RESEARCH LETTER

Change in Saliva RT-PCR Sensitivity Over the Course of SARS-CoV-2 Infection

While real-time reverse transcriptase–polymerase chain reaction (RT-PCR) on nasopharyngeal swabs is the current standard for SARS-CoV-2 detection, saliva is an attractive alternative for diagnosis and screening due to ease of collection and minimal supply requirements.[1,2] Studies on the sensitivity of saliva-based SARS-CoV-2 molecular testing have shown considerable variability.[3] We conducted a prospective, longitudinal study to investigate the testing timeframe that optimizes saliva sensitivity for SARS-CoV-2 detection.

Supplemental content

Methods | Between June 17, 2020, and February 15, 2021, a convenience sample of individuals exposed to a household member with RT-PCR–confirmed SARS-CoV-2 within 2 weeks were recruited from Children's Hospital Los Angeles and nearby community testing sites into the Household Exposure and Respiratory Virus Transmission and Immunity Study (HEARTS). Paired nasopharyngeal and saliva samples were collected every 3 to 7 days for up to 4 weeks or until 2 negative nasopharyngeal test results. RT-PCR for SARS-CoV-2 N1 and N2 genes was performed; cycle threshold less than 40

Table. Characteristics Predicting Higher Odds of Saliva RT-PCR Positivity at COVID-19–Positive Time Points[a]

Participant characteristics (n = 256)	No. (%)	Odds ratio (95% CI)	*P* value
Sex			
Female	148 (57.8)	1 [Reference]	.02
Male	108 (42.2)	1.78 (1.09-2.91)	
Age			
Adult (≥18 y old)	181 (70.7)	1 [Reference]	.60
Child (<18 y old)	75 (29.3)	1.16 (0.66-2.04)	
Ethnicity[b]			
Hispanic/Latinx	239 (93.4)	0.19 (0.03-1.48)	.11
Non-Hispanic/Latinx	17 (6.6)	1 [Reference]	
Race[b]			
Asian	9 (3.5)	0.33 (0.04-3.09)	.33
Black	0		
White	245 (95.7)	1 [Reference]	
Multiple	2 (0.8)	0.10 (0.02-0.62)	.01
Comorbidities[c]			
No	210 (82.0)	1 [Reference]	.26
Yes	46 (18.0)	1.49 (0.75-2.94)	
Smoker[d]			
No	243 (94.9)	1 [Reference]	.005
Yes	13 (5.1)	3.18 (1.43-7.09)	
Characteristic at time of sample collection (n = 524)			
COVID-19–associated symptom presentation[e]			
No	402 (76.7)	1 [Reference]	<.001
Yes	122 (23.3)	2.84 (1.58-5.11)	
Nasopharyngeal swab viral load[f]			
Low	131 (25.0)	1 [Reference]	<.001
High	393 (75.0)	5.16 (2.87-9.28)	
Sample collection timing			
Days since COVID-19 onset at time of specimen collection	524 (100)	0.94 (0.91-0.96)[g]	<.001

Abbreviation: RT-PCR, reverse transcriptase–polymerase chain reaction.

[a] From all nasopharyngeal-positive paired samples (n = 524), generalized estimating equations analysis (goodness of fit quasilikelihood information criterion, 570.9) were used to determine different likelihoods of saliva SARS-CoV-2 PCR positivity. The odds ratio of having a positive RT-PCR result in saliva while holding all other variables constant is shown.

[b] Race and ethnicity were self-reported by the participants with the groups provided. Participants who identified with more than 1 race are reported in the "multiple" category.

[c] Comorbid conditions included preexisting lung, heart, kidney, liver, or neurologic disease; diabetes; cancer; or other immunosuppression.

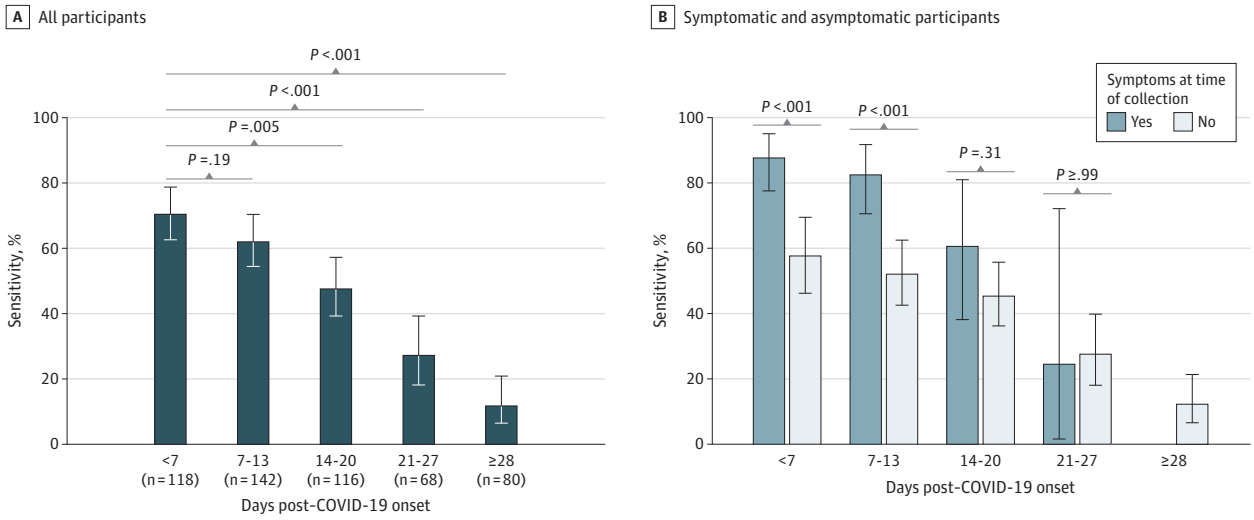
[d] Smoking status refers to self-reports of current use of tobacco, marijuana, or vaping products.

