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New Manual Quantitative Polymerase Chain Reaction Assay Validated on Tongue Swabs Collected and Processed in Uganda Shows Sensitivity That Rivals Sputum-based Molecular Tuberculosis Diagnostics

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Background. Sputum-based testing is a barrier to increasing access to molecular diagnostics for tuberculosis (TB). Many people with TB are unable to produce sputum, and sputum processing increases assay complexity and cost. Tongue swabs are emerging as an alternative to sputum, but performance limits are uncertain.

Methods. From June 2022 to July 2023, we enrolled 397 consecutive adults with cough >2 weeks at 2 health centers in Kampala, Uganda. We collected demographic and clinical information, sputum for TB testing (Xpert MTB/RIF Ultra and 2 liquid cultures), and tongue swabs for same-day quantitative polymerase chain reaction (qPCR) testing. We evaluated tongue swab qPCR diagnostic accuracy versus sputum TB test results, quantified TB targets per swab, assessed the impact of serial swabbing, and compared 2 swab types (Copan FLOQSWAB and Steripack spun polyester).

Results. Among 397 participants, 43.1% were female, median age was 33 years, 23.5% were diagnosed with human immunodeficiency virus, and 32.0% had confirmed TB. Sputum Xpert Ultra and tongue swab qPCR results were concordant for 98.2% (95% confidence interval [CI]: 96.2–99.1) of participants. Tongue swab qPCR sensitivity was 92.6% (95% CI: 86.5 to 96.0) and specificity was 99.1% (95% CI: 96.9 to 99.8) versus microbiological reference standard. A single tongue swab recovered a 7-log range of TB copies, with a decreasing recovery trend among 4 serial swabs. Swab types performed equivalently.

Conclusions. Tongue swabs are a promising alternative to sputum for molecular diagnosis of TB, with sensitivity approaching sputum-based molecular tests. Our results provide valuable insights for developing successful tongue swab–based TB diagnostics.

Keywords. tuberculosis; diagnosis; nonsputum; tongue swab; oral swab.

Tuberculosis was responsible for 1.3 million deaths in 2022, with disruptions to diagnosis and treatment caused by the coronavirus disease 2019 (COVID-19) pandemic. TB is curable and preventable. However, diagnosis remains the largest gap in the care cascade, with the number of new notifications falling 18% from 2019 to 2020 and missed diagnosis for an additional 4.2 million people during the pandemic, highlighting the urgent need for improved access to diagnostic services [1–3].

Currently, many TB diagnostics in high-burden countries rely on sputum, which is a challenging specimen for several reasons. Children, people with human immunodeficiency virus (HIV; PWH), and others who lack a productive cough are often unable to provide quality samples [4, 5]. Sputum production may release infectious bioaerosols, putting healthcare workers and nearby patients at risk [6–11], often resulting in reluctance to order sputum-based tests [12]. Laboratory-based diagnostics, such as sputum smear microscopy, have suboptimal sensitivity, while sputum culture's long turnaround time (TAT) often results in missed or delayed treatment and consequently continued transmission [5, 13, 14].

The World Health Organization (WHO) has recommended molecular diagnostics such as Xpert MTB/RIF Ultra (Cepheid) and Truenat MTB Plus (Molbio) due to improved accuracy and shorter TAT, but barriers to access remain. In Uganda, the

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estimated cost per patient tested with Xpert Ultra is more than $21.00, 80% of which is for equipment and cartridges costs [8, 15].

Oral swabbing is a compelling alternative to sputum collection that may increase access to molecular diagnostics for TB [16]. The COVID-19 pandemic highlighted the acceptability and efficacy of swab-based approaches because they are inexpensive, amenable to self-collection, and noninvasive [17]. The reduced complexity of an oral matrix may lend itself to extraction-free sample preparation methods, reducing TAT and the need for additional consumables and equipment.

Publications on tongue swab clinical studies, methods, and outcomes metrics, including sensitivity and specificity, have varied [16, 18–24], but they hint at keys to increasing performance of swab-based testing. For instance, it was reported that *Mycobacterium tuberculosis* (MTB)–containing biofilms form on tongue papillae, with higher MTB recovery reported from the tongue compared with other oral sites (cheek, gum) [19, 25].

