CoV-2 detection between saliva and NPS in children.[13-15] The diagnostic value of saliva appears promising although further validation, including by age group, is needed.

In Australia, respiratory clinics were established in general practice (GP) and hospitals to provide free SARS-CoV-2 testing to anyone meeting testing criteria.[16] We compared the concordance and acceptability of saliva to standard-of-care ONS testing in primary care and paediatric settings.

Methods

We conducted a multi-centre diagnostic validation study with participants recruited from three respiratory clinics in Melbourne, Victoria, in paediatric and general practice. Recruitment coincided with Victoria's 'second wave' of SARS-CoV-2 infections.[17] The study uses the STARD 2015 guidelines for reporting.[18]

Eligibility. Patients of any age who met SARS-CoV-2 testing criteria at a participating respiratory clinic were eligible. Following informed consent, participants provided a saliva specimen in addition to the

standard-of-care diagnostic ONS. Recruiting close contacts of known COVID-19 cases was prioritised and some known COVID-19 cases were recruited.

Royal Children's Hospital, Melbourne (recruitment period 21/07/2020–18/09/2020): Research nurses undertook recruitment and specimen collection. Saliva was collected using a SalivaBio Swab and Storage Tube (Stratech Scientific APTY LTD) for children aged under five years. People aged five years and older were asked to dribble at least 2mL of saliva into a collection pot without transport media. All participants received a standard-of-care ONS (dry FLOQSwabs®, Copan, Brescia, Italy). The specimens were tested at the Royal Children's Hospital diagnostic molecular microbiology laboratory. Swabs were eluted into 500µL of phosphate buffered saline (PBS). Saliva collected using the SalivaBio system was extracted neat and all other saliva samples were diluted 1:1 with PBS. Nucleic acids from 200µL of the saliva and swab samples were extracted using the Roche MagNA Pure 96 extraction system (Roche, Basel, Switzerland). Extracts were tested using the LightMix® Modular SARS and Wuhan CoV E-gene kit (TIB Molbiol, Berlin, Germany) on the Roche LightCycler 480 II Real-Time PCR System. Initial E-gene positive sample extracts were confirmed using the AusDiagnostics Respiratory Pathogens 16-well assay (AusDiagnostics, Mascot, Australia) system (targeting ORF-1 and ORF-8 genes), on the AusDiagnostics High-Plex 24.

General practice: Recruitment occurred at cohealth West Melbourne, a fixed-site respiratory clinic (27/07/2020–18/09/2020), and Circle Health Altona North, a GP respiratory clinic with drive-through facility (18/09/20–2/10/20). Clinic staff recruited participants, performed a standard-of-care ONS, and instructed participants to dribble at least 2mL of saliva into a collection pot without transport media. Saliva and ONS underwent SARS-CoV-2 testing at Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL) within 48 hours of collection. Saliva was diluted 1:4 in saline and dry swabs were resuspended into 3ml saline for testing using the Aptima SARS-CoV-2 assay and/or Panther Fusion SARS-CoV-2 assay (Hologic, Marlborough, Massachusetts, USA).

Study surveys. Participants and/or their guardians were invited to complete a survey regarding symptoms and saliva and ONS collection preference. Surveys were administered by research nurses at Royal Children's Hospital, who also collected exposure risk factor data. At GP sites, an online REDCap survey was accessed via a Quick Response (QR) code (cohealth West Melbourne) or texted to participants' mobile phones (Circle Health Altona North).

Study definitions. A specimen was classified as positive if two or more different SARS-CoV-2 gene targets were detected, consistent with national guidelines.[1] A COVID-19 case was defined as a participant with positive saliva and/or positive ONS; all positive tests were considered true positives.

Specimens with an indeterminate, invalid or missing result (including samples with insufficient saliva volume for testing) were classified as 'non-assessable' and excluded from analysis.

Statistical analysis. The target sample size was 38 COVID-19 cases, based on a required precision of ±9% and an assumed kappa statistic of 0.84.[19] The percent positive agreement [PPA] was reported as the proportion of all COVID-19 cases with SARS-CoV-2 detected on each specimen type (saliva and ONS). Concordance of SARS-CoV-2 detection between specimen pairs was reported as kappa and overall percentage agreement. Among cases, ONS and saliva positive proportions and exact binomial 95% CIs were calculated and compared using McNemar's test, both overall and by subgroup. Ratios of cases detected using paired samples (ONS and saliva) versus a single sample (ONS or saliva) were also reported with 95% CIs and p-values obtained from McNemar's test.

