

Accuracy of GenoQuick MTB test in detection of *Mycobacterium tuberculosis* in sputum from TB presumptive patients in Uganda

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Abstract

Objective: The objective of the study was to determine the diagnostic performance of the GenoQuick MTB test on heated sputum against the conventional Lowenstein–Jensen *Mycobacterium tuberculosis* culture as the reference method for tuberculosis diagnosis.

Introduction: Fast, reliable, and easy-to-use tests for tuberculosis diagnosis are essential to achieving the Sustainable Development Goal of diagnosing and treating 90% of tuberculosis patients by 2030. We evaluated the diagnostic performance of the GenoQuick MTB, a polymerase chain reaction–lateral flow test, in Uganda, a resource-constrained, high tuberculosis- and HIV-burden setting.

Methods: Fresh sputum samples from presumptive tuberculosis patients at Mulago Hospital were tested for *M. tuberculosis* using smear microscopy, GenoQuick MTB test, and Lowenstein–Jensen culture. For the GenoQuick MTB test, mycobacterial DNA was extracted by heating sputum at 95°C for 30 min while DNA amplification and detection were done following the manufacturer’s protocol (Hain Lifescience, Nehren, Germany). Sensitivity, specificity, and kappa agreements were calculated against Lowenstein–Jensen *M. tuberculosis* culture as a reference test using STATA V12.

Results: Of the 86 tested samples, 30.2% had culture-confirmed pulmonary tuberculosis. Overall, sensitivity was higher for GenoQuick MTB (81%, 95% confidence interval: 60%–93%) than for smear microscopy (69%, 95% confidence interval: 48%–86%). Among people living with HIV, sensitivity was identical for GenoQuick MTB and smear tests (75%, 95% confidence interval: 42%–95%). Contrastingly, smear had a higher overall specificity (98%, 95% confidence interval: 91%–100%) than for GenoQuick MTB (92%, 95% confidence interval: 81%–97%). A similar trend of specificity was observed among the people living with HIV for smear microscopy (100%, 95% CI: 87%–100%) and for GenoQuick MTB (96%, 95% confidence interval: 81%–100%).

Conclusion: The GenoQuick MTB test could be a potential tuberculosis diagnostic test given its higher sensitivity. Evaluation of this test in larger studies is recommended.

Keywords

Diagnosis, *Mycobacterium tuberculosis*, GenoQuick MTB, nucleic acid amplification, diagnostic accuracy, and resource-limited settings

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Introduction

Tuberculosis (TB) is a contagious airborne disease affecting mostly young adults in their productive years, especially those living in poverty.¹ Approximately 95% of TB deaths occur in resource-constrained settings and the majority of TB-related deaths (1.2 million) occur among people living with HIV (PLWH).^{1,2} In Uganda, about 86,000 new TB cases are reported annually and 40% occur among PLWH.³ HIV-TB co-infection is a challenge for TB control programs because most of these patients present with low mycobacterial load, making the most widely available diagnostic tools like sputum smear microscopy insensitive.⁴

One of the Sustainable Development Goals (SDGs) is to reduce TB incidence and TB-related deaths by 80% and 90%, respectively, by the year 2030.¹ To achieve this, timely and accurate diagnosis of TB using sensitive, rapid, and easy-to-use diagnostics are critical to facilitate early initiation of TB treatment and curb TB transmission.¹ Nucleic acid amplification tests (NAATs) have enormous potential to help achieve these goals. These tests allow sequences of *Mycobacterium tuberculosis* (Mtb) DNA that may be present in very few copies to be amplified in vitro using a target-specific amplification technique,⁵ thereby providing high sensitivity and high specificity. The 2009 TB diagnostic guidelines from the US Centers for Disease Control and Prevention (CDC) recommended that NAAT be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary tuberculosis independent of the smear microscopy results.⁶ Several NAATs have been developed and recommended for TB detection but with varying sensitivities and specificities.^{7–11} These NAATs were developed to work with TB presumptive cases and thus require a Biosafety Level 2 (BSL-2) laboratory for sputum processing prior to testing, which makes the whole process expensive and thus less feasible in peripheral health units in a country such as Uganda. The Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) is the only test that allows sputum processing in a BSL-1 laboratory.¹⁰ Indeed, this test was approved by the World Health Organization (WHO) in 2010 and has now been widely distributed in resource-constrained settings for TB detection in sputum. However, in Uganda, the test is only offered at referral health center IVs and is not available at peripheral laboratories where patients can easily access it. Because of this limitation, additional accurate, point-of-care tests are necessary for TB diagnosis in these settings.