We hypothesized that the sensitivity of tongue swab–based molecular tests could be enhanced by optimizing key components while simultaneously revealing the quantity of MTB that can be recovered from the tongue. There are opportunities for technical gains in sample preparation because MTB is resistant to conventional bacterial lysis techniques due to the complex structure of its cell envelope, which is comprised of lipophilic molecules that include long-chain mycolic acids and polysaccharides [26]. Low lysis efficiency may yield artificially low sensitivity, and its optimization may increase detection of MTB [27].

In addition, previous studies used DNA concentration and purification of tongue swab specimens [16], which may be a source of recovery losses. To circumvent these steps, we identified downstream amplification and detection techniques that are inhibitor-tolerant and input volumes that are high enough to limit stochastic sampling error [28].

Our primary objective was to evaluate the diagnostic accuracy of the novel quantitative polymerase chain reaction (qPCR) assay in comparison to sputum Xpert Ultra. In addition, we aimed to quantify MTB copies recovered from a single tongue swab (substudies 1–3), evaluate loss of MTB detection with serial swabbing (substudy 1), compare MTB detection with Copan FLOQSWAB and Steripack spun polyester swabs (substudy 2), and evaluate the performance of shorter duration of swabbing (substudy 3).

METHODS

Study Participants

We enrolled consecutive consenting adolescents and adults (aged >12 years) who presented with at least 2 weeks of cough to 2 health centers in Kampala, Uganda, between 28 June 2022 and 24 July 2023. We excluded people who had been treated for TB in the last 12 months, had taken antimycobacterial antibiotics in the last 2 weeks, or were unable or unwilling to return for follow-up or provide informed consent.

The Makerere University School of Medicine Research and Ethics Committee and the Ugandan National Council on Science and Technology approved the study. Clinical and laboratory staff were blinded to TB status during collection and processing, and participant identification numbers were not known to anyone outside the research team.

Specimen Collection Procedures

We collected detailed demographic data and clinical history using a standardized case report form. Prior to sputum collection, up to 4 tongue swabs were collected from each participant. The first swab collected was always a Copan FLOQSWAB (520CS01) to ensure a common thread for diagnostic accuracy and copy number calculations.

For substudy 1 (serial swabbing), 3 additional FLOQSWABs were sequentially collected (4 identical swabs per participant). For substudy 2 (swab type comparison), 2 additional swabs were collected. The first swab was always a whole-tongue FLOQSWAB, followed by 2 half-tongue swabs collected for 30 seconds each using the centerline of the tongue as a guide. One side of the tongue was swabbed with a FLOQSWAB and the other side with a Steripack swab (60564RevC). Collection order was alternated daily. For substudy 3, 1 FLOQSWAB was collected. For each swab collection, firm pressure was applied to the swab handle while swabbing the entire length and width of the anterior three-quarters of the tongue dorsum. For substudies 1 and 2, we swabbed for 30 seconds to ensure sampling uniformity. For substudy 3, swab timing was shortened to 15 seconds with a focus on the posterior portion of the anterior three-quarters of the tongue dorsum.

After collection, swab heads were inserted into gasketed screw cap tubes that contained 500 µL 1× Tris-EDTA with a pH of 7.4 or pH 8.0 (preferred). Tubes were transported in a cooler box that contained ice packs for same-day analysis.

Process control swabs were taken by swabbing the air for 30 seconds at each site once per week and then processed identically to clinical samples to ensure clinical workspaces and laboratory processes were free of contamination.

In addition to tongue swabs, all participants had finger prick or venous blood collected for HIV testing and CD4 count (if HIV-positive) and up to 3 spot sputa collected for reference standard testing that included Xpert Ultra (with repeat testing if the initial result was trace-positive, invalid, or indeterminate) and 2 cultures in liquid *Mycobacterium* growth indicator tube media.

