1   Diagnostic accuracy of the Cobas® MTB and Cobas® MTB/RIF-INH assays on sputum and the

2   Cobas® MTB assay on tongue swabs for *Mycobacterium tuberculosis* complex detection in

3   symptomatic adults in South Africa

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11   **Abstract**

12   Accurate and rapid detection of *Mycobacterium tuberculosis* complex (MTBC) and drug

13   resistance is essential for effective tuberculosis (TB) management, particularly in high-burden

14   settings. The Cobas® MTB and Cobas MTB/RIF-INH assays are moderate-complexity nucleic

15   acid amplification tests that detect MTBC and resistance to rifampicin (RIF) and isoniazid (INH).

16   This study evaluated clinical diagnostic performance of the Cobas assays on sputum, using

17   liquid culture as the reference standard and Xpert MTB/RIF Ultra (Xpert Ultra) for comparison.

18   Diagnostic accuracy of the Cobas MTB assay on tongue swabs (TS) was also assessed.

19   In a study population (n=354) with 56% HIV prevalence, the overall sensitivity and specificity

20   of Cobas MTB on sputum was 93.8% (95% CI: 84.8-98.3) and 100% (95% CI: 98.7-100)

21   compared with culture. The assay showed almost perfect agreement with Xpert Ultra (Cohen's

22   kappa = 0.904). Among HIV-positive participants, sensitivity was 88.2% (95% CI: 97.8-100). RIF

23   resistance profiling by Cobas MTB/RIF-INH was fully concordant with culture and Xpert Ultra.

24   Three INH-resistant cases were missed, likely due to genotypic-phenotypic discordance.

25   Although specimen numbers were small, TS demonstrated better diagnostic accuracy when

26   using a diluted (66%) Microbial Inactivation Solution.

27   The Cobas MTB and MTB/RIF-INH assays demonstrated high diagnostic accuracy compared to

28   culture and Xpert Ultra on sputum. Findings support TS as an alternative specimen type for

29   MTBC detection using an optimized protocol. These findings underscore the potential of the

30   Cobas assays as reliable alternatives for TB and resistance diagnostics, particularly in settings

31   where rapid, accurate detection of MTBC and RIF or INH resistance is crucial.

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33   **Importance**

34   The study demonstrates high diagnostic accuracy of the Cobas® MTB and MTB/RIF-INH assays,

35   on sputum, when compared with culture and Xpert Ultra while offering critical advantages,

36   namely, INH resistance detection and scalability via high-throughput platforms. Preliminary

37   findings on tongue swabs indicate potential for additional specimen types with modified pre-

38   processing protocols. This research supports the incorporation of the Cobas assays into

39   diversified TB testing strategies to improve detection and management in high-burden

40   settings.

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Introduction

46 The Cobas® 6800/8800 Systems (Roche Molecular Systems Inc., Pleasanton, CA) have been

47 integral to South Africa's diagnostic landscape. These high-throughput platforms were

48 introduced in 2015 (1) for testing of the Cobas® HIV-1 quantitative nucleic acid amplification

49 test (NAAT), and has since played a critical role in HIV management in a country with one of

50 the largest populations of people living with HIV (PLHIV). The Cobas systems have additionally,

51 demonstrated versatility by supporting alternative specimen types, such as plasma separation

52 cards (2), thereby providing an option to testing conventional plasma samples.

53 It is not surprising that a country with such high HIV rates also ranks as one of the countries

54 with the highest burden of tuberculosis (TB) and multi-drug resistant TB (MDR-TB), globally

55 (3). For TB diagnosis, the Xpert MTB/RIF (Xpert) assay was used as the initial diagnostic since

56 2011, followed by the Xpert MTB/RIF Ultra (Xpert Ultra) assay in 2017. Shortage of Xpert Ultra

57 cartridges experienced during (and after) the COVID-19 pandemic highlighted the risks with

58 reliance on a single supplier. This shortage together with the growing diagnostic pipeline (4) as