A p-value of <0.05 was considered statistically significant. Analyses were undertaken using STATA version 16.1 (Statacorp, Texas).

Ethics. Ethics approval was obtained from the Royal Children's Hospital Human Research Ethics Committee (HREC/65175/RCHM-2020; 26/06/2020).

Results

Non-assessable test results and exclusions: 1,165 participants were recruited, of whom 115 (10%) were excluded due to a non-assessable result: 110 (9%) saliva specimens were non-assessable, including 86/110 (78%) with insufficient volume for testing and four (4%) which leaked (Supplementary Figure).

Participant baseline data: Of 1,050 included participants, 176 (17%) were children under 10 years-old and 749 (71%) were recruited from general practice (Table 1). Fifty-four participants (5%) had SARS-CoV-2 detected in saliva and/or ONS and were classified as COVID-19 cases. Cases' median age was 27.6 years (range 6 months–65 years), 30 (56%) were male, and 22/38 (58% of those with risk factor data) were a close contact of a confirmed COVID-19 case. At the time of specimen collection 19/38 (50% of cases with symptom data) reported symptoms, most commonly cough (9/38, 24%; Table 1). Of the 11 symptomatic cases with known symptom duration, 10 (91%) had symptom duration of less than 4 days at specimen collection (median 2 days [range 0–12 days]).

Percent positive agreement: The overall PPA was 72% [95%CI 58–84%] for saliva and 63% [95%CI 49–76%] for ONS (p=0.398) (Table 2, Supplementary Table). For cases aged 10 years and older the PPA was 86% [95%CI 70–95%] for saliva and 63% [95%CI 45–79%] for ONS (p=0.059). For the 19

paediatric cases aged under 10 years, the PPA was 47% [95%CI 24–71%] for saliva and 63% [95%CI 38–84%] for ONS (p=0.467).

Comparison of paired vs. single specimen for COVID-19 case detection: Saliva and ONS tests were concordant in 19/54 (35%) COVID-19 cases (Table 1); saliva alone was positive for 20 (37%) and ONS alone was positive for 15 (28%) cases. Adding a paired saliva specimen to standard-of-care ONS testing increased total case detection by 59% [95%CI 29–95%] (Figure), and by 90% [95%CI 24–191%] among asymptomatic cases and 100% [29–210%] among female cases. Adding a paired ONS to a single saliva specimen increased total case detection by 38% [95%CI 17–63%] overall but only by 17% [95%CI 2–34%] among cases aged 10 years and older.

Paediatric COVID-19 cases: Among the 19 paediatric cases aged under 10 years, seven (37%) were symptomatic at the time of specimen collection, including 4/9 (44%) with positive saliva and 4/12 (33%) of children with positive ONS. Only 2 (11%) cases aged under 10 years had concordant results (Table 1); total case detection among children aged under 10 years was increased by 58% [95%CI 12–123%] with addition of paired saliva to standard-of-care ONS and 111% [95%CI 31–239%] with addition of paired ONS to saliva testing (Figure). Of the 21 cases aged under 18 years, two (10%) had concordant results; 8 (38%) had positive saliva only and 11 (52%) had positive ONS only (Table 1). Among the four COVID-19 cases aged under 1 year, SARS-CoV-2 was detected from ONS for all four cases but from saliva in only one (25%) case (p=0.250).

Analysis of test concordance: Overall, 1,015/1,050 (97%) paired test results were concordant, with a Cohen's kappa statistic of 0.50 (95% CI: 0.36–0.65). The kappa statistic for children under 10 years-old was 0.14 (95% CI: -0.09–0.37), compared to 0.64 (95% CI: 0.49–0.80) for people aged 10 years and older.

Specimen preference: Saliva was preferred to ONS by 79% of all participants and 92% of children aged under 10 years (Table 3). Overall, 16% of participants had no preference and 9% preferred ONS.

Discussion

Accurate identification of individuals with COVID-19 is essential for case management and preventing transmission. We found that addition of saliva to the standard-of-care ONS increased case detection by 59%. Saliva had similar PPA to ONS overall, however saliva testing alone would have missed 53% of cases aged under 10 years.