Index Test

Sample Preparation

Tongue swab samples were vortexed for 15 seconds and heated at 95°C for 30 minutes to inactivate nucleases and prevent

growth of MTB. It was subsequently determined that 10 minutes of heat at 95°C is sufficient (Supplementary Material 4). After heating, samples were vortexed again for 15 seconds and centrifuged for 3 seconds. Maximum sample volume (approximately 375 µL) was sterilely transferred to flat-bottom tubes (VWR 76417-214) that contained 150 mg of 0.1-mm glass beads (RPI Corp 9830). Tubes were balanced in a bead beater (BioSpec 607EUR) and subjected to three 1-minute beating cycles with 1-minute rests in between. Tubes were centrifuged for 3 seconds, and 320 µL were removed into a fresh tube for qPCR testing.

Bioinformatics Analysis

Oligonucleotide sequences were generated in Geneious Prime version 2020.0.3 and screened for unfavorable folding and oligomerization using AutoDimer version 1.022 with the following parameters: minimum SCORE requirement: 3; Na+ 0.085 M; temp for dG calc 37°C; total strand conc 1.0 µM. Sequences were screened for specificity to the *Mycobacterium tuberculosis* complex with National Center for Biotechnology Information Blast blastn.

qPCR Testing

Five 50-µL aliquots of crude lysate per sample were added to a PCR plate that contained 10× KAPA3G (Roche 09160914103) and oligos targeting an MTB complex–specific 85-bp region of the IS6110 insertion sequence, a 90-bp region of the IS1081 insertion sequence, and a 65-bp region of human RNaseP, which was used as a sample adequacy control (Supplementary Table 1). No template controls were run on each plate. Samples were processed on a QuantStudio5 0.2-mL thermal cycler. MTB insertion element quantities were interpolated from aggregated MTB H37Rv DNA (American Type Culture Collection 25618DQ) standard curves. Samples were considered positive if any of 5 wells were positive.

Standard operating procedures are listed in Supplementary Material 2. Additional assay optimization methods, including TB H37Ra cell-line culture methods and contrived sample generation, are listed in Supplementary Material 3.

Reference Standard Definitions

We used a composite microbiological reference standard (MRS) to define TB status. Participants were considered to have active TB if they had a positive sputum Xpert Ultra result (very low or higher semiquantitative categories reported by Xpert Ultra), 2 sputum Xpert Ultra trace-positive results, and/or a positive sputum culture result. Participants were considered negative for active TB if they had a negative sputum Xpert Ultra result and 2 negative culture results. Participants who did not meet either criterion (eg, due to culture contamination) had an indeterminate TB status. We also considered concordance with sputum Xpert Ultra results with and without including trace-positive results.

Data Analyses

To assess the serial swabbing impact on MTB recovery, we fit a linear 2-level mixed effects model for repeated measures to account for recovery differences among participants (random effect), with swab number as a fixed effect. We included MRS-positive participants and excluded participants with MTB detected from fewer than 4 swabs. We calculated mean

Table 1. Demographic and Clinical Characteristics of Study Participants

Characteristic		Overall	Serial Swabbing (Substudy 1)	Copan Versus Steripack (Substudy 2)	Shortened Swab Time (Substudy 3)
Total with valid quantitative polymerase chain reaction result		378	97	133	148
Sex at birth	Male	215 (56.9%)	53 (54.6%)	79 (59.4%)	83 (56.1%)
	Female	163 (43.1%)	44 (45.4%)	54 (40.6%)	65 (43.9%)
Median age (interquartile range), y		33 (26–43)	32 (26–42)	36 (28–43)	32 (25–42)
People living with human immunodeficiency virus		89 (23.5%)	22 (22.7%)	35 (26.3%)	32 (21.6%)
Prior TB		55 (14.6%)	19 (19.6%)	16 (12.0%)	20 (13.5%)
Xpert Ultra sputum result	TB negative	262 (69.3%)	64 (66.0%)	90 (67.7%)	108 (73.0%)
	TB positive	113 (30.0%)	32 (33.0%)	41 (30.8%)	40 (27.0%)
	Trace	3 (0.7%)	1 (1.0%)	2 (1.5%)	0 (0%)
Xpert semiquantitative grade	Trace	3 (0.7%)	1 (1.0%)	2 (1.5%)	0 (0%)
	Very low	5 (1.3%)	0 (0%)	3 (2.3%)	2 (1.4%)
	Low	36 (9.5%)	6 (6.2%0	9 (6.8%)	11 (7.4%)
	Medium	33 (8.7%)	12 (12.4%)	11 (8.3%)	10 (6.8%)
	High	49 (13.0%)	14 (14.4%)	18 (13.5%)	17 (11.5%)
Microbiologic reference standard	TB Negative	233 (61.6%)	62 (63.9%)	74 (55.6%)	97 (65.5%)
	TB Positive	121 (32.0%)	33 (34.0%)	46 (34.6%)	42 (28.4%)
	Indeterminate	24 (6.3%)	2 (2.1%)	13 (9.7%)	9 (6.1%)

