Large parallel screen of saliva and nasopharyngeal swabs in a test center setting proofs utility of saliva as alternate specimen for SARS-CoV-2 detection by RT-PCR

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Key points: Comparison with nasopharyngeal swabs in a large test center-based study shows that saliva is a reliable and convenient material for the detection of SARS-CoV-2 by RT-PCR in adults and children.

Abstract

Background

A high volume of testing followed by rapid isolation and quarantine measures is critical to the containment of SARS-CoV-2. RT-PCR of nasopharyngeal swabs (NPS) has been established as sensitive gold standard for the detection of SARS-CoV-2 infection. Yet, additional test strategies are in demand to increase and broaden testing opportunities. As one attractive option, saliva has been discussed as an alternative to NPS as its collection is simple, non-invasive, suited for children and amenable for mass- and home-testing.

Methods

Here, we report on the outcome of a head-to-head comparison of SARS-CoV-2 detection by RT-PCR in saliva and nasopharyngeal swab (NPS) of 1187 adults and children reporting to outpatient test centers and an emergency unit for an initial SARS-CoV-2 screen.

Results

In total, 252 individuals were tested SARS-CoV-2 positive in either NPS or saliva. SARS-CoV-2 RT-PCR results in the two specimens showed a high agreement (Overall Percent Agreement = 98.0%). Despite lower viral loads in saliva, we observed sensitive detection of SARS-CoV-2 in saliva up to a threshold of Ct 33 in the corresponding NPS (Positive Percent Agreement = 97.7%). In patients with Ct above 33 in NPS, agreement rate dropped but still reaches notable 55.9%.

Conclusion

The comprehensive parallel analysis of NPS and saliva reported here establishes saliva as a reliable specimen for the detection of SARS-CoV-2 that can be readily added to the diagnostic portfolio to increase and facilitate testing.

Introduction

The current gold standard for the diagnosis of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection relies on the detection by quantitative reverse-transcription polymerase chain reaction (RT-qPCR) in nasopharyngeal swabs. A range of RT-qPCRs methods have been developed and proven highly sensitive, accurate and reliable [1, 2]. Nasopharyngeal swabs (NPS) are considered the optimal material for detection, particularly in early infection [2]. However, viral load in the nasopharynx can wane in later disease stages, while the virus remains detectable in alternate specimen such as bronchoalveolar lavage or sputum, necessitating a validation of diagnostics tests in these specimens [3-5]. In addition, to overcome limitations in mass screening for early detection of SARS-CoV-2, saliva has been considered as alternate material to NPS [6-10]. NPS collection requires trained personnel while saliva collection is comparatively easy, needs little instruction and is amenable for self-collection. Importantly, saliva collection is non-invasive and it does not create discomfort for the patient. Saliva would thus be of particular advantage for testing children, for whom often parents and pediatricians refrain from testing due to the need to conduct a nasopharyngeal swab. Likewise, the possibility to switch to saliva would also be a relief for adults when frequent testing or large scale screens are required, respectively. Further, considering the current high level of SARS-CoV-2 testing by RT-PCR and antigen tests, which both require nasopharyngeal swabs, shortage in swab supplies may occur. Establishing the possibility to switch to saliva collection in this situation to allow RT-PCR testing to continue is thus highly advisable.

Several recent studies have evaluated saliva as alternate specimen [6-29]. While these studies generally agree that detection of SARS-CoV-2 in saliva is possible, comparative analyses came to different conclusions, with some studies noting a better performance of saliva, while others found a substantially lower sensitivity. With few exceptions, patient cohorts tested thus far were in most studies relatively small and often included both hospitalized individuals with advanced SARS-CoV-2 infection as well as outpatients who were newly screened for infection, leaving uncertainty in which situation saliva may be best used. The overall sensitivity and thus utility of saliva in comparison to NPS remains thus differentially debated and needs to be defined. To resolve these issues, we embarked on a large-scale head-to-head comparison of saliva and NPS in a test center setting. The high number of individuals tested (N = 1187) and the high number of positives detected (N = 252), paired with a true-to-life screening in test centers, empowered a highly controlled analysis of agreement and supports the applicability of saliva in routine testing.