59 well as the WHO recommendation for use of a new category of diagnostics, the moderate

60 complexity assays in 2021 (5), led to the diversification of the molecular platforms used in the

61 South African National TB program (6). Since 2023, alongside the Xpert Ultra assay, the Cobas

62 MTB and MTB RIF/INH assays (Roche Molecular Systems Inc., Pleasanton, CA) and the MAX

63 MDR-TB (Becton, Dickinson and Company, Sparks, MD, USA) assays have been incorporated

64 into the TB testing algorithm. Since the Cobas systems were already in use for the HIV program

65 with established in-country support, integrating a test utilizing the same technology provided

66 a strategic advantage.

67 The Cobas MTB assay is an automated, qualitative, real-time PCR test designed to detect

68 *Mycobacterium tuberculosis* complex (MTBC) DNA in respiratory specimens. If a MTBC-

69 positive result is detected on the assay, the specimen can be reflexed to the Cobas MTB/RIF-

70 INH assay which detects rifampicin (RIF)-resistance associated mutations of the *rpoB* gene, and

71 isoniazid (INH)-resistance associated mutations in the *katG* and *inhA* genes. Both assays' were

72 previously evaluated by our group, first in an analytical evaluation using spiked sputum

73 specimens and culture isolates (7) and then in a multi-country clinical performance evaluation

74 (8). However, the clinical evaluation study design excluded individuals unable to produce ≥2

75 mL of sputum, effectively omitting those who typically present for TB investigation in SA with

76 sputum volumes around 1 mL. We previously evaluated the Cobas MTB assay (9) but the

77 comparator used in that study was Xpert, not the currently used Xpert Ultra.

78 Other studies have assessed performance of the Cobas TB assays using N-acetyl-l-cysteine-

79 sodium hydroxide (NALC-NaOH)-treated specimens (10) or culture isolates and spiked sputum

80 (11) but data on raw sputum is limited.

81 In this clinical performance evaluation on sputum, we assessed the performance of the Cobas

82 MTB and Cobas MTB-RIF/INH assays for the detection of MTBC, RIF and INH resistance

83 compared to a liquid culture reference standard and to Xpert Ultra as a comparator. Given our

84 group's focus on exploring tongue swabs (TS) as an additional specimen type for TB diagnosis,

85 we collected TS in parallel with sputum and evaluated the diagnostic accuracy of the Cobas

86 MTB assay for MTBC detection in TS specimens.

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Materials and Methods

Study Design and procedures

We performed a cross sectional, prospective diagnostic study in which the accuracy of the Cobas MTB assay performed on sputum was assessed using the Mycobacterial Growth Indicator Tube (MGIT) (Becton Dickinson, Sparks, MD, USA) liquid culture reference standard. Cobas MTB assay performance was additionally compared to that of the Xpert Ultra assay. Additionally, concordance of the Cobas MTB assay on tongue swabs (TS) was evaluated using Xpert Ultra, liquid culture, and Cobas MTB results from sputum as comparators.

Symptomatic adults (≥18 years) attending the Hillbrow Community Health Centre (HCHC), in Johannesburg, Gauteng, South Africa, and being investigated for TB, were approached and invited to enroll in the study. Recruitment occurred from 20 October 2021 to 11 July 2023. The research nurse performed the WHO-recommended four-symptom screen (W4SS)—cough, fever, weight loss, and night sweats. Personal characteristics such as age, weight, height, HIV status and previous TB history were also recorded. Weight and height measurements were used to calculate Body Mass Index (BMI) which was used as an indicator of nutritional status. Participants with a BMI below 18.5 kg/m² were classified as underweight, suggesting possible malnutrition. Study inclusion criteria included participant willingness to return for a second visit, provision of the required number of study specimens and absence of any TB treatment within six months prior to enrolment. Specimen collection was performed for routine and research testing, over two visits (Figure 1). Research nurses used two Copan FLOQSwab (Copan, Brescia, Italy) swabs for tongue collection from each participant using the procedure detailed by Andama *et al.* (12). Collection was performed by swabbing the dorsum of the tongue for 30 seconds, as far back on the tongue as possible without initiating a gag reflex.

