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Letter to the Editor

Saliva collected in universal transport media is an effective, simple and high-volume amenable method to detect SARS-CoV-2

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To the Editor,

During the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-Co-V-2) pandemic we have encountered unprecedented testing volumes and constant supply chain disruptions, including swabs. These challenges could be somewhat alleviated by using saliva as a sample type because it is easy to collect, does not use swabs and is amenable to high-volume collection. In this issue, Skolimowska *et al.* argued that saliva is not an appropriate sample type for SARS-Cov-2 PCR [1], but others have shown that crude saliva can be as sensitive as a nasopharyngeal (NP) swab [2]. However, crude saliva is not amenable to high-volume and automated processing because saliva can be difficult to pipette due to its viscosity [2], and in our experience, it can congeal shortly after collection. Furthermore, processing crude saliva requires many manual manipulations including the addition of media at the laboratory, careful mixing and adding proteinase K and/or centrifugation steps [1–3]. Crude saliva added to universal/viral transport media (VTM/UTM) at the point of collection is amenable to high-volume automated processing because the saliva arrives at the laboratory suspended in liquid form. An added benefit of UTM is its ubiquitous use and the extensive experience with this medium for molecular and culture-based diagnostics of viruses and other pathogens. Most assays and laboratory equipment are validated using this medium and have regulatory agency approval for specimens collected in UTM.

We initially investigated saliva collection for SARS-CoV-2 diagnosis via a saline gargle and spit method but found it to be substantially inferior to a NP swab. In this issue, Pasomsub *et al.* report on a low number of patients ($n = 21$ positives), finding a sensitivity of 84.2% for saliva collected in UTM compared with NP and oropharyngeal/throat (OP) swabs for the detection of SARS-CoV-2 [4]. We therefore decided to test the method of Pasomsub *et al.* in a larger number of patients.

PCR-positive individuals admitted to the hospital or in the community diagnosed by a NP or OP swab gave their consent (University of Calgary Health Research Ethics Board #20-0444). For the saline gargle and spit method, participants took 3 mL of 0.85% sterile saline into their mouth, moved it back and forth to each side of the mouth twice, then gargled for 3 s and spat into a sterile polypropylene urine collection container (~4 cm wide). For the UTM method, participants accumulated saliva for 1–2 min (no specific volume) and spat into an empty urine container, and then 3 mL of Copan Italia UTM-RT (Code 330C) was immediately added. A NP swab (Flexible Mini-tip FloqSwab in 3 mL of UTM, Copan) was concomitantly collected from all patients by an infectious disease physician or a paramedic trained in NP swab collections. Study samples were tested upon receipt at the laboratory using an E gene

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RT-PCR [5]. Six participants in the gargle study group had the saliva and NP swab tested using the E gene and RdRP multiplex PCR [5]. E gene Ct values ≤35 were considered positive, and eluates of samples with an E gene Ct > 35 were repeated twice and considered positive if at least 2/3 were positive.

Twenty-nine participants (58.6% hospitalized) had a saline gargle sample collected. Using a reference standard of either sample positive ($n = 24$), the positive agreement (95% confidence interval (CI)) for the saline gargle was 70.8% (50.8–85.1) and NP swab 95.8% (79.8–99.8). In one case, only saliva was positive. The mean time from symptom onset and study collection was 11.6 days (range 3–44).

Seventy-five patients (9.5% hospitalized) had saliva in UTM collected. The positive agreement for saliva was 84.1% (73.7–90.9) and NP swab 91.3% (82.3–95.9) (Table 1), which is similar to the results of Pasomsub *et al.* using a composite reference standard (sensitivity of 85.7% for saliva and 90.7% for NP/OP). The mean time from symptom onset to study collection was 6.59 days (range 0–18). The mean (95% CI) cycle threshold (Ct) value for matched pairs was 26.5 (24.8–28.1) for NP swabs and 29.3 (27.8–30.9) for saliva in UTM ($p < 0.0017$, Wilcoxon test, Prism8, version 8.4.3 (416), GraphPad Software, San Diego, CA). When a NP swab was positive and saliva negative, the mean Ct value was 33.5 (30.23–36.76) and for saliva-positive and NP-negative specimens 33.7 (29.97–37.5). The STARlet (Hamilton Co., Reno, NV) robotic liquid handler processed saliva in UTM and saline from healthy volunteers without issues.

Adding UTM to saliva at the point of collection results in acceptable sensitivity (within ~5% of NP swabs). This method is also amenable to high-volume automated testing and is preferable to the saline gargle method because of its superior sensitivity and has little risk of generating aerosols, which requires air moving over a layer of fluid. Like our saline gargle results, Jamal *et al.* reported a sensitivity of 70% when phosphate-buffered saline (PBS) was added to saliva immediately after collection [6] (samples were frozen before testing), suggesting saline or PBS may be suboptimal saliva transport media.

It is noteworthy that our UTM test group were primarily outpatients and all in Pasomsub *et al.*, whereas 59% were hospitalized in our gargle group and 100% in Jamal *et al.* [6]. Hospitalized patients may have altered saliva composition and production due to co-morbidities and/or multiple medications lowering saliva production and altering saliva composition [7]. Controlling for variation in saliva production by collecting specific volumes of saliva may improve the UTM method sensitivity, which is not possible with the gargle method. An additional factor that may have contributed to the differences observed between groups is the mean time from symptom onset to study sampling, which was longer in our gargle test group than our UTM test group.

Alternatives to UTM such as saline have been necessary due to supply chain problems in the SARS-CoV-2 pandemic. However, due to the utility of UTM for the detection of other respiratory viruses and the benefits for the detection of SARS-CoV-2 we demonstrated, ensuring secure production and a supply chain of UTM are needed for this current pandemic and for respiratory virus seasons.

Although collection of NP swab alone (or with an OP swab) and saliva in UTM may provide higher sensitivity than saliva alone, saliva in UTM offers many advantages over a NP swab because it is non-invasive, convenient (especially for children), involves minimal labour and lends itself well to mass screening programmes.

Table 1
SARS-CoV-2 E gene PCR results from a NP swab and saliva collected in UTM from known COVID-19 patients

	NP swab (Copan FloqSwab)		
		Pos	Neg
Saliva in UTM	Pos	52	6
	Neg	11	6

Transparency declaration

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Author contributions

B. Berenger: Conceptualization, Methodology, Formal Analysis, Data Curation, Writing: Original and Draft, Supervision, Project Administration. J Conly: Investigation, Methodology, Writing: Review and Editing. Jia Hu: Investigation, Supervision, Project administration, Writing: Review and Editing. K Fonseca: Methodology, Writing: Review and Editing. Thomas Louie:Investigation, Writing: Review and Editing. A Schneider: Investigation, Writing: Review and Editing. T Singh: Investigation, Writing: Review and Editing. W Stokes: Conceptualization, Methodology, Investigation, Writing: Review and Editing. L Ward: Investigation, Writing: Review and Editing. Nathan Zelyas: Conceptualization; Methodology; Writing: Review and Editing.

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