A Comparison of Less Invasive SARS-CoV-2 Diagnostic Specimens in Nursing Home Residents — Arkansas, June–August 2020

Paige Gable[1], Jennifer Y. Huang[1], Sarah E. Gilbert[1], Susan Bollinger[1], Amanda K. Lyons[1], Sarah Sabour[1], Diya Surie[1], Caitlin Biedron[1], Tafarra Haney[3], Elizabeth Beshearse[1,2], Christopher J. Gregory[1], Kathryn A. Seely[3], Nakia S. Clemmons[1], Naveen Patil[3], Atul Kothari[3], Trent Gulley[3], Kelley Garner[3], Karen Anderson [1], Natalie J. Thornburg [1], Alison L. Halpin [1], L. Clifford McDonald[1], Preeta K. Kutty[1], Allison C. Brown[1], and the CDC COVID-19 Laboratory Task Force*

[1]COVID-19 Response Team, CDC, Atlanta, Georgia, USA

[2]Epidemic Intelligence Service, CDC, Atlanta, Georgia, USA

[3]Arkansas Department of Health, Little Rock, Arkansas, USA

* CDC COVID-19 Laboratory Task Force listed in acknowledge section

Corresponding author: Paige Gable, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30329 (woz8@cdc.gov).

Key Points: Anterior nasal swabs (AN), oropharyngeal swabs (OP), and saliva were 80–88% RT-PCR concordant. Saliva and OP remained RT-PCR-positive longer than AN. AN outperformed OP by viral culture. AN and saliva were effective specimens for repeat testing in this population.

ABSTRACT

Background

SARS-CoV-2 testing remains essential for early identification and clinical management of cases. We compared the diagnostic performance of three specimen types for characterizing SARS-CoV-2 in infected nursing home residents.

Methods

A convenience sample of 17 residents were enrolled within 15 days of first positive SARS-CoV-2 result by real-time reverse transcription polymerase chain reaction (RT-PCR) and prospectively followed for 42 days. Anterior nasal swabs (AN), oropharyngeal swabs (OP), and saliva specimens (SA) were collected on the day of enrollment, every 3 days for the first 21 days, then weekly for 21 days. Specimens were tested for presence of SARS-CoV-2 RNA using RT-PCR and replication-competent virus by viral culture.

Results

Comparing the three specimen types collected from each participant at each time point, the concordance of paired RT-PCR results ranged from 80–88%. After the first positive result, SA and OP were RT-PCR–positive for ≤48 days; AN were RT-PCR–positive for ≤33 days. AN had the highest percentage of RT-PCR–positive results (81%; 21/26) when collected ≤10 days of participants' first positive result. Eleven specimens were positive by viral culture: nine AN collected ≤19 days following first positive result and two OP collected ≤5 days following first positive result.

Conclusions

AN, OP, and SA were effective methods for repeated testing in this population. More AN than OP were positive by viral culture. SA and OP remained RT-PCR–positive longer than AN, which could lead to unnecessary interventions if RT-PCR detection occurred after viral shedding has likely ceased.

Keywords: SARS-CoV-2, Diagnostics, Specimens, Repeat testing, Noninvasive

BACKGROUND

Rapid and accurate detection of SARS-CoV-2, the virus that causes COVID-19, is essential to controlling the ongoing pandemic. This has become crucially important, as rates of COVID-19 among the 1.3 million people currently living in U.S. nursing homes increased to 26.1 cases per 1,000 resident-weeks in January 2021[1,2]. As of February 2021, the CDC's National Healthcare Safety Network reported over 635,300 confirmed COVID-19 cases and more than 128,200 COVID-19 deaths among nursing homes residents[2].

Residents living in these facilities are typically older, and many have underlying conditions, making them at high risk for disease and death associated with COVID-19[1,3]. Existing chronic conditions and possible atypical presentations can make it difficult to differentiate COVID-19 symptoms from other symptoms[4,5]. Compared with persons residing in the surrounding community, residents are also more likely to be immunocompromised and can remain infective for longer periods[4,5,6]. Some adults with severe illness may shed replication-competent virus (which may serve as an indicator of infectivity) >10 days, and a subset of these can shed >20 days due to severe immunocompromise[7]. Additionally, SARS-CoV-2 can spread quickly within congregate settings such as long-term care facilities[1,3].

