Feasibility and accuracy of a novel saliva sampling method for large-scale SARS-CoV-2 screening in children < 12 years of age

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Abstract

Objectives

Children have been disproportionately affected during the COVID-19 pandemic. Novel test strategies are urgently needed to ensure safe operation of schools and childcare institutions and to avoid prolonged closures.

Methods

A weekly SARS-CoV-2 sentinel study in primary schools, kindergartens and childcare facilities over a 12-week-period was conducted. In total, 3123 concurrent oropharyngeal and saliva samples were processed for SARS-CoV-2 rRT-PCR testing in both children (n=2104) and staff (n=1019). Saliva sampling was optimised, and a novel sampling system was introduced and assessed for feasibility, the Salivette® system.

Results

For children across all age groups a mean of 1.18 ml saliva could be obtained with this easy-to-handle system. Using 1293 concurrent oropharyngeal swabs from children, staff and participants of a positive control cohort as reference, the Salivette testing method could be assigned an overall specificity of 99.8% and sensitivity of 95.1%. Of note, ‘clinical sensitivity’, defined as detection of positive cases with an oropharyngeal-swab derived Ct-value < 33, was 100%. Comparative analysis of Ct-values derived from saliva vs. oropharyngeal swabs demonstrated a significant difference (mean difference 4.23 (95% CI 2.48–6.00).

Conclusions

The Salivette system is an easy-to-use, safe and feasible collection method for saliva sampling and subsequent SARS-CoV-2 testing in children aged 3 years and above. In view of the excellent sensitivity and specificity documented in our study, this novel testing approach is a very reliable and much more pleasant alternative to oropharyngeal swab based testing, particularly in children and for self-testing in the home setting.
Introduction

The ongoing pandemic caused by Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) is a major challenge for all medical specialties and global society as a whole. Despite the fact that children, in particular the group of <12-year-olds, have been shown to be at reduced risk for contracting SARS-CoV-2 and for suffering from COVID-19, they have been substantially affected by closure of schools, kindergartens and childcare facilities (1, 2). Hence scientists and public health leaders alike have been exploring options for coronavirus testing approaches in order to ensure safe operation of these respective institutions. Accumulating evidence points towards a rather low and stable transmission risk in educational institutions despite rising incidence rates in the population, as long as preventative hygiene measures are in place (3). The ideal system to allow for large-scale test operations would be easy to handle, safe to perform, include its use in younger children, and ideally allow for self-sampling at home or at the appropriate child-care institution without the help of a medical professional. Various groups have explored different sampling methods from a range of clinical specimens while naso-/ oropharyngeal swabs are considered the gold standard (4–7). SARS-CoV-2 is known to replicate in the oropharynx and to affect the oral cavity as demonstrated by taste loss and mucosal lesions (i.e. macules, enanthema). Of particular note, just very recently it has been confirmed that the virus infects both the oral mucosa and salivary glands and that viral burden of the patient’s saliva correlated with COVID-19 symptoms, including taste loss (8). Thus, obtaining saliva samples appears to be a very sensible and valuable alternative to oropharyngeal swabs and has been frequently assessed in recent months (9–12). It is a less unpleasant procedure holding great potential, in particular for the pediatric population and for self-sampling at home. Numerous reports have described saliva sampling in adults as a reliable non-invasive method for SARS-CoV-2 testing with a sensitivity of 83.2% and a specificity of 98.9% compared to naso-/oropharyngeal swabs according to a recent metaanalysis (13). However, all these reports
either do not explicitly address or remain unclear about the important relevant pre-analytic aspects (11, 14, 7). For example, only Wyllie and colleagues reported the actual amount of saliva collected from each individual tested in their study (11). Similarly, it remains unclear whether saliva samples were processed as neat material or diluted with a respective buffer or normal saline in the laboratory before rRT-PCR testing. Finally, these studies were primarily conducted in adults and the proposed protocols are impractical for screening children since they include fasting over several hours or are technically complex (11, 14). Overall, data from pediatric cohorts is scarce. Thus, Skolimowska et al. have rightly concluded that further investigations to optimise saliva collection methodology to improve sample adequacy and sensitivity would be required (14). Hence, the aim of our sub-study accompanying a weekly sentinel program was to establish a practical, safe and easy-to-use system for saliva collection and subsequent rRT-PCR testing for SARS-CoV-2 for children aged 3 years and above that may enable communities to role out large-scale test operations in schools and insitutions of childcare without the help of on-site medical professionals.

