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Original article

Diagnostic accuracy of self-collected tongue swabs for *Mycobacterium tuberculosis* complex detection in individuals being assessed for tuberculosis in South Africa using the Xpert MTB/RIF Ultra assay

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ABSTRACT

Objectives: Tongue swabs (TS) have shown potential for detecting *Mycobacterium tuberculosis* complex (MTBC) through downstream molecular testing. Analytical performance varies, depending on the processing protocol and the molecular test used. This study aimed to first investigate the ease of use of TS collection in addition to acceptability by individuals being assessed for tuberculosis and second to determine the performance of self-collected TS on the Xpert MTB/rifampicin (RIF) Ultra (Ultra) assay (Cepheid, Sunnyvale, CA, USA) for MTBC and RIF resistance detection.

Methods: The ease of use of TS collection and acceptability by study participants was assessed through completion of a survey questionnaire. Analytical performance of self-collected TS on the Ultra assay was determined by comparing results with a liquid culture reference result and with Ultra on sputum. Results were additionally stratified by HIV and smear status.

Results: Of the 399 survey respondents, all were happy with the TS collection procedure with minimal discomfort reported. Data analysis was performed on 321 specimens. The sensitivity of the Ultra TS assay for MTBC detection was 78.1% (95% CI, 66.0–87.5%), whereas the specificity was 100% (95% CI, 98.6–100%). Test performance was better in individuals with a higher bacillary load, with some variability. On HIV-positive individuals, TS performance is ~30% lower than that on sputum but also slightly better than smear microscopy, whereas the performance of TS among HIV-negative individuals is similar to sputum (93% vs. 97%). For RIF resistance profiling, the Ultra TS assay showed 41/41 (100%) concordance with phenotypic drug-susceptibility testing.

Discussion: : Although self-collected TS have lower sensitivity compared with sputum, their ease of use and high acceptability make them a valuable additional specimen type for molecular tuberculosis testing. Self-collection can reduce the burden on healthcare workers and increase access to testing, particularly for individuals who are too busy to visit healthcare facilities or fear stigma. **Anura David, Clin Microbiol Infect 2025;▪:1**

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Introduction

WHO recommends rapid molecular diagnostics as the frontline testing option for people showing signs and symptoms of tuberculosis (TB) [1,2], but smear microscopy is still widely used with culture remaining the reference standard. Diagnosis of pulmonary TB including the above-mentioned methods requires sputum for testing. However, obtaining sputum can be difficult and costly, especially in paediatrics and people living with HIV (PLHIV), many of whom are unable to expectorate [3]. Another concern is rejection of a sputum specimen by the laboratory due to insufficient volume and specimen leakage because of incorrect container closure or container quality defects, which in South Africa (SA) has been reported to be as high as 8.3% in 2015 [4]. A further concern as demonstrated by Zimba et al. [5] is that sputum quality and quantity are not predictive of bacteriologically positive results by Xpert MTB/rifampicin (RIF) (Cepheid, Sunnyvale, CA, USA), or smear. Among the seven transitions to transform TB diagnosis suggested by Pai et al. [6] is the use of non-sputum specimens which when paired with rapid, accurate community-based TB screening and diagnosis may be the answer to closing the diagnostic gap [7]. The oral swab (OS) was proposed for specimen collection for *Mycobacterium tuberculosis* complex (MTBC) detection, in 2015 [8]. Since then, evaluation studies have used different swab types, sampling locations, and preprocessing steps with different back-end technologies [9–11] in attempts to optimize TB testing using swabs. A summary on performance data was published in a systematic review by Church et al. [12] which included 15 studies in adults, where the sensitivity of OS was shown to range from 36% to 91%, depending on sample collection and analysis methodology. In terms of sampling location in the mouth, it has been demonstrated that tongue swabs (TS) produce the best sensitivity together with the Copan FLOQ swab (FLOQSwab 501CS01; Copan Italia S.p.A., Brescia, Italy) [13,14].