Depending on county incidence, nursing homes may be required to conduct screening for SARS-CoV-2 as often as twice a week; facility-wide testing is also required in the event of an outbreak[8,9]. Therefore, as widespread transmission continues in nursing homes, the development of methods to improve the speed and sensitivity of SARS-CoV-2 testing, while decreasing resident discomfort, remains necessary for early identification, clinical management, and initiation of transmission-based precautions in residents[5,7,10].

Currently, the gold standard specimen for SARS-CoV-2 detection is the nasopharyngeal swab (NP)[11-13]. However, this specimen type has several disadvantages. It

poses a risk of infection to healthcare personnel (HCP) collecting the specimen, requires technical skill to optimize specimen quality and maximize collection safety, and is often uncomfortable especially for medically fragile patients[12,14,15]. As tests utilizing different sample types were authorized, many institutions switched from NP to more easily collected samples, such as anterior nasal swabs (AN), for increased patient comfort[16-18]. AN are less invasive and can be collected by HCP or self-collected[19]. Tests utilizing oropharyngeal swabs (OP) were authorized for diagnostic use early in the pandemic and results from OP had 95% concordance with NP results[20]. Saliva specimens (SA) are another convenient, non-invasive, and readily available specimen type that yields results comparable to NP results[21-23]. Yet, given the frequent occurrence of dry mouth (xerostomia) from medical conditions and medications taken by residents, saliva collection might be less feasible. With the broader use of alternative specimen types, there is a need for direct comparison of SA, AN, and OP results to determine the interchangeability of these specimen types for SARS-CoV-2 detection.

Another key aspect of controlling the COVID-19 pandemic is containing the spread of the virus. Specimens that facilitate detection of replication-competent virus are key to identifying and isolating infectious individuals. The comparative performance of less invasive types of specimen collection on viral culture may inform our understanding of which patients are most at risk for infecting others.

Using matched specimens collected serially through infection and recovery in a cohort of nursing home residents, we compared AN, OP, and SA for SARS-CoV-2 detection by RT-PCR and viral culture to help determine the usefulness of such specimens for diagnostic purposes, and thereby assist in the timely implementation of appropriate infection control measures.

METHODS

Assessment population and participant enrollment

As previously described by Surie et al, a prospective cohort assessment was conducted between July 7 and August 28, 2020 in a 105-bed Arkansas nursing home that reported 90 SARS-CoV-2 positive cases[6]. Briefly, we enrolled a convenience sample of residents ≤15 days of their first positive SARS-CoV-2 result by RT-PCR, using an AN or NP tested by either a commercial laboratory or the Arkansas State Public Health Laboratory. Residents were excluded if they could not provide informed consent. This activity was reviewed by CDC and conducted in accordance with applicable federal law and CDC policies pertaining to public health emergencies[1].

Specimen collection and processing

Matched respiratory and saliva specimens were collected mid-day and simultaneously at 11 timepoints from each participant: on the day of enrollment, every 3 days for the first 21 days, and then weekly for 21 days. Specimens were obtained by CDC field staff following CDC guidelines and device manufacturer instructions for each unique specimen type[11]. AN and OP were collected using flocked swabs (Copan Diagnostics, CA, USA) and placed in individual viral transport media containers (Becton, Dickson and Company, NJ, USA). SA were collected using the OMNIgene saliva [OM-505] kits (DNA Genotek Inc., Ontario, Canada) no sooner than 30 minutes after any food or drink.

Specimens were stored and transported at 2-8°C. AN and OP were tested ≤72 hours after collection in a CDC laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). Per manufacturer instructions, the OMNIgene saliva specimens were processed ≤3 weeks from the collection date[24]. Specimens were processed at CDC in accordance with the FDA Emergency Use Authorization (EUA)[25]. Viral RNA

[1] See e.g., 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.

was extracted from specimens using the QIAGEN EZ1 Advanced XL (QIAGEN, Germantown, MD), Roche MagNA Pure 96 (Roche Diagnostics, Indianapolis, IN), or Promega Maxwell RSC 48 (Promega, Madison, WI), and then tested for the presence of SARS-CoV-2 RNA according to the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel instructions for use[25]. RT-PCR assays were performed using the Thermo Fisher Scientific TaqPath™ 1-Step RT-qPCR Master Mix on the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument. Each assay contained a primer and probe set of fluorescently labelled DNA oligonucleotides (2019-nCoV nucleocapsid [N] gene; N1 and N2), which bind to the SARS-CoV-2 RNA. A second primer/probe set was included to detect the RNase P gene (an internal amplification control; RP) in all specimens and control samples. Human specimen controls and no template controls were added. Thermal cycling conditions were: 2 minutes at 25°C, 15 minutes at 50°C, 2 minutes at 95°C, and 45 cycles of 3 seconds at 95°C, and 30 seconds at 55.0°C. All processed specimens and extracted RNA were stored at ≤-70°C.