Methods and study concept

Between June and November 2020, we conducted a weekly SARS-CoV-2 sentinel study in primary schools, kindergartens and childcare facilities in Munich (3). A total of 3123 concurrent oropharyngeal and saliva samples were processed for SARS-CoV-2 real time reverse transcription PCR (rRT-PCR) testing in both children (n=2104) and staff (n=1019) (Figure 1). Phase 1 of the study used standard urine pots for collection of saliva samples. Children and staff were asked to spit into the pots prior to having an oropharyngeal swab taken. In phase 2 a novel sampling system was introduced and assessed for feasibility, the Salivette® system (SARSTEDT AG & Co, Germany). In addition, a total of 50 individuals, both adults and children, known to be infected with SARS-CoV-2 were recruited and consented into a positive control cohort (PCC). Again, concurrent oropharyngeal swabs and
saliva samples were obtained and run on our rRT-PCR systems. PCC participants were either recruited in the hospital inpatient setting or by visiting quaranteened individuals in the private home. All individuals had not taken in any food nor drink prior to saliva sampling. Participants of the PCC were asked to leave the device’s absorbent cotton pad in their mouth for a minimum of 2 minutes; subsequently each individual replaced the saliva-absorbing pad into the Salivette® collection tube and closed it with the topper. The concurrent oropharyngeal swab was taken immediately after the saliva sampling. Following collection, saliva samples and swabs were transferred into the study laboratory. For Salivette samples, tubes were centrifuged for 5 minutes at 3000rpm to harvest saliva. All saliva specimens and swabs were processed using the ampliCube Coronavirus SARS-CoV-2 (Mikrogen, Germany) on a Bio-Rad CFX96 Touch rRT-PCR Detection System (Bio-Rad, Germany). Single gene results were retested with Xpert Xpress SARS-CoV-2 (Cepheid, USA). For methodological comparison between swab and saliva sampling we referred to semi-quantitative cycle threshold (Ct) values of corresponding SARS-CoV-2 gene locus. Statistical analysis was done using R-studio software, version 4.0.2.

**Results**

Though collecting saliva spitting samples during phase 1 of our study was very easy, the volume obtained was minimal requiring the addition of normal saline for processing and running the samples on the rRT-PCR system. In addition, urine pots (height: 5.5 cm, width: 4.5 cm) required substantial room for storage making transport of study samples and processing in the laboratory rather difficult. Finally, spitting into a urine pot poses a substantial risk for SARS-CoV-2 transmission making routine use of this method in a school or kindergarten setting challenging. Therefore, the novel Salivette® system (SARSTEDT AG & Co, Germany), a medical diagnostic saliva sampling device approved for patients aged 3 years and above, was introduced in phase 2 of the study (15, 16). This collection system
proved to be substantially more suitable for large-scale usage in our school and kindergarten sentinel setting requiring only minimal storage space (height: 9.7 cm, width: 1.7 cm). Furthermore, it improved processing in the laboratory since the tubes are compatible with centrifuges. In addition to the minimal sample volume required for nucleic acid extraction (200 ul), the respective dead storage volume of nucleic acid extraction machines (800 - 1000 ul) needed to be accounted for; thus, samples yielding less than 1 ml of saliva needed topping up with normal saline.

Once the Salivette® sampling system was established in participating institutions, a total of 875 individual samples (574 children, 301 staff) were subjugated to accurate measurements of saliva volume to explicitly address pre-analytic aspects of this method. We found that for children across all age groups a mean of 1.18 ml saliva could be obtained with this easy-to-handle system. For staff of the participating institutions a mean of 1.34 ml saliva could be collected (Table 1).