Although the use of swabs for disease diagnosis gained significant attention during the COVID-19 pandemic, they were previously used in the molecular diagnosis of various diseases [15]. For COVID-19, molecular testing using upper respiratory tract swab specimens (nasopharyngeal and oropharyngeal) for detection of SARS-CoV-2 was initially recommended during the COVID-19 pandemic [16]. However, these specimen types were difficult to collect with adverse events reported in some people [17]. Additionally, the need to rapidly test an increasing number of people necessitated the investigation of alternative, less-invasive specimen types, such as nasal swabs [18,19], which were eventually used for self-collection [20] and even self-testing [21].

Similarly, addressing the TB epidemic requires innovation and OS self-collection should be considered. Two studies which explored self-collected OS for MTBC detection showed comparable performance to healthcare worker (HCW)-collected samples and acceptance of self-collection by HCWs [22,23].

The aim of this study is first to investigate the ease of use of TS collection (among other specimen types) in addition to acceptability by individuals being assessed for TB and second to determine the analytical performance of self-collected TS on the Xpert MTB/RIF Ultra (Ultra) assay (Cepheid) compared with a liquid culture reference result. In addition, Ultra sputum was used as a comparator assay.

Methods

Participant recruitment and study procedures

In this cross-sectional study, symptomatic, eligible adults (≥18 years), under investigation for TB, were prospectively enrolled from 20 October 2021 to 11 July 2023 at Hillbrow Community Health Centre (HCHC), Johannesburg, SA. HCHC is a primary healthcare facility providing outpatient services to the densely populated and urban Hillbrow neighbourhood. In addition to the WHO-recommended four-symptom screen (cough, fever, weight loss, and night sweats), data on HIV status, TB history, and demographics were collected. CD4 counts were retrieved from patient files for PLHIV. Participants had to agree to return for a second visit and perform self-collection of TS. Those who had received TB treatment within 6 months before enrolment were excluded. Specimen collection occurred over two visits (Fig. 1). At visit 1, participants were provided with a swab for self-collection on the morning of visit 2 (within 7 days). Although self-collected, participants were instructed to follow the same procedure as the HCW. This required swabbing the back and top of the tongue in back-front and left-right motions for 30 seconds while rotating the swab and avoiding a gag reflex, before brushing their teeth or eating. Swabs were transported dry to the research laboratory at 2–8°C.

Specimen processing

All specimens were transported to the Wits Diagnostic Innovation Hub laboratory in Braamfontein (Johannesburg) for routine and research testing (Fig. 1). Routine testing and resulting was performed by clinical trial laboratory staff, who were blinded to clinical information and the Ultra TS results, as per the National TB Management Guidelines 2014 [24]. TS were stored dry at −80°C until batch testing by research staff who had access to routine test results but were blinded to clinical information. Cepheid's sample reagent liquefies sputum and reduces bacilli infectiousness. Because TS do not require liquefaction and contain fewer bacilli than sputum, alternate buffers (phosphate buffer [PB; Media Mage, Johannesburg, SA] and Tris–EDTA [TE] buffer [10 mM Tris–HCl containing 1 mM EDTA Na_2, pH 8.0; Merck, Johannesburg, SA]) were investigated for this study. Initially, PB was used for swab preprocessing, but testing on contrived swabs showed improved

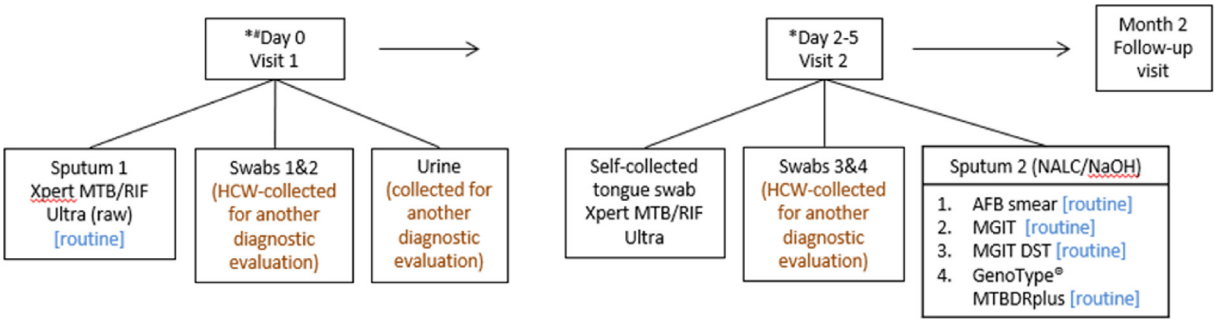

Fig. 1. Description of study outline indicating clinic visits and specimen laboratory pathways. AFB, acid-fast bacilli; DST, drug-susceptibility testing; HCW, healthcare worker; MGIT, Mycobacterial Growth Indicator Tube; MTB, *Mycobacterium tuberculosis*; NALC/NaOH, N-acetyl-L cysteine–sodium citrate–sodium hydroxide; RIF, rifampicin.