For each specimen, RT-PCR results were determined "positive" if targets N1 and N2 crossed the cycle threshold (Ct) line within 40 cycles (Ct <40). Results were considered "negative" if RP was detected but no amplification was observed (Ct ≥40) from either N1 or N2. Results were considered "inconclusive" if all controls passed and only one target (N1 or N2, but not both) crossed the Ct line within 40 cycles. Repeat testing was performed if initial RT-PCR results were inconclusive, per the instructions for use. If repeat testing was inconclusive, results were reported as inconclusive; specimens could not be recollected because the corresponding time point had already passed. Based upon CDC internal data that specimens with higher Ct values are near uniformly viral culture negative, only RT-PCR–positive AN and OP specimens with Ct values ≤34 for N1 and N2 targets were submitted for

viral culture within 4 weeks of RT-PCR testing[26]. Specimens awaiting viral culture were stored at ≤-70°C.

Viral culture was conducted by placing 100 µL of specimen in a 96-well plate in serum-free DMEM supplemented with 2x penicillin-streptomycin and 2x amphotericin B (Sigma-Aldrich, St. Louis, MO, USA). Vero CCL-81 cells were trypsinized and resuspended in DMEM + 10% FBS + 2x penicillin-streptomycin + 2x amphotericin B at 2.5 x 10^5 cells/ml. A 100 µL cell suspension was added directly to the specimen dilutions. The inoculated cultures were grown in a humidified 37°C incubator with 5% CO2. When cytopathic effect was observed visually, presence of SARS-CoV-2 was reconfirmed by RT-PCR. SA could not be assessed by culture because the OMNIgene RNA stabilization solution inactivates the virus[24].

Data Management and Statistical Analysis

Specimen collection forms from each time point were entered into CDC's electronic information system. Testing results from each type of laboratory test were compiled to create an analytical dataset. Ct values for amplification of the N1 target gene were evaluated in this analysis. Descriptive statistics of RT-PCR results were calculated. Chi-square, paired t-test, Pearson's R, and Wilcoxon signed-rank test were used to evaluate statistical differences where appropriate. Statistical significances were defined as p-value <0.05 and noted if significant. Frequencies of concordance and discordance results were computed for paired specimen types. Sensitivity was calculated to assess diagnostic performance of AN, OP, and SA. Data management processes and statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

We collected 434 specimens from 17 participating residents over 42 days. Among all specimens submitted for CDC testing, five were rejected due to damage in transit and 429 generated results by RT-PCR: 152 AN, 143 OP, and 134 SA. One participant was unable to open their mouth because of an unrelated health condition, preventing OP collection. Some participants were at times unable to produce enough saliva on demand for testing due to xerostomia. Five participants were hospitalized or died during the follow up period and thus did not complete all 11 collection timepoints. Further clinical information including symptoms, comorbidity, and correlation with additional diagnostic testing results was previously summarized by Surie et al[6].

SARS-CoV-2 RNA was detected in 131(31%) tested specimens; 289(67%) were RNA-negative and 9(2%) had inconclusive results. The 131 RT-PCR–positive specimens were comprised of 45(34%) AN, 42(32%) OP, and 44(34%) SA collected from 16 participants. Of specimens collected ≤10 days of the first RT-PCR–positive test, 81% (21/26) of AN, 75% (18/24) of OP, and 72% (18/25) of SA were positive; after 10 days, 19% (24/126) of AN, 20% (24/119) of OP, and 24% (26/109) of SA were positive. When defining the duration of RNA detection as days from the first RT-PCR–positive to the last known positive specimen collected, and excluding participants who died during the evaluation, the median duration of RNA detection differed by specimen type: 12.5 days (range 4–33 days) for AN, 16 days (range 4–48 days) for OP, and 12 days (range 4–48 days) for SA (Figure 1).