Providing saliva was preferred to ONS by more than three-quarters of participants including more than 90% of children aged under 10 years. Saliva was considered more comfortable, more convenient, and preferable to self-collected NPS in a Singaporean study.[20] An online discrete choice experiment survey of 4,793 adults reported that NPS may be a deterrent to testing, which could be mitigated by using saliva specimens.[21] Saliva testing may be of particular benefit for people unwilling or incapable of providing an ONS and in settings where repeat testing is desired, such as higher-risk workplaces.

We estimated the PPA for ONS and saliva as a proxy for test sensitivity in the absence of a gold standard. Test concordance in our study was low (35%), with a lower PPA for saliva and upper respiratory swabs than in many other studies. The comparator specimen in our study was ONS, consistent with Australia's national guidelines,[22] rather than NPS used in many studies. The lower ONS PPA in our study might be due lower sensitivity than NPS, although one meta-analysis indicated pooled throat and nasal swabs had a sensitivity of 97% [95% CI 93–100] compared to NPS.[6] Our positive concordance was very low (10%) among cases aged under 18 years. Our positive concordance among adult cases (52%) was similar to a Canadian study of 70 adult cases testing saliva and either ONS/NPS (49%), but lower than a UK study with ONS as the comparator (74%).[23, 24]

Our study has important differences to other paediatric studies, including a different comparator respiratory specimen. In a study of 11 children with COVID-19 diagnosed on NPS, overall sensitivity of saliva was 73%, falling from 80% (week 1) to 11% (week 3).[9] A study of 18 children with COVID-19 diagnosed on NPS demonstrated peak sensitivity of saliva (52.9%) at days 4–7.[10] The denominator for these sensitivity calculations was children with a positive NPS, assumed to be a "gold-standard" test. As our study included 176 suspected COVID-19 cases aged under 10 years (and 212 aged under 18 years) we could more fully elucidate the role of saliva for diagnosing COVID-19. We included cases who tested positive on ONS and/or saliva in our denominator when calculating PPA, acknowledging that there is no gold-standard SARS-CoV-2 test. Among children aged under 10 years, 37% of COVID-19 cases would be missed if a paired saliva specimen was not added to ONS testing, which reflects some previous research findings in children.[13, 14]

Several studies using NPS as the comparator reported higher PPA among paediatric COVID-19 cases compared to our study for saliva (80.0–93.9%[11, 12, 15] versus 47%) and upper respiratory swab (86.7% versus 63%).[11] Of note, we included 14 cases under 4 years-old while nearly all cases in other studies were aged 4 years and older. We found addition of ONS to saliva more than doubled case detection among children aged under 10 years. Our findings suggest the best role for saliva

among paediatric suspected cases is in addition to, rather than in place of, standard-of-care ONS. Among people aged 10 years and older, addition of ONS to saliva testing increased case detection by only 17%, supporting the use of saliva in place of standard-of-care ONS in this age group.

Our study was implemented rapidly at all three recruiting centres, including a drive-through testing clinic. An important limitation was that 9% of saliva specimens were non-assessable, most frequently due to inadequate saliva volume. A prior study showed at least one-third of pure saliva specimens were difficult to pipette.[25] A much lower invalid specimen rate (0.03%) was observed when saliva was collected using a straw-like device, with centrifugation and addition of proteinase K.[26] Other options to reduce the invalid specimen rate include participant education, marking the required volume on the collection pot, or using alternative collection devices.[27] Where saliva volume was adequate for testing, it is possible that the higher SARS-CoV-2 detection related to the larger sample volume compared to ONS.

This was a 'real life' study assessing saliva collection and testing among symptomatic and asymptomatic individuals in multiple settings. In low-incidence settings, it can be necessary to use targeted sampling to increase the number of cases and increase study power. As COVID-19 incidence was dropping quickly during our recruitment period, we targeted known cases and their close contacts for recruitment to achieve our required sample size. It was not possible to synchronise procedures/recruitment between sites due to staff workloads and the embedded nature of specimen processing and different PCR platforms in the study laboratories. RT-PCR analysis occurred in two separate laboratories using different assay platforms. Results from assays with different detection parameters were pooled. We assumed all participants with SARS-CoV-2 detected were COVID-19 cases and did not account for the possibility of false positive results. Our saliva collection methods differed for RCH participants aged under 5. This limits the comparability of findings between study sites for this age group. The small number of paediatric cases aged 5–17 years (n=6) is insufficient to inform COVID-19 testing guidelines for this age-group. Further validation regarding the role of saliva in COVID-19 diagnosis in children is warranted.

