Title: Performance of saliva specimens for the molecular detection of SARS-CoV-2 in the community setting: does sample collection method matter?

Running title: Saliva for detection of SARS-CoV-2

Authors: Marta Fernández-González1*, Vanesa Agulló1*, Alba de la Rica2, Ana Infante3, Mar Carvajal1, José Alberto García1, Nieves Gonzalo-Jiménez2, Claudio Cuartero3,Montserrat Ruiz-García2, Carlos de Gregorio4, Manuel Sánchez4, Mar Masiá1,5#, Félix Gutiérrez1,5#

Affiliations:

1Infectious Diseases Unit, Hospital General Universitario de Elche, Spain
2Microbiology Service, Hospital General Universitario de Elche, Spain
3Microbiology Service, Hospital Universitario de San Juan, San Juan de Alicante, Spain
4Department 20th Health Authority, Consellería de Sanitat, Generalitat Valenciana, Spain
5Clinical Medicine Department, Universidad Miguel Hernández, San Juan de Alicante, Spain

*Marta Fernández-González and Vanesa Agulló contributed equally to the work. Author order was determined by drawing straws.

#Joint senior authors

Key words: COVID-19, saliva, SARS-CoV-2, PCR, diagnostics

Corresponding author: Prof. Félix Gutiérrez, Universidad Miguel Hernández, Avda de la Universidad S/N, 03202, Elche, Alicante, Spain. E-mail: gutierrez_fel@gva.es
Alternate corresponding author: Dr. Mar Masiá, Universidad Miguel Hernández, Avda de la Universidad S/N, 03202, Elche, Alicante, Spain. E-mail: mmasia@umh.es
Abstract

Background: Data on the performance of saliva specimens for diagnosing COVID-19 in ambulatory patients are scarce and inconsistent. We assessed saliva-based specimens for detecting SARS-CoV-2 by RT-PCR in the community setting and compared three different collection methods.

Method: Prospective study conducted in three primary care centres. RT-PCR was performed in paired nasopharyngeal swabs (NPS) and saliva samples collected from outpatients with a broad clinical spectrum of illness. To assess differences in collection methods, saliva specimens were obtained in a different way in each of the participating centres: supervised collection (SVC), oropharyngeal washing (OPW) and self-collection (SC).

Results: NPS and saliva pairs of samples from 577 patients (median age 39 years, 44% men, 42% asymptomatic) were collected and tested, and 120 (20.8%) gave positive results. The overall agreement with NPS and kappa coefficients (K) for SVC, OPW and SC were 95% (K=0.85), 93.4% (K=0.76), and 93.3% (K=0.76), respectively. The sensitivity (95% CI) of the saliva specimens varied from 86% (72.6-93.7) for SVC to 66.7% (50.4-80) for SC samples. The sensitivity was higher in samples with lower cycle threshold (Ct) values. The best performance of RT-PCR was observed for SVC, with sensitivity (95% CI) for Ct values ≤30 of 100% (85.9-100) in symptomatic, and 88.9% (50.7-99.4) in asymptomatic individuals.

Conclusions: Saliva is an acceptable specimen for the detection of SARS-CoV-2 in the community setting. Specimens collected under supervision perform comparably to NPS and can effectively identify individuals with higher risk of transmission in real life conditions.
Introduction

SARS-CoV-2 infection is usually detected by real-time reverse transcriptase polymerase chain reaction (RT-PCR) on RNA extracted from upper respiratory tract specimens, typically nasopharyngeal swabs (NPS). However, collection of NPS is uncomfortable for the patient and may induce cough and sneezing, which may expose health care provider to infectious aerosols. Therefore, alternative sampling has been investigated, including easy-to-obtain specimens with the potential for patient self-collection such as saliva. Although initial studies investigating the use of saliva suggested that this specimen may be a good alternative sample to NPS, mixed results have been reported with sensitivities in the range of 30 to 100% [1–12].