We matched specimens collected on the same date from the same participant to create specimen pairs. When comparing the specimens collected from each participant at the same time point, the overall concordance of RT-PCR results was 80–88% (Table 1). Overall, AN and OP had the highest concordance of specimen comparisons and an equal number of positives among their discordant results. When comparing AN and OP to SA, there was lower

concordance (82% and 80%, respectively), largely because SA was positive when AN or OP were negative or inconclusive (74% and 67%, respectively). Among 24 discordant findings comparing SA against AN or OP, SA was positive and both the paired AN and OP were negative or inconclusive in 12(50%) of these instances.

Ct values varied by specimen type and timing of collection since first RT-PCR diagnosis (Figure 1). Among positive specimens, the median Ct values and ranges for the N1 target were as follows: 31.3 (IQR: 26.5–35.3) and a range 18.3–38.6 for AN, 33.6 (IQR: 29.4–35.5) and a range 22.6–38.7 for OP, and 31.4 (IQR: 25.3– 34.4) and a range 15.5–36.5 for SA. The differences in the overall Ct values from SA compared to AN and OP were lower and statistically significant (Figure 1).

Viral culture data were analyzed to compare the performance of AN and OP by time and Ct value. Among the 83 RT-PCR–positive specimens qualified for viral culture, (i.e. Ct values ≤34 for N1 and N2 targets), 44(53%) were AN and 39(47%) were OP. Virus was isolated from 11(13%) specimens collected from 9 participants. Among culture-positive specimens, 9(82%) were AN, collected as far as 19 days since first RT-PCR–positive, and 2 were OP collected 4 and 5 days since first RT-PCR–positive (Figure 2). The culture-positive specimens collected after day 10 were all from one severely immunocompromised participant. Viral culture-positive specimens had N1 Ct values ranging from 18–28 for AN and 22–23 for OP.

CONCLUSIONS

Public health surveillance and containment strategies for SARS-CoV-2 are predicated on rapid case detection and patient isolation. This assessment sought to better understand the temporal dynamics and diagnostic performance of three less invasive specimen types for SARS-CoV-2 detection and characterization. Comparing SARS-CoV-2 RT-PCR results

across specimen types, we found that AN, OP, and SA usually produced concordant results but performed differently, despite being collected at the same time from the same patient.

In our assessment, viral RNA was detectable throughout the course of infection and persisted for up to 48 days after first RT-PCR detection. AN had the highest proportion of RT-PCR–positives when collected ≤10 days of participants' first RT-PCR–positive specimen and were RT-PCR–positive for the shortest duration. AN were also more sensitive than OP for detecting replication-competent virus by culture.

Our data showed that overall viral RNA detection rates in SA were higher than both AN and OP among the concordant pairs. SA also had a long duration of RT-PCR positivity, with lower Ct values across the evaluation period, which suggests higher viral loads in SA (inversely related to Ct value). Several recent studies demonstrated saliva to be more sensitive than NP or OP for SARS-CoV-2 testing[22,27–30]. The performance of SA in our assessment mirrors the findings reported by previous studies[31,32], including those of Yee et al. who reported SARS-CoV-2 detection in SA up to 43 days after initial diagnosis, compared to 32 days for NP[33]. By collecting SA, we identified 12 additional SARS-CoV-2–positive specimens that were negative by AN and OP. However, given that these additional positives were all collected >10 days of the first RT-PCR–positive result, and data showing infectivity is extremely rare after this time (in the absence of severe immunocompromise) [7,34], the increased detection of SARS-CoV-2 in these later SA (and OP) appears likely to unnecessarily increase infection control burden by identifying RT-PCR–positive individuals who may no longer be infectious.

Indeed, not all reports have found saliva to be the most adequate specimen. Several studies have shown greater performance of NP and other respiratory specimens than SA for SARS-CoV-2 detection[22,35–37]. This variability in performance across studies could be due to variations in saliva collection and SARS-CoV-2 testing methods. We used a general

spitting method to collect saliva in a commercial saliva collection kit containing an RNA stabilization solution. This kit preserved the viral RNA, facilitating transport and testing. It also had restricted collection times, prevented viral culture due to inactivation reagents, and cost more than other saliva collection containers.

In this assessment, RT-PCR positivity in AN and OP was prolonged and did not correlate closely with culture positivity. Replication-competent virus was recovered from AN and OP specimens with N1 Ct values <29 and from specimens collected <20 days post-diagnosis. These findings are consistent with results from other institutions that recovered virus only from specimens with Ct values <34 and as far as day 18 post-diagnosis[26,30,34]. Among our specimens, AN yielded replication-competent virus more often across a wider range of Ct values and for longer periods of time from first RT-PCR–positive than OP (Figure 2). Although we are not certain why viral culture might be less sensitive for OP than AN, despite similar Ct values and being closer in time from first RT-PCR–positive, one possibility is the detection of non-infectious viral RNA present in the oral cavity[38].