When testing for SARS-CoV-2, using paired saliva and ONS offers the best chance to detect cases, particularly among children aged under 10 years. In people aged 10 years and older, testing saliva alone might be appropriate due to individual's preferences for saliva testing and adequacy of SARS-CoV-2 detection using saliva.

Table 1. Results of SARS-CoV-2 testing for all participants, by subgroup and specimen type

Swab is an oropharyngeal and bilateral deep nasal swab (ONS); 1. Percent of all participants with included data; 2 Percent of participants with that feature present; 3 Percent of cases with that feature present; †Participants recruited from Royal Children's Hospital only, no participants reported recent international travel; ‡ less frequent symptoms include: myalgia 31 (6.0%), diarrhoea 15 (2.9%), dyspnoea in 12 (2.3%) participants; anosmia 4 (0.8), confusion 3 (0.6%), and dysgeusia 2 (0.4%)

Table 2. Positive percent agreement (PPA) of saliva and swabs among COVID-19 cases

Swab is a combined oropharyngeal and bilateral deep nasal swab (ONS)
p-values describe the difference in positive proportions between saliva and swab test results using McNemar's test. Exact binomial 95% confidence intervals are provided for each positive proportion
* one-sided 95% confidence interval

Table 3. Participant preference regarding specimen type

Swab is a combined oropharyngeal and bilateral deep nasal swab (ONS)

Figure 1. Ratio of cases detected by paired specimens [saliva and ONS] vs. single specimen (1a. paired specimens vs. ONS alone; 1b. paired specimens vs. saliva alone)

Swab is a combined oropharyngeal and bilateral deep nasal swab (ONS)
Bars represent 95% confidence intervals.
95% confidence intervals and p-values obtained from McNemar's test.

Supplementary Table. 2x2 tables of SARS-CoV-2 PCR results for swabs and saliva, by age-group and testing laboratory

Swab is a combined oropharyngeal and bilateral deep nasal swab (ONS)

Supplementary Figure. Participant recruitment and reasons for exclusion

Swab is a combined oropharyngeal and bilateral deep nasal swab (ONS)

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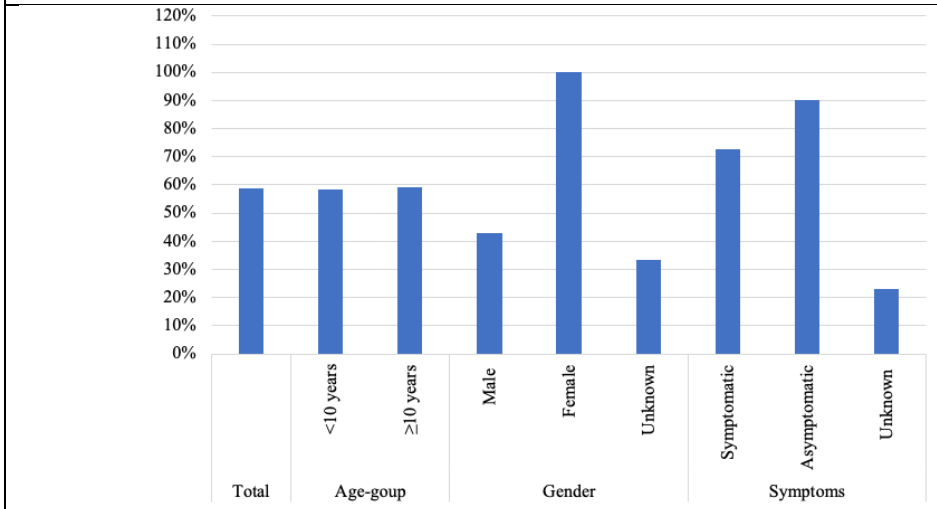
Table 1. Results of SARS-CoV-2 testing for all participants, by subgroup and specimen type