Most of the studies that have evaluated SARS-CoV-2 detection in saliva were conducted in patients admitted to hospital with known COVID-19 infection and some of them were limited by the lack of simultaneous collection of NPS and saliva specimens and by the reduced composition of the cohorts, including mainly adults and symptomatic patients, all of which may limit the overall generalizability of the findings.

Additionally, the procedure for collecting saliva specimens has varied substantially among the studies from enhanced collection under direction or supervision by the clinician [1-5] to unsupervised self-collection by the participants [10-12]. Variation in saliva sampling may be an explanation for the varying results of the published studies.

The use of saliva specimens in the ambulatory setting may be particularly appealing due to ease of collection and reduced equipment required, but data are scarce and inconsistent. While findings from a study on 45 patients support its potential for detecting SARS-CoV-2 from outpatients [5], a reduced sensitivity relative to NPS has
been reported in a recent community-based cohort, raising concerns on the use of saliva samples in this setting [12,13]. Therefore, to clarify the role of saliva as an alternate specimen type for the detection of SARS-CoV-2 in the community setting, larger clinical studies are needed.

The present study aimed to evaluate the performance of saliva-based specimens for detecting SARS-CoV-2 by RT-PCR in a prospective study of adults and children with suspected COVID-19. In this investigation, we performed RT-PCR in paired NPS and saliva samples collected from outpatients with a broad clinical spectrum of illness, including asymptomatic cases, undergoing SARS-CoV-2 testing, and compared three different collection methods.

**Methods**

**Setting and study subjects**

This prospective, observational study was carried out at the Departments of Health 17 and 20 of the province of Alicante, Spain. The study was approved by the Hospital General Universitario de Elche COVID-19 Institutional Advisory Board. Patients enrolled in the study were those presenting for SARS-CoV-2 testing as requested by their providers, at three primary care centres (PCC) facilities. Both symptomatic patients and asymptomatic subjects that had been exposed to SARS-CoV-2 were invited to participate in the investigation by providing saliva samples immediately preceding collection of the NPS. After obtaining written consent, demographic and clinical findings were recorded.
Specimen collection and processing

Saliva specimens were collected into a 100 ml sterile empty container without transport medium. To assess differences in collection methods, saliva specimens were obtained in a different way in each of the participating PCC by random. In centre A, saliva specimens were obtained under the supervision of a healthcare worker (Supervised collection, SVC); in centre B, saliva specimens were obtained after oropharyngeal washing (OPW) consisting in rinsing the mouth with 2 ml of saline solution and spitting the solution into the collection pot; and in centre C, saliva specimens were collected independently by the individual providing the sample following written instructions (Self-collection, SC); the instructions read as follows: “To collect saliva, bow your head forward to allow saliva to pool in the front of your mouth and spit up to a minimum of 1 ml of saliva (half a teaspoon) into the collection pot”. In the SVC centre, subjects were instructed to produce and pool saliva in their mouth for 1-2 minutes moving the mouth and the tip of the tongue across the cheeks and gums, and then repeatedly spit a minimum of 1 ml saliva into the collection pot in the presence of a healthcare worker.

In the three PCC, nasopharyngeal samples were taken by qualified nurses following the same procedure and the same kind of NPS. The flexible, mini-tip swab was passed through the patient’s nostril until the posterior nasopharynx was reached, left in place for several seconds to absorb secretions, then slowly removed while rotating. Swabs were placed in 3 ml of sterile transport media containing guanidine salt (Mole Bioscience, SUNGO Europe B.V., Amsterdam, Netherlands). To reduce variability in specimens transport, storage and processing, the procedures were standardized in the
three centres. Nasopharyngeal and saliva specimens were transported within 2 hours of sample collection to the clinical microbiology laboratory for molecular analysis by RT-PCR. NPS samples were analyzed immediately and saliva specimens frozen (-20 °C) and analyzed within two weeks after collection. Containers with saliva or OPW were thawed at room temperature, sample was homogenized with the tip of the automatic pipette and 300 µl were taken to the automated extraction plate.