Abbreviation: TB, tuberculosis.

Table 2. Diagnostic Accuracy of Tongue Swabs Compared With Xpert Ultra and Microbiological Reference Standard

	Samples With Valid Paired Xpert and qPCR Results			Excluded qPCR	
Category	Xpert (Sputum)	qPCR (Tongue Swab)	Percent Agreement (95% CI)	Invalid	Not Tested
Total enrolled = 397	378	378		13 (3.3%)	6 (1.5%)
Overall agreement (without trace)	375	370	98.7 (96.9–99.4)		
Overall agreement (with trace)	378	371	98.2 (96.2–99.1)		
Negative	262	259	98.9 (96.7–99.7)	10 (3.8%)	5 (1.2%)
Positive (excluding trace)	113	111	98.2 (93.8–99.7)	3 (2.7%)	0
Positive (including trace)	116	112	96.6 (91.5–98.7)	3 (2.7%)	1 (0.09)%
Xpert Ultra semiquantitative					
Very low	5	3	60.0 (23.1–92.9)	1	
Low	26	26	100.0 (86.7–100.0)		
Medium	33	33	100.0 (89.8–100.0)	1	
High	49	49	100.0 (92.6–100.0)	1	
Trace	3	1	33.3 (1.3–69.9)		1

	Samples With Valid Paired MRS and qPCR Results			Excluded qPCR	
Category	MRS (Sputum)	qPCR (Tongue Swab)	Percent Agreement (95% CI)	Invalid	Not Tested
Total with valid results and culture data	354	354		13 (3.3%)	6 (1.5%)
MRS-negative (culture negative)	233	231	99.1 (96.9–99.8)	8	2
MRS-positive (culture plus Xpert)	121	112	92.6 (86.5–96.0)	4	1
Total	354	343	96.9 (94.5–98.3)		
Indeterminate	24			1	3
Pending culture results	0				

	Number of Samples With Valid Matched MRS and Xpert Results		
Category	MRS (Sputum)	qPCR (Tongue Swab)	Percent Agreement (95% CI)
Total with valid results and culture data	369	369	
MRS-negative (culture negative)	243	242	99.6 (97.7–100.0)
MRS-positive (culture plus Xpert)	126	118	93.7 (88.0–96.7)
Total	369	360	97.6 (95.4–98.7)
Indeterminate	28		
Pending culture results	0		

Category	Culture Positive	Culture Negative	Indeterminate
Xpert Ultra trace	2	1	1

Abbreviations: CI, confidence interval; MRS, microbiological reference standard; qPCR, quantitative polymerase chain reaction.

MTB copy number from 5 technical replicates. We log-transformed mean MTB copy number to account for the highly skewed distribution of the data.

To evaluate the difference in MTB target recovery from Copan and Steripack swabs, we performed a ratio paired *T* test. To understand whether differences between swabs were due to the abundance of MTB cells on the swabs, we also performed a Bland–Altman analysis to calculate the percent difference of the tests divided by mean MTB IS6110 target recovery. We calculated mean MTB copy number per swab by transforming the mean copy number calculated per 50-µL well using the function $Y = 10 * Y$.

