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Accuracy of Tongue Swab Testing Using Xpert MTB-RIF Ultra for Tuberculosis Diagnosis

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ABSTRACT Tongue dorsum swabs have shown promise as alternatives to sputum for detecting *Mycobacterium tuberculosis* (MTB) in patients with pulmonary tuberculosis (TB). Some of the most encouraging results have come from studies that used manual quantitative PCR (qPCR) to analyze swabs. Studies using the automated Cepheid Xpert MTB/RIF Ultra qPCR test (Xpert Ultra) have exhibited less sensitivity with tongue swabs, possibly because Xpert Ultra is optimized for testing sputum, not tongue swab samples. Using two new sample preprocessing methods that demonstrated good sensitivity in preliminary experiments, we assessed diagnostic accuracy and semi-quantitative signals of Xpert Ultra performed on tongue swabs collected from 183 adults with presumed TB in Kampala, Uganda. Relative to a sputum Xpert Ultra reference standard, the sensitivity of tongue swab Xpert Ultra was 77.8% (95% confidence interval [CI] 64.4–88.0) and specificity was 100.0% (95% CI, 97.2–100.0). When compared to a microbiological reference standard (MRS) incorporating both sputum Xpert Ultra and sputum mycobacterial culture, sensitivity was 72.4% (95% CI, 59.1–83.3) and specificity remained the same. Semi-quantitative Xpert Ultra results were generally lower with tongue swabs than with sputum, and cycle threshold values were higher. None of the eight sputum Xpert Ultra "trace" or "very low" results were detected using tongue swabs. Tongue swabs should be considered when sputum cannot be collected for Xpert Ultra testing, or in certain mass-screening settings. Further optimization of tongue swab analysis is needed to achieve parity with sputum-based molecular testing for TB.

KEYWORDS diagnostics, oral swab analysis, tongue swab, tuberculosis

Each year, an estimated 10 million people develop tuberculosis (TB) and over 1.5 million die of TB despite the availability of effective treatment for most forms of the disease (1). Historically, TB diagnosis has relied on passive case findings, in which people with symptoms self-report to a health care facility for further evaluation. Diagnostic testing most often involves microbiological or molecular analysis of sputum for the presence of *Mycobacterium tuberculosis* (MTB) cells or DNA to confirm diagnosis (2). The production of sputum can be burdensome for those providing the specimen and hazardous to health workers and others

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when adequate safety measures are not in place. Moreover, children and people living with HIV are often unable to produce adequate sputum (3, 4). These limitations have spurred efforts to identify alternative sample types for TB diagnosis that are easier, less invasive, and safer to collect.

Tongue swabbing, also known as oral swab analysis (OSA), has emerged as a potential alternative to sputum-based molecular testing (5), which is now the first-line test recommended test for diagnosis of pulmonary TB (6–8). Compared to sputum collection, OSA is faster, easier, and safer. Because tongue swabs are easy to collect from any person in any setting, OSA may be especially useful for contact investigation and community-based screening. We have previously optimized and clinically validated OSA procedures for manual DNA extraction and IS6110-targeted qPCR (9–13). Sensitivity in adults relative to positive sputum Xpert MTB/RIF (Xpert, Cepheid; Sunnyvale, CA, USA) or positive sputum culture has ranged from 88% to 93%, with specificity ranging from 79% to 92% (10, 12). Given the widespread use of sputum Xpert for TB diagnosis in high burden countries, it offers an opportunity to quickly bring a new sampling method to scale.

To date, only a few studies have evaluated OSA in conjunction with Xpert Ultra testing in adults. Success has been limited, with reported sensitivities ranging from ~45% relative to sputum culture (14) to ~51% relative to sputum Xpert Ultra (15). Although these studies utilized different swabbing and sample handling methods, the results underscore the need to optimize and standardize protocols for performing Xpert Ultra testing using tongue swabs.

In this study, we sought to identify and characterize improved methods, and to provide updated guidance and protocols for use of Xpert Ultra to detect MTB in tongue swab specimens. We began by evaluating methods using volunteer tongue swab samples spiked with standardized cultured MTB cells. We then evaluated the diagnostic accuracy of the Xpert Ultra tongue swab when using the updated protocols among adults being evaluated for pulmonary TB at two outpatient clinics in Kampala, Uganda.