The GenoQuick MTB test (Hain Lifescience, Nehren, Germany) is a rapid, molecular genetic test for detection of Mtb complex from decontaminated patient specimens. Sputum processing is done using the NALC-NaOH technique in a BSL-2 laboratory, followed by DNA extraction using the commercially available Geno-Lyse-Kit reagents, polymerase chain reaction (PCR) amplification in an ordinary thermocycler, and detection done using a dipstick strip

in a lateral flow format, similar to the conventional pregnancy tests.

In 2012, the GenoQuick MTB test was evaluated in Spain for Mtb detection on NALC-NaOH-processed stored clinical samples and it was found to have an overall sensitivity of 85% in smear positive and 78% in smear negative patients.¹² This test is rapid and up to 96 samples can be run every 3 h. However, despite its potential diagnostic relevancy, there is no information about its performance using fresh sputum samples and in resource-constrained, high TB- and HIV-burdened settings.

In this study, we evaluated the diagnostic performance of GenoQuick MTB test using fresh sputum samples from TB presumptive patients. We assessed the possibility of using directly heated sputum for GenoQuick MTB testing instead of using NALC-NaOH-processed sputum as per the manufacturer's protocol, to make the test easier and cheaper to perform in resource-constrained setting laboratories which lack biosafety cabinets for sputum processing.

Methods

Setting and study design

This study was nested within a bigger study, the Mulago Inpatient Noninvasive Diagnosis of Pneumonia-International HIV-associated Opportunistic Pneumonias (MIND-IHOP) study^{13,14} conducted on the Pulmonology (TB) Ward of Mulago National Referral Hospital, located in Kampala City, Uganda. We carried out a prospective, cross-sectional study of consecutive adults (age ≥ 18) enrolled in MIND-IHOP study from February to November 2015.

Study participants

The study enrolled participants with self-reported persistent and productive cough for at least 2 weeks, and evaluated them for TB, as previously described.^{12,13} The participants signed a written informed consent (Ethical approval number REC REF No. 2006-017), after which collection of demographic and clinical information was done by the study nurses. Participants were excluded from the study if they were already on TB treatment or if their hemoglobin levels were less than 7.0 g/dL. They also underwent voluntary HIV counseling and testing,¹⁵ and those confirmed to be living with HIV had their CD4+ T-cell counts measured (FACS Calibur; BD, San Jose, CA, USA). For the GenoQuick MTB study, a total of 89 study participants were enrolled.

Specimen collection

Sputum samples were collected by trained laboratory technicians in 50 mL falcon tubes from every patient for smear microscopy using LED illuminator, for Lowenstein-Jensen (LJ) solid media culture, and for the GenoQuick MTB study.

For GenoQuick MTB study, 1 mL of sputum was collected and transported in a cool box to MBN Clinical Laboratories for testing. Laboratory technicians performing this test were blinded to the clinical characteristics of the participants and their prior sputum results.