We analyzed differences in tongue swab IS6110 recovery by Xpert Ultra semiquantitative categories using a Brown–Forsythe and Welch analysis of variance test to correct for unequal standard deviations among groups and used a Dunnett T3 test to correct for multiple comparisons given the small (<50) sample size per group. Analyses were performed using GraphPad Prism version 9.3.0. and Stata Version 17 (StataCorp, College Station, TX).

RESULTS

Participant Characteristics

Between 28 June 2022 and 24 July 2023, 397 participants who met eligibility criteria were enrolled for all 3 parts of this study. Tongue swabs were not collected from 6 (1.5%) participants who presented to the clinic too late for same-day sample processing, and 13 (3.3%) participants had invalid tongue swab qPCR results. Of the remaining 378 participants, 43.1% were female, median age was 33 years (interquartile range, 26–43), 23.5% were PWH, and 32.0% had MRS-confirmed TB (Table 1).

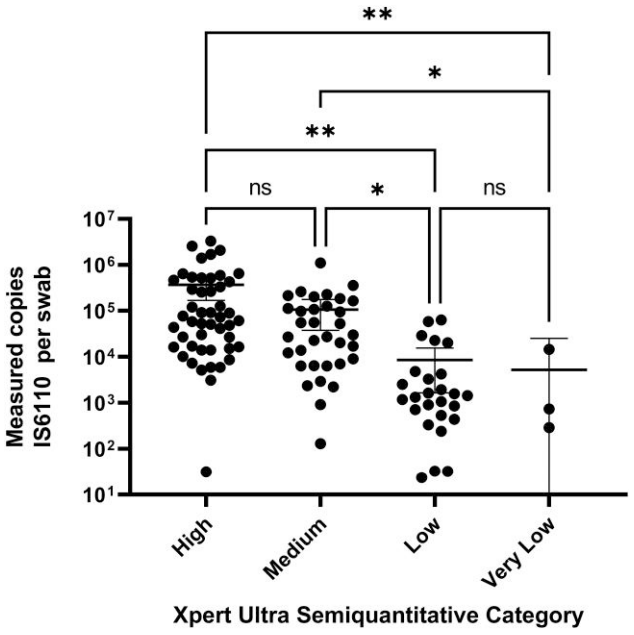

Figure 1. Measured mean log copies of *Mycobacterium tuberculosis* IS6110 per swab per participant by Xpert semiquantitative grade. Abbreviation: ns, not significant,

Diagnostic Accuracy of Tongue Swab qPCR Testing

Among 378 participants with valid qPCR results, concordance between sputum Xpert Ultra and the first tongue swab collected and processed using qPCR testing was 98.7% (95% confidence interval [CI]: 96.9 to 99.4) when Xpert Ultra trace results were excluded and 98.2% (95% CI: 96.2 to 99.1) when Xpert Ultra trace results were included.

In comparison to the MRS, tongue swab PCR sensitivity was 92.6% (95% CI: 86.5 to 96.0) and specificity was 99.1% (95% CI: 96.9 to 99.8). Results were similar for sputum Xpert Ultra (sensitivity: 93.7%; 95% CI: 88.0 to 96.7 and specificity: 99.6%; 95% CI: 97.7 to 100.0; Table 2).

In comparison to sputum Xpert Ultra results alone, sensitivity of tongue swab qPCR was 98.2% (95% CI: 93.8 to 99.7) and specificity was 98.9% (95% CI: 96.7 to 99.7). When Xpert Ultra trace-positive results were included, sensitivity decreased to 96.6% (95% CI: 91.5 to 98.7).

Quantification of MTB Copies Recovered From a Single Tongue Swab

Detectable quantities of MTB were present on 112 of 121 swabs (swab 1) collected from participants with positive MRS results. The number of MTB IS6110 targets per swab spanned a 7-log range and correlated with Xpert Ultra semiquantitative categories (trace, very low, low, medium, and high), an indicator of bacillary load (Figure 1). Mean IS6110 copies observed from tongue swabs were 369844, 106841, 8592, and 5200 compared with high, medium, low, and very low sputum Xpert Ultra semiquantitative categories, respectively. There were no significant differences between means of high and medium ($P = .0908$) or low and very low categories ($P = .9864$), though the latter finding may be skewed by the low number of participants with a very low sputum result.