[e] Participants were considered symptomatic for COVID-19 if they reported at least 1 of the following: fever, chills, headache, fatigue, muscle aches, runny nose, congestion, cough, sore throat, shortness of breath, wheeze, altered smell, altered taste, vomiting, diarrhea, or abdominal pain.

[f] A high nasopharyngeal swab viral load was defined as cycle threshold ≤34 and a low viral load as cycle threshold >34 in the SARS-CoV-2 N1 gene.[4]

[g] For each day after COVID-19 onset, the odds of saliva RT-PCR positivity decreased by a factor of 0.94.

Figure. Saliva Sensitivity by Collection Timing After COVID-19 Onset Overall and in Symptomatic and Asymptomatic Individuals



Saliva sensitivity in all 524 nasopharyngeal-positive paired samples from 256 participants (A) and participants who were symptomatic vs asymptomatic at time of specimen collection (B) grouped by collection timing after COVID-19 onset, defined as the earliest of either first symptom or first reverse transcriptase–polymerase chain reaction positivity. Error bars indicate 95% CIs.

defined a positive result. A nasopharyngeal N1 cycle threshold of 34 or less was defined as high viral load.[4] Detailed specimen collection and RT-PCR methods are reported in eMethods in the Supplement.

Saliva sensitivity was calculated using nasopharyngeal-positive RT-PCR as the reference standard. COVID-19 onset was defined as the earlier date between first symptom (collected by questionnaire daily) or first RT-PCR positivity. Pre- and post-symptomatic were defined as asymptomatic time points before and after a symptomatic interval, respectively. Saliva sensitivity by week of collection and between symptomatic and asymptomatic individuals were compared using χ^2 test or Fisher exact test. Generalized estimating equations were used to determine clinical characteristics (**Table**) associated with saliva sensitivity in nasopharyngeal-positive pairs while accounting for repeated samples from the same individuals. Analyses were performed using SPSS Version 27.0 (IBM Corp) with a 2-sided $P < .05$ considered significant. Written informed consent was obtained from participants. The study was approved by the institutional review board of Children's Hospital Los Angeles.

Results | We tested 889 paired nasopharyngeal swab-saliva samples from 404 participants, of which SARS-CoV-2 was detected in 524 nasopharyngeal (58.9%) and 318 saliva (35.7%) specimens. SARS-CoV-2 was detected in both specimens in 258 pairs (29.0%). Of the 256 nasopharyngeal SARS-CoV-2-positive participants (63.4%), the mean age was 28.2 years (range, 3.0-84.5); 108 (42.2%) were male. Participants returned for a median of 3 visits (interquartile range, 2-4). Ninety-three participants (36.3%) were asymptomatic throughout their infection; 126 (77.3%) of 163 symptomatic individuals reported mild severity.

Saliva sensitivity was highest in samples collected during the first week of infection at 71.2% (95% CI, 62.6%-78.8%) but decreased each subsequent week (**Figure**, A). Participants who presented with COVID-19-associated symptoms on the specimen collection day during week 1 of infection had significantly higher saliva sensitivity compared with asymptomatic participants (88.2% [95% CI, 77.6%-95.1%] vs 58.2% [95% CI, 46.3%-69.5%]; $P < .001$). Saliva sensitivity remained significantly higher in symptomatic participants in week 2 (83.0% [95% CI, 70.6%-91.8%] vs 52.6% [95% CI, 42.6%-62.5%]; $P < .001$). No difference was observed more than 2 weeks after COVID-19 onset (Figure, B). Sensitivities did not significantly differ for never-symptomatic (34.7% [95% CI, 27.3%-42.7%]), presymptomatic (57.1% [95% CI, 31.7%-80.2%]), and postsymptomatic (42.9% [95% CI, 36.8%-49.1%]) time points ($P = .26$).

For each day after COVID-19 onset, the odds ratio for saliva detection was 0.94 (95% CI, 0.91-0.96) compared with the previous day ($P < .001$) (Table). Participants presenting with COVID-19–associated symptoms at the time of specimen collection or with high nasopharyngeal viral loads had 2.8 (95% CI, 1.6-5.1; $P < .001$) and 5.2 (95% CI, 2.9-9.3; $P < .001$) higher odds of having a saliva-positive RT-PCR result compared with those with asymptomatic presentation or low nasopharyngeal viral loads, respectively.

Discussion | Saliva was sensitive for detecting SARS-CoV-2 in symptomatic individuals during initial weeks of infection, but sensitivity in asymptomatic SARS-CoV-2 carriers was less than 60% at all time points. As COVID-19 testing strategies in workplaces, schools, and other shared spaces are optimized, low saliva sensitivity in asymptomatic infections must be considered.[5] This study suggests saliva-based RT-PCR should not be used for asymptomatic COVID-19 screening.

This study has limitations. Samples were collected following household exposure; therefore, pretest probability was high. Nasopharyngeal swab testing was the reference standard, but this is not a perfect test for SARS-CoV-2 infection, and a positive RT-PCR result from any sample past 10 days of infection may not be predictive of viral replication or infectivity.[6]

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