113   One TS was collected before sputum collection and the second after sputum collection.

114   Participants abstained from consuming any food or beverages for at least 30 minutes prior to

115   specimen collection. Both swabs were transported "dry" (without any transport buffer) to the

116   laboratory for testing.

117   Routine and research testing was performed at the Wits Diagnostic Innovation Hub (WitsDIH)

118   research laboratory in Braamfontein (Johannesburg).
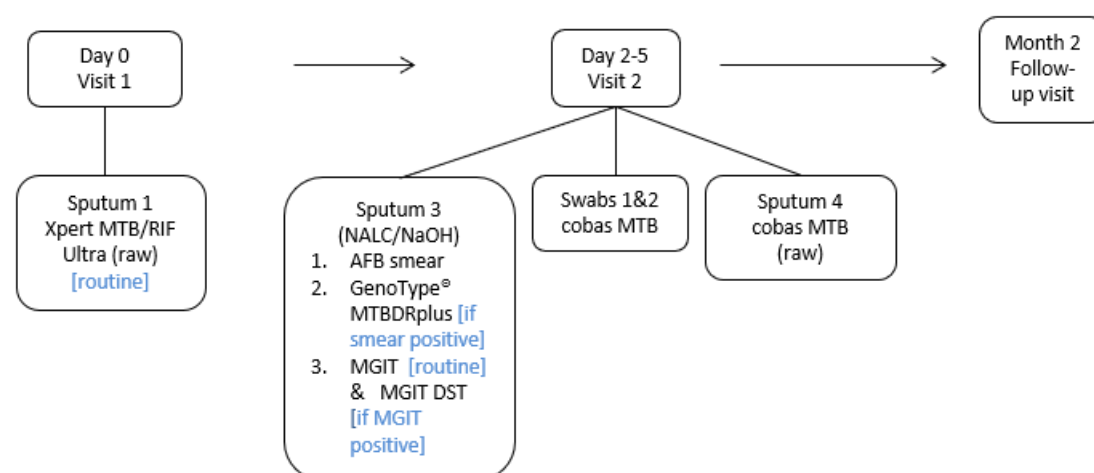
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121   Figure 1: Description of study outline indicating clinic visits and specimen laboratory pathways

122   Specimens for routine and study testing were collected over two visits approximately 2-5 days apart.

123   AFB, acid-fast bacilli; MGIT, Mycobacterial Growth Indicator tube; DST, drug-susceptibility testing; NALC/NaOH,N-acetyl-L-

124   cysteine–sodium citrate–sodium hydroxide; BD, Becton Dickinson

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128   *Ethics statement*

129   Ethics approval for this study was obtained from the University of the Witwatersrand Human

130   Research Ethics Committee (M1911150). The trial was registered with the South African

131   National Clinical Trials Registry (DOH-27-052021-5442). All participants provided written

132   informed consent.

133   *Laboratory testing*

134   Genotype® MTBDRplus line probe assay (LPA) (Hain Lifescience/Bruker, Nehren, Germany)

135   testing was performed on all smear-positive sputum and also used to speciate liquid cultures

136   that were acid-fast bacilli (AFB) positive. Routine testing and result reporting was performed

137   by laboratory staff in accordance with the National Tuberculosis Management Guidelines (13).

138   Staff performing routine testing were blinded to Cobas MTB results.

139   Cobas MTB testing on sputum was performed according to manufacturer instructions. Sputum

140   was stored at -20°C until batch testing. Microbial Inactivation Solution (MIS) (Roche Molecular

141   Systems Inc., Pleasanton, CA) was added to raw sputum in a 2:1 ratio of solution to specimen.

142   This mix was incubated at room temperature for 60 minutes, followed by sonication and a 5-

143   minute centrifugation. Testing was performed on the Cobas 6800 System. Results are reported

144   as MTB Positive, MTB Negative or Invalid (test was unable to produce a reliable or interpretable

145   result due to issues with the specimen or the testing process). Any specimen which produced

146   an MTB Positive result, was reflexed for RIF and INH testing using the Cobas MTB/RIF-INH

147   assay. Research staff performed Cobas testing and were blinded to routine TB results.