Materials and Methods

Study population

Adults and children (N = 1187) opting for a voluntary SARS-CoV-2 test at one of five participating test centers were included. Four centers were dedicated test centers for outpatients and one was an emergency department. The study population comprised individuals with SARS-CoV-2 related symptoms based on Swiss testing criteria and asymptomatic individuals with relevant exposure to a SARS-CoV-2 index case. Hospitalized patients were not included. Individuals were included without further selection to avoid skewing. Information on symptomatic or asymptomatic status was collected as part of the regular procedure for SARS-COV-2 testing and reporting based on self-evaluation (asymptomatic/mild/strong) by the participants, as they did not see a physician in the test center setting.

Ethical approval

The Zurich Cantonal Ethics Commission waived the necessity for a formal ethical evaluation based on the Swiss law on research on human subjects, as the collection of saliva in parallel to a scheduled nasopharyngeal swab induces no risk and no additional personal data beyond the usual information on symptoms and duration required by the FOPH for all SARS-CoV-2 tests in Switzerland was collected (Req-2020-00398). Due to the ethics waiver no informed consent had to be collected.

Sample collection

Test centers were advised to use their regular swab and virus transport medium (VTM)/universal transport medium (UTM) for nasopharyngeal sampling. Transport media used by the centers included Cobas PCR Medium (Roche), Liquid amies preservation medium (Copan), Virus Preservative Medium (Improviral), and in-house VTM (HEPES, DMEM, FCS, antibiotics, antimycotics).

Collection kits for saliva were supplied to the test centers: one tube for saliva collection (Sarsted 62.555.001) and a separate tube with 3 ml VTM (Axonlab AL0607). The procedure for saliva collection was described in an instruction leaflet (Figure 1). In Study Arm 1, “Basic”, individuals were asked to clear the throat thoroughly and collect saliva one or two times into the same tube (N = 835). As a guidance for the volume of saliva to be sampled, participants were instructed by study teams to collect 0.5 – 1 ml (approx. a teaspoon full). To investigate a possible influence on SARS-CoV-2 detection in saliva through differences in saliva collection, a subset of patients (N = 352) in Study Arm 2, “Enhanced”, was asked to clear their throat three times thoroughly and collect saliva into the same tube. Emphasis in this study arm was on enhanced throat clearing to ascertain sampling material from the posterior oropharynx. Immediately after saliva collection, VTM was added to the crude saliva and the content mixed through gentle twisting. Saliva was collected directly after NPS and both specimens immediately sent for SARS-CoV-2 RT-PCR testing.

Quantitative SARS-CoV-2 PCR

NPS and Saliva were processed identically using the procedures established for NPS in the diagnostics laboratory of the Institute of Medical Virology. 500 ul of NPS or saliva in VTM were diluted in 500 ul of Nuclisens easyMAG Lysis Buffer (BioMérieux), centrifuged (2000 rpm, 5 min) and analyzed with the Cobas SARS-CoV-2 IVD test (Roche) on a Cobas 6800. All testing for NPS and saliva was done in parallel on the same day. SARS-CoV-2 detection was further quantified using SARS-CoV-2 Frankfurt 1 RNA as calibrator (European Virus Archive, 004N-02005) allowing to report both Ct and genome equivalents.

Verification by in-house SARS-CoV-2 E-gene and GAPDH PCR

Discordant results of the Cobas SARS-CoV-2 test between NPS and saliva were re-analyzed using an in-house RT-qPCR targeting the E-gen based on Corman et al. [1]. GAPDH was measured as input control as described [30]. Both assays used AgPath-ID One-Step RT-PCR chemistry (Ambion, ThermoFisher).

Data analysis

E-gene Ct values were used for comparison. If E-gene reported negative but ORF1 reported positive by the Cobas SARS-CoV-2 IVD test, the ORF1 result was considered and the respective sample rated positive for SARS-CoV-2. This was the case for one saliva sample.

Data was analyzed using R (version 4.0.2) [31]. 95% confidence intervals were calculated with the epiR package (version 1.0.15). Method comparison and regression analysis (Passing-Bablok Regression [32] and Bland-Altman Plot [33]) was performed with the mcr package (version 1.2.1).