MATERIALS AND METHODS

Protocol optimization experiments. (i) Participants. The optimization experiments enrolled healthy volunteers (>18 years) recruited from the University of Washington, School of Public Health, Department of Environmental and Occupational Health Sciences between November 2019 and February 2021. Participants were assumed to be TB-negative based on low risk of exposure and lack of symptoms. Inclusion criteria and screening protocols have been described previously by Wood et al. (11). Procedures for collection of swab samples from human participants were approved by the University of Washington Human Subjects Division (STUDY00001840).

(ii) Sample collection. We used Copan FLOQSwab tongue swabs, which were shown to increase tongue dorsum biomass collection, relative to other swab products (12). Participants were asked to self-swab along the breadth of the mid-tongue dorsum, firmly pressing and rolling the swab head for approximately 10 s. Participants repeated the procedure up to 10 times with different swabs. In order to protect the safety of participants and study personnel, study staff left the room while collection took place. After collection, swab heads were immediately spiked with 10 μL of serially-diluted MTB strain H37Ra cells stored in 1× phosphate-buffered saline with 15% glycerol and 0.05% Tween 80 (PBSGT), or with blank PBSGT. Swab heads were then snapped off into 5 mL polypropylene transport tubes containing 800 μL sterile 1× Tris-EDTA (TE) buffer (10 mM Tris-HCl containing 1 mM EDTA•Na2, pH 8.0). A total of 15 participants were enrolled and sampled. Participants were contacted for repeated sampling as needed.

(iii) Processing methods and experiments. We evaluated three strategies for improving the sensitivity of Xpert Ultra performed on tongue swabs. The limit of detection (LoD) of each method was compared to that of manual methods similar to those reported previously (10, 12). Methods are presented in detail in the Supplemental Information and summarized here. In Method 1 (single swab SR), a single FLOQSwab was processed with Cepheid Sample Reagent (SR) using a protocol similar to that recommended by the manufacturer for sputum. In Method 2 (double swab SR), two FLOQSwabs were collected and combined into a single tube and processed as in Method 1. In Method 3 (boil method), a single FLOQSwab was processed by boiling, incubation, and mixing (without using SR). Methods 1 and 2, using SR, were assessed because they closely resemble sputum processing protocols currently recommended by Cepheid for Xpert Ultra. The double swab SR method tested the hypothesis that the processing steps applied to one FLOQSwab could be applied to two FLOQSwabs to improve the sensitivity of TB detection. A recent study demonstrated that repeated flocked swab sampling (10 swabs) does not significantly deplete bacterial ribosomal rRNA gene, a biomarker of bacterial biomass (12). Therefore, if two flocked swabs are collected in succession, suspended in buffer at the same volume as a single swab, and tested as a single sample, then the collection of MTB bacilli from TB patients may be doubled on average relative to single swabbing. The boil method was assessed for this study because it showed the highest sensitivity in contrived samples and resembled the first steps of our previous manual methods (10–13).

LoDs of the experimental methods were quantified using serial dilutions as described in the Supplemental Information. LoDs are reported on a CFU per swab basis, which is the same as the CFU

per sample amount for the single swab SR and boil methods, but is half of the CFU per swab basis for the double swab SR method, which included 2 swabs each spiked with the same number of bacilli as a single swab. Exploratory runs at some dilutions (e.g., 100 CFU/swab) increased the number of runs at those dilutions, relative to other dilutions. Results of all experiments were used in LoD calculations. This minimized bias that could have resulted from the designation of specific runs as exploratory.

Clinical evaluation procedures. (i) Design and participants. We conducted a cross-sectional study between April and September 2021 at outpatient clinics at Mulago National Referral Hospital and Kisenyi Health Center IV in Kampala, Uganda. We screened consecutive adults (>18 years), presenting to the health centers for any reason, for cough lasting more than 2 weeks. We excluded patients who 1) had completed latent or active TB treatment within the past 12 months; 2) had taken medication with antimycobacterial activity (including fluoroquinolones) for any reason within 2 weeks of study entry; 3) resided >20km from the study site or were unwilling to return for follow-up visits; or 4) were unwilling or unable to provide informed consent. The Makerere University School of Medicine Research and Ethics Committee, the Uganda National Council for Science and Technology, and the University of California, San Francisco Committee on Human Research reviewed and approved the study. The study was conducted and reported in accordance with the Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines (2).