SARS-CoV-2 detection

Nucleic acid extraction was performed using 300 µL of specimen (NPS or saliva) on Chemagic™ 360 Nucleic Acid Purification Instrument (PerkinElmer España SL, Madrid, Spain). Then, 10 uL of eluate was used for real-time RT-PCR assay targeting the E-gene (LightMix® Modular SARS-CoV (COVID19) E gene, TIB MOLBIOL, Berlin, Germany, distributed by Roche). Testing was performed according to the manufacturer’s guidelines on Cobas z 480 Analyzer (Roche, Basilea, Suiza). The number of cycles of amplification in RT-PCR (cycle threshold, Ct) value was assessed as a surrogate measure of the RNA concentration. Per the manufacturer, a Ct value of ≤40 by PCR is considered a positive result.

Statistical methods

Continuous variables were expressed as median ± 25th and 75th percentiles (Q1, Q3), and categorical variables as percentages. Wilcoxon or Student’s t-test were used to compare two continuous variables and Kruskal Wallis anova for three or more, and the chi-square or Fisher’s exact test for categorical variables comparison. The percent agreement (positive, negative and overall) (PPA, NPA and OPA) for saliva specimens...
obtained by SVC, OPW and SC was calculated using the results of the RT-RCR test in NPS as reference standard. Performance agreement was evaluated using kappa coefficients (κ). Positive results of either NPS or saliva specimens were considered true positives for calculations of sensitivity. Patients with an undetermined result in RT-PCR for NPS or saliva specimen were not considered for calculations of agreement. Performance of saliva specimens was also evaluated stratifying by the presence of symptoms. Given the increasing evidence of a positive relationship between low Ct values and likelihood of culturing live virus and therefore infectious potential, we also evaluated the performance of saliva specimens according to Ct values in NPS with different cut-off levels that have been associated with higher probability of positive viral culture (≤25 and ≤30) [14].

Statistical analyses were performed with R version 4.0.3 (2020-10-10) software. Percent positive or negative agreement and kappa coefficients (K) were calculated using the package caret [15]. For the graphical analysis, the ggplot2 package [16] was used.

We planned to include 133 patients per arm assuming a sensitivity of 95% and a confidence of 95%. With this sample size the study would have a statistical power of 80% to detect a 10% difference in sensitivity between the different collection methods.

Results

Patient characteristics and positivity rates
A total of 634 patients (103 children) were invited to take part in the study between 15th September and 29th October 2020. Fifty-four (median age 5.8 [4-10] years; 44 children) were unable to provide saliva specimens and 3 (0.5%) specimens had insufficient sample for laboratory analysis. 577 pairs of samples (229 SVC, 140 OPW, 208 SC) were included in the analyses. Flow chart of the patients is depicted in Supplemental Figure S1. Demographic and clinical characteristics of the patients according to the collection method of saliva specimens are shown in Table 1. There were 120 (20.8%) positive results for SARS-CoV-2 by either NPS or saliva, 50 (21.8%) in the SVC, 28 (20%) in the OPW and 42 (20.2%) in the SC group. NPS and saliva samples from 2 (0.3%) and 9 (1.6%) patients, respectively, generated invalid transcription-mediated amplification results due to internal control failure.

Sensitivity of the different specimens for SARS-CoV-2 detection and concordance between saliva specimens and NPS

Table 2 shows the qualitative positive results for SARS-CoV-2 RNA obtained from NPS and saliva specimens according to the collection method, and Figure 1 the concordance of positive test results between NPS and the different saliva specimen types. The sensitivity for NPS specimens was 95% (95% CI, 88.9-97.9). Among saliva specimens, SVC showed the best case detection rate (43 of 50 infected patients) with significantly higher sensitivity than SC samples (86% [95% CI, 72.6-93.7] versus 66.7% [50.4-80]; p=0.027). OPW detected 21 of 28 individuals (sensitivity, 75% [95% CI], 54.8-88.6) (Table 1). The greatest sensitivity was obtained by combining NPS sampling with saliva collected under supervision (sensitivity 97.5% [95% CI, 92.3-99.3]). Table 3 shows the agreement of the three different saliva specimens with NPS. The best agreement with
NPS was found for the specimens of the SVC group with a kappa coefficient of 0.85. For the OPW and SC groups, the kappa coefficient was 0.76.