A total of 1243 concurrent oropharyngeal swabs and Salivette® samples from both children and staff were collected during our sentinel study (1241 negative and 2 positive sample pairs).

Since our sentinel study yielded only two positive pairs of samples in week 12 (3) we undertook an additional approach to be able to accurately assess both sensitivity and specificity of the Salivette® testing method using results from oropharyngeal swabs as reference. The two positive sample pairs from the sentinel study and concurrent oropharyngeal swabs and saliva samples from additional 50 individuals known to be infected with SARS-CoV-2 were processed in the PCC (n=52). Median age of this group was 45.0 years (range 3 to 87 years, male/female ratio 1.3) and assessment of saliva volume per Salivette® showed a mean of 1.70 ml (range: 0.75 – 2.75 ml). A total of nine individuals tested negative in both saliva and oropharyngeal swab samples. Thirty-nine individuals showed a positive test result from both sampling materials, including the two sentinel study participants. Finally, two individuals demonstrated a discordant negative/positive (“false
negative”) and two additional individuals showed a discordant positive/negative (“false positive”) result for saliva and oropharyngeal swab samples, respectively. For negative saliva samples Ct values from corresponding oropharyngeal swab samples were 33.17 and 33.72, while for negative oropharyngeal swab samples Ct values from corresponding saliva samples read 37.49 and 37.68, respectively.

Based in these figures derived from 1293 sample pairs, our Salivette® testing method could be assigned a specificity of 99.8% (95% CI 99.4 - 100) and a sensitivity of 95.1% (95% CI 82.2 - 99.2) in comparison to the oropharyngeal swab as gold standard (Table 2). In order to assess the effect of natural dilution in saliva sampling compared to oropharyngeal swabs, we assessed Ct-values of individual sample pairs. Figure 2 and 3 visualise person-matched saliva and swab SARS-CoV-2 rRT-PCR Ct-values for respective 39 corresponding sample pairs. Wilcoxon signed rank test with continuity correction showed a significant difference between Ct-value measurements derived from saliva vs. oropharyngeal swabs (p-value = 0.032). In addition, Bland-Altmann graphical comparison showed an agreement between the two sampling methods with saliva-derived Ct-values being systematically higher than Ct-values derived from oropharyngeal swabs: Mean difference 4.23 (95% CI 2.48–6.00), upper limit of agreement 14.85 (95% CI 17.87 – 11.82) and lower limit of agreement -6.38 (95% CI -9.41 – -3.35) (17). To further assess ‘clinical sensitivity’ of the testing method we analysed results of corresponding samples pairs for individuals with a high likelihood of being infectious based on a Ct-values from oropharyngeal swab samples (Ct < 33; high to moderate viral load). In this group of individuals sensitivity of our novel saliva sampling algorithm was 100%.

