Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Evaluating Diagnostic Accuracy of Saliva Sampling Methods for Severe Acute Respiratory Syndrome Coronavirus 2 Reveals Differential Sensitivity and Association with Viral Load

Pieter Mestdagh,*† Michel Gillard,‡ Sharonjit K. Dhillon,§ Jean-Paul Pirnay,¶ Jeroen Poels,‖ Jan Hellemans,* Veronik Hutse,** Celine Vermeiren,‡ Maxime Boutier,‡ Veerle De Wever,‡ Patrick Soentjens,††‡‡ Sarah Djebara,†† Hugues Malonne,‖§§¶¶ Emmanuel André,‖‖ Marc Arbyn,§ John Smeraglia,‡ and Jo Vandesompele*†

From the Biogazelle, Zwijnaarde; the Department of Biomolecular Medicine,† Ghent University, Ghent; the UCB,‡ Braine-l'Alleud; the Unit of Cancer Epidemiology,§ Belgian Cancer Centre, and the Service of Viral Diseases,** Operational Directorate Infectious Diseases in Humans, Sciensano, Brussels; the Laboratory for Molecular and Cellular Technology¶ and the Center for Infectious Diseases,†† Queen Astrid Military Hospital, Brussels; the Federal Agency for Medicines and Health Products,‖ Brussels; the Department of Clinical Sciences,‡‡ Institute of Tropical Medicine, Antwerp; the Unit of Pharmacology, Pharmacotherapy and Pharmaceutical Care,§§ Faculty of Pharmacy, Université Libre de Bruxelles, Brussels; the Department of Biomedical Sciences,¶¶ Namur Research Institute for Life Sciences, University of Namur, Namur; and the Laboratory of Clinical Bacteriology and Mycology,‖‖ KU Leuven, Leuven, Belgium

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Address correspondence to Jo Vandesompele, Ph.D., Technologiepark 82, Zwijnaarde B-9052, Belgium. E-mail: jo.vandesompele@biogazelle.com.

Nasopharyngeal swabs are considered the preferential collection method for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostics. Less invasive and simpler alternative sampling procedures, such as saliva collection, are desirable. We compared saliva specimens and nasopharyngeal (NP) swabs with respect to sensitivity in detecting SARS-CoV-2. A nasopharyngeal and two saliva specimens (collected by spitting or oral swabbing) were obtained from >2500 individuals. All samples were tested by RT-qPCR, detecting RNA of SARS-CoV-2. The test sensitivity was compared on the two saliva collections with the nasopharyngeal specimen for all subjects and stratified by symptom status and viral load. Of the 2850 patients for whom all three samples were available, 105 were positive on NP swab, whereas 32 and 23 were also positive on saliva spitting and saliva swabbing samples, respectively. The sensitivity of the RT-qPCR to detect SARS-CoV-2 among NP-positive patients was 30.5% (95% CI, 1.9%—40.2%) for saliva spitting and 21.9% (95% CI, 14.4%—31.0%) for saliva swabbing. However, when focusing on subjects with medium to high viral load, sensitivity on saliva increased substantially: 93.9% (95% CI, 79.8%—99.3%) and 76.9% (95% CI, 56.4%—91.0%) for spitting and swabbing, respectively, regardless of symptomatic status. Our results suggest that saliva cannot readily replace nasopharyngeal sampling for SARS-CoV-2 diagnostics but may enable identification of the most contagious cases with medium to high viral loads. *(J Mol Diagn 2021, 23: 1249—1258; https://doi.org/10.1016/j.jmoldx.2021.07.017)*

Massive RT-qPCR—based testing for the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA is a key element in the strategy to control the current coronavirus disease 2019 (COVID-19) pandemic. Currently, collecting samples from the upper respiratory tract is recommended for diagnostic testing by the World Health Organization and (American and European) Centers for

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Disease Control and Prevention, with nasopharyngeal (NP) swabs being considered the standard collection procedure[1,2] (*https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html*, last accessed April 8, 2021). Although extremely sensitive, this sampling procedure is relatively invasive, causing discomfort and anxiety in individuals undergoing the procedure, and relies on trained health care workers wearing full personal protective equipment to obtain samples.

Before the outbreak of SARS-CoV-2, several studies have reported on the utility of saliva as a diagnostic specimen for testing respiratory viruses.[3–6] In addition, studies related to SARS-CoV-2 have shown that the virus binds to angiotensin-converting enzyme 2 receptors that are present in epithelial cells of the oral mucosa, suggesting the use of saliva as a potential sample for SARS-CoV-2 detection. The noninvasive nature of saliva collection in a simple container makes this specimen a valuable biomaterial. Besides, saliva sampling could be a solution in resource-limiting settings with respect to health care personnel, and could reduce the amount of contact required between a health care provider and the patient, lowering the risk of transmission and personal protective equipment use. As saliva sampling is patient friendly, it can also be of value when testing in children during a SARS-CoV-2 outbreak in schools.

Although several recent studies have documented the potential utility of saliva for diagnostic testing of SARS-CoV-2,[7–13] these studies experience one or more limitations (ie, nonpaired study design, small cohorts, and testing in biased populations, such as previously confirmed positive cases and/or hospitalized patients). Herein, we set out to prospectively evaluate the potential use of saliva samples for diagnostic testing of SARS-CoV-2 using a large population of >2500 individuals in triage centers in Belgium. Individuals were sampled using two saliva collection devices and a matching nasopharyngeal swab, and samples were analyzed by two test laboratories to independently verify conclusions (Figure 1).