148   Swabs were stored at -80°C until batch testing. For processing of TS, 1.8 mL of MIS was added

149   to the "dry" swab and 1.5 mL was loaded on the Cobas 6800 System. Processing of TS was

150   performed according to the standard sputum protocol using neat MIS, with the exception that

151   the centrifugation step was excluded (n=239). However, parallel testing on contrived swabs

152   showed improved sensitivity using a diluted (66%) MIS buffer, hence 66% MIS buffer was used

153   to process the remaining swabs (n=99).

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155   *Outcomes and statistical analysis*

156   For diagnostic accuracy performance, sputum results from the Cobas MTB assay were

157   compared with liquid culture for MTBC detection and to phenotypic drug susceptibility testing

158   (pDST) (performed using the MGIT960 SIRE kit (Becton Dickinson, Sparks, MD, USA)) for RIF

159   and INH resistance detection. Data analysis included calculation of sensitivity, specificity,

160   positive predictive value (PPV) and negative predictive value (NPV) for MTBC detection and

161   concordance for RIF and INH resistance detection, with 95% CIs calculated using the Wilson

162   score method (first including all participants and then excluding participants with an Ultra

163   "trace" result). Cobas MTB results were additionally compared with Xpert Ultra. For Cobas

164   MTB assay performance on TS, concordance with Xpert Ultra, liquid culture and Cobas MTB

165   sputum is reported. The target sample size was 400 participants which was derived using the

166   formula described below with population proportion estimated at 50% and 95% confidence

167   interval set at 5%. Sample size (n) = $(Z)^2 * p(1-p) / x^2$, Where Z = 1.96, & where p = population

168   proportion & where x = confidence interval as a proportion. In determining the sample size,

169   an allowance was made for participants who do not meet the inclusion criteria. To determine

170   the performance of the Cobas MTB assay, only specimens that generated valid results across

171   all tests (Cobas MTB, Ultra and MGIT) were included in the statistical analysis.

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174 **Results**

175 *Characteristics of the study population*

176 A total of 554 participants were screened for the study (Figure 2). Of these, 133 were excluded

177 during the recruitment process, and 421 participants were consented and enrolled. Of those

178 enrolled, 67 were excluded from the analysis, resulting in a final sample size of 354 participants

179 included in the statistical analysis. Characteristics of the study population are shown in Table

180 1. The average age of participants was 39 years and most (64%) were male. As indicated by

181 their BMI, a total of 28/66 (42%) participants diagnosed with active TB were malnourished.

182 Among the 351 participants with a known HIV status, 57% (199/351) were positive. Forty-two

183 reported a previous TB episode. Tuberculosis was microbiologically confirmed on liquid culture

184 in 64/354 (18%) of participants. A total of 290 participants did not have microbiologically

185 confirmed tuberculosis (TB) and were consequently classified as not having active TB disease.

186 The culture contamination rate was 11% (43/404); of these, the Ultra and Cobas MTB assays

187 detected one MTBC-positive specimen, and the participant reported an improvement in

188 symptoms after having received treatment. Sixty-six participants were microbiologically

189 confirmed to have TB using the Xpert Ultra assay.

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194   Figure 2: Data description for statistical analysis

195   Of 554 individuals screened, 133 were excluded (132 with dry cough and 1 unwilling to remove a mask

196   for specimen collection). A total of 421 participants were enrolled and consented. Of these, 67 were

197   excluded from analysis due to loss to follow-up (n = 17) or unsuccessful test results (n = 50). The final

198   dataset included 354 participants for statistical analysis.