GenoQuick MTB test

DNA extraction from fresh sputum. Conventionally, the Hain Lifescience DNA extraction protocol using the Geno-Lyse-Kit requires prior sputum decontaminated/processing in a BSL-2 laboratory. In our study, that protocol was modified to remove the requirement of a BSL-2 laboratory and allowed DNA extraction in a BSL-1 laboratory facility as a strategy for potentially easing the DNA extraction steps. In the modified protocol, Mtb was killed and mycobacterial DNA was extracted directly from sputum by heating it at 95°C for 30 min. Briefly, approximately 500 µL of raw sputum was placed into a 1.5 mL screw cap cryo-tube and an equal volume of sterile phosphate buffered saline (PBS) added. It was then heat incubated at 95°C for 30 min on a heat block. The sample was then centrifuged at 14,000 r/min for 15 min to separate supernatant (DNA) from pellet (debris). The supernatant which contains the DNA was then stored at -20°C for a maximum time of 24 h if not used immediately for amplification.

Amplification. Amplification was done as per the manufacturer's instructions, using commercial reagents that were supplied in the GenoQuick MTB kit (Hain Lifescience). Briefly, 45 µL of prepared master mix (35 µL of primer nucleotide mix, 5 µL of 10× PCR buffer containing 15 mM MgCl₂, 5 µL of 25 Mm MgCl₂ solution leading to a final concentration of 4 mM, and 0.2 µL of 5 unit(s) thermo-stable hot-start DNA polymerase) was put into a PCR tube and 5 µL of extracted DNA solution was added, leading to a final volume of 50.2 µL. A positive control DNA and negative control (nuclease-free water) supplied in the kit were added on each run batch from extraction to detection. The tube(s) were labeled and put in the GTQ thermocycler (Hain Lifescience). The PCR amplification parameters were initial activation of hot-start DNA polymerase for 10 min at 95°C for 1 cycle, denaturation for 30 s at 95°C, annealing for 40 s at 55°C, and extension for 30 s at 72°C for 40 cycles, followed by 2 min at 95°C for 1 cycle, 5 min at 20°C for 1 cycle, and, finally, for infinite at 8°C.

Lateral flow dipstick detection of amplified products. To detect Mtb, 10 µL of the amplicons was added to 100 µL of GenoQuick MTB hybridization buffer supplied in the kit and mixed. The GenoQuick MTB strip was then introduced into the amplicon-buffer mixture in the vial and allowed to stand/hybridize at room temperature for 10 min in a dipstick format. After 10 min, the reactive zones were read visually and the results were scored. The GenoQuick MTB strip has three

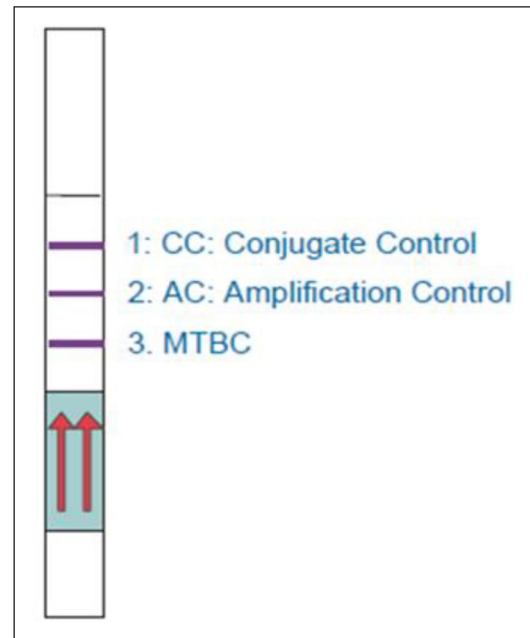


Figure 1. The three reaction zones of the GenoQuick dipstick (Hain Lifescience).

reaction zones: (1) which indicates the presence of the MTB complex in the patient specimen, (2) the amplification control (AC) which ensures that the test is performed correctly, and (3) the conjugate control (CC) which proves the efficiency of conjugate binding as shown in Figure 1.

Statistical analysis

Analysis was done using STATA Version 12.0 (Stata Corp., College Station, TX, USA). Contingency tables were used to obtain true positives, false positives, false negatives, and true negatives. Sensitivity and specificity for the GenoQuick MTB test and smear microscopy for diagnosis of pulmonary tuberculosis (PTB) were calculated with 95% confidence interval (95% CI) using LJ culture as reference.