In addition to the reliable performance of AN by RT-PCR and culture in this nursing home population, AN also appeared to be a more feasible collection method than OP or SA. The CDC field team collected AN at each time point from each available participant. In contrast, participants weren't always able to produce a sufficient volume of saliva for testing and one participant was never able to provide an OP. These findings, although perhaps unique to this population, support the use of AN and other nasal specimens for SARS-CoV-2 diagnostics.

Our results and experience with this nursing home population indicate that AN are convenient, non-invasive, and adequate for the detection of SARS-CoV-2 by RT-PCR. AN collected in this assessment yielded more information on the presence of infectious virus while also having a shorter post-shedding duration of RT-PCR positivity. Together, these

indicators demonstrate that AN have the potential to minimize the inadvertent detection of previously resolved infections while still capturing those most likely capable of transmitting virus. In nursing home settings, AN may provide more accurate results than other specimen types during acute SARS-CoV-2 infection and allow facilities to better allocate public health resources appropriately. The utility of AN is particularly notable given the current recommendations and focus on rapid point-of-care antigen tests, which also require nasal specimens, albeit the composition of the swab and use of transport medium varies[39].

This assessment was subject to limitations. We had a small cohort size and, due to rolling enrollment of participants 4-13 days post-RT-PCR diagnosis, we were unable to describe specimen performance across the entirety of participants' infectious period. Additionally, not all specimens could be collected from each participant at each time point, particularly later in infection because some participants were hospitalized or died. This assessment was conducted before the widespread availability of antigen tests; therefore, our analysis does not include a side-by-side analysis of these three specimens by RT-PCR and antigen test. RT-PCR and culture methods vary among institutions and findings, such as Ct value cutoffs and culture yield, may not be generalizable. And although identifying replication-competent virus with culture is a correlate of infectiousness, culture-negative findings do not necessarily prove the absence of viral shedding. Finally, the OMNIgene preservative prevented culture attempts on saliva specimens.

Our findings contribute to current discussions on appropriate diagnostic specimens for detecting SARS-CoV-2 in nursing homes. AN and SA have the potential to be used in settings where repeated testing may be needed or in outbreak investigations that require widespread screening. Our findings may inform efforts to guide fit-for-purpose specimen choice when repeat testing is required for different populations in different settings where convenience must be balanced with test performance.

NOTES

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TABLE 1. Comparative RT-PCR performance of paired specimens collected from SARS-CoV-2-infected nursing home residents, Arkansas, 2020