(ii) Procedures. For all patients enrolled, we obtained a detailed demographic and medical history using a standardized form and collected blood for HIV testing and CD4 count (if HIV-positive). Trained research staff collected up to 3 tongue swabs (COPAN FLOQswab) using the same procedure described for healthy volunteers in Washington. Two tongue swabs were placed in a single tube with 800 μL of Tris-EDTA (TE) buffer and processed within 1 h for Xpert Ultra testing using the double swab SR method. In a subset of patients, an additional single swab stored at -80°C in a tube with 800 μL of TE buffer was processed for Xpert Ultra testing using the boil method. Tongue swab Xpert Ultra results were not provided to clinicians and were not used to inform patient management.

For reference standard testing, all participants provided three expectorated sputum specimens (3–5 mL each) following collection of tongue swabs. One sputum sample was used to perform Xpert Ultra testing for all patients (an additional sample was collected and tested if the initial Xpert Ultra result was trace-positive). Sputum sample processing for Xpert testing involves the addition of Cepheid's proprietary Sample Reagent (SR). This reagent liquefies and decontaminates the sample without lysing bacilli. Once the treated sample is loaded into the cartridge and the run started, the sample is drawn through a membrane filter, trapping intact bacilli. After wash steps, a sonic horn lyses the bacilli on the membrane and releases their DNA. The DNA is then subjected to a nested quantitative polymerase chain-reaction (qPCR), targeting the rifampicin resistance determining region (RRDR) of the rpoB gene. A mixed two-insertion sequence (IS1081 and IS6110) probe is also included in the latest version of the assay, Xpert MTB/RIF Ultra (Xpert Ultra), to enhance detection of low bacillary load samples (6, 16). If the initial or repeat sputum Xpert Ultra was negative or invalid, the remaining two sputum samples were digested, decontaminated and cultured in liquid media (Bactec Mycobacterial Growth Indicator Tube [MGIT] 960, Becton Dickinson). In 32 participants for whom MGIT tubes were not available at the time of enrollment, solid 7H10 media was used for culture. In the event of a negative 7H10 culture result, leftover sputum pellet was stored at 80°C for MGIT culture when available. Speciation testing was performed to confirm the presence of *M. tuberculosis* (MTB) using the SD MPT64TB Ag kit (SD Bioline).

(iii) Reference standard and index test definitions. We considered patients to be TB-positive if they had a positive result on sputum Xpert Ultra (grade very low, low, medium, high), two trace-positive sputum Xpert Ultra results or a culture result positive for MTB. We considered patients not to have TB if they were not TB positive and had two negative cultures results (TB status was considered to be indeterminate if one or both cultures were contaminated).

Staff performing tongue swab Xpert Ultra (the index test) were blinded to reference standard TB test results. For both the double swab SR and boil methods, the primary analysis considered all trace-positive tongue swab Xpert Ultra results to be positive. Secondary analyses followed the WHO recommendation for interpreting sputum Xpert Ultra results, with trace-positive results considered positive for people living with HIV only.

Data analysis. We summarized demographic and clinical data using appropriate descriptive statistics. We calculated sensitivity and specificity of tongue swab Xpert Ultra performed using Methods 2 and 3 in reference to sputum Xpert Ultra results alone, and in reference to sputum Xpert Ultra and culture results (with TB status defined as described above). The difference in sensitivity between the double swab SR and boil methods was assessed using McNemar's test, and the differences in cycle threshold (Ct) values for sputum Xpert Ultra, the double swab SR method, and the boil method were assessed using the Wilcoxon signed rank test. We used STATA 15 (StataCorp, College Station, TX, USA) for all analyses.