Materials and Methods

Study Design

This study has been reported using the Standards for Reporting of Diagnostic Accuracy Studies guidelines 2015.[14] As part of the Belgian national testing platform, asymptomatic and symptomatic individuals suggestive of COVID-19 at centralized triage centers in Belgium were prospectively enrolled. More than 2500 individuals were tested in these triage centers from June 2020 to July 2020, at the end of the first infection wave. All individuals aged ≥18 years who presented at triage centers were considered eligible.

Sample and Data Collection

Study samples were collected by trained mobile teams. Individuals were sampled using three different procedures: i) a nasopharyngeal swab sample representing the standard comparator for SARS-CoV-2 diagnostics, ii) a saliva sample collected through self-sampling with a commercial saliva spitting device (Saliva RNA Collection and Preservation Device; Norgen Biotek, Thorold, ON, Canada), and iii) a saliva sample collected through self-sampling with a commercial oral swabbing device (Oracollect RNA; DNA Genotek, Kanata, Ontario, Canada). For the saliva spitting device (Norgen Biotek), the collected volume of saliva was 2 mL. For the saliva swabbing device (DNA Genotek), the collected volume of saliva was approximately 300 μL. NP sample collection was performed using iClean NP swabs (Chenyang Global, Shenzhen, China). All samples were collected in a transport buffer that inactivates the virus and stabilizes the RNA [Norgen Biotek or DNA Genotek buffer for the respective saliva samples and 2 mL of DNA/RNA shield buffer (Zymo Research, Irvine, CA), custom prefilled in Vacuette tubes (Greinder Bio-One, Vilvoorde, Belgium) for the nasopharyngeal samples]. Participants in the study were asked not to eat, drink, smoke, or use chewing gum 30 minutes preceding saliva sampling. Saliva samples were collected according to the manufacturer's instructions. These instructions were available as an instruction sheet with each saliva collection device. Instructions were communicated to each participant by a health care professional before sampling. Participants were not instructed to produce deep throat saliva or gargle before saliva collection. For the swabbing device, participants were instructed to place the swab between the right cheek and gum, swab 10 times, and repeat for the left cheek. After sample collection, a short survey was completed and data were collected on age group, ease of use of the saliva devices, comfort of saliva sampling versus nasopharyngeal sampling, and symptomatic status. To enquire about symptomatic status, the case definition of Sciensano, the Belgian Institute for Public Health, was used. The case definition stated that a possible case of COVID-19 had at least one of the following main symptoms that occurred acutely without other plausible cause: cough, dyspnea, thoracic pain, anosmia, or dysgeusia; or at least two of the following symptoms that occurred without other plausible cause: fever, muscle strain, fatigue, rhinitis, sore throat, headache, anorexia, watery diarrhea, acute confusion, or sudden fall; or worsening of chronic respiratory symptoms (chronic obstructive pulmonary disease, asthma, or chronic cough) without other plausible cause. This study was approved by the ethical review committee of the University Hospital of Leuven on May 29, 2020, as S64125., Vilvoorde, Belgium.

Test Methods

SARS-CoV-2 testing was performed by two independent test laboratories, applying different RNA extraction and RT-qPCR workflows (see below). Note that, because of logistics reasons, not all samples were analyzed by both laboratories. After sample collection, samples were shipped to one of the laboratories, where the required volume of sample for RNA extraction was removed from the sample collection tube.

Sample collection tubes were subsequently shipped to the other test laboratory for analysis. Before sample transfer, samples were vortex mixed and centrifuged. For highly viscous samples, aspiration was performed at low speed.

Nucleic Acid Extraction and RT-qPCR in Laboratory 1

RNA was extracted using the Total RNA Purification Kit (Norgen Biotek; number 24300), according to the manufacturer's instructions, using 200 μL viral transport medium [for the nasopharyngeal swab, DNA/RNA Shield (number R1100-250; Zymo Research) or 200 μL saliva collected with the spitting or swabbing device (ie, saliva mixed with transport buffer)] as input in the 96-well filter plate. Samples were supplemented with 200 μL lysis buffer, 200 μL ethanol, 4 μL of a proprietary 700-nucleotides spike-in control RNA (5000 copies, produced through *in vitro* transcription), and carrier RNA [200 ng of yeast tRNA (Roche, Vilvoorde, Belgium; number 10109517001)]. Filter plates were further processed with a centrifuge (5810R with rotor A-4-81; both from Eppendorf [Aarschot, Belgium]). RNA was eluted from the filter plates using 50 μL elution buffer (nuclease-free water), resulting in approximately 45 μL eluate. RNA extractions were simultaneously performed for 94 patient samples and 2 negative controls (nuclease-free water). To the eluate of one of the negative control wells, 7500 (digital PCR value assigned) RNA copies of positive control RNA (Synthetic SARS-CoV-2 RNA Control 2; Twist Biosciences, San Francisco, CA; number 102024) were added to serve as positive PCR control.

RNA eluate (6 μL) was used as input for a 20-μL duplex RT-qPCR in a CFX384 real-time quantitative PCR instrument using 10 μL iTaq one-step RT-qPCR mastermix (Bio-Rad, Temse, Belgium; number 1725141), according to the manufacturer's instructions. Reactions were set up using 400 nmol/L final concentration of primers and 250 nmol/L of a hydrolysis probe. Primers and probes were synthesized by Integrated DNA Technologies (Coralville, IA) using clean-room GMP production. For detection of the SARS-CoV-2 virus, the Charité *E* gene assay was used (FAM)[15]; for the internal control, a proprietary hydrolysis probe assay (HEX) was used. Quantification cycle (Cq) values were generated using the FastFinder software version 3.300.5 (UgenTec, Hasselt, Belgium). The FastFinder software was also used to call a sample positive or negative for SARS-CoV-2. Only batches with a clean negative control and a positive control in the expected range were approved. Proper RNA extraction and RT-qPCR was confirmed by observing spike-in RNA signal in each sample well in the expected range.