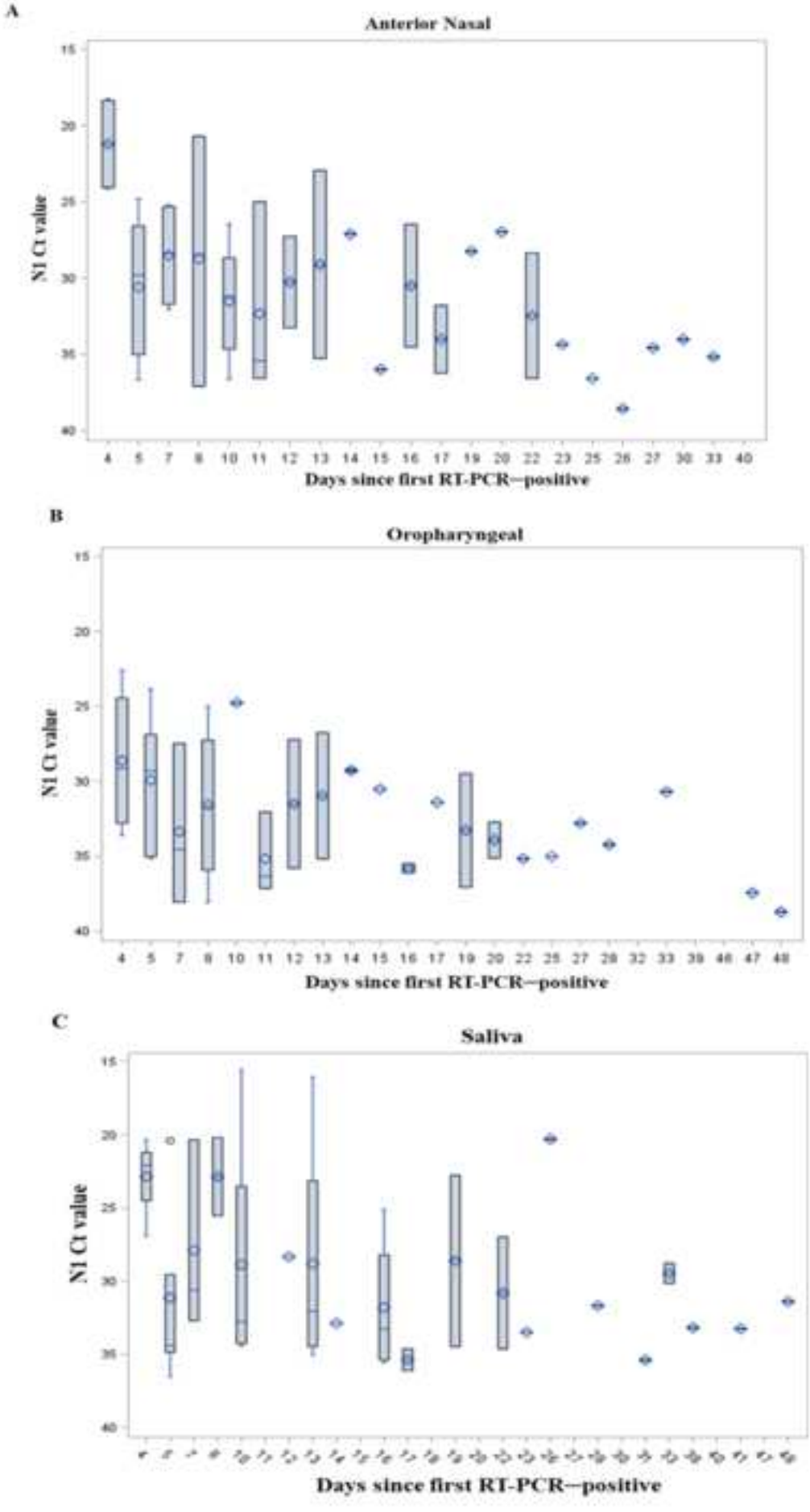
Paired RT-PCR Results	**AN and OP N=135**	**AN and SA N=130**	**OP and SA N=121**
	n (%)	n (%)	n (%)
Concordant	**119 (88)**	**107 (82)**	**97 (80)**
+/+	29 (24)	24 (22)	22 (23)
-/- or i/i	90 (76)	83 (78)	75 (77)
Discordant	**16 (12)**	**23 (18)**	**24 (20)**
AN+ and OP-/i	8 (50)		
AN-/i and OP+	8 (50)		
AN+ and SA-		6 (26)	
AN-/i and SA+		17 (74)	
OP+ and SA-			8 (33)
OP-/i and SA+			16 (67)