Results

Head-to-head comparison of saliva and nasopharyngeal swabs as material for SARS-CoV-2 detection by RT-PCR

In our protocol we advised participants to collect approx. 0.5 ml saliva into a wide (30 ml, 30 mm diameter) tube (Figure 1). Initial attempts in a pilot experiment at the participating emergency department with smaller tubes (15 ml, 17 mm diameter) showed that spitting into narrower tubes is problematic for some participants, leading to a contamination of the outside of the tube with saliva in some cases. Sampling with the wider tubes was in contrast unproblematic and thus deemed safe. Saliva sampling in children was found equally unproblematic, children were collaborating and able to expectorate.

Our study included five different test sites to ensure that data are not skewed due to specific procedures at one site. In Study Arm “Basic” (N = 835) saliva sampling was done with one-time throat clearing followed by expectorating saliva one to two times. In Study Arm “Enhanced” (N = 352) participants

cleared their throat 3x times followed by spitting. Saliva was mixed with VTM immediately after collection. The thus diluted material was unproblematic for further processing in the laboratory, no complications in pipetting or invalid results due to the intrinsic viscosity of saliva or congealing were observed.

High positive predictive agreement of SARS-CoV-2 detection in saliva and nasopharyngeal swabs

Adults and children that qualified for a regular SARS-CoV-2 test according to the FOPH and reported to one of the participating test centers or emergency units were enrolled from October 20, 2020 to November 4, 2020. In total 1187 individuals (male 54.8%/female 45.2%) were included (Table1). Median age was 35 with an age range of 5 – 98 years. 89 participants were under the age of 18. The majority of participants were symptomatic 71.9%. Median Days of symptoms ranged from 1 to 30 with a median of 2 days. The overall daily positivity rate of SARS-CoV-2 tests by RT-PCR during the study period at our diagnostics unit ranged between 14% and 22%. The positivity rate amongst study participants was 21%.

Across both study arms NPS and saliva results showed a high overall percent agreement (OPA = 98%) and good positive percent agreement (PPA = 91.9%, Table 2 and 3). In only 24 cases discordant results were observed, with 20 saliva samples and 4 NPS showing a negative results when the other specimen tested positive (Figure 2, Table 2). To investigate if discordant results are due to inadequate sampling, detection problems in the RT-PCR, or reflect true negatives in the respective sample material, all discordant pairs were retested using an in-house RT-PCR for the E-gene in conjunction with a GAPDH measurement to control for input. Mean levels for GAPDH input were Ct = 24.6 (SD = 2.7) for NPS and Ct = 24.7 (SD = 2.1) for saliva. One false-negative saliva sample (E-gene Ct 19.7 in NPS) did not contain any material (GAPDH Ct > 40). Excluding this sample, the PPA in the NPS Ct 15 – 20 range reaches 100% (Table 4).

Re-assessment with an in-house E-gene PCR confirmed all discordant results. For one case with a negative NPS, a second swab was collected the following day. This sample showed a high viral load, confirming an unsuccessful swab collection the day earlier.

Of note, in our head-to-head comparison both NPS (N = 1) and saliva (N = 5; N = 4 excluding the sample that did not contain saliva) produced false-negative results in cases where the other specimen showed a high viral load (Ct < 30) highlighting variability in collection for both specimens.

SARS-CoV-2 loads in saliva and nasopharyngeal swab correlate

Correlation analysis of sample pairs that both tested positive (N = 228) confirmed that saliva and NPS results are in good agreement (Figure 3A). Notably, Ct values in saliva were on average 4.79 higher than the corresponding Ct in NPS. This corresponds to a factor 28 lower viral load (Figure 3B). Notably though, at high Ct values, this difference was less pronounced possibly adding to the high PPA of detection in saliva at low viral load in the corresponding NPS.

Detection of SARS-CoV-2 in saliva from symptomatic and asymptomatic individuals

Our study recorded severity of symptoms (asymptomatic/mild/strong) at the sampling time point by self-evaluation (Figure 4A). We observed a good positive percent agreement of saliva and NPS in symptomatic individuals (PPA = 92.3%). In line with a trend to lower viral loads, i.e. higher Ct values in absence of symptoms (asymptomatic median Ct 28.4; mild symptoms median Ct 23.7; strong symptoms median Ct 21.6), the PPA was lower in asymptomatic participants (PPA = 84.2%). We observed decreasing viral loads with ongoing symptomatic infection in both saliva and NPS, highlighting a transient window of detection in the upper respiratory tract. Interestingly, changes in saliva were overall less dynamic than in NPS (Figure 4B).