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sensitivity with TE buffer prompting a switch to TE. However, increased error rates led to a return to PB. In total, 96 specimens were processed with TE and 303 with PB. For pre-processing, 2.3 mL of buffer was added to the frozen swab, followed by vortexing for 10 seconds and a 5-minute room temperature incubation. The liquid (~2 mL) was aspirated and added to the Ultra cartridge, and testing proceeded per manufacturer instructions.

Swab acceptability questionnaire

At the end of their second visit, participants were requested to complete a questionnaire on the ease of use of specimen collection (sputum, urine and TS [self-collection and HCW collected]) for TB investigation.

Outcomes and statistical analysis

Ultra TS results were compared with Mycobacterial Growth Indicator Tube (MGIT) (Becton Dickinson, Sparks, MD, USA) (reference standard) for MTBC detection and with phenotypic drug-susceptibility testing (pDST) (performed using the MGIT960 SIRE kit [Becton Dickinson, Sparks, MD, USA]) for RIF resistance detection. Ultra sputum was used as a comparator assay. Data analysis included calculation of sensitivity, specificity, positive predictive value, and negative predictive value for MTBC detection and concordance for RIF resistance detection, with 95% CIs calculated using the Wilson score method (first including all participants and then excluding participants with an Ultra 'trace' result). The target sample size was 400 participants which was derived using the formula described below with population proportion estimated at 50% and 95% CI set at 5%. Sample size $(n) = (Z)^2 * p(1-p)/x^2$, where $Z = 1.96$, and where $p =$ population proportion and where $x =$ CI as a proportion. In determining the sample size, allowance was made for patients who do not meet the inclusion criteria. To determine the performance of the Ultra TS assay, only specimens that generated valid results across all assays (Ultra and MGIT) were included in the statistical analysis.

Follow-up visit

Approximately 8 weeks after enrolment, participants were contacted by phone for a health status update through a series of questions. If the participant felt unwell, they were asked to return to the HCHC for follow-up sputum collection, if possible. Routine tests (smear, MGIT, and other confirmatory tests) were performed.

Ethics statement

Ethics approval for this study was obtained from the University of the Witwatersrand Human Research Ethics Committee (M1911150). The trial was registered with the South African National Clinical Trials Registry (DOH-27-052021-5442).

Protocol availability

The study protocol is available upon request from the corresponding author.