		SARS-CoV-2 test result		SARS-CoV-2 detection among cases		
		Not detected	Detected (case)	Concordant positives	Saliva only (Swab false-negative)	Swab only (Saliva false-negative)
	N (%)[1]	N (%)[2]	N (%)[2]	N (%)[3]	N (%)[3]	N (%)[3]
Total	1,050 (100)	996 (95)	54 (5)	19 (35)	20 (37)	15 (28)
Laboratory / site						
RCH lab / Paediatric site	301 (28.7)	261 (87)	40 (13)	11 (28)	17 (43)	12 (30)
MDU lab / Primary care site	749 (71)	735 (98)	14 (2)	8 (57)	3 (21)	3 (21)
Primary care clinic type (n=749)						
fixed site	575 (77)	563 (98)	12 (2)	7 (58)	2 (17)	3 (25)
drive-through	174 (23)	172 (99)	2 (1)	1 (50)	1 (50)	0 (0)
Age-group, years						
<5	129 (12)	114 (88)	15 (12)	2 (13)	5 (33)	8 (53)
5–9	47 (5)	43 (92)	4 (9)	0 (0)	2 (50)	2 (50)
10–17	36 (3)	34 (94)	2 (6)	0 (0)	1 (50)	1 (50)
≥18	838 (80)	805 (96)	33 (4)	17 (52)	12 (36)	4 (12)
Gender						
Male	375 (36)	345 (92)	30 (8)	12 (40)	9 (30)	9 (30)
Female	432 (41)	412 (95)	20 (5)	4 (20)	10 (50)	6 (30)
Unknown	243 (23)	239 (98)	4 (2)	3 (75)	1 (25)	0 (0)
Risk factors (n=298[†])						

Close contact	168 (56)	146 (87)	22 (13)	3 (14)	10 (46)	9 (41)
Healthcare worker in household	12 (4)	9 (75)	3 (25)	0 (0)	2 (67)	1 (33)
Recent positive test	22 (7)	12 (55)	10 (46)	6 (60)	1 (10)	3 (30)
No identified risk factor	107 (36)	101 (94)	6 (6)	0 (0)	6 (100)	0 (0)
Symptoms (n=465)[‡]						
No	199 (43)	180 (91)	19 (10)	1 (5)	9 (47)	9 (47)
Yes - any	266 (57)	247 (93)	19 (7)	8 (42)	8 (42)	3 (16)
Sore throat	107 (23)	101 (94)	6 (6)	3 (50)	2 (33)	1 (17)
Cough	103 (22)	94 (91)	9 (9)	6 (67)	2 (22)	1 (11)
Runny nose (n=298[†])	93 (31)	88 (95)	5 (5)	2 (40)	3 (60)	0 (0)
Fatigue	55 (12)	51 (93)	4 (7)	1 (25)	2 (50)	1 (25)
Fever	53 (11)	47 (89)	6 (11)	3 (50)	2 (33)	1 (17)
Headache (n=298[†])	30 (10)	26 (87)	4 (13)	3 (75)	1 (25)	0 (0)

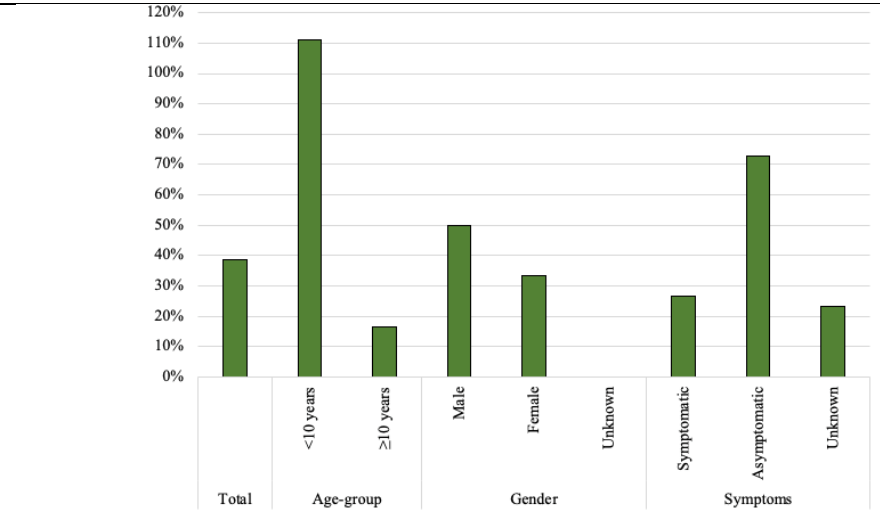
Figure 1. Percent increase in case detection by addition of a second specimen to the baseline specimen