Intensified throat clearing with saliva collection is favorable

To investigate if the intensity of saliva collection has an impact, we analyzed the two study arms of saliva collection separately. Participants were either asked to clear the throat thoroughly ("Basic", N = 835) or in an intensified protocol to clear it three times ("Enhanced", N = 352) and collect about 0.5 – 1 ml of saliva. We found that intensified saliva collection appears favorable for samples with low viral load. With the enhanced sampling protocol, PPA with NPS of ct >33 reached 66.7% (CI 35% - 90%), compared to 50.0% (CI 28% - 72%) with the basic protocol (Figure 5 and Table 5). Differences were, however, not statically significant, highlighting robust detection of SARS-CoV-2 in saliva in the two collection procedures tested.

Discussion

In the present study we sought to devise and evaluate a saliva sampling strategy that provides i) representative sampling of virus containing material, ii) easy and safe sampling in adults and children, iii) possibility for home collection, iv) straight forward processing in the laboratory.

We opted for a saliva collection procedure where participants clear their throat to first generate saliva from the back of the throat and then expectorate the saliva into an empty container. We considered clearing the throat important to sample material from the posterior oropharynx where SARS-CoV-2 sampling by oropharyngeal swabs is known to be efficient [34, 35]. While gargling with saline or buffer solutions has been suggested as a possibility to sample saliva from the deep throat [36, 37], we rated this procedure as less operable as the gargling solution would need to be optimized for taste to be accepted by individuals, could not include preservatives, and gargling itself may potentially generate aerosols. In addition, gargling is not practicable for many smaller children for whom we in particular sought to create increased possibilities for SARS-CoV-2 testing as NPS collection for children is often not practical.

Our study demonstrates an excellent agreement of saliva in the head-to-head comparison with NPS and thus recommends saliva as alternate material for SARS-CoV-2 detection by RT-PCR. Up to a Ct 33 (equivalent to approximately 26'000 genome copies/ml) in the corresponding NPS, a notably high PPA

(97.6%) is reached. Of note, virus loads in an even lower range are considered to impose a marginal risk for transmission as suggested by contact tracing and in vitro culturing studies [38-40].

Considering the observed PPA in detection, saliva may safely be envisaged as substitute for NPS detection in a range of settings. Possible scenarios include i) sampling of children, ii) home collection in quarantine, iii) test centers without trained medical personnel (e.g. schools, universities, companies), iv) non-irritating alternative for persons that need frequent testing due to their occupation or health status, v) fast large-scale screens in institutions (e.g. elderly homes). In situations where besides SARS-CoV-2 other respiratory viruses, e.g., Influenza and RSV, need to be excluded, NPS should, however, remain the standard material of choice as it allows rapid detection with multiplex-PCR from a single specimen. In addition, if SARS-CoV-2 infection has to be ruled out with highest possible sensitivity (e.g. in transplantation), NPS should remain the standard procedure.

The majority of SARS-CoV-2 in saliva represents likely virus secreted from infected cells in the nasopharynx and is not locally produced. Collecting material from the posterior oropharynx is thus important. This is also highlighted in our study as the collection protocol with intensified throat clearing shows a trend to increased PPA at low viral loads.

It remains possible that eating or drinking shortly before collection may decrease viral content in the oral cavity and throat. In the present study, neither eating, drinking nor smoking was controlled as study subjects came for an elective analysis by NPS and thus could only be informed about the saliva sampling on site immediately before the collection. Abstaining from food and beverage uptake shortly (1h) before saliva collection could be considered in forth-coming applications of saliva as test material, as it may increase the efficacy of SARS-CoV-2 detection in saliva even further.

In summary, our analysis rates saliva as valid alternate specimen for SARS-CoV-2 detection by RT-PCR. Saliva collection is non-invasive, thus not strenuous for patients, does not need trained personnel, allows collection at any location, and allows self-collection. Importantly, as we show here, saliva collection does not require any adjustments in the diagnostics tests; established RT-qPCR can be used. Combined with the high reliability in detecting SARS-CoV-2 infection as demonstrated in our head-to-head comparison with the standard NPS, increasing and facilitating test efforts by monitoring SARS-CoV-2 infection in saliva is rapidly attainable and needs to be considered.