Nucleic Acid Extraction and RT-qPCR in Laboratory 2

RNA extraction was performed using a magnetic bead–based RNA extraction method developed by University of Liège (CoRNA kit) and according to the recommended protocol. For nasopharyngeal swab samples, 200 μL of sample was transferred to 11 μL of a proteinase K solution (20 mL/mL). For both saliva devices, 100 μL of saliva was transferred to 175 μL of a lysis buffer mix [11:164 (vol/vol) of proteinase K solution (20 mg/mL) + lysis buffer]. All samples were spiked with the MS2 phage as internal control [10 μL, concentration proprietary information from supplier Thermo Fisher Scientific (Waltham, MA) kit A47814], and in presence of carrier RNA (10 μL; 20 ng/μL; Merck/Roche, Vilvoorde, Belgium; number 10109517001) to increase RNA extraction efficiency. The multiplex RT-qPCR was performed on 5 μL of RNA eluate using TaqPath COVID-19 Combo Kit (comprising *ORF1ab*, *N* gene, *S* gene, and MS2 as internal control; number A47814; Thermo Fisher Scientific), TaqPath positive control kit (containing a stock of 10^4 copies SARS-CoV-2/μL; number A47816; Thermo Fisher Scientific), and TaqPath 1-Step Multiplex Master Mix (no ROX; number A28523; Thermo Fisher Scientific), following the manufacturer's instructions. Positive control was diluted to 25 copies/μL in control dilution buffer, and 2 μL (50 copies) was further diluted in 3 μL nuclease-free water, which was added to the well of the RT-PCR plate. Cq values were generated using the FastFinder software version 3.300.5. The FastFinder software was also used to call a sample positive or negative for SARS-CoV-2. Results were approved when a clean negative control and a positive control in the expected range were obtained. Correct RNA extraction and RT-qPCR setup was also confirmed by controlling MS2 amplification in each sample well (applying an MS2 Cq cutoff of 33).

Digital PCR Quantification of Positive Control RNA Samples

Digital PCR was performed on a QX200 instrument (Bio-Rad) using the One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad; number 1864022), according to the manufacturer's instructions. Briefly, 22 μL prereactions were prepared, consisting of 5 μL 4× supermix, 2 μL reverse transcriptase, 6 μL positive control RNA (see further), 15 mmol/L dithiothreitol, 900 nmol/L of each forward and reverse primer, and 250 nmol/L *E*-gene hydrolysis probe (FAM).[15] A total of 20 μL of the prereaction was used for droplet generation using the QX200 Droplet Generator (Bio-Rad), followed by careful transfer to a 96-well PCR plate for thermocycling: 60 minutes at 46 °C for reverse transcription, 10 minutes at 95 °C for enzyme activation, 40 cycles of 30 seconds of denaturation at 95 °C and 1 minute of annealing/extension at 59 °C, and finally 10 minutes at 98 °C for enzyme deactivation. Droplets were analyzed by the QX200 Droplet Reader (Bio-Rad) and QuantaSoft software (Bio-Rad).

With an input of 4000 RNA copies per reaction (Armored RNA Quant SARS-CoV-2 Panel; Asuragen, Austin, TX; number 52036), the digital PCR result was 875 cDNA copies (or 21.88% of the expected number). Of note, RNA was not extracted on the Armored RNA material; instead, a

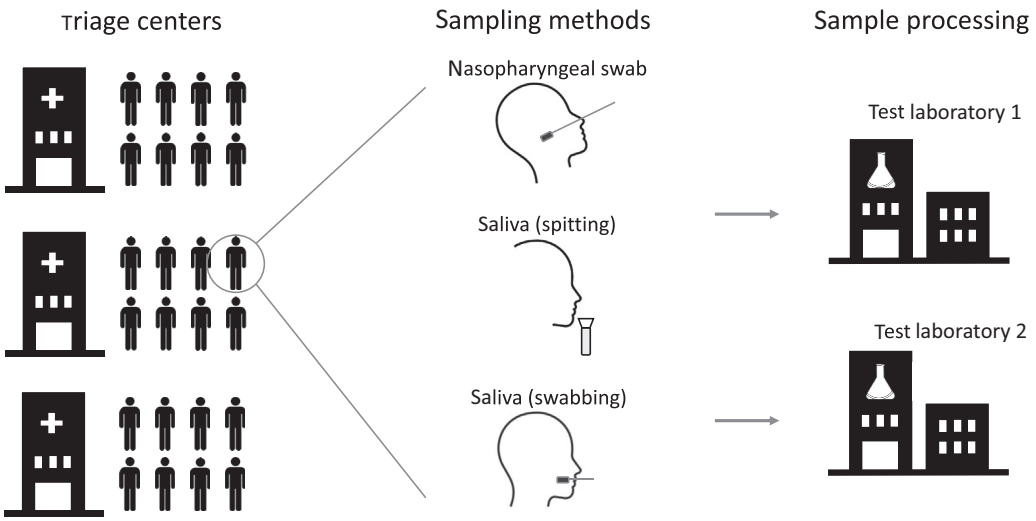

Figure 1 Overview of study design. Study participants were sampled at triage centers using a nasopharyngeal swab and two different saliva collection devices. Samples were processed at two different test laboratories using independent sample processing workflows.

short heat release of RNA was done per the manufacturer's instructions. With an RNA input of 750 copies per reaction (Synthetic SARS-CoV-2 RNA Control 2; Twist Biosciences; number 102024), the digital PCR result was 150 cDNA copies (or 20% of the expected number).