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209   Table 1: Cohort characteristics for participants used in the statistical analysis

Characteristic	All (n=354)
Demographics	
Age, mean (range), years	39 (18-70)
Male sex, *n* (%)	213 (63.6)
BMI kg/m^2, *n* (%)	
<18.5 (Underweight)	94 (26.6)
18.5-24.9 (Healthy weight)	186 (52.5)
25-29.9 (Overweight)	44 (12.4)
>30 (Obese)	30 (8.5)
HIV-related information	
HIV-positive, *n* (%)	199 (56.2)
HIV-negative, *n* (%)	152 (42.9)
Status unknown, *n* (%)	3 (0.9)
TB History	
Previously diagnosed with TB, *n* (%)	42 (11.9)
Clinical signs and symptoms of TB at presentation	
Cough (any duration), n (%)	353 (99.7)
Unexplained weight loss, n (%)	265 (74.9)
Nights sweats, n (%)	263 (74.2)
Fever, n (%)	236 (66.9)
*Other, n (%)	257 (72.6)
Bacteriological confirmation (sputum 3), n (%)	
Smear and culture positive	44 (12.4)
Smear negative and culture positive	20 (5.6)
Smear and culture negative	284 (80.2)
Smear positive and culture negative	6 (1.7)
Xpert MTB/RIF Ultra (sputum 1)	66
High	24
Medium	8
Low	19
Very low	8
Trace	7

BMI, body-mass index; *Other symptoms include body aches, loss of appetite, hemoptysis, shortness of breath, dizziness and vomiting

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213   *Clinical performance evaluation of the Cobas MTB assay on sputum*

214   The performance of the Cobas MTB assay on raw sputum was compared with the reference

215   method of liquid culture and, additionally, stratified by HIV and smear status, as outlined in

216   Table 2 for 354 specimen results. The assay exhibited high sensitivity (94%) and 100%

217   specificity, with improved performance observed when Xpert Ultra "trace" results were

218   excluded. Assay sensitivity among HIV-positive individuals was 88%, while perfect sensitivity

219   (100%) and specificity (100%) was achieved in HIV-negative individuals. The Cobas MTB assay

220   produced 1/404 (<1%) invalid results. Unsuccessful tests were not repeated due to cost

221   considerations.

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233 Table 2: The performance of smear microscopy, Xpert Ultra and Cobas MTB assays compared

234 to the reference standard (liquid culture) for MTBC detection on sputum

Variable	Smear microscopy	Xpert MTB/RIF Ultra	Cobas MTB (raw sputum)
All (including trace) (n=354)			
Sensitivity, % (95% CI)	68.8 (55.9-79.8)	93.8 (84.8-98.3)	93.8 (84.8-98.3)
Specificity, % (95% CI)	97.9 (95.6-99.2)	97.9 (95.6-99.2)	100 (98.7-100)
PPV, % (95% CI)	88.2 (76.1-95.6)	90.9 (81.3-96.6)	100 (94.0-100)
NPV, % (95% CI)	93.4 (90.0–95.9)	98.6 (96.5-99.6)	98.6 (96.6-99.6)
All (excluding trace) (n=347)			
Sensitivity, % (95% CI)	70.5 (57.4-81.5)	93.4 (84.1-98.2)	96.7 (88.7–99.6)
Specificity, % (95% CI)	97.9 (95.5-99.2)	99.3 (97.5–99.9)	100 (98.7–100)
PPV, % (95% CI)	87.8 (75.2-95.4)	96.6 (88.3–99.6)	100 (93.9–100)
NPV, % (95% CI)	94.0 (90.6–96.4)	98.6 (96.5–99.6)	99.3 (97.5–99.9)
Specimens from HIV-positive individuals (n=199)			
Sensitivity, % (95% CI)	58.8 (40.7-75.4)	91.2 (76.3-98.1)	88.2 (72.5–96.7)
Specificity, % (95% CI)	98.2 (94.8-99.6)	97.6 (93.9-99.3)	100 (97.8–100)
PPV, % (95% CI)	87.0 (66.4–97.2)	88.6 (73.3-96.8)	100 (88.4-100)
NPV, % (95% CI)	92.0 (87.0–95.6)	98.2 (94.7-99.6)	97.6 (94.1–99.4)
Specimens from HIV-negative individuals (n=152)			
Sensitivity, % (95% CI)	82.1 (63.1-93.9)	96.4 (81.7-99.9)	100 (87.7–100)
Specificity, % (95% CI)	97.6 (93.1-99.5)	98.4 (94.0-99.8)	100 (97.1–100)
PPV, % (95% CI)	88.5 (69.8-97.6)	93.1 (77.2-99.2)	100 (87.7–100)
NPV, % (95% CI)	96.0 (91.0-98.7)	99.1 (95.3–100)	100 (97.1–100)
Smear microscopy negative specimens (n=304)			
Sensitivity, % (95% CI)	n/a	85.0 (62.1-96.8)	85.0 (62.1-96.8)
Specificity, % (95% CI)		97.9 (95.5-99.2)	100 (98.7–100)
PPV, % (95% CI)		73.9 (51.6-89.8)	100 (80.5–100)
NPV, % (95% CI)		98.9 (96.9-99.8)	99.0 (97.0–99.8)