Performance of the different saliva specimens for SARS-CoV-2 detection by cycle threshold values and presence of symptoms

Median Ct values were significantly lower in NPS than in their paired saliva specimens (p=0.035), although in 19 (15.8%) patients the saliva showed a lower Ct than the corresponding NPS. Median Ct values in the three saliva specimen types were not significantly different from each another (p=0.962) (Figure 2). Figure 1 displays the concordance for SARS-CoV-2 detection between paired NPS and saliva samples, and Ct values of the discordant positive results. Median (Q1-Q3) Ct values for NPS-positive-only or saliva-positive-only specimens were 33 (31-34) and 32 (29-33.5), respectively.

Sensitivity of the different specimens for SARS-CoV-2 detection according to Ct values is depicted in Figure 3. The sensitivity of the saliva specimens was higher in the samples with lower Ct values. For Ct values ≤ 25, median (95% CI) sensitivities of SVC, OPW and SC sampling reached 100% (85.9-100), 93.3% (66-99.7) and 93.3% (66-99.7), respectively, and decreased only minimally, to 97.4% (84.9-99.9), 81% (57.4-93.7) and 91.3% (70.5-98.5), respectively, for Ct ≤ 30. Supplemental Figure S2 shows the sensitivity by sample type according to Ct values and the presence of symptoms.

There were no significant differences in the sensitivity between patients with and without active symptoms for the same Ct values across the different specimens. The
best performance of RT-PCR was observed for NPS, closely followed by supervised collected saliva, with sensitivity for Ct values ≤ 30 of 98.4% (90.2-99.9) and 100% (85.9-100), respectively, in symptomatic individuals, and 95.2% (74.1-99.8) and 88.9% (50.7-99.4), respectively, in asymptomatic individuals.
Discussion

We confirmed that saliva is an acceptable specimen for molecular detection of SARS-CoV-2 in the community setting and can effectively identify individuals with the highest risk of transmission in real life conditions. The study revealed that the collection method may be critical for improving sensitivity. Saliva specimens obtained under supervision outperforms self-collected samples and show higher sensitivity in symptomatic and asymptomatic patients.

As expected, the sensitivity of the saliva specimens increased in samples with low Ct values. Indeed, supervised collected specimens performed almost as well as nasopharyngeal samples with sensitivities well above 90% in patients with low Ct values, who are considered to have the greatest potential to spread the virus [14,17]. For Ct values ≤ 25, the sensitivities of SVC, OPW and SC saliva specimens reached 100%, 93% and 93%, respectively, and they were 97%, 81% and 91% in patients with Ct values ≤ 30. Among NPS-only positive individuals we found only one case of saliva collected under supervision who had Ct value ≤ 30. Therefore, the use of saliva specimens in general, and particularly when is obtained under supervision, allowed the detection of SARS-CoV-2 in the vast majority of the patients with significant risk of transmission.

In contrast to most of the previous studies, largely focused on inpatient populations, in the present investigation we included outpatients with a broad clinical spectrum of the illness, comprising children and asymptomatic cases. Like Williams et al. [11] we evaluated casual saliva specimens without previous fasting or enhancement techniques such as strong sniffing or coughing, used in other studies [4]. Prior investigations
comparing different samples for molecular detection of SARS-CoV-2 in the community setting have reported lower sensitivity rates with saliva specimens than with NPS, ranging from 30% to 85% [11-13]. The reduced performance has been attributed to the milder symptoms in outpatients, with reduced viral load relative to more severe cases [18,19] and to differences in temporal dynamics in viral shedding in upper respiratory locations versus saliva [20,21] with lower viral loads in saliva samples [11]. We did not find prominent differences in the performance between those with or without active symptoms but, in line with other studies [8,22,23] we detected higher Ct values indicating lower viral loads in saliva specimens than in the corresponding NPS, which suggests differences in viral shedding between the two compartments. In addition, the significant differences in the performance of the specimen types evaluated in our study suggest that variation in saliva sampling may have contributed to the disparities in sensitivity observed in previous investigations.