RESULTS

Limits of detection of tongue swab processing methods for Xpert Ultra testing. LoDs are summarized in Table 1, and Fig. S1 plots the LoD curves of each of the described methods. Raw dose-response results are presented in Table S1. The single swab SR method, with 69 spiked samples, was relatively insensitive, with an LoD of 101.7 CFU/swab (95% CI, 64.5–144.0). With 64 spiked samples, the double swab SR method improved sensitivity, with a LoD of 76.5 CFU/swab (95% CI, 54.2–104.1). The boil method demonstrated the highest sensitivity (Table 1). With a total of 38 spiked samples represented in the dose-response data, the boil method exhibited an LoD of 22.3 CFU/swab (95% CI, 15.3–34.3). For purposes of comparison,

TABLE 1 Comparison of Methods 1–3 and manual qPCR method LoDs

Method	Description	H37Ra LoDs in CFU/swab (95% CI)[a]
Method 1	1 FLOQSwab, SR[b], Xpert Ultra ("single swab SR")	101.7 (64.5–144.0)
Method 2	2 FLOQSwabs, SR, Xpert Ultra ("double swab SR")	76.5 (54.2–104.1)
Method 3	1 FLOQSwab, boil w/o SR[b], Xpert Ultra ("boil method")	22.3 (15.3–34.3)
Manual (Reference)[c]	1 FLOQSwab, Qiagen extraction and EtOH precipitation, manual IS6110 qPCR	53.5 (36.9–73.0)

[a]LoDs, limits of detection. Contrived samples were tongue swabs from healthy volunteers spiked with dilution series of cultured MTB H37Ra.
[b]SR, GeneXpert sample reagent.
[c]Method used in Luabeya et al. (10) and Wood et al. (12).

with 40 spiked samples, the LoD of our clinically-validated manual qPCR method was 53.5 CFU/swab (95% CI, 36.9–73.0). This value fell between those of the double swab SR and boil methods (Table 1). The double swab SR method was selected for the main clinical evaluation due to its ease and comparable sensitivity to the clinically-validated manual qPCR method. Of note, there were no false positives among negative control tongue swab samples (n = 27) with any of the tongue swab processing methods. Additionally, there were no false-positive rifampin resistance determinations (n = 61).

Study population demographics for clinical evaluation. Between April 14 and September 9, 2021, 184 eligible patients were enrolled and underwent tongue swab Xpert Ultra testing using the double swab SR method. One patient had an indeterminate tongue swab Xpert Ultra result and was excluded from this analysis. Among the remaining 183 patients, median age was 33 years (inter-quartile range [IQR], 26-43); median BMI was 21.4 (IQR, 19.3–23.9); 36 (19.7%) were underweight, 76 (41.5%) were female, 58 (31.7%) were HIV-positive, and 22 (12.0%) had previously been diagnosed with TB (Table S2). TB symptoms were common, with 142 (77.6%) experiencing weight loss, 137 (74.9%) fever, 122 (66.7%) night sweats, 115 (62.8%) decrease in appetite, 26 (14.2%) bumps in neck, armpit, or groin, and 25 (13.7%) had hemoptysis. Overall, 58 (31.7%) patients were diagnosed with TB, of whom 54 (93.1%) were positive by sputum Xpert Ultra.

Diagnostic accuracy. Relative to sputum Xpert Ultra, the sensitivity of tongue swab Xpert Ultra was 77.8% (n/N = 42/54, 95% CI 64.4–88.0), and specificity was 100.0% (n/N = 129/129; 95% CI, 97.2–100.0) (Table 2). Sensitivity fell to 72.4% (n/N = 42/58; 95% CI, 59.1–83.3) against the microbiological reference standard (incorporating both sputum Xpert Ultra and sputum culture), but specificity remained 100% (n/N = 119/119; 95% CI, 96.9–100). In the secondary analysis, classifying tongue swab Xpert Ultra trace results as positive for HIV-positive patients only, sensitivity was 68.5% (n/N = 37/54; 95% CI, 54.4–80.5) with no impact on specificity (Table 2). For both the primary and secondary analyses, sensitivity and specificity stratified by HIV status are show in Table S3. Sensitivity of tongue swab Xpert Ultra was consistently higher among patients with a higher sputum mycobacterial load, as assessed using sputum

TABLE 2 Diagnostic accuracy of tongue swab Xpert Ultra (double swab SR method)[a]