Viral Load Cuffoff Concentration Determination

The Cq value cutoff for viral load classification was determined on the basis of the Cq correlation between NP and saliva samples. The cutoff represents the NP viral load above which saliva samples show highest sensitivity for SARS-CoV-2 detection in NP positive samples, and below which sensitivity for SARS-CoV-2 detection in saliva decreases to almost 0%. This analysis resulted in an *E*-gene Cq cutoff of 24.5 and an *N*-gene Cq cutoff of 25.5 for laboratory 1 and 2, respectively.

To convert the laboratory 1 Cq value cutoff to SARS-CoV-2 RNA copies/mL of viral transport medium, a six-point 10-fold serial dilution of Armored RNA was generated in triplicate (from 2.19×10^8 to 2.19×10^3 digital PCR value assigned copies/mL), followed by RNA extraction and RT-qPCR (using the laboratory 1 method). On the basis of the slope of -3.381, the y intercept of 45.387, and the r^2 value of 0.995, the laboratory 1 Cq value cutoff corresponds to 1.51×10^6 copies/mL viral transport medium. This viral copy number corresponds to a viral load that is typically associated with infectious individuals in literature. For instance, van Kampen et al[16] demonstrated that the probability of isolating infectious SARS-CoV-2 was <5% when the viral load was <6.63 log10 RNA copies/mL. Therefore, this was referred as high viral load.

Sample Size Calculation

The sample size was computed for assessment of a hypothesis on noninferior SARS-CoV-2 positivity on saliva compared with on nasopharyngeal specimen in paired testing, as proposed by Tang et al,[17] using target values from a systematic review.[18] A confidence of 95%, a power of 80%, a sensitivity of the test in NP samples of 95%, a proportion of saliva-negative/NP-positive samples of 5%, and 0.90 were accepted as benchmark for the relative positivity rate (saliva/NP), which yielded 84 SARS-CoV-2–positive subjects needed. These could be found in a study population of 841 to 8410 subjects, assuming a prevalence of 1% to 10%. Given the substantially larger contrast in test positivity between saliva and NP specimens, study enrollment was stopped after reaching 2850 inclusions.

Data Analysis

All results presented in the article are based on data generated by laboratory 1, unless stated otherwise. Only patients with available results for the three specimens were included. Patient paired data were used to construct 2×2 contingency tables. The sensitivity of SARS-CoV-2 testing was defined by the proportion of saliva-positive patients (index positive) among those who were positive on NP swab (reference positive). The test positivity ratio was also computed as the proportion with a positive index test/the proportion with a positive reference or comparator test. The 95% CIs for binomial data were computed as well as for ratios of paired proportions. Three separate analyses were performed: one comparing spit samples with NP samples, a second comparing swab samples with NP samples, and a third comparing spit and swab samples. NP samples were considered the standard comparator or reference. In addition, the estimations were stratified by viral load (categorized as high and low) and symptoms (categorized as symptomatic and asymptomatic).

All statistical analyses were performed using Stata statistical software version 14.2 (Stata, College Station, TX). Statistical significance was defined at $P < 0.05$.