a) Increase in cases detected due to addition of saliva to standard-of-care swab



	Total	Age-goup		Gender			Symptoms		
N	54	19	35	30	20	4	19	19	16
Detected by swab	34	12	22	21	10	3	11	10	13
Detected by saliva alone	20	7	13	9	10	1	8	9	3
p-value	<0.001	0.008	<0.001	<0.001	<0.001	1.000	0.003	0.001	0.226

b) Increase in cases detected due to addition of swab to saliva



	Total	Age-group		Gender			Symptoms		
N	54	19	35	30	20	4	19	19	16
Detected by saliva	39	9	30	20	15	4	15	11	13
Detected by swab alone	15	10	5	10	5	0	4	8	3
p-value	<0.001	<0.001	0.054	0.001	0.047		0.105	0.003	0.226

Swab is an oropharyngeal and bilateral deep nasal swab (ONS

Table 2. Positive percent agreement (PPA) of saliva and swabs among COVID-19 cases

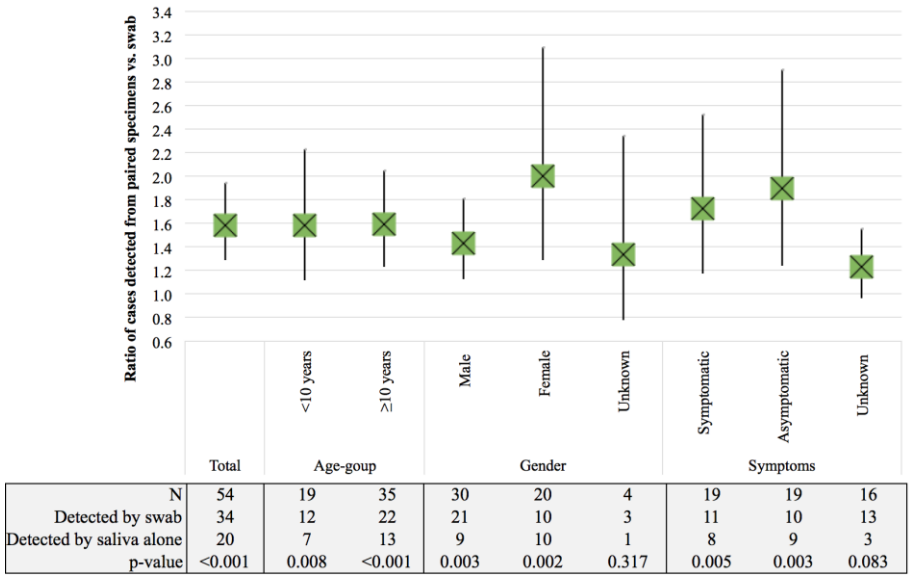
	N	Saliva positive			Swab positive			p-value
		n	PPA	[95% CI]	n	PPA	[95%CI]	
Total	54	39	72%	[58–84%]	34	63%	[49–76%]	0.398
Age-group, years								
Under 10	19	9	47%	[24–71%]	12	63%	[38–84%]	0.467
10 and over	35	30	86%	[70–95%]	22	63%	[45–79%]	0.059
Gender								
Male	30	21	70%	[51–85%]	21	70%	[51–85%]	1.000
Female	20	14	70%	[46–88%]	10	50%	[27–73%]	0.317
Unknown	4	4	100%	[40–100%]*	3	75%	[19–99%]	0.317
Laboratory								
RCH	40	28	70%	[53–83%]	23	58%	[41–73%]	0.353
MDU	14	11	79%	[49–95%]	11	79%	[49–95%]	1.000
Symptoms								
Symptomatic	19	16	84%	[60–97%]	11	58%	[33–80%]	0.132
Asymptomatic	19	10	53%	[29–76%]	10	53%	[29–76%]	1.000
Unknown	16	13	81%	[54–96%]	13	81%	[54–96%]	1.000

Table 3. Participant preference regarding specimen type

	No preference	Swab preferred	Saliva preferred
	N (%)	N (%)	N (%)
Age-group, years			
<5	6 (6)	4 (4)	100 (91)
5–9	0 (0)	2 (5)	41 (95)
10–17	4 (16)	3 (12)	18 (72)
≥18	59 (22)	31(12)	176 (66)
Gender			
Male	21 (12)	12 (7)	148 (82)
Female	32 (14)	22 (10)	169 (76)
Total	53 (13)	34 (8)	317 (79)

