



RESEARCH ARTICLE

REVISED **The utility of repeat Xpert MTB/RIF testing to diagnose tuberculosis in HIV-positive adults with initial negative result [version 2; peer review: 1 approved, 1 approved with reservations]**

Yasmeen Hanifa ¹, Katherine L. Fielding¹, Violet N. Chihota ^{2,3}, Lungiswa Adonis⁴, Salome Charalambous^{2,3}, Nicola Foster ⁵, Alan Karstaedt^{6,7}, Kerrigan McCarthy², Mark P. Nicol^{8,9}, Nontobeko T. Ndlovu², Edina Sinanovic⁵, Faieza Sahid^{6,7}, Wendy Stevens^{9,10}, Anna Vassall¹, Gavin J. Churchyard^{1-3,11}, Alison D. Grant ^{1,3,12}

¹TB Centre, London School of Hygiene & Tropical Medicine, London, UK

²The Aurum Institute, Johannesburg, South Africa

³School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

⁴Mamelodi Hospital, Pretoria, South Africa

⁵Health Economics Unit, School of Public Health and Family Medicine, University of Cape Town, Cape Town, South Africa

⁶Department of Medicine, Chris Hani Baragwanath Hospital, Johannesburg, South Africa

⁷University of the Witwatersrand, Johannesburg, South Africa

⁸Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

⁹National Health Laboratory Service, Johannesburg, South Africa

¹⁰Department of Molecular Medicine and Haematology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

¹¹Advancing Care and Treatment for TB/HIV, South African Medical Research Council Collaborating Centre for HIV and TB, Johannesburg, South Africa

¹²Africa Health Research Institute, School of Nursing and Public Health, University of KwaZulu-Natal, Durban, South Africa

v2 **First published:** 26 Apr 2018, 2:22
<https://doi.org/10.12688/gatesopenres.12815.1>
Latest published: 25 Feb 2022, 2:22
<https://doi.org/10.12688/gatesopenres.12815.2>

Abstract

Background: Amongst HIV-positive adults in South Africa with initial negative Xpert results, we compared the yield from repeating Xpert MTB/RIF ("Xpert") on sputum to guideline-recommended investigation for tuberculosis (TB).

Methods: A systematic sample of adults attending for HIV care were enrolled in a cohort exploring TB investigation pathways. This substudy was restricted to those at highest risk of TB (CD4<200 cells/mm³ or unknown) who had a negative initial Xpert result. At attendance for the Xpert result, a repeat sputum sample was stored, and further investigations facilitated per national guidelines. Participants were reviewed monthly, with reinvestigation if indicated,

Open Peer Review

Approval Status ? ✓

1

2

version 2

(revision)

25 Feb 2022

version 1

26 Apr 2018

?

[view](#)

✓

[view](#)

1. **Colleen F. Hanrahan**, Johns Hopkins

Bloomberg School of Public Health,

for at least three months, when sputum and blood were cultured for mycobacteria, and the stored sputum tested using Xpert. We defined TB as “confirmed” if Xpert, line probe assay or *Mycobacterium tuberculosis* culture within six months of enrolment were positive, and “clinical” if TB treatment was started without microbiological confirmation.

Results: Amongst 227 participants with an initial negative Xpert result (63% female, median age 37 years, median CD4 count 100 cells/mm³), 28 (12%) participants had TB diagnosed during study follow-up (16 confirmed, 12 clinical); stored sputum tested positive on Xpert in 5/227 (2%). Amongst 27 participants who started TB treatment, the basis was bacteriological confirmation 11/27 (41%); compatible imaging 11/27 (41%); compatible symptoms 2/27 (7%); and unknown 3/27 (11%).

Conclusions: Amongst HIV-positive individuals at high risk of active TB with a negative Xpert result, further investigation using appropriate diagnostic modalities is more likely to lead to TB treatment than immediately repeating sputum for Xpert. TB diagnostic tests with improved sensitivity are needed.

Keywords

Tuberculosis, Diagnostic Test, HIV infection, South Africa

Baltimore, USA

2. **Tow Keang Lim**, National University Hospital, Singapore, Singapore

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Yasmeen Hanifa (yhanifa@hotmail.com)

Author roles: **Hanifa Y:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Fielding KL:** Conceptualization, Data Curation, Formal Analysis, Methodology, Software, Supervision, Visualization, Writing – Review & Editing; **Chihota VN:** Conceptualization, Methodology, Project Administration, Resources, Writing – Review & Editing; **Adonis L:** Resources, Writing – Review & Editing; **Charalambous S:** Conceptualization, Methodology, Project Administration, Resources, Writing – Review & Editing; **Foster N:** Conceptualization, Writing – Review & Editing; **Karstaedt A:** Conceptualization, Resources, Writing – Review & Editing; **McCarthy K:** Conceptualization, Writing – Review & Editing; **Nicol MP:** Conceptualization, Writing – Review & Editing; **Ndlovu NT:** Investigation, Project Administration, Writing – Review & Editing; **Sinanovic E:** Conceptualization, Writing – Review & Editing; **Sahid F:** Resources, Writing – Review & Editing; **Stevens W:** Conceptualization, Writing – Review & Editing; **Vassall A:** Conceptualization, Writing – Review & Editing; **Churchyard GJ:** Conceptualization, Resources, Writing – Review & Editing; **Grant AD:** Conceptualization, Funding Acquisition, Investigation, Methodology, Project Administration, Supervision, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Bill and Melinda Gates Foundation [OPP1034523].