Results

Study participants

Of the 89 presumptive pulmonary TB participants studied, 86 were included in the analysis and three excluded due to contamination on LJ culture. Participants had a median age of 34.5 (interquartile range, IQR: 27–42) years and the majority were male (n=52, 60.5%) (Table 1). A total of 39 (45.3%) participants were living with HIV with a median CD4+ T-cell count of 109 cells/µL (IQR: 58–283). Overall, 32 of 39 (82.1%) HIV-positive participants were taking antiretroviral therapy at the time of admission. All patients had a cough ≥ 2 weeks, and of these, 22 (26%) participants had hemoptysis. Seventy (81%) reported fever/chills/night

Table 1. Demographic and clinical characteristics of the study population.

Characteristic	Frequency/value	Proportion (%)
Median age, years	34.5 [27–42]	NA
Gender: male	52	60.5
Patients living with HIV	39	45.3
CD4+ cell count, median [IQR], cells/ μ L	109 [58–283]	NA
ART use on admission	32	82
Clinical presentation	Frequency/value	Proportion (%)
Cough \geq 2 weeks	86	100
Weight loss \geq 3 kg	78	91
Fever/chills/night sweats	70	81
Hemoptysis	22	26
Previous TB diagnosis	9	10
Sputum characteristics	Frequency/value	Proportion (%)
Salivary	9	10
Mucoid	67	78
Purulent	5	6
Blood stained	5	6
Sputum characteristics	Frequency of GenoQuick positivity	Proportion
Salivary (n = 9)	2	22.2
Mucoid (n = 67)	19	28.4
Purulent (n = 5)	2	40
Blood stained (n = 5)	3	60

HIV status was obtained using the Ministry of Health (MOH)–recommended HIV rapid testing algorithm in Uganda. CD4 counts were measured using FACSCalibur (BD, San Jose, CA, USA). Sputum smears were stained using Auramine and read at 400 \times with LED-FM. ART: antiretroviral therapy; IQR: interquartile range; TB: tuberculosis.

sweats within the past 7 days and 78 (91%) reported weight loss \geq 3 kg. Of all participants, nine (10%) had a previous diagnosis of TB.

Performance of the GenoQuick MTB test

Among the 86 sputum samples, Mtb was isolated by LJ culture from 26 (30.2%) specimens (Figure 2). The overall sensitivity was higher for GenoQuick MTB at 81% (95% CI: 60%–93%) compared with smear microscopy at 69% (95% CI: 48%–86%) (Table 2). Among individuals without HIV, the sensitivity was also higher for GenoQuick MTB at 86% (95% CI: 57%–98%) than for smear microscopy at 64% (95% CI: 35%–87%). Among people living with HIV, however, the sensitivity was identical at 75% (95% CI: 42%–95%) for both GenoQuick MTB and smear microscopy.

On the contrary, a total of 60 (69.8%) samples did not show any visible growth on culture. The specificity of GenoQuick MTB of 92% (95% CI: 81%–97%) was lower than that of smear microscopy (98%) (95% CI: 91%–100%) (Table 2). Among HIV-positive patients, the specificity of 96% (95% CI: 81%–100%) for GenoQuick MTB was approximately close to that of smear microscopy (100%) (95% CI: 87%–100%). Among HIV-seronegative patients,

however, the specificity of 88% (95% CI: 71%–97%) for GenoQuick MTB was still lower than the 97% (95% CI: 84%–100%) for smear microscopy.

The GenoQuick MTB test had a sensitivity of 100% among smear-positive participants and a specificity of 93% among smear-negative participants.

A total of five samples were positive on GenoQuick MTB test but negative on LJ culture.