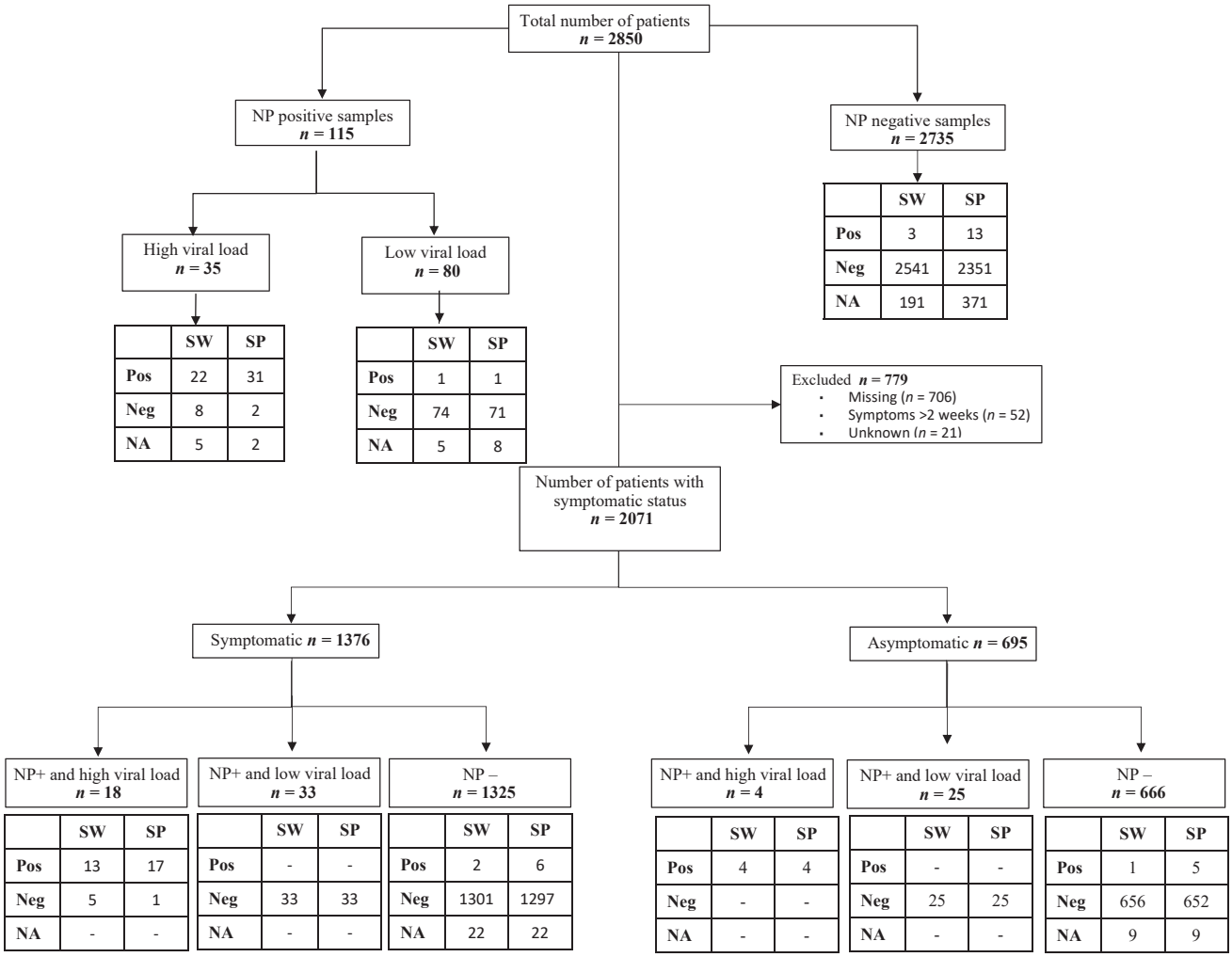

Figure 2 Study flow chart and results of RT-qPCR of patients enrolled in the saliva SARS-CoV-2 diagnostic test accuracy study by specimen type, viral load level, and symptomatic status (laboratory 1). NA, not available; Neg, negative; NP, nasopharyngeal samples; Pos, positive; SP, spit sample; SW, swab samples.

Data Availability

RT-qPCR data from test laboratory 1 and test laboratory 2 as well as the survey data are provided in Supplemental Tables S1, S2, and S3.

Results

Patient Characteristics

In total, 2954 individuals were recruited between June 2020 and July 2020. Data were excluded from 104 (3.5%) participants because of missing NP results. A total of 2268 individuals were sampled with a nasopharyngeal swab and both saliva collection methods, whereas 2469 and 2649 matched samples were available for spit and swab samples, respectively. The median age group of participants was between 31 and 40 years old, and symptomatic status data were available for 2071 individuals.

Comfort and Ease of Saliva Self-Sampling Devices

Although saliva sampling was generally perceived as more comfortable than nasopharyngeal sampling (Supplemental Figure S1A), study participants scored the ease of use of the saliva swabbing device significantly higher than that of the saliva spitting device ($P < 0.0001$, U-test) (Supplemental Figure S1B).

Saliva Sampling Identifies Individuals with Medium to High Viral Load

Of 2850 nasopharyngeal swab samples analyzed by laboratory 1, 115 (4.0%) were SARS-CoV-2 positive (Figure 2). In positive NP samples, 30.4% (35/115) showed high viral load. There were 105 of 115 nasopharyngeal-positive samples for which a matching saliva spitting sample was available, and 105 of 115 nasopharyngeal-positive samples for which a matching saliva swabbing sample was available