235 PPV, positive predictive value; NPV, negative predictive value; CI, Confidence interval

236 *Comparison of the Cobas MTB assay to Xpert Ultra for Detection of MTBC*

237 The Cobas MTB and Xpert Ultra assays demonstrated a high level of concordance, with an

238 overall agreement of 97.5% (344/354) and a Cohen's kappa coefficient of 0.904 (95% CI:

239 0.8447–0.9623).

240 Among the discordant results, eight specimens were positive by Xpert Ultra but negative by

241 Cobas MTB; all eight had "trace" or "very low" semi-quantitative results on Ultra, and two of

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242 these were culture-positive. Conversely, two specimens were positive by Cobas MTB but

243 negative by Xpert Ultra; both were also confirmed positive by liquid culture.

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245 *Clinical performance evaluation of the Cobas MTB/RIF-INH assay on sputum*

246 Of the 60 raw sputum which produced an MTB Positive result on the Cobas MTB assay and

247 were reflex tested on the Cobas MTB/RIF-INH assay, reportable resistance results were

248 available for 52 (87%) specimens. Unsuccessful tests were not repeated due to insufficient

249 specimen volumes.

250 For RIF resistance detection, the Cobas MTB/RIF-INH assay accurately identified correct

251 resistance profiles in 47/47 (100%) sputum samples with valid results (Table 3). Of the

252 specimens tested, 8/60 (13%) yielded invalid results. Where pDST was unsuccessful, the assay

253 successfully assigned resistance profiles to five specimens, as confirmed by the LPA.

254 Table 3: Cobas RIF resistance results, compared to phenotypic DST

pDST result	Cobas MTB-RIF/INH result		
	RIF-R not detected	RIF-R detected	Invalid
RIF sensitive (n=54)	46	0	8
RIF resistant (n=1)	0	1	0
Invalid (n=5)	5	0	0

255 RIF, rifampicin; RIF-R, rifampicin resistance; pDST, phenotypic drug susceptibility testing

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257 For INH resistance detection, the Cobas MTB/RIF-INH assay correctly identified resistance

258 profiles in 44/47 (94%) sputum samples with valid results (Table 4). In three cases, the assay

259 did not detect INH resistance that was identified by pDST, with resistance also undetected by

260 LPA in 1/3 (33%) of these cases. Additionally, 8/60 (13%) specimens produced invalid results;

261 however, the assay successfully assigned resistance profiles to five specimens, as confirmed by

262 LPA, where pDST was unsuccessful.

263 Table 4: Cobas INH resistance results, compared to phenotypic DST

pDST result	Cobas MTB-RIF/INH result		
	INH-R **not detected**	**INH-R** **detected**	**Invalid**
INH sensitive (n=50)	43	0	7
INH resistant (n=5)	3	1	1
Invalid (n=5)	5	0	0

264 INH, isoniazid; RIF-R, isoniazid resistance; pDST, phenotypic drug susceptibility testing

265 *Diagnostic performance of tongue swabs on the Cobas MTB assay*

266 When compared to Xpert Ultra, TS detected MTBC across all semi-quantitative categories,

267 from "high" to "very low." Using undiluted MIS, MTBC detection was observed in three

268 participants on TS collected after sputum, compared to those collected before (Table 5).