Discussion

To our knowledge, this is the first large-scale feasibility and qualitative study introducing the Salivette® system in combination with rRT-PCR for SARS-CoV-2 testing in children and specifically addressing the issue of pre-analytic methodology in saliva sampling and of its
practicability. So far only few studies reported the use of this testing system in adults (15, 16, 18). Its use for home-sampling in 201 adults over a 2-week period has been evaluated, comparing rRT-PCR results from saliva and oropharyngeal swabs (16). However, feasibility of this system as a screening tool over a longer period of time and in the paediatric population has not been demonstrated. The Salivette® system is an easy-to-use, safe and feasible collection method licensed for saliva sampling in children aged 3 years and above. The mean difference in Ct-values between oropharyngeal swabs and saliva collected in the Salivette® system was significant. Still, overall test sensitivity of 95.1% and specificity of 99.8% demonstrated in this study was excellent and ‘clinical sensitivity’ for detecting individuals with a high likelihood of being infectious turned out to be 100%. Accumulating evidence indicates that rRT-PCR Ct-values of 33 characterize an individual who is no longer infectious (19). Hence, the parameter of ‘clinical sensitivity’ appears to be of practical relevance. Other studies have demonstrated lower sensitivity and specificity of saliva testing methods, but this is most likely due to inadequate pre-sampling conditions and sample volumes (16). It is very likely that saliva test results are influenced by prior fluid or food intake, smoking or other habits such as chewing gums, thus further increasing the inherent dilution effect on SARS-CoV-2 of this biological sample compared to oropharyngeal swabs. Therefore, we took great care to ensure that saliva samples in our study were not confounded by these factors. In fact, sampling was only performed after a minimum of 30 minutes had elapsed since the individual had last taken in any food or drink. One may speculate that the best sampling window would be when self-sampling is integrated as an early-morning, pre-breakfast and pre-toothbrushing routine procedure in the home setting. The findings in our study are further supported by a recent systematic review and meta-analysis comparing saliva and nasopharyngeal swabs for rRT-PCR testing for SARS-CoV-2 and demonstrating that both methods yield similar sensitivity and specificity across all 16 studies included in the analysis (13). Interestingly and on a slightly different note, some recently published data even point
towards SARS-CoV-2 viral load in saliva as a dynamic unifying correlate of COVID-19 severity, further highlighting the relevance and diagnostic potential of this biological specimen (20). Other alternative non-oropharyngeal swab approaches have also been explored and may be practical for both adults and children. For example, Willeit and colleagues have reported results from gargling samples (21). While this method may be feasible in adults and older children, it cannot be used in younger children. In addition, gargling involves external fluid or buffer while the Salivette® allows collection of a substantial and suitable amount of neat saliva, so that an undiluted clinical specimen can be directly used for laboratory processing. Furthermore, while the Salivette-based saliva collection is a safe and closed system, gargling methods might generate aerosols and thus appear to be less suitable from an infection-control point of view. In view of recent evidence that SARS-CoV-2 also infects salivary glands and oral mucosa, saliva must be regarded as an optimal specimen of SARS-CoV-2 testing (8). While our study clearly demonstrates both feasibility and highly reliable test performance of the Salivette® saliva collection method in conjunction with subsequent rRT-PCR testing for SARS-CoV-2 in a large group of adult staff and children aged 3 years and above, the positive control group for comparison of Ct-values from concurrent sample pairs (oropharyngeal swab and saliva) was rather small. Still, and of great practical significance, the Salivette® is highly suitable for self-testing without posing any risk for virus transmission to healthcare workers or friends and family nearby. Of note, as little as 200 µl of neat saliva obtained from a Salivette® can be used for SARS-CoV-2 testing provided laboratory personal opts for a manual pipetting approach avoiding to account for large dead storage volumes of extraction robots. In addition, in situations where test capacities are limited the Salivette®-collected individual saliva samples can easily be pooled in the laboratory to assess 5 or more single samples in one rRT-PCR run (22). Depending on respective local incidence rates, these pools could be adapted to more or less individual samples per pool. With adequate local and IT-logistics and rapid processing in the laboratory,
turn-around time (time-span form taking the sample until the final result) can be as short as 12 hours, ensuring ‘same day results’. In view of these findings and since the Salivette collecting method is a much more pleasant sampling technique independent of medically trained personel, we propose it as the prefered method for routine rRT-PCR-based SARS-CoV-2 testing in months and years to come, particularly in children and for self-testing at home. Additional studies to further assess this novel test approach on an even larger scale and in the home setting (in a pre-breakfast, pre-toothbrushing routine) as well as the respective pooling options adjusted to local incidence rates will be needed and are already underway.

**Conflict of interest:** All authors declare that there are no conflicts of interest.

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**Author’s contributions**
MH, TS, SV and UvB developed the concept and designed the study, TS and UvB wrote study protocol. LK, TS and UvB obtained ethics approval. MH, SV, UE, LK, VG, SK, ARH,
MMB and UvB contributed to data acquisition. UE, NA, AS, BL, MMB and VF supervised laboratory procedures. MH, SV, UE, LK, VG and UvB analyzed the data. MH, SV, LK, JH, TS and UvB interpreted the data. MH, SV and UvB wrote the manuscript. MH, SV, LK and UvB supervised development of illustrating figures and tables. All authors read and approved the manuscript and take full responsibility of its content.