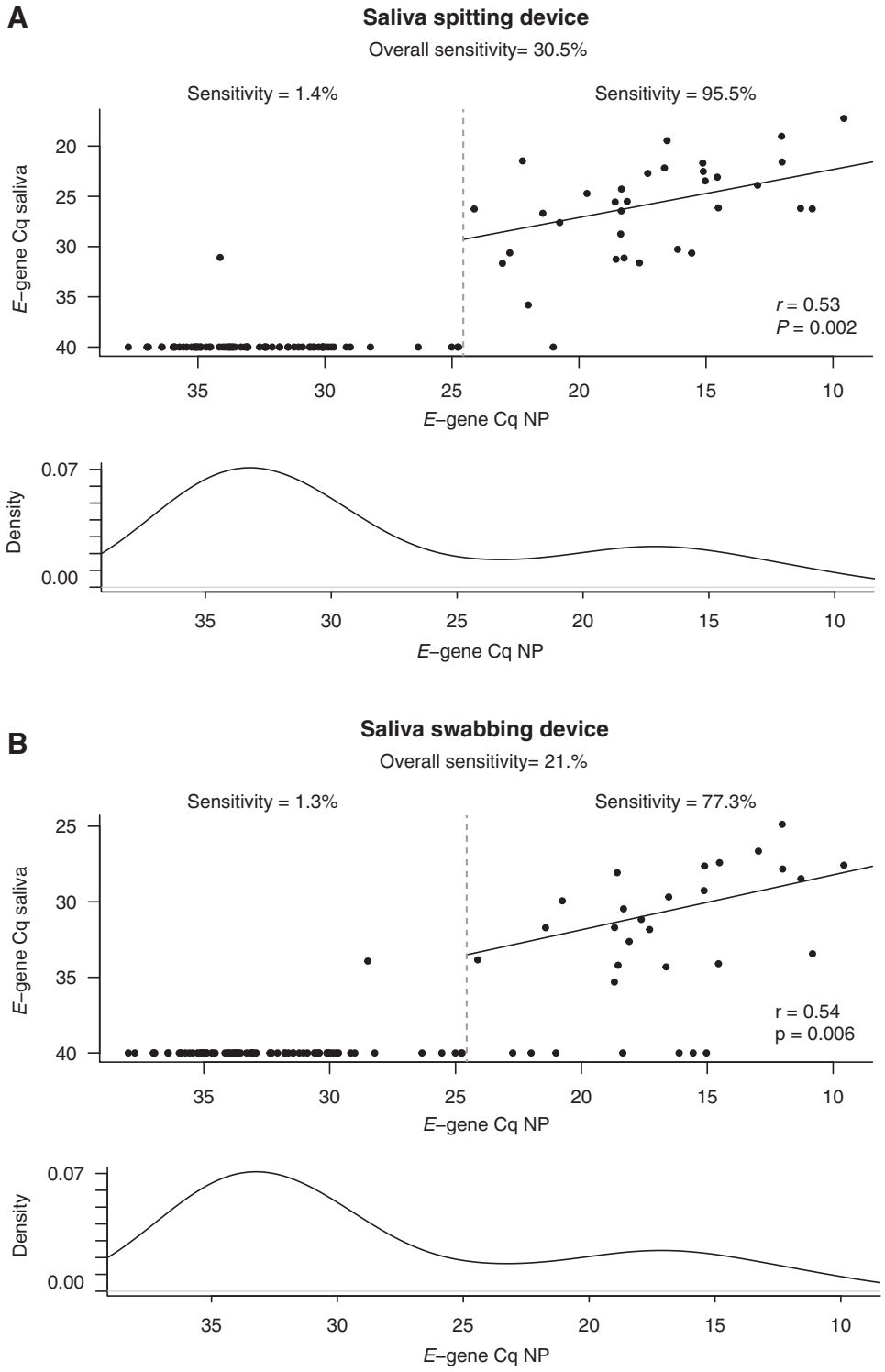

Figure 3 *E*-gene quantification cycle values from laboratory 1 in SARS-CoV-2–positive nasopharyngeal samples (NP) and matching saliva samples. **A:** Results obtained with the saliva spitting device. **B:** Results obtained with the saliva swabbing device. Plots indicate the correlation coefficient (*r*) value and *P* value (Pearson correlation test) calculated using only those samples with a nasopharyngeal *E*-gene Cq value of <24.5 (threshold marked with a **dashed line**). Bottom graphs in each panel represent the *E*-gene Cq-value distribution in the NP-positive samples (*y* axis represents kernel density estimate).

(Figure 2). We observed 32 of 105 (sensitivity, 30.5%; 95% CI, 21.9%–40.2%) in the spitting sample and 23 of 105 (sensitivity, 21.9%; 95% CI, 14.4%–31.0%) in the swabbing samples that were SARS-CoV-2 positive, indicating reduced overall sensitivity in saliva for SARS-CoV-2 detection (Figures 2 and 3 and Table 1).

However, a significantly higher nasopharyngeal viral load was observed in patients with a true-positive saliva sample

Table 1 Sensitivity and Test Positivity Ratios of SARS-CoV-2 Testing on NP Specimens versus Saliva (Collected by Swabs or Spitting) and on Swab Saliva Samples versus Spitting Saliva Samples by Viral Load Level and Symptom Status (Laboratory 1)

Comparisons				Reference positive		Reference negative		Sensitivity, %		Test positivity ratio	
Index	Reference	Viral load categories	Symptom status	Index positive	Index negative	Index positive	Index negative	TP/(TP + FN)	95% CI	(TP and FP)/(TP + FN)	95% CI
Swab	NP specimen	All	All	23	82	3	2541	21.9	14.4–31.0	0.25	0.15–0.31
Spit	NP specimen	All	All	32	73	13	2351	30.5	21.9–40.2	0.43	0.23–0.41
Swab	Spit	All	All	23	14	0	2231	62.2	44.8–77.5	0.62	0.48–0.80
Swab	NP specimen	All	Yes	13	38	2	1301	25.5	14.3–39.6	0.29	0.16–0.41
Spit	NP specimen	All	Yes	17	34	6	1297	33.3	20.8–47.9	0.45	0.23–0.49
Swab	Spit	All	Yes	15	8	0	1309	65.2	42.7–83.6	0.65	0.48–0.88
Swab	NP specimen	All	No	4	25	1	656	13.8	3.9–31.7	0.17	0.06–0.34
Spit	NP specimen	All	No	4	25	5	652	13.8	3.9–31.7	0.31	0.06–0.34
Swab	Spit	All	No	5	4	0	668	55.6	21.2–83.6	0.56	0.31–1.00
Swab	NP specimen	High	All	22	8	0	0	73.3	54.1–87.7	0.73	0.59–0.91
Spit	NP specimen	High	All	31	2	0	0	93.9	79.8–99.3	0.94	0.86–1.02
Swab	Spit	High	All	20	6	0	2	76.9	56.4–91.0	0.77	0.62–0.95
Swab	NP specimen	High	Yes	13	5	0	0	72.2	46.5–90.3	0.72	0.54–0.96
Spit	NP specimen	High	Yes	17	1	0	0	94.4	72.7–99.9	0.94	0.81–1.06
Swab	Spit	High	Yes	13	4	1	0	76.5	50.1–93.2	0.82	0.59–1.00
Swab	NP specimen	High	No	4	0	0	0	100.0	39.8–100.0	1.00	1.00–1.00
Spit	NP specimen	High	No	4	0	0	0	100.0	39.8–100.0	1.00	1.00–1.00
Swab	Spit	High	No	4	0	0	0	100.0	39.8–100.0	1.00	1.00–1.00

FN, false negative; FP, false positive; NP, nasopharyngeal; TP, true positive.

compared with patients with a false-negative saliva sample (spitting device: $\log_2$ fold change = 14.89, $P = 3.79 \times 10^{-15}$; swabbing device: $\log_2$ fold change = 14.7, $P = 3.67 \times 10^{-12}$; *U*-test) (Supplemental Figure S2).