Discussion

In this study, the diagnostic performance of the GenoQuick MTB test was assessed, alongside smear microscopy and using LJ culture as the reference standard. While previous studies used NALC-NaOH-processed sputum for GenoQuick MTB testing,^{12,13} in this study we assessed the possibility of using directly heated sputum for GenoQuick MTB testing to make the test easier and cheaper to perform from BSL-1 laboratories in resource-limited settings, which lack biosafety cabinets for sputum processing. Results from our study showed that unprocessed sputum yielded higher sensitivity, though lower specificity, as compared with microscopy. In this study, the sensitivity of 81% for GenoQuick MTB was in the range with 85.4% of a previous study.¹¹

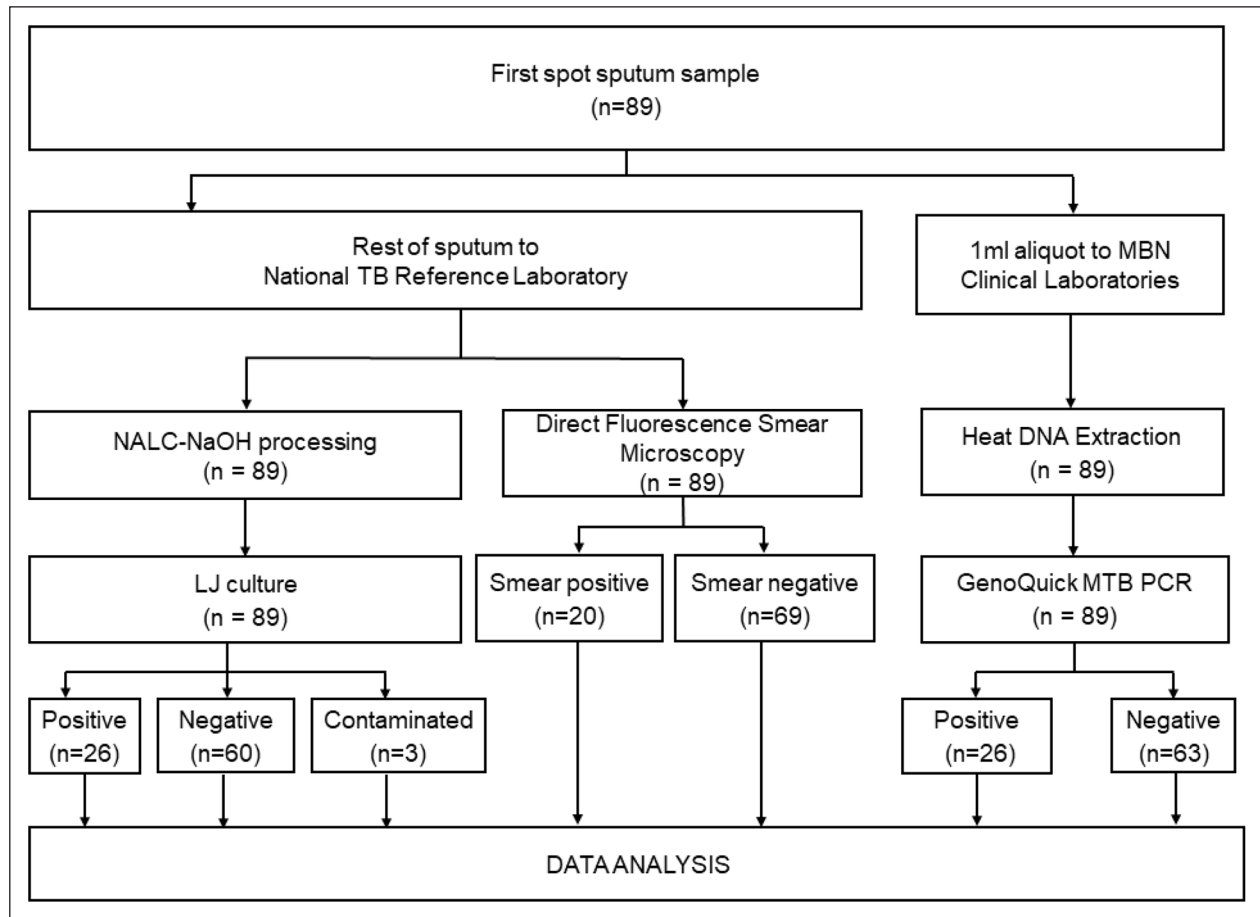


Figure 2. Sample flow chart.