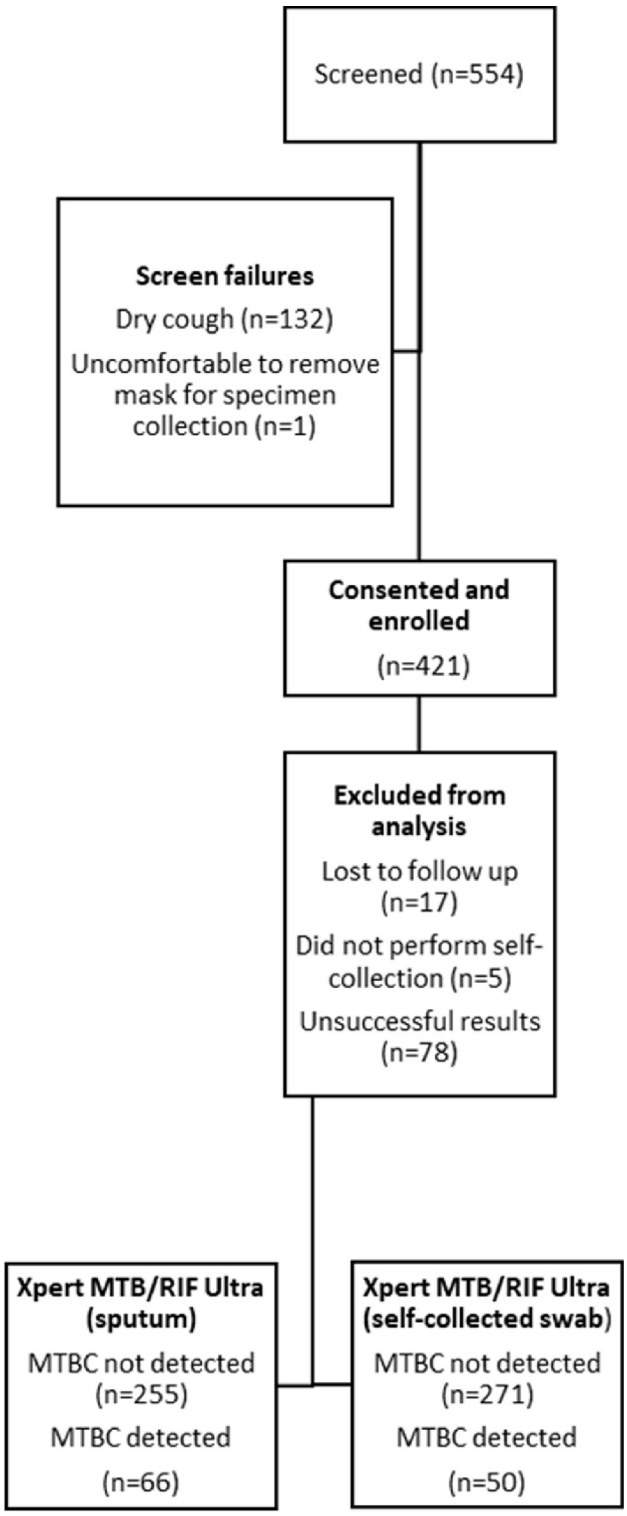

Fig. 2. Data description for statistical analysis. MTB, *Mycobacterium tuberculosis*; MTBC, *Mycobacterium tuberculosis* complex; RIF, rifampicin.

Results

Study population characteristics

Of all participants screened, 24% were ineligible, mostly because of non-productive coughs (Fig. 2). Informed consent was obtained from 421 participants but 22 were excluded due to TS unavailability for testing. Of the 399 enrolled, 78 produced unsuccessful results (Ultra or MGIT), with similar failure numbers for Ultra TS ($n = 33$) and MGIT ($n = 33$). Statistical analysis was performed on 321 participants. The average age was 39 years, 64% were male, and 56% were HIV positive (Table 1). Cough was the most common symptom and fever the least. Bacteriological classification using culture was performed on sputum 2 (Table 1). All smear-positive/culture-

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Table 1
Cohort characteristics for participants used in the statistical analysis.

Characteristic	All ($n = 321$)
Demographics	
Age (y), mean (range)	39 (18–70)
Male sex, *n* (%)	205 (63.8)
HIV-related information	
HIV-positive, *n* (%)	179 (55.8)
HIV-negative, *n* (%)	139 (43.3)
Status unknown, *n* (%)	3 (0.9)
CD4[a] count (cells/μL), mean (range)	295 (1–1072)
CD4[a] count (<200 cells/μL), *n* (%)	49 (37)
TB history	
Previously diagnosed with TB, *n* (%)	34 (10.6)
Clinical signs and symptoms of TB at presentation	
Unexplained weight loss, *n* (%)	239 (74.5)
Nights sweats, *n* (%)	241 (75.1)
Fever, *n* (%)	215 (67.2)
Cough (any duration), *n* (%)	320 (99.7)
Other,[b] *n* (%)	233 (72.6)
Bacteriological confirmation (sputum 2), *n* (%)	
Smear and culture positive	45 (14.0)
Smear negative and culture positive	19 (5.9)
Smear and culture negative	251 (78.2)
Smear positive and culture negative	6 (1.9)
Xpert MTB/RIF Ultra (sputum 1) (semi-quantitative result), *n*	66
High	24
Medium	8
Low	20
Very low	8
Trace	6

MTB, *Mycobacterium tuberculosis*; RIF, rifampicin; TB, tuberculosis.
[a] CD4 counts were only available for 133 participants.
[b] Other symptoms include body ache, loss of appetite, haemoptysis, shortness of breath, dizziness, and vomiting.