Serial Swabbing

MTB was recovered from all 4 Copan swabs for 32 participants who were MRS-positive. We excluded 1 MRS-positive participant from serial swabbing analysis for whom MTB was only detected on the initial swab.

Considering variation in MTB recovery across participants, we observed a decrease in MTB recovery with each sequential swab when compared with the initial swab (swab 2 regression coefficient: −0.52; 95% CI: −.75 to −.30; swab 3 coefficient: −0.76; 95% CI: −.99 to −.53; swab 4 coefficient: −1.04; 95% CI: −1.27 to −.82; Figure 2). Some variation was observed by Xpert semiquantitative grade (Figure 2*C*).

To confirm that MTB target identification was not due to differences in swabbing technique between swabs, we performed a delta delta C_t calculation, enabling normalization to the RNaseP human gene. The delta delta C_t also showed a decreasing trend of MTB yield by swab (Supplementary Material 2).

Copan and Steripack Swab Comparison

We recovered MTB from the first whole-tongue Copan swab taken from 39 MRS-positive participants enrolled during substudy 2. Of these, 37 participants had positive qPCR results for half-tongue Copan and Steripack swabs and were included in the analysis. Two participants were excluded because only 1 swab had a positive result. We observed 2 MRS-negative participants with weakly positive tongue swab results, and 3 participants were excluded due to negative process control contamination.

We used a ratio paired *T* test to evaluate recovery from the 2 swab types due to large differences in bacillary load among participants. Copan log mean MTB recovery was 3.950 (95% CI: 3.555 to 4.344), and Steripack log mean MTB recovery was 3.954 (95% CI: 3.600 to 4.308). The test indicated there is no statistically significant difference in MTB recovery between types ($P = .9516$). The geometric mean of the ratios was 0.9902, and the pairing was significantly effective ($r = 0.9354$; $P = <.0001$). Normality of residuals was confirmed. A Bland–Altman analysis of the same data demonstrated no difference between Copan and Steripack recovery, regardless of the mean quantity of MTB targets per swab (Figure 3).

DISCUSSION

We created and evaluated a sensitive tongue swab qPCR method to better understand the limits of performance for tongue swab–based molecular assays under "best-case-scenario" circumstances. Overall sensitivity of 92.6% and specificity of