Eighty-nine sputum samples were studied; all had a smear and LJ culture done at National TB Reference Laboratory; 26 turned positive on LJ, 60 negative and 3 were contaminated. N represents number.

Table 2. Performance of GenoQuick MTB and smear microscopy tests against Lowenstein–Jensen MTB culture method as reference for TB diagnosis in fresh sputum.

	Overall (n = 86)		HIV positive (n = 39)		HIV negative (n = 47)	
	GenoQuick MTB	Smear microscopy	GenoQuick MTB	Smear microscopy	GenoQuick MTB	Smear microscopy
True positive	21	18	9	9	12	9
False positive	5	1	1	0	4	1
False negative	5	8	3	3	2	5
True negative	55	59	26	27	29	32
Sn, % (95% CI)	81 (60–93)	69 (48–86)	75 (42–95)	75 (42–95)	86 (57–98)	64 (35–87)
Sp, % (95% CI)	92 (81–97)	98 (91–100)	96 (81–100)	100 (87–100)	88 (71–97)	97 (84–100)
kappa agreement	0.72	0.73	0.75	0.81	0.71	0.68

CI: confidence interval; MTB: *Mycobacterium tuberculosis*; N: number of samples; Sn: sensitivity; Sp: specificity.

When comparing GenoQuick MTB with other NAATs like Xpert MTB/RIF, which has been adopted globally for TB detection and rifampicin resistance especially in resource-limited settings, a systematic review of the Xpert MTB/RIF test involving 86 studies reported a pooled sensitivity of 85% and specificity of 98%.¹⁶ A sensitivity of 98% was reported among the smear-positive, culture-confirmed

TB cases and 67% among the smear-negative, culture-confirmed TB cases. In this same review, a sensitivity of 88% was reported among HIV-positive patients and 81% among HIV-negative patients.¹⁰ These results are in range with the results of this study. The small differences in sensitivities between this review and our study could be explained by the differences in study designs and sample size between studies;

in our study, the sensitivity data we presented herein are from a small number of participants based on TB presumptive samples from symptomatic patients whose smear status was unknown. On the contrary, the Xpert MTB/RIF meta-analysis presented results from many studies that assessed test performance on either known smear-positive or smear-negative sputum samples with bigger sample sizes. Moreover, in our study, we observed a total of five samples that were positive on GenoQuick MTB but negative on LJ culture. Although the study did not identify the non-tuberculous mycobacteria (NTMs), it is possible that the “false positives” in this study could have resulted from the low performance of LJ culture compared with the GenoQuick MTB.^{17,18} We further explored this possibility and found that three of the five patients were also diagnosed with TB using the Xpert MTB/RIF test (results not shown), one was diagnosed with TB using a second spot sample on LJ culture, and one was treated for bronchopneumonia. If the first four samples had been detected by the LJ culture, the reference test in our study, the true positive results would have increased from 21 to 25, and the sensitivity of the GenoQuick MTB would have jumped to 96%, which is clearly comparable with the Xpert MTB/RIF test’s reported performance of 97%. With these results, one could conclude that GenoQuick MTB test has the potential to identify and diagnose TB diseased patients and exclude MTB faster and may be suitable for use in settings with conventional PCR testing services.

In this study, we also explored the use of a simple sputum processing method where DNA is directly extracted from sputum using heat, instead of the conventional method where sputum is processed with NALC-NaOH, and later DNA is extracted from the pellet using commercially available GenoLyse (Hain Lifescience), which is a time-consuming and expensive method. Although this study did not aim to compare the two methods of sputum processing, findings from our study are comparable with previous studies of GenoQuick MTB where the conventional method of processing was done. For this test, only ordinary conventional PCR thermocyclers, which are currently widely available or easy to purchase as they are much cheaper than a real-time PCR analyzer, and easy to setup in many laboratories in resource-limited settings, are needed.