269 However, with 66% MIS, detection improved across all semi-quantitative categories, with

270 comparable detection rates between TS collected before and after sputum. Similarly, when

271 compared to liquid culture or Cobas MTB sputum and processed with 66% MIS, TS showed

272 improved MTBC detection and comparable detection rates on Cobas MTB. Regardless of the

273 comparator used, assay specificity remained consistent between undiluted and 66% MIS.

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274   Table 5: Performance of the MAX MDR-TB assay on tongue swabs compared to Xpert Ultra,

275   liquid culture and MAX MDR-TB sputum results

Comparator test result (sputum)	Cobas MTB TS result, n/N (%)			
	Neat MIS (TS 1)	Neat MIS (TS 2)	66% MIS (TS 1)	66% MIS (TS 2)
Xpert Ultra positive	25/53 (47.2)	28/53 (52.8)	9/13 (69.2)	9/13 (69.2)
High	*16/18 (88.9)*	*15/18 (83.3)*	*4/6 (66.7)*	*4/6 (66.7)*
Medium	*2/6 (33.3)*	*3/6 (50.0)*	*1/2 (50.0)*	*1/2 (50.0)*
Low	*6/15 (40.0)*	*6/15 (40.0)*	*4/4 (100)*	*4/4 (100)*
Very low	*1/7 (14.2)*	*4/7 (57.1)*	*0/1 (0)*	*0/1 (0)*
Trace	*0/7 (0)*	*0/6 (0)*	-	-
Xpert Ultra negative	215/217 (99.1)	216/217 (99.5)	70/71 (98.6)	71/71 (100)
Liquid culture positive	26/52 (50)	29/52 (55.8)	9/12 (75.0)	9/12 (75.0)
Liquid culture negative	217/218 (99.5)	218/218 (100)	71/72 (98.6)	72/72 (100)
Cobas MTB sputum positive	26/48 (54.2)	29/48 (60.4)	9/12 (75.0)	9/12 (75.0)
Cobas sputum negative	221/222 (99.5)	222/222 (100)	71/72 (98.6)	72/72 (100)

276   MIS, microbial inactivation solution; TS, tongue swab; TS 1 was collected before sputum collection and TS 2 after

277   sputum collection

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Discussion

This study investigated the clinical performance of the Cobas MTB and Cobas MTB/RIF-INH assays for MTBC, RIF and INH resistance detection using raw sputum against a liquid culture reference standard and to Xpert Ultra as a comparator. In addition, we investigated the diagnostic accuracy of the Cobas MTB assay on TS compared with Xpert Ultra sputum, liquid culture and Cobas MTB sputum.

In this study population with an HIV prevalence of 57%, the Cobas MTB assay demonstrated high sensitivity (94%) compared to culture, comparable to the Xpert Ultra assay (94%), with a specificity of 100%. Among HIV-positive participants, the Cobas MTB assay showed a sensitivity of 88%, compared to 91% for Xpert Ultra. In this subset, when participants with "trace" semi-quantitative Xpert Ultra results were excluded from the analysis, the sensitivity of the Cobas MTB assay improved to 97%. The assay exhibited perfect sensitivity (100%) within the HIV-negative subset of participants. There was almost perfect agreement (14) between the Cobas MTB and Xpert Ultra assays, reflecting a high degree of concordance with both assays demonstrating similar unsuccessful rates of <1%. Thus, in terms of performance, both assays perform comparably which supports use of the Cobas MTB assay in South Africa's TB testing diversification.