The study has limitations. The investigation focused on comparing three specific methods for collection of saliva samples and was powered to detect rather large differences among groups. The sample size does not allow to draw conclusions on the performance in particular subgroups, including children and patients tested at different time point of illness. Other potential weaknesses include the possibility that differences in performance of the collection methods could be confounded by differences in the populations and sampling among the three PCC. However, the populations were comparable in terms of gender, age and prevalence of SARS-CoV-2 RNA positivity, and to reduce variability in specimens’ transport, storage and processing, the procedures were standardized in the three participating centres. Noteworthy, a substantial proportion of the children recruited, most of them very young children, were unable to provide the minimum of 1 ml of saliva sample after explaining the procedure,
suggesting that this specimen might be less suitable for this group. In addition, we used a particular detection system (Cobas z 480 Analyzer), other platforms may have yielded different results. Strengths of the study are that it was population-based and carried out in real-life conditions, enrolling consecutive outpatients of all ages presenting for testing due to symptoms and asymptomatic people who had come into contact with confirmed cases.

In conclusion, our results indicate that the adequate collection of samples may be essential for the molecular diagnosis of COVID-19 when using saliva specimens. Saliva specimens obtained under supervision perform comparably to NPS and should be considered as a reliable sample for the diagnosis in the community setting in both symptomatic and asymptomatic individuals, particularly to detect individuals with significant risk of transmission. Although self-collected saliva would be the most advantageous way of sampling if mass testing were considered, these specimens had less sensitivity in our study. Further research is needed to determine whether other strategies of instruction, for example through videos or telematic means, can substitute for the direct supervision of a health professional.
294 References


Funding

This work was supported by the RD16/0025/0038 project as a part of the Plan Nacional Research + Development + Innovation (R+D+I) and cofinanced by Instituto de Salud Carlos III - Subdirección General de Evaluación y Fondo Europeo de Desarrollo Regional; Instituto de Salud Carlos III (Fondo de Investigaciones Sanitarias [grant number PI16/01740; PI18/01861; CM 19/00160, COV20-00005]).

Conflicts of Interest

All authors: No conflict

Acknowledgements

Members of the COVID19-Elx-Rapid Diagnostic Tests Study Group

Félix Gutiérrez, Mar Masiá, Sergio Padilla, Guillermo Telenti, Lucia Guille, María Andreo, Fernando Lidón, Vladimir Ospino, José López, Marta Fernández, Vanesa Agulló, Gabriel Estañ, Javier García, Cristina Martínez, Leticia Alonso, Joan Sanchís, Ángela Botella, Paula Mascarell, María Selene Falcón, Sandra Ruiz, José Carlos Asenjo, Carolina Ding, Mar Carvajal, Inmaculada Candela, Jorge Guijarro, Cristina la Moneda, Cristina Jara, Raquel Mora, Juan Manuel Quinto, Sergio Ros, Daniel Canal, Pascual Pérez, Carolina Garrido, Manuel Sánchez, Jaime Sastre, Carlos de Gregorio, Francisco Carrasco, Juan Navarro, Andrés Navarro, Nieves Gonzalo, Clara Pérez, Adoración Alcalá, José Luis Rincón, Montserrat Ruiz, José Antonio Gutiérrez.
Table 1. Demographic and clinical characteristics of the patients according to the collection method of saliva specimens.