In sub-Saharan Africa, over 90% of peripheral laboratories have for decades relied on microscopy for TB diagnosis, yet the test has lower sensitivity.^{13,18} In 2009, the Global TB diagnostic guidelines recommended that NAATs be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary tuberculosis independent of the smear microscopy results,⁶ and in response, the Xpert MTB/RIF test by itself a real-time PCR test has increasingly become available in sub-Saharan Africa. Conventionally, the Xpert MTB/RIF test is rather costly.¹⁹ However, the WHO/Global Fund and other agencies have been subsidizing the price of cartridges for poor countries and thus keeping the testing services running in those

settings.²⁰ However, long-term reliance on this mechanism of TB tests funding can be challenging. No cost data were collected in this study but with the promising performance of the GenoQuick MTB test as shown in this study, a cost comparison study is warranted to find out whether the GenoQuick MTB test could be cheaper, and so adoptable for long-term self-reliance in terms of financing the TB diagnostics in the resource-limited settings.

We included smear microscopy in this study since it is one of the points-of-care tests used for TB diagnosis in many resource-limited settings.²¹ With a sensitivity of only 69% (48%–86%) vs 81% (60%–93%) with the GenoQuick MTB test, the latter test has the capacity to detect more patients who would have gone undiagnosed if only smear microscopy was used, though no statistical test was done to compare the two. However, it should be noted that the smear test had a slightly higher specificity, meaning it provides a low chance of reporting false-positive results.

Study limitations

This being a pilot study, there was no formal calculation of sample size, and power analysis for sample size was not done. Our intention was to determine the performance of the test for TB diagnosis using heated sputum. The results of the pilot study, however, provide an important insight about the potential role of GenoQuick MTB test for TB diagnosis using heated sputum, and with these results, a study with a bigger sample size can be recommended in future. In addition, this study used LJ culture as reference standard for which confirmation of positive culture was traditionally based on colony characteristics like shape, size, color, and consistence of the colonies formed. We also report that there was no identification of NTMs and this could have been the reason for appearance of LJ culture positives but GenoQuick MTB negatives since the latter PCR test detects only members of the MTB complex and not NTMs. Finally, GenoQuick MTB uses manual DNA extraction and detection of amplified products, hence the potential for cross contamination of samples leading to false-positive results. To overcome this shortage, trained technical staff and a lab setup that allows the different steps such as DNA extraction, amplification, and detection to be carried out in separate self-contained rooms are required.

Conclusion

Our findings showed that diagnosis of TB using GenoQuick MTB test together with an easy-to-perform heat MTB DNA extraction method from sputum had a high MTB yield. The method cannot replace the use of Gene Xpert due to its higher sensitivity and ability to diagnose rifampicin resistance. Our method, however, may be feasible for peripheral facilities with no biosafety hoods or lack of Xpert machines for TB diagnosis particularly among new TB patients where rifampicin resistance is very low at less or equal to 1%. The

method has a short turnaround time of 3 h, a key attribute required in speeding-up initiation of TB treatment.

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Authors' contribution

S.K. planned and coordinated the study, and prepared the manuscript. E.M. helped with data analysis and manuscript preparation. A.S. and P.B. reviewed the manuscript. I.S. and J.Z. identified and enrolled all patients, collected all the clinical data, and also reviewed the manuscript. L.H. funded the main study under which this study was performed. W.W., and L.H., oversaw the conduct of the study and assisted with preparation and technical revision of the manuscript. A.O. performed the GenoQuick MTB tests at MBN. F.B. planned and oversaw the technical conduct of the study, data analysis, and performed technical revision of the manuscript. All authors approved the submitted version of the manuscript.

Data availability

All applicable data are within the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from Makerere University, College of health sciences, School of Medicine Research ethics committee board (REC REF No. 2006-017).

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Informed consent

Written informed consent was obtained from all participants before the study.

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