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Tables

Table 1: Participant demographics

Total	**1187**
Male/Female (%)	650 (54.8%)/537 (45.2%)
Age median (range)	35 (5 – 98)
Symptomatic mild (%)	764 (64.4%)
Symptomatic strong (%)	89 (7.5%)
Asymptomatic (%)	291 (24.5%)
No information on symptoms (%)	43 (3.6%)
Median days of symptoms (range)	2 (1 – 30)

Table 2: Contingency table full cohort

	NPS positive	**NPS negative**	**Total**
Saliva positive	228	4	232
Saliva negative	20	935	955
Total	248	939	1187

Table 3: Agreement and Predictive Values

Saliva and NPS Agreement and Predictive Values (reference standard NPS, 95% CI)	
Positive Percent Agreement (PPA)	**91.9% (87.8% - 95.0%)**
Negative Percent Agreement (NPA)	**99.6% (98.9% - 99.8%)**
Overall Percent Agreement (OPA)	**98.0% (97.0% - 98.7%)**

Table 4: Positive Percent Agreement (PPA) stratified by NPS E-gene Ct-values

NPS (Ct)	**>10-15**	**>15-20**	**>20-25**	**>25-30**	**>30-33**	**>33-35**	**>35-40**
NPS positive	1	54	90	56	13	13	21
Saliva false negative	0	1 (0*)	2	2	0	5	10
PPA	100%	98.1% (100%*)	97.8%	96.4%	100%	61.5%	52.4%

*Excluding one sample that did not contain saliva as defined by GAPDH measurement.

372 *Table 5: Positive Percent Agreement (PPA) stratified by NPS E-gene Ct-values and saliva sampling*

	Full cohort (N = 1187)			Basic Sampling (N = 835)			Enhanced Sampling (N = 352)		
NPS (Ct)	**all**	**>10-33**	**>33-40**	**all**	**>10-33**	**>33-40**	**all**	**>10-33**	**>33-40**
NPS positive	248	214	34	183	161	22	65	53	12
Saliva false negative	20	5	15	16	5	11	4	0	4
PPA	91.9%	97.7%	55.9%	91.3%	96.9%	50.0%	93.8%	100%	66.7%

373

Figures

Figure 1: Instruction leaflet for saliva collection

Participants were asked to clear the throat and collect saliva into a collection tube (A). VTM was added to the crude saliva immediately after collection (B), and the content was mixed through gentle twisting (C).

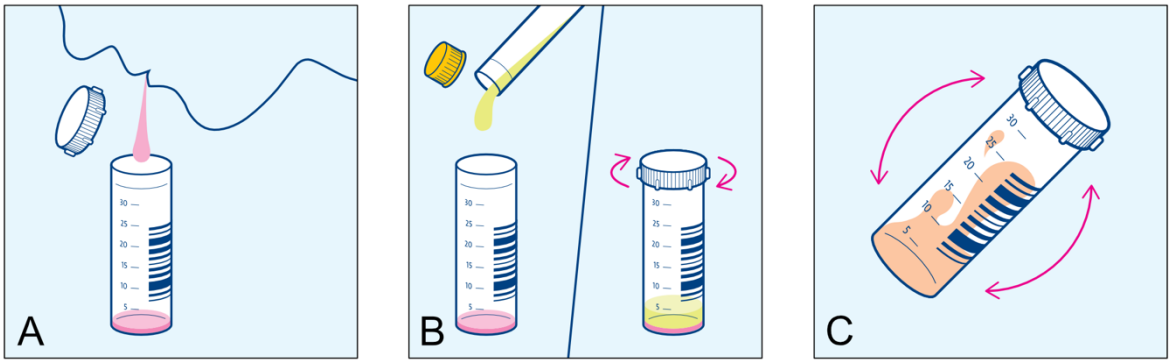

Figure 2: High agreement of SARS-CoV-2 detection in saliva and nasopharyngeal swabs

Summary of the full cohort (N = 1187 study participants). Roche Cobas E-Gene Ct values of paired NPS and saliva samples are depicted. neg = PCR negative; red dashed line equals identity.

