

Diagnostic Performance of Smear Microscopy and Xpert MTB/RIF Versus MGIT Culture in Republic of Congo

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Abstract

In the Republic of the Congo, tuberculosis (TB) remains a major public health concern. Although the GeneXpert MTB/RIF assay is the WHO-recommended first-line diagnostic test, smear microscopy is still used for treatment monitoring and in facilities where molecular testing is limited. Evaluating the diagnostic accuracy of smear microscopy compared to GeneXpert and MGIT culture is essential to guide diagnostic strategies and strengthen TB control in the country. A cross-sectional study was conducted among 92 presumptive pulmonary TB patients at Makelekele Hospital. Sputum samples were analyzed by smear microscopy, GeneXpert MTB/RIF, and MGIT culture. Sensitivity, specificity, positive and negative predictive value were calculated for smear microscopy and GeneXpert, using culture as the reference standard. Culture detected more *Mycobacterium tuberculosis* than microscopy (49% vs. 32%, $P < 0.001$). Smear microscopy showed a sensitivity of 58% (95% CI: 43–71%) and specificity of 92% (95% CI: 80–97%). GeneXpert detected more MTB (62% vs. 49%, $P < 0.001$) with a sensitivity of 98% (95% CI: 89–100%) and specificity of 72% (95% CI: 58–83%). GeneXpert showed superior sensitivity for TB detection, while microscopy remained specific. Expanding GeneXpert testing across the Republic of the Congo will improve TB management.

Introduction

Tuberculosis (TB) remains one of the major infectious diseases worldwide, representing a significant public health problem, particularly in sub-Saharan Africa [1,2]. According to the latest World Health Organization, 2024 report, approximately 10.8 million people developed TB in 2023, with 1.25 million deaths, including a substantial proportion among people living with HIV [1]. Early and accurate detection of *Mycobacterium tuberculosis* (MTB) is crucial to reduce TB-related morbidity and mortality. Diagnostic methods in use include smear microscopy, molecular tests such as Xpert MTB/RIF, and culture, which is considered the gold standard due to its high sensitivity and ability to provide a definitive diagnosis [3,4].

For decades, direct smear microscopy has been the most widely available initial diagnostic test in resource-limited settings such as the Republic of Congo [5]. In this country, TB is also a major concern, with cases of multidrug-resistant TB (MDR-TB) and frequent HIV co-infections, complicating disease management and control [6,7]. While its technical simplicity and low cost are operational advantages, its notoriously low sensitivity, particularly in HIV patients or those with paucibacillary disease, likely results in a substantial number of missed diagnoses [8]. However, the technical complexity, long turnaround time, and biosafety requirements of culture limit its utility for rapid clinical decision-making at the point of care [3].

To bridge this diagnostic gap, the WHO recommends the Xpert MTB/RIF assay as the initial diagnostic test for TB because of its ability to rapidly detect MTB complex DNA and rifampicin resistance [4,9]. In the Republic of Congo, this recommendation has driven a significant shift in diagnostic policy, with Xpert now widely adopted as the first-line diagnostic tool in more than 40% of testing centers [7].

However, critical reliance on microscopy persists for patient follow-up to monitor treatment response, even in centers equipped with GeneXpert. Moreover, in many centers, microscopy remains the only on-site tool for both initial diagnosis and follow-up. This continued, widespread reliance on direct smear microscopy for both initial diagnosis and treatment monitoring underscores the need for a contemporary evaluation of its performance in the Congolese context, especially in high-risk populations such as people living with HIV [5,6,10]. Generating such local evidence is essential to validate current practices, identify potential risks, and guide the evolution of national TB diagnostic strategies.

Methodology

Ethical Approval

The study protocol was approved by the Institutional Ethics Committee of the Congolese Foundation for Medical Research (015/CIE/FCRM/May 30, 2018). All procedures were conducted in accordance with the Declaration of Helsinki and national ethical guidelines. Written informed consent was obtained from adult participants, while assent from minors was obtained along with consent from their legal guardians.

Type and population of study

This is a cross-sectional study, conducted from October 2018 to October 2019 and targeted presumptive pulmonary tuberculosis participants and who had never received treatment recruited. Eligible participants were between 8 and 70 years of age, presenting TB clinical signs, without prior anti-TB treatment, voluntarily consented and assented to HIV testing, and residing in Brazzaville during the study period. Participants with diseases such as cancer, advanced HIV-AIDS, severe malaria and extra-pulmonary TB were excluded.