Individuals with an *E*-gene Cq >24.5 in the nasopharyngeal sample (corresponding to 1.51×10^6 copies/mL viral transport medium, as determined by digital PCR and further referred to as low viral load) almost always presented with a negative saliva sample [sensitivity, 1.4% (95% CI, 0.07%–7.5%); and sensitivity, 1.3% (95% CI, 0.07%–7.2%) in the saliva spitting and saliva swabbing sample, respectively] (Figure 3). In contrast, for individuals with a high viral load (*E*-gene Cq < 24.5 in the nasopharyngeal sample), concordance between the nasopharyngeal and matching saliva sample improved dramatically, especially for the saliva spitting device, resulting in high sensitivity in this subgroup [sensitivity, 95.5% (95% CI, 77.2%–99.9%); and sensitivity, 77.3% (95% CI, 54.6%–92.2%) for the saliva obtained by spitting and swabbing, respectively] (Figure 3 and Table 1). In addition, a significant positive correlation was observed between *E*-gene Cq values in the nasopharyngeal and saliva samples for those individuals with high viral load (spitting device: $r = 0.53$, $P = 0.002$; swabbing device: $r = 0.54$, $P = 0.006$; Pearson correlation). Notably, similar findings were obtained on the basis of test results generated by laboratory 2 (Supplemental Figure S3 and Supplemental Table S4). In the medium to high viral load subgroup, saliva spitting resulted in a sensitivity of 96.9% (95% CI, 83.8%–99.9%), whereas saliva swabbing resulted in a sensitivity of 60.7% (95% CI, 40.6%–78.5%).

To assess whether the poor sensitivity in saliva for SARS-CoV-2 detection could be due to sampling issues during saliva collection, the human gene *RPS18* was quantified using RT-qPCR on a representative set of RNA samples used for SARS-CoV-2 detection. No differences in *RPS18* levels were observed between saliva-positive and saliva-negative samples for any of the saliva sampling devices, suggesting that sampling issues do not explain the false-negative saliva samples (Supplemental Figure S4).

SARS-CoV-2 Detection in Saliva, according to Symptom Status

Presence or absence of symptoms of COVID-19 in 2123 study participants was registered. From these participants, 1376 (64.8%) were symptomatic, 695 (32.7%) were asymptomatic, and 52 (2.5%) indicated they experienced symptoms 2 weeks before the test. The latter group was excluded from further analyses because of the limited number of individuals.

The proportions of individuals who were SARS-CoV-2 positive in the nasopharyngeal sample were similar in the symptomatic and asymptomatic groups (3.7% and 4.2%, respectively); however, the symptomatic group was enriched with high viral load samples (*E*-gene Cq < 24.5; $P = 0.042$; Fisher exact test). As a result, sensitivity in saliva for SARS-CoV-2 detection was higher among symptomatic cases

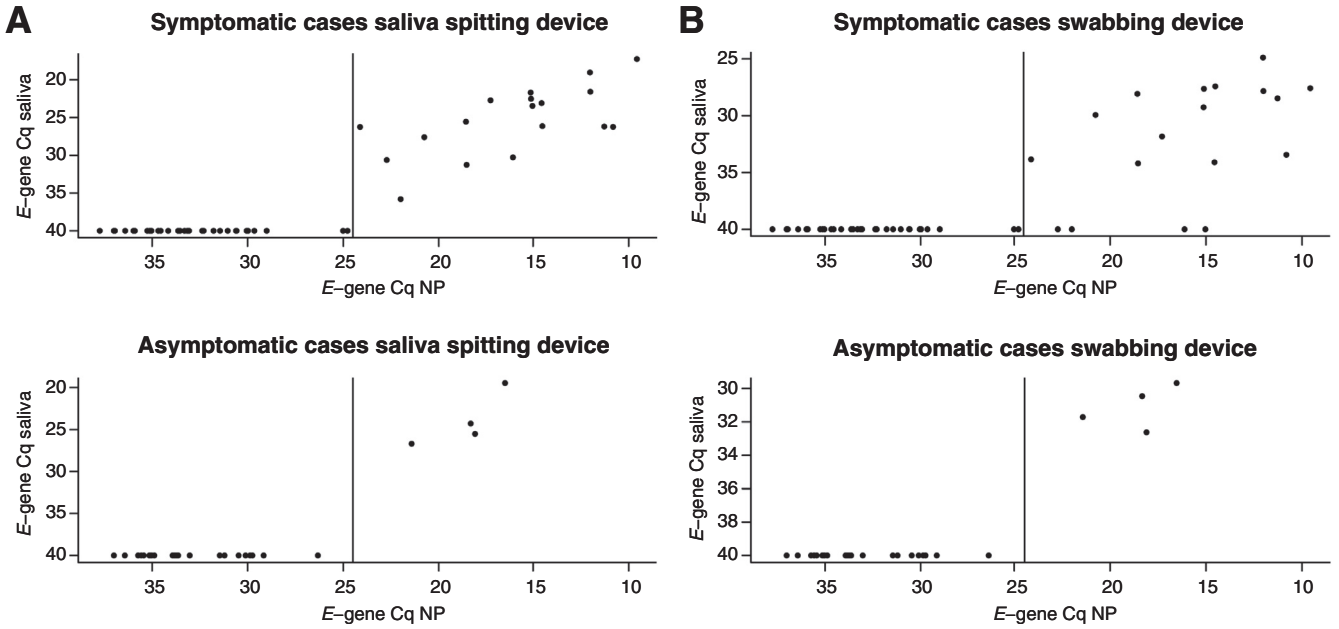

Figure 4 *E*-gene quantification cycle values from laboratory 1 in nasopharyngeal-positive samples and matching saliva samples from symptomatic and asymptomatic cases. **A:** Results obtained with the saliva spitting device. **B:** Results obtained with the saliva swabbing device. NP, nasopharyngeal samples.