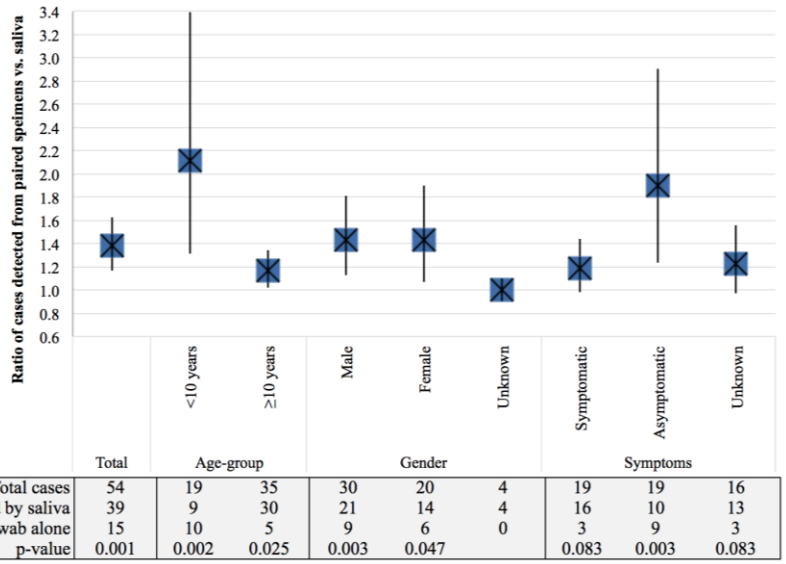
Figure 1. Ratio of cases detected by paired specimens [saliva and oropharyngeal/deep nasal swab] versus single specimen (1a. paired specimens vs. swab alone; 1b. paired specimens vs. saliva alone)