Primary analysis[b] (N = 183)	Sputum Xpert Ultra reference standard (estimate, 95% CI)	Microbiologic reference standard[c] (estimate, 95% CI)
Sensitivity	42/54, 77.8 (64.4–88.0)	42/58, 72.4 (59.1–83.3)
Specificity	129/129, 100 (97.2–100)	119/119, 100 (96.9–100)
PPV	42/42, 100 (91.6–100)	42/42, 100 (91.6–100)
NPV	129/141, 91.5 (85.6–95.5)	119/135, 88.1 (81.5–93.1)

Secondary analysis[d] (N = 183)	Sputum Xpert Ultra reference standard (estimate, 95% CI)	Microbiologic reference standard[c] (estimate, 95% CI)
Sensitivity	37/54, 68.5 (54.4–80.5)	37/58, 63.8 (50.1–76.0)
Specificity	129/129, 100 (97.2–100)	119/119, 100 (96.9–100)
PPV	37/37, 100 (90.5–100)	37/37, 100 (90.5–100)
NPV	129/146, 88.4 (82.0–93.1)	119/140, 85.0 (78.0–90.5)

[a]CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.
[b]Primary analysis classifies all tongue swab Xpert Ultra trace-positive results as positive.
[c]Excluding 6 indeterminate MRS results (<2 negative cultures due to contamination).
[d]Secondary analysis classifies tongue swab Xpert Ultra trace-positive results as positive for HIV-positive patients only.

TABLE 3 Sensitivity of tongue swab Xpert Ultra by sputum Xpert Ultra semiquantitative grade

Sputum Xpert Ultra semiquantitative grade	Sensitivity of Method 2		Sensitivity of Method 3	
	Primary analysis[a]	Secondary analysis[b]	Primary analysis[a]	Secondary analysis[b]
Trace	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
Very Low	0/6 (0%)	0/6 (0%)	1/5 (20.0%)	0/5 (0%)
Low	6/12 (50.0%)	4/12 (33.3%)	5/9 (55.6%)	3/9 (33.3%)
Medium	15/15 (100%)	13/15 (86.7%)	11/11 (100%)	9/11 (81.8%)
High	21/21 (100%)	20/21 (95.2%)	10/10 (100%)	10/10 (100%)
All	42/56 (75.0%)	37/56 (66.1%)	27/37 (73.0%)	22/37 (59.5%)

[a]Primary analysis classifies all tongue swab Xpert Ultra trace-positive results as positive.
[b]Secondary analysis classifies tongue swab Xpert Ultra trace-positive results as positive for HIV-positive patients only.

Xpert Ultra semi-quantitative results (Table 3). Semi-quantitative Xpert Ultra results were generally lower with tongue swabs than with sputum samples, with none of the eight sputum Xpert Ultra "trace" or "very low" results being detected by tongue swabs using Method 2 (Table 4).

Median IS6110 Ct values were 19.9 (IQR 17.6–22.1, $n = 42$) for tongue swab Xpert Ultra and 16.4 (IQR 16.2–17.6, $n = 56$) for sputum Xpert Ultra. The Ct value was higher for tongue swab Xpert Ultra than for sputum Xpert Ultra among 41/42 patients who had a positive result on both tests, with 1 patient having the same Ct value on both tests (Ct value 16.3, sputum Xpert Ultra grade High, tongue swab Xpert Ultra grade Low). The median difference in Ct value was 3.6 (95% CI, 1.3–5.6, $P < 0.0001$).

In a subset of 37 patients with trace-positive (N = 2) or higher grade (N = 35) positive sputum Xpert Ultra results, frozen tongue swab samples were also processed and tested using the boil method (Table 5). The two participants with sputum Xpert Ultra trace-positive results were not detected by either tongue swab method. Among the remaining 35 patients with very low or higher semi-quantitative sputum Xpert Ultra positive results, 24 (68.6%) were identified by both swab methods, three were identified by the boil method and missed by the double swab SR method, and one was identified by the double swab SR method but missed by the boil method (sensitivity difference 5.7% [95% CI, -19.6–8.2%], $P = 0.63$). In secondary analyses when tongue swab Xpert Ultra trace-positive results were considered positive for HIV-positive patients only, the boil method identified two positives missed by the double swab SR method, and the double swab SR method identified two positives missed by the boil method, resulting in no difference in sensitivity. Among the 24 patients who were identified by both tongue swab methods, Ct values were not significantly different ($P = 0.48$).