Table 3
The performance of smear microscopy and Xpert Ultra assays compared with the reference standard (MGIT) for MTBC detection.

Variable	Smear microscopy, % (95% CI)	Xpert MTB/RIF Ultra (TS), % (95% CI)	Xpert MTB/RIF Ultra (sputum), % (95% CI)
All (including trace) ($n = 321$)			
Sensitivity	70.3 (57.6–81.1)	78.1 (66.0–87.5)	95.3 (86.9–99.0)
Specificity	97.7 (95.0–99.1)	100 (98.6–100)	98.1 (95.5–99.4)
PPV	88.2 (76.1–95.6)	100 (92.9–100)	92.4 (83.2–97.5)
NPV	93.0 (89.2–95.7)	94.8 (91.5–97.1)	98.8 (96.6–99.8)
All (excluding trace) ($n = 315$)			
Sensitivity	72.1 (59.2–82.9)	82.0 (70.0–90.6)	95.1 (86.3–99.0)
Specificity	97.6 (94.9–99.1)	100 (98.6–100)	99.2 (97.2–99.9)
PPV	88.0 (75.7–95.5)	100 (92.9–100)	96.7 (88.5–99.6)
NPV	93.6 (89.9–96.2)	95.8 (92.7–97.9)	98.8 (96.6–99.8)
Specimens from HIV-positive individuals ($n = 179$)			
Sensitivity	60.6 (42.1–77.1)	63.6 (45.1–79.6)	93.9 (79.2–99.3)
Specificity	97.9 (94.1–99.6)	100 (97.5–100)	97.9 (94.1–99.6)
PPV	87.0 (66.4–97.2)	100 (83.9–100)	91.2 (76.3–98.1)
NPV	91.7 (86.2–95.5)	92.4 (87.1–96.0)	98.6 (95.1–99.8)
Specimens from HIV-negative individuals ($n = 139$)			
Sensitivity	82.8 (64.2–94.2)	93.1 (77.2–99.2)	96.6 (82.2–99.9)
Specificity	97.3 (92.2–99.4)	100 (96.7–100)	98.2 (93.6–99.8)
PPV	88.9 (70.8–97.6)	100 (87.2–100)	93.3 (77.9–99.2)
NPV	95.5 (89.9–98.5)	98.2 (93.7–99.8)	99.1 (95.0–100)
Smear microscopy negative specimens ($n = 270$)			
Sensitivity	n/a	57.9 (33.5–79.7)	89.5 (66.9–98.7)
Specificity		100 (98.5–100)	98.0 (95.4–99.4)
PPV		100 (71.5–100)	77.3 (54.6–92.2)
NPV		96.9 (94.0–98.7)	99.2 (97.1–99.9)

MGIT, Mycobacterial Growth Indicator Tube; MTB, *Mycobacterium tuberculosis*; MTBC, *Mycobacterium tuberculosis* complex; NPV, negative predictive value; PPV, positive predictive value; RIF, rifampicin; TS, tongue swab; n/a. not applicable.

negative cases were detected as 'Trace' on Ultra, with only one reporting previous TB. The culture contamination rate was 11% (42/399); one MTBC-positive specimen was detected on Ultra sputum, and the participant reported symptom improvement after treatment.

Swab acceptability by users

Of all survey respondents, 399/399 (100%) were happy with TS collection and 74% preferred the TS for TB investigation (Table 2). A slightly higher proportion of participants preferred TS collection to be performed by the HCW (52%) to self-collection (48%).

Diagnostic performance of the Xpert Ultra TS assay for MTBC detection

Assay performance was compared with the MGIT and, additionally, stratified by HIV and smear status, as outlined in Table 3. Sixty-six sputum specimens tested positive for MTBC on the Ultra assay, spanning the semi-quantitative ranges (Table 1). When compared with Ultra on sputum, TS was able to detect MTBC across the semi-quantitative range, with some variability, even among those categorized as high (Table 4). Swabs did not detect MTBC on any trace sputa ($n = 6$). Of these, 3/6 (50%) were bacteriologically confirmed using culture.