<table>
<thead>
<tr>
<th></th>
<th>Overall cohort</th>
<th>Supervised collection (SVC)</th>
<th>Oropharyngeal washing (OPW)</th>
<th>Self-collection (SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
<td>Positive SARS-Cov-2 RNA</td>
<td>Positive SARS-Cov-2 RNA</td>
<td>Positive SARS-Cov-2 RNA</td>
</tr>
<tr>
<td></td>
<td>577 (120 (20.8)</td>
<td>229 (50 (21.8))</td>
<td>140 (28 (20))</td>
<td>208 (42 (20.2))</td>
</tr>
<tr>
<td>Gender male, no. (%)</td>
<td>251 (43.5)</td>
<td>91 (39.7)</td>
<td>66 (47.1)</td>
<td>94 (45.2)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39 (24-51)</td>
<td>28 (12.2)</td>
<td>16 (11.4)</td>
<td>15 (7.2)</td>
</tr>
<tr>
<td>0-14 years, no. (%)</td>
<td>59 (10.2)</td>
<td>9 (3.9)</td>
<td>11 (7.9)</td>
<td>25 (12)</td>
</tr>
<tr>
<td>15-50, no. (%)</td>
<td>368 (63.8)</td>
<td>152 (66.4)</td>
<td>87 (62.1)</td>
<td>120 (62)</td>
</tr>
<tr>
<td>50-65, no. (%)</td>
<td>105 (18.2)</td>
<td>40 (17.5)</td>
<td>26 (18.6)</td>
<td>39 (18.8)</td>
</tr>
<tr>
<td>&gt;65, no. (%)</td>
<td>45 (7.8)</td>
<td>9 (3.9)</td>
<td>11 (7.9)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>42 (7.3)</td>
<td>17 (7.4)</td>
<td>12 (8.6)</td>
<td>13 (6.2)</td>
</tr>
<tr>
<td>Dyslipidemia, no. (%)</td>
<td>37 (6.4)</td>
<td>17 (7.4)</td>
<td>11 (7.9)</td>
<td>9 (4.3)</td>
</tr>
<tr>
<td>Diabetes, no. (%)</td>
<td>19 (3.3)</td>
<td>10 (4.4)</td>
<td>4 (2.9)</td>
<td>5 (2.4)</td>
</tr>
<tr>
<td>Cardiomyopathy, no. (%)</td>
<td>17 (2.9)</td>
<td>9 (3.9)</td>
<td>7 (5)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Obesity, no. (%)</td>
<td>25 (4.3)</td>
<td>9 (3.9)</td>
<td>8 (5.7)</td>
<td>8 (3.8)</td>
</tr>
<tr>
<td>Presence of symptoms the day of sample collection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic, no. (%)</td>
<td>336 (58.2)</td>
<td>145 (63.3)</td>
<td>68 (48.6)</td>
<td>123 (59.1)</td>
</tr>
<tr>
<td>Days with symptoms, median (Q1-Q3)</td>
<td>4 (3-6)</td>
<td>3 (2-5)</td>
<td>4 (3-5)</td>
<td>4 (3-6)</td>
</tr>
<tr>
<td>Sensitivity of saliva specimens, % (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global</td>
<td>76.7% (67.9-83.7)</td>
<td>86% (72.6-93.7)*</td>
<td>75% (54.8-88.6)</td>
<td>66.7% (50.4-80)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>77.9% (67.4-85.9)</td>
<td>89.2% (73.6-96.5)**</td>
<td>70.6% (44-88.6)</td>
<td>68.8% (49.9-83.3)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>73.5% (55.3-86.5)</td>
<td>76.9% (46-93.8)</td>
<td>81.8% (47.8-96.8)</td>
<td>60% (27.4-86.3)</td>
</tr>
<tr>
<td>Ct ≤ 25</td>
<td>96.7% (87.5-99.4)</td>
<td>100% (85.9-100)</td>
<td>93.3% (66-99.7)</td>
<td>93.3% (66-99.7)</td>
</tr>
<tr>
<td>Ct ≤ 30</td>
<td>91.6% (82.9-96.3)</td>
<td>97.4% (84.9-99.9)</td>
<td>81% (57.4-93.7)</td>
<td>91.3% (70.5-98.5)</td>
</tr>
<tr>
<td>Ct &gt; 30</td>
<td>43.2% (27.5-60.4)</td>
<td>45.5% (18.1-75.4)</td>
<td>57.1% (20.2-88.2)</td>
<td>36.8% (17.2-61.4)</td>
</tr>
</tbody>
</table>

Ct, cycle threshold of RT-PCR for nasopharyngeal swabs (or saliva specimens in cases with negative nasopharyngeal swab and positive saliva).