Sample Collection and study design

The study design was based on a previously published survey conducted among the same group of presumptive TB patients [6]. From October 2018 to October 2019, sputum samples were collected from patients with presumed pulmonary tuberculosis hospitalized at the Makelekele Hospital located in Brazzaville, Republic of Congo. Sociodemographic, behavioral, and clinical data were recorded using a standardized, structured questionnaire.

After informed consent was obtained, a 5 mL blood sample was taken for HIV screening, preceded by a pre-test interview for HIV. Two sputum samples of 3 mL each were collected according to national guidelines: a single sample on the first day in the hospital and a morning sample on the second day at home, sent the same day to the mycobacteria laboratory at Makélékélé Reference Hospital.

All samples were transported at 4–8°C to the mycobacteria laboratory at the Christophe Mérieux Center for Research on Infectious Diseases (CeRMI-CM). Sputum samples were decontaminated using the BD BBL® MycoPrep™ kit before being cultured.

Blood was used for HIV screening, while sputum was analyzed for *Mycobacterium tuberculosis* detection using microscopy (Ziehl–Neelsen staining), Xpert MTB/RIF test (GeneXpert Cepheid), and culture in MGIT BACTEC 960 liquid medium [2,8,12,11].

Mycobacterial Culture

Decontaminated sputum samples were inoculated into BACTEC MGIT 960 culture tubes (Becton Dickinson, United States of America) supplemented with oleic acid, albumin, dextrose, and catalase, together with polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin as antimicrobial agents [11,13]. The tubes were incubated in the MGIT 960 system [11]. Positive cultures were confirmed by Ziehl–Neelsen staining and checked for sterility on blood agar [8]. Cultures were considered negative after forty-two days without growth [3,11].

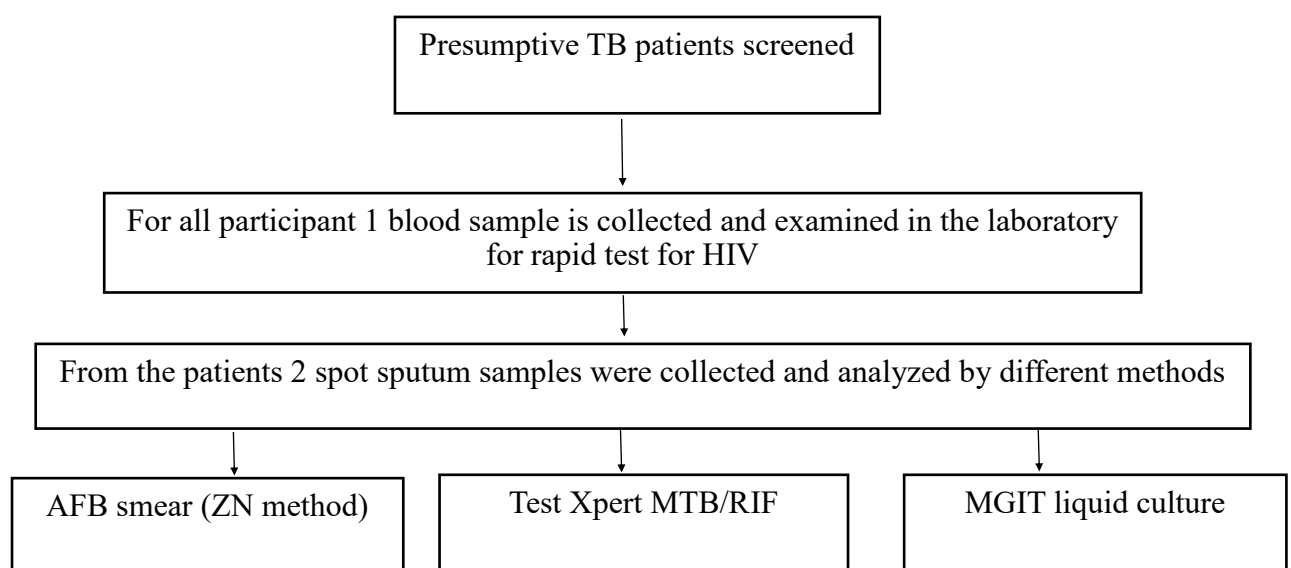


Figure 1. Flowchart of patient recruitment at Makelekele Referral Hospital and laboratory methods used.

Statistical analysis

Participant characteristics were summarized using standard descriptive statistics. Graph Pad Prism (Version 8.0.2) was used for statistical analyses. The count data were expressed as rate (%), Fisher. Two-sided test, P value <0.05 indicated a statistically significant difference. Microscopy and GeneXpert sensitivity and specificity to detect MTB in sputum were determined using following formula. Sensitivity = true positive / (true positive + false negative) × 100% Specificity = true negative / (true negative + false positive) × 100% PPV = true positive / (true positive + false positive) × 100% NPV = true negative / (true negative + false negative) × 100%.