Table 4
Comparison of Ultra sputum semi-quantitative results with Ultra TS.

Ultra sputum semi-quantitative result	Ultra TS results	
	n/*N*	Percent detection
High	23/24	95.8
Medium	7/8	87.5
Low	14/20	60.0
Very low	5/8	62.5
Trace[a]	0/6	0

TS, tongue swab.
[a] $n = 3$ were culture positive.

Errors produced on the Ultra TS assay

Of all tests performed on TS Ultra assay, 42/399 (11%) produced errors (Table 5). A higher proportion of errors were produced when

Table 2
Summary of participant responses to tongue swab collection ($n = 399$ [100%]).

Specimen type	Question	Response			
Tongue swab	Did you experience any discomfort during tongue swab sampling?	No (88%)		Yes (12%)	
	Is the specimen collection shorter for sputum or the tongue swab?	Tongue swab (95%)		Sputum (5%)	
	Who would you prefer to collect the specimen?	Self (48%)		HCW (52%)	
	What do you think of the overall sampling procedure?	Easy (98%)		Difficult (2%)	
	How do you rate your satisfaction for tongue swab collection?	Happy or very happy (100%)		Unhappy or very unhappy (0%)	
Overall	Based on your experience in this study, do you have a preferred specimen: tongue swab, sputum or urine?	Tongue swab (74%)	Urine (3%)	Sputum (4%)	All of the above (19%)

HCW, healthcare worker.

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Table 5
Error type according to buffer used for TS processing.

Buffer used for specimen processing, *n*	Error code				
	1002	2008	5007	1001	2126
TE ($n = 96$)	3	5	12	—	—
PB ($n = 303$)	—	7	13	1	1

Errors 1001 and 1002: temperature or heater failure; error 2008: abnormal pressure detected; error 5007: probe check failed; error 2126: power supply issue.
PB, phosphate buffer; TE, Tris–EDTA.

Table 6
Rifampicin resistance results, per assay, compared with phenotypic DST.

Phenotypic RIF result	Ultra RIF result (sputum)		Ultra RIF result (TS)		
	Resistance detected	Resistance not detected	Resistance detected	Resistance not detected	Unsuccessful
Resistance detected ($n = 1$)	1	0	1	0	0
Resistance not detected ($n = 40$)	0	40	0	40	0
Unsuccessful ($n = 4$)	0	4	0	2	2[a]

DST, drug-susceptibility testing; RIF, rifampicin; TS, tongue swab.
[a] Reported as 'trace'.

the TS was processed using TE buffer. The most frequent error was a probe check error (error 5007), occurring in 25/42 (60%) specimens.

RIF resistance detection using the TS

Concordance for RIF resistance between the Ultra TS assay and pDST was assessed for 41 specimens (Table 6). The agreement was 100% (Cohen's kappa = 1.00; 95% CI, 1.00–1.00).

Follow-up results

During the 8-week telephonic follow-up, the research nurse contacted 368/399 (92%) participants or next-of-kin. Of these, 309 reported an improvement in symptoms, 46 had no improvement, and next-of-kin reported that 13 participants passed away (not study related). Of those still unwell, 21/46 (46%) returned for follow-up sputum testing. MTBC was not detected in 19/21 (90%) cultures, whereas non-tuberculosis mycobacteria were detected in the cultures of the other two participants.

Discussion

This study assessed the acceptability and ease of use of the TS collection procedure and evaluated the analytical performance of self-collected TS on the Ultra assay. In comparison to MGIT, assay sensitivity (78%) is comparable with the sensitivity of 79% reported by Wood et al. [23]. Although overall Ultra TS sensitivity was lower than using sputum (78% vs. 95%), performance was better in individuals with a higher bacillary load, with some variability. Similar findings were reported by Lima et al. [10] and Wood et al. [23]. In this study, for PLHIV, including those with advanced HIV disease (CD4 count <200 cells/μL), TS sensitivity was demonstrated to be ~30% lower than that on sputum but slightly better than smear microscopy, whereas TS sensitivity among HIV-negative individuals is similar to sputum (93% vs. 97%). This was not surprising because the Ultra sputum semi-quantitative results suggested lower bacterial load in PLHIV compared with the HIV-negative population. Sensitivity using TS was better than that seen using sputum smear microscopy (78% vs. 70%) which is an advantage using this specimen type. Ultra TS RIF susceptibility results showed perfect agreement with pDST and additionally provided resistance profiles in two cases with inconclusive pDST results.