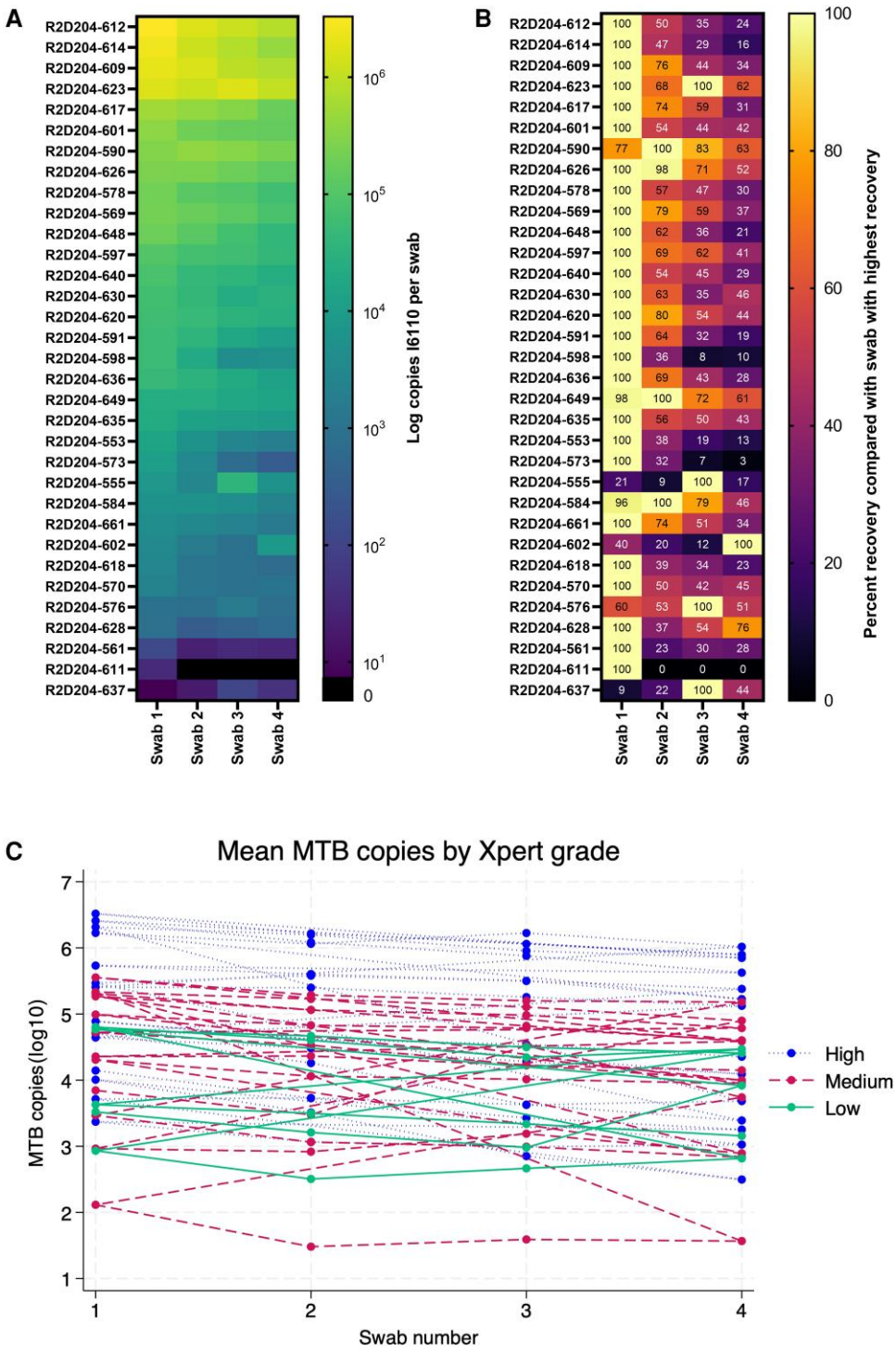

Figure 2. Quantity of MTB IS6110 copies present on 4 sequentially collected swabs. *A*, Heat map demonstrating decreasing recovery with each sequentially collected swab. *B*, Normalized MTB targets recovered from all swabs calculated as a percentage of the highest recovery condition ("100%"). *C*, Recovery of 4 swabs by Xpert semi-quantitative status. Abbreviation: MTB, *Mycobacterium tuberculosis*.

99.1% were nearly equivalent to sputum Xpert Ultra when compared with MRS, suggesting MTB is typically present on the tongues of people with pulmonary TB. The results also meet the minimum diagnostic sensitivity (>90%) and specificity (>95%) requirements in the WHO's target product profile for nonsputum testing for pulmonary tuberculosis [29], which should encourage test developers. However, participants in this study presented with TB symptoms, and of Xpert