Results

Sociodemographic, clinical, risk behavior, and comorbidity characteristics of patients enrolled in the study

A total of 92 patients were included in the study. The gender distribution was balanced, with 47 women (51.1%) and 45 men (48.9%). The mean age of the participant was 38.2 ±15 years. Most were in the 18–44 years age group (55.4%), followed by those aged ≥45 years (35.9%) and under 18 years (8.7%). Regarding marital status, single patients predominated. In addition, a large proportion of participants had secondary or higher education. Concerning risk behaviors, 43 patients (46.7%) reported alcohol consumption, 20 (21.7%) reported tobacco use, and 5 (5.4%) reported cannabis use. Twenty-two patients (23.9%) were HIV-positive, while 70 (76.1%) were HIV-negative. The dominant clinical signs included: chronic cough in 66 cases (71.7%), acute cough in 28 (30.4%), fever in 72 (78.3%), weight loss in 82 (89.1%), physical asthenia in 27 (29.3%), and anorexia in 19 (20.7%), as shown in Table 1.

Table 1. Sociodemographic, clinical, risk behavior, and comorbidity characteristics of patients enrolled in the study.

Characteristics	Number of patients (n=92)	Percentage (%)
Sociodemographic		
Age group (years)		
<18	8	8.7
18–44	51	55.4
≥45	33	35.9
Median age	38.2	
Interquartile range		23.0–49.7
Gender		
Female	47	51.1
Male	45	48.9
Marital status		
Single	81	88.0
Cohabiting	11	12.0
Education level		
None / Primary	16	17.4
Secondary / Higher	76	82.6

Season of recruitment		
Rainy season	57	62.0
Dry season	35	38.0
Tuberculosis risk behaviors		
Alcohol use	43	46.7
Tobacco use	20	21.7
Cannabis use	05	05.4
HIV comorbidity		
Negative	70	76.1
Positive	22	23.9
Clinical signs		
Chronic cough	66	71.7
Acute cough	28	30.4
Fever	72	78.3
Anemia	10	10.9
Neurological signs	04	04.3
Night sweats	15	16.3
Physical asthenia	27	29.3
Anorexia	19	20.7
Weight loss	82	89.1

Table 2. Performance parameters of MTB detection microscopy compared to the culture

		Culture		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		Positive	Negative				
Microscopy	Positive	26	4	58	91.5	86.7	70
	Negative	19	43	(43.3–71)	(80.1–96.6)	(70.3–94.7)	(58–79.4)

PPV: Positive Predictive Value; NPV: Negative Predictive Value; CI: Confidence Interval.

Performance of microscopy in TB diagnosis using culture as the gold standard

The performance of smear microscopy for the detection of pulmonary TB was evaluated using culture as the gold standard (Table 2).

Compared to the culture, smear microscopy showed a lower rate of MTB detection ($P < 0.001$) (49% vs 32%). Considering culture as the gold standard, smear microscopy demonstrated a sensitivity of 57.8% (95% CI: 43.3–71.0%) and a specificity of 91.5% (95% CI: 80.1–96.6%). The positive predictive value was 86.7% (95% CI: 70.3–94.7%), and the negative predictive value was 69.4% (95% CI: 57.0–79.4%). The Fisher’s exact test showed a statistically significant association between microscopy and culture results (Fisher’s exact test, $p < 0.0001$, two-sided).

Table 3. Performance parameters of MTB detection Xpert MTB/RIF compared to the culture.

		Culture		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
		Positive	Negative				
Xpert MTB/RIF	Positive	44	13	97.8	72.3	77.1	97.1
	Negative	1	34	(88.4–99.9)	(58.1–83)	(64.7–86)	(85.4–99.8).

PPV: Positive Predictive Value; NPV: Negative Predictive Value; CI: Confidence Interval.

Performance of Xpert MTB/RIF for TB diagnosis using culture as the gold standard

The performance of Xpert MTB/RIF was also assessed using culture as the gold standard (Table 3).

In contrast to microscopy Xpert MTB/RIF detected more MTB cases than culture (62% vs 49%, $p < 0.001$). Using culture as the reference, Xpert MTB/RIF demonstrated a sensitivity of 97.8% (95% CI: 88.4–99.9%) and a specificity of 77.2% (95% CI: 58.2–83.1%). The positive predictive value was 77.2% (95% CI: 64.8–86.2%), and the negative predictive value was 97.1% (95% CI: 85.5–99.9%). The association between GeneXpert and culture results was also statistically significant (Fisher's exact test, $p < 0.0001$, two-sided).