TS performance in this study (sensitivity of 78% and specificity of 100%) meets the 2014 WHO Target Product Profiles for a rapid, non-sputum-based test for detecting TB [25] and meets the indicative criteria outlined in 2024 [26] for a low-complexity assay, for SA. Another consideration for the use of TS is supported by recent modelling for TB diagnostics which illustrates that a hypothetical test with 70% sensitivity and 98% specificity that is able to reach 75% of the population requiring testing results in more than double the number of people diagnosed when compared with a hypothetical test with 90% sensitivity and 99% specificity that is only able to reach 25% of the population [27,28].

The overall Ultra TS error rate was 11%, with the most frequent error being 5007, typically indicating a viscous sample, insufficient volume, or introduction of air bubbles during preparation, suggesting the use of a suboptimal buffer or pre-processing protocol. Use of PB for TS pre-processing reduced the error rate to 7% (22/303), whereas TE buffer had a higher error rate of 21% (20/96). PB was thus preferred for TS processing on Ultra in this study. However, using PB instead of the provided SR buffer introduces additional costs to specimen pre-processing, which is a limitation of this protocol.

This study demonstrated that TS self-collection is convenient and efficient for an early morning specimen. Participants reported that they preferred to collect the sample in the privacy of their homes and found transporting a TS more discreet than transporting sputum. Self-collection caused minimal discomfort, was shorter than sputum collection, provided a high rate of satisfaction for specimen collection, and was the preferred specimen type among urine, TS, and sputum. Self-collected swabs are also attractive for initiatives such as Targeted Universal Testing for TB which investigates TB in people who are in high-risk groups [29]. Since self-collection can be performed at any time, it enables testing access for those unable to take leave from work or hesitant to visit healthcare facilities because of stigma. These options will not only assist with finding the 'missing millions' but will also alleviate the burden on public health systems.

Non-productive cough, observed in 23.8% (132/554) of screened participants, supports using TS as an additional specimen type. These participants were deemed ineligible for this evaluation study but warrant inclusion in future studies.

Recruitment took place during the fourth and fifth COVID-19 waves in SA which could be a possible explanation for the unusually high number of deaths (13/399) reported during the follow-up visit.

Study results confirmed the potential of self-collected TS for molecular testing of TB, but the reduced sensitivity and invalid rates using different buffers, on an assay designed for sputum suggested that protocol optimization was required. The aggregated data from this study and other groups that make up a swab consortium led to the suggested protocol of using a diluted manufacturer buffer for TS pre-processing [30] for the Ultra assay.

In addition to centralized testing, self-collected TS can also be paired with point-of-care (POC) tests or near POC tests. POC TB screening is looking more realistic with the growing diagnostic pipeline which includes the development of OS-based molecular tests [26]. The availability of these platforms will allow fast, accurate, and perhaps affordable testing using an easy-to-collect specimen. Hence, an optimized protocol using a self-collected TS tested by a molecular assay has the potential to transform TB diagnostics by increasing access to testing thereby assisting initiatives to end TB.

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Author contributions

A.D. contributed to study conception, methodology, validation, formal analysis, data curation, project administration, visualization, writing - original draft, and writing – review and editing. L. Singh, K.P.-S., Z.N., and V.M. contributed to investigation and writing – review and editing. G.C. and P.d.S. contributed to methodology and writing – review and editing. W.S. contributed to resources, supervision, funding acquisition, writing – review and editing. L. Scott contributed to study conception, methodology, resources, supervision and writing – review and editing.

Transparency declaration

Potential conflict of interest

The authors declare the following conflicts of interest: L. Scott reports that she owns shares and receives royalties from Smartspot Quality (Pty) Ltd, consultancy fees from Foundation for Innovative New Diagnostics for performance of new TB diagnostic technologies and the receipt of royalties for patent 'A method for identifying bacteria in a sample' via the University of the Witwatersrand. All other authors declare no conflicts of interest related to this work.

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