DISCUSSION

OSA is a simpler alternative to sputum collection for TB testing, but standardized preprocessing methods for pairing tongue swabs with widely available molecular testing platforms are needed. In laboratory spiking experiments, we showed that the LoD is comparable to manual DNA extraction and qPCR when two tongue swabs are processed for Xpert Ultra testing using a protocol similar to that used for sputum-based testing. In clinical evaluation, this method resulted in three-quarters of patients with TB being detected by Xpert

TABLE 4 Semiquantitative results

Sputum Xpert Ultra[a]	Tongue swab Xpert Ultra (Double swab SR[b] method)					
	Negative	Trace	Very low	Low	Medium	Total
Negative	127	0	0	0	0	127
Trace	2	0	0	0	0	2
Very low	6	0	0	0	0	6
Low	6	3	0	3	0	12
Medium	0	3	5	7	0	15
High	0	1	4	14	2	21
Total	141	7	9	24	2	183

[a]Highest semiquantitative grade from sputum Xpert Ultra; test repeated if the first resulted trace-positive or indeterminate.
[b]SR, GeneXpert sample reagent.

TABLE 5 Diagnostic accuracy of tongue swab Xpert Ultra (boil method)[a]

Primary analysis[b] (N = 37)	Sputum Xpert Ultra reference standard (estimate, 95% CI)	Microbiologic reference standard (estimate, 95% CI)
Sensitivity	27/35, 77.1 (59.9–89.6)	27/35, 77.1 (59.9–89.6)
Specificity	2/2, 100 (15.8–100)	2/2, 100 (15.8–100)
PPV	27/27, 100 (87.2–100)	27/27, 100 (87.2–100)
NPV	2/10, 20.0 (2.5–55.6)	2/10, 20.0 (2.5–55.6)

Secondary analysis[c] (N = 37)	Sputum Xpert Ultra reference standard (estimate, 95% CI)	Microbiologic reference standard (estimate, 95% CI)
Sensitivity	22/35, 62.9 (44.9–78.5)	22/35, 62.9 (44.9–78.5)
Specificity	2/2, 100 (15.8–100)	2/2, 100 (15.8–100)
PPV	22/22, 100 (84.6–100)	22/22, 100 (84.6–100)
NPV	2/15, 13.3 (1.7–40.5)	2/15, 13.3 (1.7–40.5)

[a]CI: confidence interval, PPV: positive predictive value, NPV: negative predictive value.
[b]Primary analysis classifies all tongue swab Xpert Ultra trace-positive results as positive.
[c]Secondary analysis classifies tongue swab Xpert Ultra trace-positive results as positive for HIV-positive patients only.

Ultra testing of tongue swabs while retaining high specificity (100%, 95% CI; 95.8–100). These data highlight that OSA should be considered an alternative when sputum cannot be collected. The data also highlight the need for further optimization of OSA-based TB diagnosis to detect pauci-bacillary disease.

Compared with previous studies of OSA-based Xpert Ultra testing, we show a marked improvement in sensitivity while retaining high specificity. Sensitivity was 45% (95% CI, 29–62) in a study from Peru that collected a single cheek swab using one of three swab types and storage buffers, and processed swabs for Xpert Ultra testing in a manner similar to Methods 1 and 2 used in our study (14). Based on our previous studies, more bacterial biomass is present on the tongue dorsum than on cheeks, and FLOQSwabs pick up more biomass than the swab types used in the Peru study. In a study of mass TB screening among prisoners in Brazil, sensitivity was 51% overall (95% CI, 43–60) when testing two tongue swabs sequentially and ranged from 38% to 90% in patients with very low/low to high semi-quantitative sputum Xpert Ultra results (15). Sensitivity was modestly higher in our study (78%, 95% CI; 64–88), especially among patients with higher mycobacterial load, supporting the use of our protocol involving the use of FLOQswabs and simultaneous testing of two swabs processed with GeneXpert SR. However, our results showed the same pattern of inability to detect paucibacillary disease.