Xpert Ultra has the advantage of providing a MTBC result and an upfront RIF resistance profile within a shorter period and fewer pre-processing steps compared to the Cobas MTB assay (15). However, the Cobas assay, despite requiring reflex resistance testing can provide an INH result which the Xpert Ultra cannot. This has implications for patient management, as a South African prevalence survey (2012–2014) found that INH-monoresistant TB accounted for more than 5% of cases in all provinces (16). Since testing is performed on the high throughput

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303   68/8800 systems, the Cobas MTB assay is suited for high-burden settings or in countries which

304   are trying to ramp up TB screening in attempts to overcome the effects of COVID-19 (17). An

305   additional advantage of the Cobas MTB assay is that resistance testing can be performed only

306   when required, which will save costs.

307   For RIF resistance detection, the Cobas MTB/RIF-INH assay showed perfect agreement with

308   liquid culture and Xpert Ultra. Both Xpert Ultra and Cobas MTB/RIF-INH yielded an

309   unsuccessful rate of 13% for RIF detection. For INH, resistance was missed in three specimens

310   (as per the pDST result); one of which was also missed by the LPA. One explanation for this is

311   that it has been shown that phenotypic resistance may sometimes be identified before the

312   associated genetic mutations can be detected since molecular assays require a higher drug

313   resistant proportion to be present. This mismatch can arise from the limited sensitivity of

314   genotypic assays, which generally focus on the most frequently occurring mutations, or from

315   resistance mechanisms that have not yet been genetically characterized (18).

316   For the Cobas MTB assay on TS, MTBC detection was comparable between TS collected before

317   and after sputum collection, suggesting that the timing of collection does not affect assay

318   performance. When evaluated against Xpert Ultra sputum semi-quantitative results, the Cobas

319   MTB assay on TS detected MTBC down to the "very low" category, with improved detection in

320   participants with higher bacterial loads, though some variability was observed. These findings

321   align with those reported by Ahls et al. (19), further supporting that MTBC detection using TS

322   improves with increasing bacterial load. Cobas MTB assay performance on TS is lower than

323   that on sputum but this is expected since the assay is designed for sputum and additional

324   specimen types will likely require protocol modification, as has been seen with other assays

325   designed for sputum but tested on TS (20). Some protocol modification was attempted in this

326   study, where a diluted MIS (66% MIS) was tested on a subset of specimens to determine if

327   assay sensitivity could be improved. While the number of MTBC positive specimens is too

328   limited to draw definitive conclusions, assay sensitivity and specificity seems improved using

329   66% MIS. Future studies should consider this diluted buffer for TS pre-processing for testing

330   on the Cobas MTB assay.

331   In conclusion, the Cobas MTB and Cobas MTB/RIF-INH assays demonstrated comparable

332   performance to liquid culture and the Xpert Ultra assay, on sputum, showcasing high

333   sensitivity and specificity in detecting MTBC. These findings underscore the potential of the

334   Cobas assays as reliable alternatives for TB and resistance diagnostics, particularly in settings

335   where rapid, accurate detection of MTBC and RIF or INH resistance is crucial. The study findings

336   further support the potential utility of TS as an additional specimen type for MTBC detection

337   on the Cobas MTB assay, particularly when using an optimized pre-processing protocol such

338   as 66% MIS. Future studies should explore application of the Cobas assays in diverse patient

339   populations and operational settings to further establish utility in global TB control efforts.

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Conflict of Interest disclosure

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360 The authors declare the following conflicts of interest: Professor Lesley Scott declares that she

361 is the inventor of the Dried Culture Spot technology (USP 8,709,712) which is licenced through

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369 **Author contributions**

370 Conceptualization: A. David and L. Scott; Data curation: A. David; Formal analysis: A. David;

371 Funding acquisition: L. Scott and W. Stevens; Investigation: A. David, L. Singh. K. Peloakgosi-

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373 administration: L. Scott and W. Stevens; Supervision: L. Scott; Validation: A. David;

374 Visualization: A. David and L. Scott; Writing—original draft: A. David; Writing—review and

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376 Molepo and W. Stevens.

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