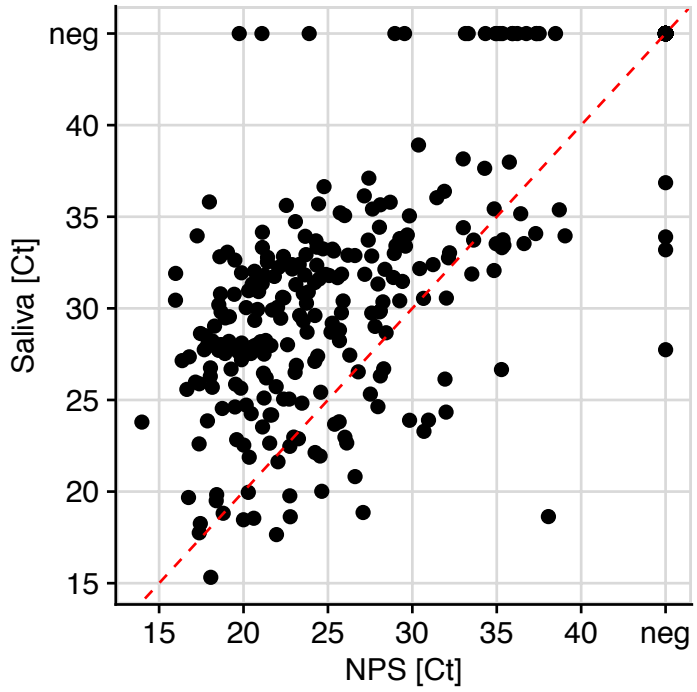

Figure 3: SARS-CoV-2 levels in saliva and nasopharyngeal swabs correlate

A) Passing-Bablok Regression of E-gene Ct-values of NPS and saliva of all positive pairs from the full cohort (N = 228; $p < 0.0001$). Red dashed line equals identity, blue line shows linear trend.

B) Bland-Altmann Plot of E-gene Ct-values of NPS and saliva of all positive pairs from the full cohort (N = 228).

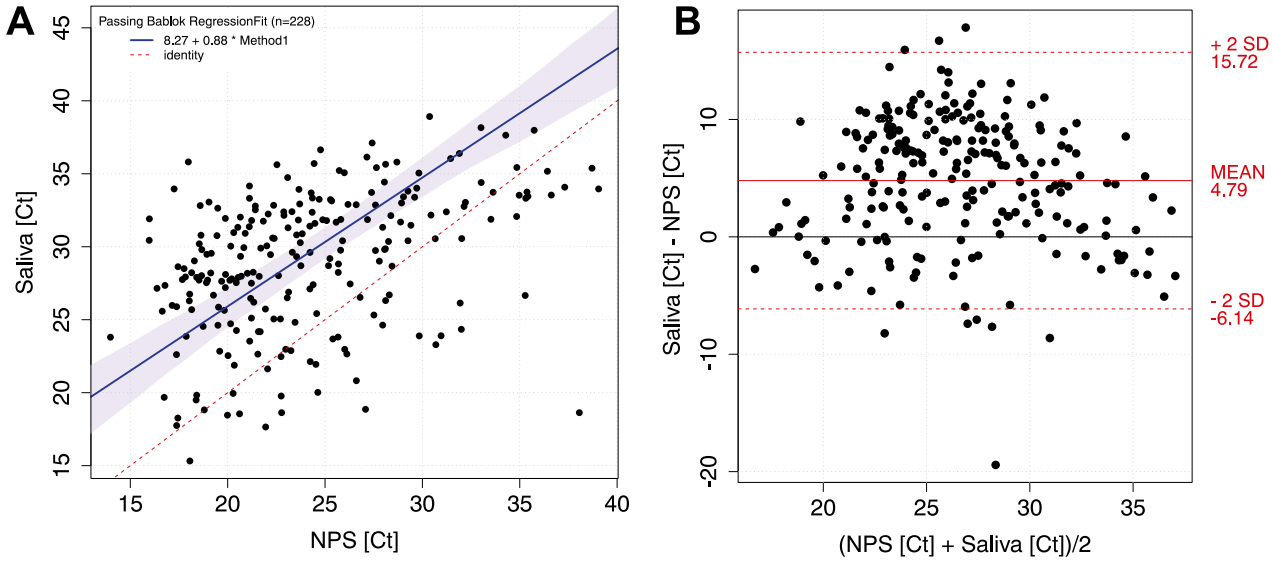

Figure 4: Viral loads in NPS and saliva decrease with ongoing infection

A) E-gene Ct-values of NPS and saliva of all positive pairs from the full cohort (N = 226).

B) Duration of symptoms in symptomatic patients (N = 836) versus E-gene Ct value in saliva and NPS.

neg = PCR negative, red dashed line equals identity, blue line shows linear trend.