Two more recent studies shed new light on potential strengths and limitations of the oral swab method. First, members of our consortium (17) reported a hybrid non-sputum strategy for people with HIV (PHIV), who often present with paucibacillary respiratory samples. When PHIV were tested for urine lipoarabinomannan (LAM) in addition to MTB DNA in oral swabs, the two samples yielded complementary results (urine LAM detected a lot of swab-negative people and vice versa). Testing both non-sputum specimens yielded significantly better sensitivity than testing either specimen alone (17). Secondly, Cox et al. reported very low sensitivity (22%) for oral swabs collected from children with TB, when the swabs were tested with Xpert Ultra (18). However, the specimen processing method used by Cox et al. was similar to Method 1 in the current report (single swabs processed with Cepheid SR). Method 1 was the least sensitive of the three methods that we evaluated (Table 1). These results illustrate the need for further research and development to adapt existing sputum-testing protocols to the new task of swab testing.

Although our data support further exploration of the use of tongue swabs when sputum cannot be collected, there remains a need to further optimize OSA-based molecular testing for TB. Our laboratory experiments showed that boiling a single swab results in lower LoD compared with processing one or two swabs with Xpert Ultra SR. However, benefits were marginal in our small clinical sub-study of the boil method. We reported recently that flocked tongue swabs collect only a small percentage of the biomass that is present on tongue dorsa (13). Therefore, efforts to optimize sensitivity are focused on the use of alternative swab products that collect more biomass.

Although the boil method used a single swab without SR and therefore would fit well in settings with Xpert testing capacity, the requirement for heat block and other accessories further reduce its advantage over the double-swab with SR. In addition, given a large proportion of laboratories in resource limited settings often have other competing priorities, we would be hesitant to recommend the boil method as a routine procedure for all users.

Key strengths of the study include the use of laboratory- and clinic-based studies to validate protocols for Xpert Ultra testing using tongue swabs, and clinical validation among a sample of patients with cough identified through clinic-based active TB case finding. However, there are also some limitations to this study. Our clinical study population is reflective of patients seeking health care in Uganda. OSA-based Xpert Ultra testing may have lower sensitivity if applied for TB screening among high-risk but asymptomatic individuals. Our clinical sub-study of the boil method was limited to a small subset of patients. A larger evaluation may confirm some benefits as predicted by our laboratory-based analysis. However, unless the difference is meaningful, the additional laboratory work required is unlikely to be taken up in routine practice. Last, we did not confirm positive sputum Xpert Ultra results with culture. However, Xpert Ultra has been shown to have very high specificity (>98%) for TB (16).

In summary, we demonstrate that Xpert Ultra testing of tongue swabs should be considered when sputum collection is not feasible. With further improvements in sample collection and processing and leveraging advances in molecular testing of swabs for SARS-CoV-2, OSA-based molecular testing has the potential to open the door to expanded TB case-finding efforts. This non-invasive, fast, and safer diagnostic specimen and processing method may provide a useful new tool in the global fight against TB.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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REFERENCES

1. World Health Organization. 2020. Global Tuberculosis Report 2020. World Health Organization, Geneva, Switzerland.
2. Cohen JF, Korevaar DA, Altman DG, Bruns DE, Gatsonis CA, Hooft L, Irwig L, Levine D, Reitsma JB, de Vet HCW, Bossuyt PMM. 2016. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open 6:e012799. https://doi.org/10.1136/bmjopen-2016-012799.
3. World Health Organization. 2015. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV: Policy guidance. https://apps.who.int/iris/handle/10665/193633.
4. Denkinger CM, Kik SV, Cirillo DM, Casenghi M, Shinnick T, Weyer K, Gilpin C, Boehme CC, Schito M, Kimerling M, Pai M. 2015. Defining the needs for next generation Assays for Tuberculosis. J Infectious Diseases 211:S29–S38. https://doi.org/10.1093/infdis/jiu821.
5. Valinetz ED, Cangelosi GA. 2021. A look inside: oral sampling for detection of non-oral infectious diseases. J Clin Microbiol 59 JCM.02360-20. https://doi.org/10.1128/JCM.02360-20.
6. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, Banada PP, Deshpande S, Shenai S, Gall A, Glass J, Krieswirth B, Schumacher SG, Nabeta P, Tukvadze N, Rodrigues C, Skrahina A, Tagliani E, Cirillo DM, Davidow A, Denkinger CM, Persing D, Kwiatkowski R, Jones M, Alland D. 2017. The new Xpert MTB/RIF Ultra: improving detection of mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. mBio 8 https://doi.org/10.1128/mBio.00812-17.
7. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing DH, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD. 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363:1005–1015. https://doi.org/10.1056/NEJMoa0907847.
8. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop JAnn, Owens MR, Rodgers R, Banada P, Safi H, Blakemore R, Lan NTN, Jones-López EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan B, Winn-Deen E, Christel L, Dailey P, Perkins MD, Persing DH, Alland D. 2010. Rapid detection of mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 48:229–237. https://doi.org/10.1128/JCM.01463-09.
9. Deviaene M, Weigel KM, Wood RC, Luabeya AKK, Jones-Engel L, Hatherill M, Cangelosi GA. 2020. Sample adequacy controls for infectious disease diagnosis by oral swabbing. PLoS One 15:e0241542. https://doi.org/10.1371/journal.pone.0241542.
10. Luabeya AK, Wood RC, Shenje J, Filander E, Ontong C, Mabwe S, Africa H, Nguyen FK, Olson A, Weigel KM, Jones-Engel L, Hatherill M, Cangelosi GA. 2019. Noninvasive detection of tuberculosis by oral swab analysis. J Clin Microbiol 57:e01847-18. https://doi.org/10.1128/JCM.01847-18.