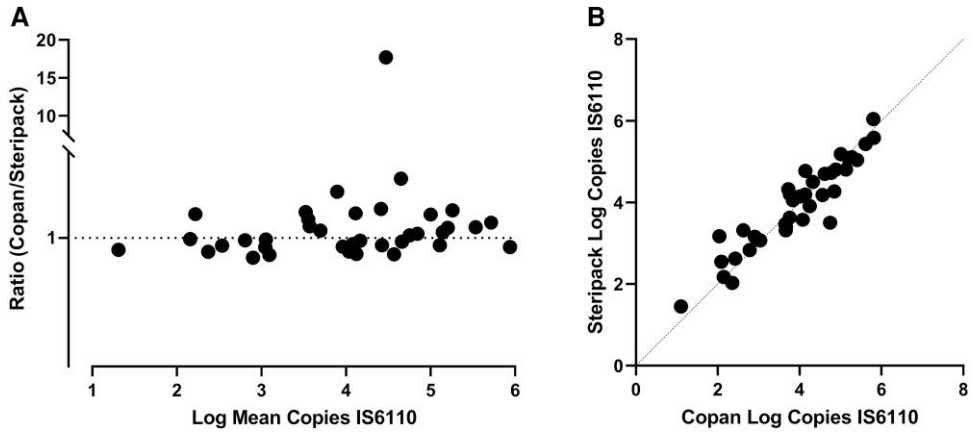

Figure 3. Recovery of IS6110 copies from Copan versus Steripack swabs. *A*, Bland–Altman ratio of measured IS6110 copies recovered by Copan versus Steripack divided by log mean measured copies of IS6110. *B*, Measured log copies 1S6110 recovered from Steripack versus Copan.

Ultra-positive participants, 93% had a sputum bacillary load that was categorized as "low" or higher. Further testing of participants with "very low" and "trace" results must be conducted to understand tongue swab yield for these groups.

For participants with measurable MTB on tongue swabs, qPCR testing revealed that most had quantities sufficient for detection with less-sensitive methods. For instance, one may be able to decrease test input volume 5-fold, reduce complexity of the lysis instrument, or use a more common polymerase and still recover detectable quantities of MTB.

We demonstrated that expensive and laborious DNA extraction steps may be removed from swab-based TB diagnostic workflows, decreasing cost, consumables, and waste; reducing user steps and TAT; and minimizing contamination risks associated with these procedures. We determined that heating for 10 minutes is sufficient for inactivation of nucleases and is mycobactericidal (Supplementary Material 4). Our findings also underscore the importance of efficient TB lysis methods. There are currently few MTB lysis tools amenable to point of care settings, and we emphasize the need for low-cost lysis devices to complement molecular assays. However, our results demonstrate that with adequate lysis, tongue swab testing may be adapted to existing near-patient platforms such as Molbio Truenat or newer swab-based molecular platforms developed for COVID-19 testing.

Serial swabbing results confirmed that additional MTB may be recovered from the tongue with more sensitive sampling tools since 4 of 4 swabs produced positive results for all but 1 participant. Studying yield from 2 swab types produced 2 key conclusions: there is flexibility in the type of swab that may be used for sampling, but we are leaving valuable MTB targets behind. For these reasons, we believe sampling innovations may increase sensitivity. Our early prototyping with 3-dimensional printed "plastic swabs" did not produce better sampling efficiency than Copan (data not shown), but refinements to surface chemistry (eg, flocking) and form (eg, scraping stringency or surface area) may increase performance.

Serial swabbing results should serve as a reminder to be cautious when designing multiswab studies to compare variables, as each swab is likely to yield varying amounts of MTB targets. We suggest randomization of swab order or collecting timed, half-tongue swabs when smaller sample sizes are desired, and we demonstrated the efficacy of this approach. While we swabbed each participant for 30 seconds to ensure uniformity between swabs during 2 of 3 substudies, we confirmed that 15 seconds of total swab time is adequate to saturate the swab, based on results from our third substudy.

The present findings provide important research tools and demonstrate the feasibility of same-day molecular testing of tongue swabs, but findings are limited to 2 clinics, and expanding to multiple sites and geographies is a top priority. Performance must still be assessed in the groups that may benefit the most from nonsputum sampling options, such as PWH, children, and household contacts of index cases. Our results quantify TB targets on the tongue for the first time and demonstrate that enhanced efficiency of collection, lysis, and amplification and detection achieve high concordance with sputum Xpert Ultra testing, even in the absence of DNA extraction. The present study reinforces the efficacy of tongue swabs for TB diagnosis and identifies keys to developing a class of novel, simpler, and lower-cost nonsputum tests.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Data sharing statement. The data generated in this study are available from the corresponding author upon reasonable request.

Potential conflicts of interest. A. C. reports participation on the Medaica Advisory Board and stock or stock options for Medaica. All remaining authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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