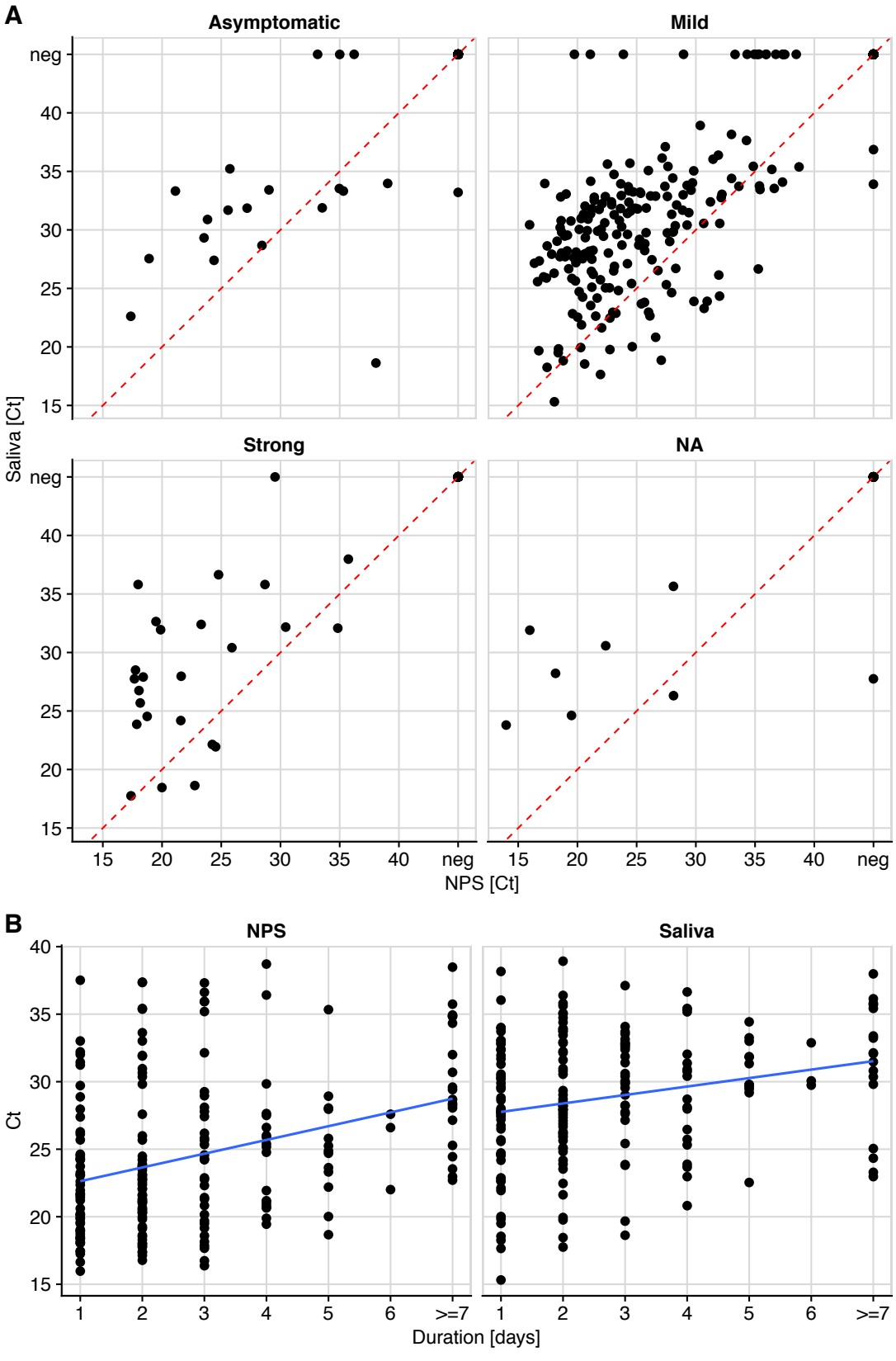

Figure 5: Intensified saliva sampling increases low level SARS-COV-2 detection in saliva.

E-gene Ct values of paired NPS and saliva samples of study arm "Basic" (1-2x saliva per tube; N = 835) and "Enhanced" saliva collection (3x saliva per tube; N = 352).