11. Wood RC, Luabeya AK, Weigel KM, Wilbur AK, Jones-Engel L, Hatherill M, Cangelosi GA. 2015. Detection of mycobacterium tuberculosis DNA on the oral mucosa of tuberculosis patients. Sci Rep 5:8668. https://doi.org/10.1038/srep08668.
12. Wood RC, Andama A, Hermansky G, Burkot S, Asege L, Job M, Katumba D, Nakaye M, Mwebe SZ, Mulondo J, Bachman CM, Nichols KP, Le Ny A-LM, Ortega C, Olson RN, Weigel KM, Olson AM, Madan D, Bell D, Cattamanchi A, Worodria W, Semitala FC, Somoskovi A, Cangelosi GA, Minch KJ. 2021. Characterization of oral swab samples for diagnosis of pulmonary tuberculosis. PLoS One 16:e0251422. https://doi.org/10.1371/journal.pone.0251422.
13. Nicol MP, Wood RC, Workman L, Prins M, Whitman C, Ghebrekristos Y, Mbhele S, Olson A, Jones-Engel LE, Zar HJ, Cangelosi GA. 2019. Microbiological diagnosis of pulmonary tuberculosis in children by oral swab polymerase chain reaction. Sci Rep 9:10789. https://doi.org/10.1038/s41598-019-47302-5.
14. Mesman AW, Calderon R, Soto M, Coit J, Aliaga J, Mendoza M, Franke MF. 2019. Mycobacterium tuberculosis detection from oral swabs with Xpert MTB/RIF ULTRA: a pilot study. BMC Res Notes 12:349. https://doi.org/10.1186/s13104-019-4385-y.
15. Lima F, Santos AS, Oliveira RD, Silva CCR, Gonçalves CCM, Andrews JR, Croda J. 2020. Oral swab testing by Xpert MTB/RIF Ultra for mass tuberculosis screening in prisons. J Clin Tuberc Other Mycobact Dis 19:100148. https://doi.org/10.1016/j.jctube.2020.100148.
16. World Health Organization. 2017. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. World Health Organization, Geneva, Switzerland.
17. Shapiro AE, Olson AM, Kidoguchi L, Niu X, Ngcobo Z, Magcaba ZP, Ngwane MW, Whitman GR, Weigel KM, Wood RC, Wilson DP, Drain PK, Cangelosi GA. 2022. Complementary non-sputum diagnostic testing using oral swabs and urine LAM testing for TB in people with HIV. medRxiv. https://doi.org/10.1101/2022.02.16.22271079.
18. Cox H, Workman L, Bateman L, Franckling-Smith Z, Prins M, Luiz J, Van Heerden J, Ah Tow Edries L, Africa S, Allen V, Baard C, Zemanay W, Nicol MP, Zar HJ. 2022. Oral swabs tested with Xpert MTB/RIF Ultra for diagnosis of pulmonary tuberculosis in children: a diagnostic accuracy study. Clin Infect Dis https://doi.org/10.1093/cid/ciac332.