References


Table & Figures

Table 1

Maximum, mean and minimum amount of saliva collected using the Salivette system in children and staff (n=875): Volume (ml).

<table>
<thead>
<tr>
<th>Volume [ml]</th>
<th>3-4 years (n=145)</th>
<th>5-6 years (n=167)</th>
<th>7-8 years (n=170)</th>
<th>9-11 years (n=92)</th>
<th>All children (n=574)</th>
<th>Staff (n=301)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum</td>
<td>2.50</td>
<td>2.50</td>
<td>3.00</td>
<td>2.50</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.04</td>
<td>1.13</td>
<td>1.21</td>
<td>1.33</td>
<td>1.18</td>
<td>1.34</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.25</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

Person-matched saliva (obtained using the Salivette collection system) and oropharyngeal swab data (rRT-PCR for SARS-CoV-2) for 1293 individuals tested for SARS-CoV-2 indicating sensitivity and specificity.

<table>
<thead>
<tr>
<th>oropharyngeal swab</th>
<th>SARS-CoV-2 detected</th>
<th>SARS-CoV-2 not detected</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>saliva (Salivette)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2 detected</td>
<td>39</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td>SARS-CoV-2 not detected</td>
<td>2</td>
<td>1250</td>
<td>1252</td>
</tr>
<tr>
<td>total</td>
<td>41</td>
<td>1252</td>
<td>1293</td>
</tr>
</tbody>
</table>

Sensitivity: 95.1 % (82.2 - 99.2 %)
Specificity: 99.8 % (99.4 - 100 %)
Figure legends:

Figure 1
Age stratification of children (n=2104) per study week tested for SARS-CoV-2 using saliva samples. Weeks 1-5 represent phase 1 (using urine pots for saliva collection) and weeks 6-12 represent phase 2 (use of the Salivette® system for saliva collection) of the study.

Figure 2
Comparison of Cycle threshold (Ct) values of SARS-CoV-2 rRT-PCR corresponding gene loci from 39 person-matched saliva and oropharyngeal swab samples. Respective p-values were calculated by Wilcoxon matched-pairs signed rank test.

Figure 3
Bland-Altman graph displaying means and mean differences of Cycle threshold (Ct) values between 39 saliva and oropharyngeal swab sample pairs including upper and lower limits of agreement.
<table>
<thead>
<tr>
<th>Age Group</th>
<th>wk 1</th>
<th>wk 2</th>
<th>wk 3</th>
<th>wk 4</th>
<th>wk 5</th>
<th>wk 6</th>
<th>wk 7</th>
<th>wk 8</th>
<th>wk 9</th>
<th>wk 10</th>
<th>wk 11</th>
<th>wk 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-11 y</td>
<td>35</td>
<td>57</td>
<td>43</td>
<td>43</td>
<td>46</td>
<td>39</td>
<td>39</td>
<td>39</td>
<td>34</td>
<td>30</td>
<td>35</td>
<td>32</td>
</tr>
<tr>
<td>7-8 y</td>
<td>53</td>
<td>38</td>
<td>50</td>
<td>54</td>
<td>47</td>
<td>57</td>
<td>47</td>
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<td>65</td>
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<td>5-6 y</td>
<td>43</td>
<td>51</td>
<td>58</td>
<td>49</td>
<td>63</td>
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<td>48</td>
<td>48</td>
<td>52</td>
<td>41</td>
<td>49</td>
<td>57</td>
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<tr>
<td>3-4 y</td>
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<td>26</td>
<td>28</td>
<td>31</td>
<td>26</td>
<td>42</td>
<td>34</td>
<td>40</td>
<td>42</td>
<td>40</td>
<td>45</td>
<td>39</td>
</tr>
</tbody>
</table>

Number of tested children (n = 2104)
The graph depicts the distribution of Ct-values for samples obtained from oropharyngeal swabs and saliva samples. The p-value indicates a statistically significant difference between the two groups, with a p-value less than 0.05. The data suggests that saliva samples generally have higher Ct-values compared to oropharyngeal swabs, which is consistent with previous studies.