a) Ratio of cases detected by paired specimens (saliva and swab) vs. swab alone

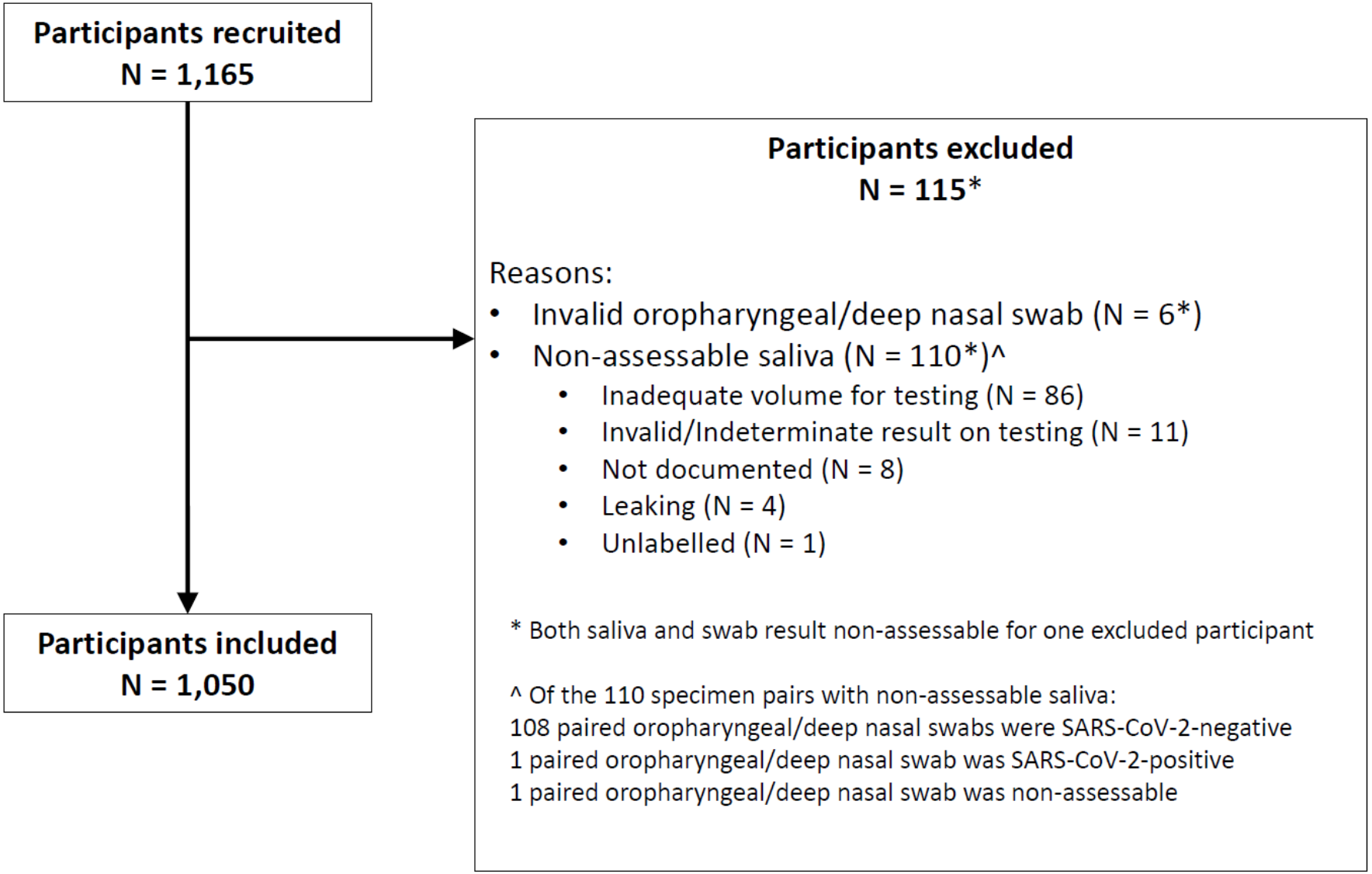


	Total	Age-goup		Gender			Symptoms		
N	54	19	35	30	20	4	19	19	16
Detected by swab	34	12	22	21	10	3	11	10	13
Detected by saliva alone	20	7	13	9	10	1	8	9	3
p-value	<0.001	0.008	<0.001	0.003	0.002	0.317	0.005	0.003	0.083

b) Ratio of cases detected by paired specimens (saliva and swab) vs. saliva alone



	Total	Age-group		Gender			Symptoms		
Total cases	54	19	35	30	20	4	19	19	16
Detected by saliva	39	9	30	21	14	4	16	10	13
Detected by swab alone	15	10	5	9	6	0	3	9	3
p-value	0.001	0.002	0.025	0.003	0.047		0.083	0.003	0.083


Participants recruited
N = 1,165
Participants excluded
N = 115*
Reasons:
• Invalid oropharyngeal/deep nasal swab (N = 6*)
• Non-assessable saliva (N = 110*)^
• Inadequate volume for testing (N = 86)
• Invalid/Indeterminate result on testing (N = 11)
• Not documented (N = 8)
• Leaking (N = 4)
• Unlabelled (N = 1)
* Both saliva and swab result non-assessable for one excluded participant
^ Of the 110 specimen pairs with non-assessable saliva:
108 paired oropharyngeal/deep nasal swabs were SARS-CoV-2-negative
1 paired oropharyngeal/deep nasal swab was SARS-CoV-2-positive
1 paired oropharyngeal/deep nasal swab was non-assessable
Participants included
N = 1,050

Supplementary Table – 2x2 tables of SARS-CoV-2 PCR results for swabs and saliva, by age-group and testing laboratory

a) Total study population

	Oropharyngeal/deep nasal swab		
Saliva	Detected	Not detected	Total
Detected	19	20	39
Not detected	15	996	1,011
Total	34	1,016	1,050

b) People aged 10 years old and older

	Oropharyngeal/deep nasal swab		
Saliva	Detected	Not detected	Total
Detected	17	13	30
Not detected	5	839	844
Total	22	852	874

c) Children aged less than 10 years old

	Oropharyngeal/deep nasal swab		
Saliva	Detected	Not detected	Total
Detected	2	7	9
Not detected	10	157	167
Total	12	164	176

d) Royal Children's Hospital laboratory

	Oropharyngeal/deep nasal swab		
Saliva	Detected	Not detected	Total
Detected	11	17	28
Not detected	12	261	273

Total	23	278	301

e) Microbiological Diagnostic Unit laboratory

	Oropharyngeal/deep nasal swab		
Saliva	Detected	Not detected	Total
Detected	8	3	11
Not detected	3	735	738
Total	11	738	749