Discussion

This study evaluated the diagnostic performance of Xpert MTB/RIF assay and smear microscopy in a cohort of Congolese patients with presumptive pulmonary tuberculosis hospitalized at the Makelelele Hospital, using culture as the gold standard. Our patient demographic, predominantly younger adults, reflects the typical population most affected by TB [1,14,15]. Clinically, the same observation was made regarding common symptoms such as chronic cough, HIV infection, and fever [16,17,7]. The overall findings confirm that while smear microscopy remains highly specific, its sensitivity is considerably lower than that of Xpert MTB/RIF, underscoring the limitation of microscopy as a primary diagnostic tool [18,2].

Smear microscopy detected fewer MTB cases than culture (32% vs 49%), with a sensitivity of only 57.8%. This means that over 40% of true TB cases confirmed by culture were missed by microscopy. The low sensitivity directly translates into a modest Negative Predictive Value (NPV) of 69.4%, which is a critical weakness in high-burden settings such as the Republic of Congo. This modest NPV indicates that if diagnosis relies solely on microscopy, a substantial proportion of true TB cases may be missed. The finding underlines the limited reliability of smear microscopy in high-burden settings, particularly where early and accurate detection is essential for reducing transmission [19,2].

In contrast, the Xpert MTB/RIF assay demonstrates a markedly improved diagnostic profile. Xpert MTB/RIF detected more MTB cases than culture (62% vs 49%) and achieved excellent sensitivity (97.8%) while maintaining a high specificity (77.2%). The high negative predictive value (97.1%) suggests that Xpert MTB/RIF is highly reliable in excluding TB in negative cases, a critical advantage in clinical practice. The apparent higher detection rate compared with culture may be due to the molecular assay's ability to detect DNA from non-viable bacilli or culture losses due to sample degradation or contamination. Despite this, the strong statistical association between Xpert MTB/RIF and culture results supports its validity as a diagnostic tool [20].

The relatively low sensitivity of microscopy observed in our study compared to Xpert MTB/RIF is consistent with several previous studies. Steingart et al. [8,21] showed that the sensitivity of Ziehl–Neelsen smear microscopy for the detection of MTB ranges between 50% and 60%, whereas the sensitivity of Xpert MTB/RIF can exceed 90%, particularly among HIV co-infected patients. This highlights the limitation of microscopy in detecting paucibacillary cases, which may go unnoticed, while Xpert MTB/RIF, based on PCR amplification, efficiently detects MTB genetic material even at low bacillary loads [12,22,23,9].

Taken together, these findings reinforce the WHO recommendation that Xpert MTB/RIF should be prioritized as the initial diagnostic test for TB [6,24], especially in high TB burden and resource-limited settings. While microscopy remains useful because of its simplicity, rapid turnaround time, and low cost, its low sensitivity significantly limits its standalone diagnostic value. Culture remains indispensable for definitive confirmation but is constrained by longer turnaround times and higher infrastructure requirements [3,11].

Limitations

The sample size was modest, which may have affected the precision of sensitivity and specificity estimates. Our future studies with larger cohorts will assess the combined use of GeneXpert, smear microscopy, and culture within diagnostic algorithms to optimize case detection and treatment initiation.

Conclusion

This study reinforces the pivotal role of molecular diagnostics in the modern TB care cascade. While smear microscopy retains utility due to its high positive predictive value and ability to indicate infectiousness, its critically low sensitivity remains a major limitation. In contrast, the Xpert MTB/RIF assay has proven to be a far more reliable tool, offering significantly superior sensitivity and an exceptional ability to rule out disease. Its implementation is indispensable for strengthening TB control efforts, as it facilitates earlier and more accurate diagnosis, enables prompt treatment initiation, and ultimately curbs community transmission.

Therefore, these findings provide robust, local evidence to support the multiple appeals made to national health authorities to equip all TB screening centers across the Republic of Congo with GeneXpert technology.

Authorship contribution statement

FN and DOEA conceived the study. DOEA and MAEN contributed to study design and sample processing or methodology. FHM, JCV, FHOO analysed the data. FN had overall responsibility for the study. AG, FHOO, FHM, and FN were involved in conceptualization, data compilation, editing, and review of drafts, as well as finalization. All authors contributed to drafting the manuscript and read and approved the final version

AI declaration: No AI tools were used for data collection, data analysis, or interpretation of study results.

Declaration of competing interest

The authors disclose no competing interests associated with the study.

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