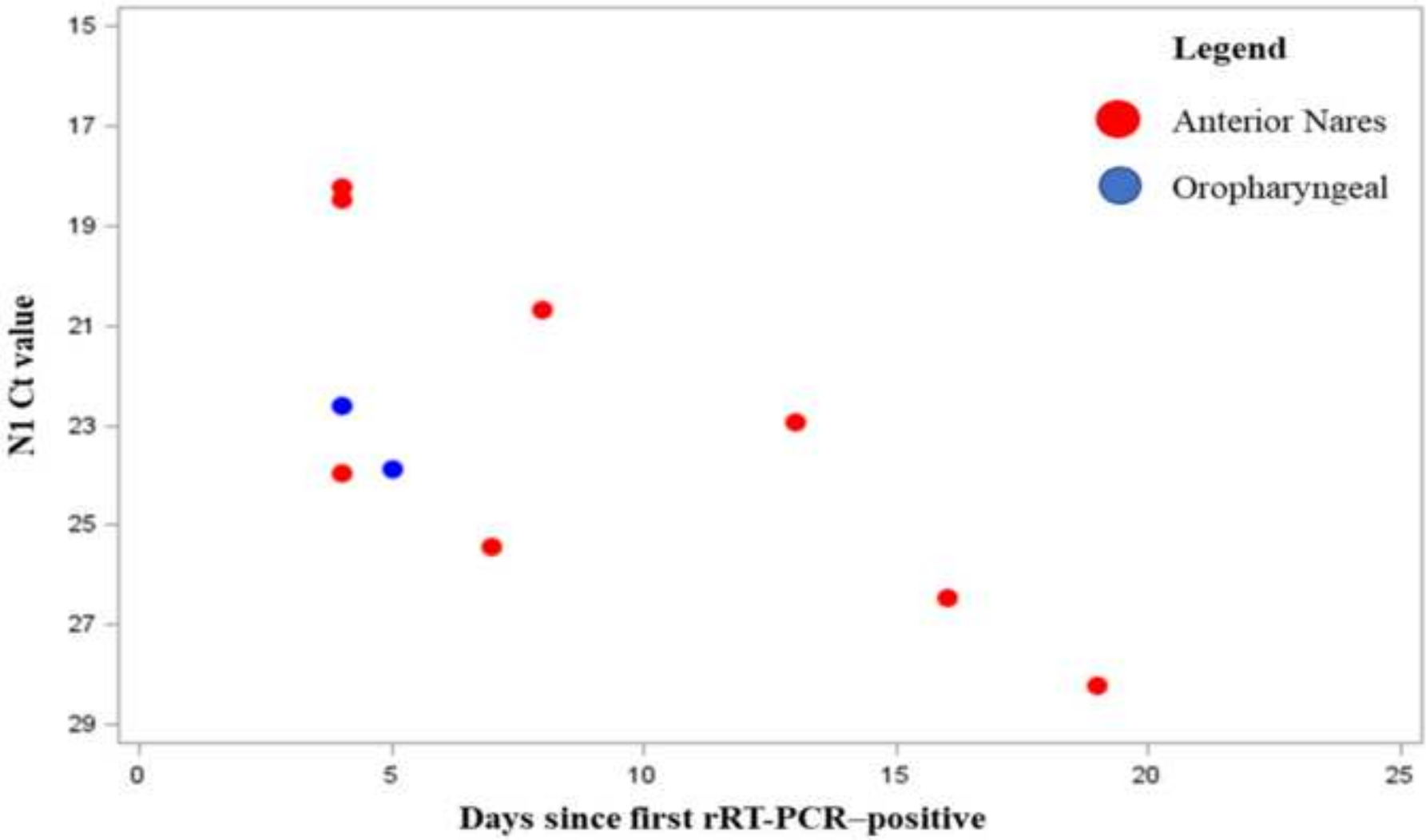
40. Abbreviations: AN, anterior nasal swab; OP, oropharyngeal swab; SA, saliva; i, inconclusive results; RT-PCR, real-time reverse transcription polymerase chain reaction.
41. Results were determined positive (+) if nucleocapsid [N] gene targets N1 and N2 crossed the cycle threshold (Ct) line within 40 cycles (Ct <40); results were determined negative (-) if RNase P gene (RP) was detected but no amplification was observed (Ct ≥40) from either N1 or N2.

FIGURE 1. Distribution of nucleocapsid [N] gene target, N1, cycle threshold (Ct) values over time among RT-PCR–positive specimens, categorized by days since first SARS-COV-2 RT-PCR–positive and specimen type. RT-PCR results were determined positive if N1 and N2 crossed the Ct line within 40 cycles (Ct <40). Boxes represent the interquartile range and median Ct values from specimens collected at each time point. Median Ct value and days since first RT-PCR–positive in (A) anterior nasal, (B) oropharyngeal, and (C) saliva specimens.

FIGURE 2. Distribution of nucleocapsid [N] gene target, N1, cycle threshold (Ct) values of viral culture-positive specimens*, by days since first RT-PCR–positive and specimen type. RT-PCR results were determined positive if N1 and N2 targets crossed the Ct line within 40 cycles (Ct <40). Ct values of all culture-positive specimens from paired anterior nasal and oropharyngeal swabs are plotted on the y-axis. Days since first RT-PCR–positive are plotted on the x-axis.

*One unpaired culture-positive AN excluded because OP was not collected at the same visit.


A
Anterior Nasal
N1 Ct value
Days since first RT-PCR–positive
B
Oropharyngeal
N1 Ct value
Days since first RT-PCR–positive
C
Saliva
N1 Ct value
Days since first RT-PCR–positive


Legend
Anterior Nares
Oropharyngeal
N1 Ct value
15
17
19
21
23
25
27
29
0
5
10
15
20
25
Days since first rRT-PCR–positive