**P=0.028 for the comparison between supervised collection and self-collection.

**P=0.035 for the comparison between supervised collection and self-collection.
Table 2. Comparison of qualitative results from nasopharyngeal swabs and saliva specimens according to the collection method.

<table>
<thead>
<tr>
<th>Saliva</th>
<th>Supervised collection (n=229)</th>
<th>Oropharyngeal washing (n=140)</th>
<th>Self-collection (n=208)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td>Und</td>
</tr>
<tr>
<td>Nasopharyngeal swab</td>
<td>39</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>171</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Pos, positive; Neg, negative; Und, undetermined.
Table 3. Agreement of the different saliva specimens with the nasopharyngeal swabs.

<table>
<thead>
<tr>
<th>Saliva type</th>
<th>Positive percent agreement (95% CI)</th>
<th>Negative percent agreement (95% CI)</th>
<th>Overall percent agreement (95% CI)</th>
<th>Performance Agreement (Kappa) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supervised collection</td>
<td>84.8% (70.5-93.2)</td>
<td>97.7% (93.9-99.3)</td>
<td>95% (91-97.4)</td>
<td>0.85 (0.76-0.93)</td>
</tr>
<tr>
<td>Oropharyngeal washing</td>
<td>72% (50.4-87.1)</td>
<td>98.2% (93.1-99.7)</td>
<td>93.4% (87.5-96.8)</td>
<td>0.76 (0.61-0.91)</td>
</tr>
<tr>
<td>Self-collection</td>
<td>66.7% (50.4-80)</td>
<td>100% (97.2-100)</td>
<td>93.3% (88.7-96.1)</td>
<td>0.76 (0.64-0.88)</td>
</tr>
</tbody>
</table>
Figure 1. Positivity for SARS-CoV-2 RNA in nasopharyngeal swabs (NPS) and in the different saliva specimens, including cycle threshold values of discordant specimens.

*One positive saliva, with NPS negative confirmed six days later.

Figure 2. Cycle threshold values for nasopharyngeal swabs and saliva specimens’ pairs from all positive individuals.

Each dot represents the Ct value (RT-PCR) for positive: nasopharyngeal swabs (NPS, n=113), saliva obtained under supervised collection (SVC, n=43), saliva obtained after oropharyngeal washing (OPW, n=21) and saliva obtained by self-collection (SC, n=28).

Figure 3. Sensitivity of the different specimens for SARS-CoV-2 detection according to Ct value in nasopharyngeal swabs*.

*In cases with negative nasopharyngeal swab and positive saliva, the Ct value of the saliva specimen was taken.
Figure 1

Saliva supervised collection

NPS (+) Saliva (+)
7 (14%)
Ct=33 (31.5-33.5)

NPS (+) Saliva (+)
39 (78%)
Ct=32 (31-32.5)

Saliva (+)
4 (8%)
Ct=30 (29-33.5)

Saliva oropharyngeal washing

NPS (+) Saliva (+)
7 (25%)
Ct=34 (29-35)

Saliva (+)
18 (64%)

Self-collection

NPS (+) Saliva (+)
14 (33%)
Ct=32.9 (31.9-33.8)

Saliva (+)
28 (67%)

" Figure 1 

Downloaded from http://jcm.asm.org/ on February 11, 2021 by guest
Figure 2

p (NPS vs Saliva specimens) = 0.035

p = 0.962
Figure 3

![Figure 3](http://jcm.asm.org/)

Sensitivity of various methods:
- Nasopharyngeal swabs
- Saliva supervised collection
- Saliva oropharyngeal washing
- Saliva self-collection

Ct value ranges:
- ≤ 25
- ≤ 30
- > 30
p (NPS vs Saliva specimens) = 0.035

p = 0.962
Performance of saliva specimens for the molecular detection of SARS-CoV-2 in the community setting: does sample collection method matter?

Supplemental Data

Figure S1. Flow chart of the study.
Figure S2. Sensitivity of the different specimens for SARS-CoV-2 detection according to Ct value and presence of symptoms.