[sensitivity, 33.3% (95% CI, 20.8%−47.9%); and sensitivity, 25.5% (95% CI, 14.3%−39.6%) for spitting and swabbing saliva device, respectively] compared with asymptomatic cases [sensitivity, 13.8% (95% CI, 3.9%−31.7%) for both the spitting and swabbing saliva device] (Figure 4).

Among individuals with high viral load in NP samples, the sensitivity in the saliva samples was high, irrespective of symptomatic status. Sensitivity was 94.4% (95% CI, 77.2%−99.9%) and 100.0% (95% CI, 39.8%−100%) in symptomatic and asymptomatic individuals in the spitting sample, whereas the sensitivity from the swabbing saliva sample was 72.2% (95% CI, 46.5%−90.3%) in symptomatic subjects and 100.0% (95% CI, 39.8%−100%) in asymptomatic subjects. The sensitivity and test positive ratio are summarized in Table 1 (and in Supplemental Table S4 for laboratory 2).

Discussion

The literature on the use of saliva for SARS-CoV-2 detection is rapidly evolving and expanding. Saliva sampling for COVID-19 diagnostics has been put forward as an alternative for nasopharyngeal sampling in several independent studies. A rapid review of the literature estimated a pooled sensitivity of SARS-CoV-2 testing on saliva versus nasopharyngeal samples as high as 97%.[18] More recently, a meta-analysis of 16 unique studies, representing 5922 patients, reported a pooled sensitivity for SARS-CoV-2 detection in saliva of 83.2%.[19] Herein, we compared the sensitivity of two different saliva collection devices with the nasopharyngeal swab in >2500 individuals who were sampled at different triage centers in Belgium.

In contrast to the current literature, a substantially lower SARS-CoV-2 test positivity rate was observed in saliva than in nasopharyngeal samples. However, when focusing on individuals with a high viral load ($>1.51 \times 10^6$ copies/mL viral transport medium), sensitivity improved dramatically, especially in saliva samples produced through spitting. There are several potential reasons for the discrepancy between results presented herein and current literature reports. First, the study population may be different. Most of the studies reported in literature predominantly include symptomatic patients (whether or not hospitalized) who are more likely to have a high viral load. Our study included individuals visiting triage centers to obtain diagnostic testing for SARS-CoV-2, because either they presented with COVID-19 symptoms or they had been in close contact with an infected individual. These individuals were not critically ill and often did not have symptoms. Second, although our study compared two different devices for saliva collection, the possibility that these devices are less suitable for saliva collection compared with what has been used in other studies cannot be excluded. Of note, differential sensitivity was observed between both devices and it was concluded that saliva sampling through spitting is more sensitive than swabbing. Whether the order of saliva collection (participants were asked to first swab, then spit) could impact these results remains to be investigated. A study conducted in British Columbia collected samples from outpatient testing centers and evaluated saline mouth rinse/gargle (alias, swish and gargle approach) to collect saliva samples compared with neat saliva collection and found that the swish/gargle method had a higher sensitivity than neat saliva, 97.5% (95% CI, 86.9%−99.9%) versus 78.8% (95% CI,

61%−91%). It is unclear why the authors observed a significant difference in the sensitivity, but the saliva collection method could influence sensitivity of the test result. Note that, in this study, saliva samples were collected without gargling or throat clearing, actions that may further improve the sensitivity of SARS-CoV-2 detection in saliva. Finally, other covariates, including time of the day of sampling, stage of infection at sampling, and timing relative to an epidemiologic wave (ie, varying reproduction number), may also impact results. With respect to the latter, patients were sampled at the end of the first (spring) wave in Belgium, a period of low prevalence and low individual viral load [compared with the beginning of the second (autumn) wave[20]]. More studies are required to further investigate the impact of these factors. Notably, testing different but biologically related samples from each patient (saliva and oral swab) provides an internal validation of our study results, as both sample types lead to highly similar conclusions.

Our study also had some limitations. First, more detailed clinical and demographic data would have been helpful to evaluate if other factors could explain the difference in sensitivity between saliva and the nasopharyngeal swab. In addition, the inclusion of hospitalized patients may have allowed a more in-depth analysis on the relation between disease severity and detection sensitivity in saliva. Finally, longitudinal saliva collections in positive individuals could shed light on the dynamics of SARS-CoV-2 detection rates in function of disease progression.

In summary, this study suggests that saliva sampling cannot replace the standard nasopharyngeal swab for the diagnosis of SARS-CoV-2 in the population studied herein. Nevertheless, because of its ease of use and compatibility with self-sampling, saliva sampling could play a role in systematic screening campaigns that aim to identify asymptomatic cases with medium to high viral loads. However, on the basis of results presented herein, such screening campaigns would fail to identify low positives. Whether, and to what extent, these low positives are capable of spreading the virus requires further investigation.

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Author Contributions

P.M., M.G., E.A., J.-P.P., J.P., V.H., H.M., J.S., and J.V. conceptualized and supervised the study. P.M., M.G., J.H., C.V., M.B., V.D.W., J.S., and J.V. generated and processed primary data. P.M., M.G., S.K.D., M.A., J.S., and J.V. analyzed the data. M.A. performed the sample size computation. S.K.D. and M.A. conducted the statistical analysis of the accuracy data. M.G. and P.M. verified the underlying data. J.-P.P., J.P., P.S., and S.D. organized and supervised sample collection at triage centers. All authors contributed to discussions of results and manuscript writing, and approved the final version of the manuscript.

Supplemental Data

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