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Hanifa Y *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Hanifa Y, Fielding KL, Chihota VN *et al.* **The utility of repeat Xpert MTB/RIF testing to diagnose tuberculosis in HIV-positive adults with initial negative result [version 2; peer review: 1 approved, 1 approved with reservations]** Gates Open Research 2022, 2:22 <https://doi.org/10.12688/gatesopenres.12815.2>

First published: 26 Apr 2018, 2:22 <https://doi.org/10.12688/gatesopenres.12815.1>

REVISED Amendments from Version 1

The manuscript has been revised in response to Reviewer comments regarding complicated presentation of the data and advice to exclude the LAM data. The major differences are 1) removal of the LAM data, 2) modification of Figure 1 to include information regarding the basis for TB treatment and additional yield of Xpert, 3) modification of the supplementary file to include a Figure 3 (new Figure 2 in supplementary material from the previous manuscript and clarification regarding the testing undertaken).

Any further responses from the reviewers can be found at the end of the article

Introduction

Since 2011 the World Health Organization (WHO) has recommended Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA) as the initial diagnostic test for individuals being investigated for HIV-associated tuberculosis (TB)¹. TB diagnosis in people living with HIV (PLHIV) is complicated by the high proportion who are smear-negative and/or have extrapulmonary disease². Although Xpert has superior sensitivity to sputum microscopy, it is less sensitive than culture, with a pooled sensitivity of 61% for smear-negative, culture-positive TB among PLHIV³.

South Africa replaced smear microscopy with Xpert starting in 2011, for all individuals with symptoms suggesting TB⁴. Further evaluation of those who are HIV-positive and Xpert-negative comprises clinical reassessment, chest radiograph if available, sputum for mycobacterial culture, and treatment with antibiotic if clinically indicated⁴. In a South African study of 394 patients investigated for TB (irrespective of presence of symptoms) prior to antiretroviral therapy (ART) initiation, the sensitivity of Xpert for smear-negative, culture-positive TB increased from 43% to 62% when a second sample collected at the first visit was tested⁵. Mathematical modelling using a decision model from South Africa⁶ suggested that replacing sputum culture with the cheaper option of a second Xpert would reduce loss to follow-up so 1% more patients would start TB treatment⁷, and save an estimated US\$17.4 million per year⁷. This model assumed, based on limited data, the same sensitivity for the second Xpert test as for the first⁷, guidelines would be correctly followed⁷, and only 1% of those with TB symptoms start TB treatment based on a clinical diagnosis⁶. The strategy of sending a repeat Xpert for HIV-positive individuals whose initial Xpert result is negative has not been evaluated empirically.

The aim of our study was to describe the diagnostic yield from an immediate repeat Xpert on sputum, compared to sequential further investigation guided by South African recommendations, amongst HIV-positive adults being investigated for TB whose initial Xpert result is negative.

Methods

This “repeat Xpert” substudy was part of “Xpert for people attending HIV/AIDS care: test or review?” (XPHACTOR), a

prospective cohort study evaluating a risk-based algorithm to prioritise Xpert testing amongst adults attending for routine HIV care in South Africa⁸.

XPHACTOR study population, recruitment and procedures

XPHACTOR study flow, procedures and algorithm are described in detail in the supplementary material. In summary, we enrolled a systematic sample of adults (aged ≥ 18 years) attending four HIV clinics in Gauteng province, irrespective of presence of symptoms suggestive of TB, in the XPHACTOR study. Patients taking anti-tuberculosis treatment within the previous three months were excluded. Patients were enrolled into three groups: “on ART” (ART-experienced); “pre-ART” (in HIV care but not taking ART); and “HIV Testing and Counselling (HTC)” (newly-diagnosed HIV-positive). At the time of the study, ART eligibility comprised CD4 ≤ 350 cells/mm³ or WHO clinical stage ≥ 3 . Research staff screened participants for TB at monthly intervals to three months, using a standardised questionnaire which incorporated the WHO symptom screen (any one of current self-reported cough, fever, weight loss or night sweats, hereafter the WHO tool). A spot sputum sample was collected for Xpert for individuals at *a priori* highest risk of active TB according to the study algorithm, which prioritised testing for those with any of: current cough, fever ≥ 3 weeks, night sweats ≥ 4 weeks, BMI < 18.5 kg/m², CD4 < 100 cells/mm³, or weight loss $\geq 10\%$; and at enrolment from all in HTC group or pre-ART with CD4 < 200 cells/mm³ (Supplementary material, Figure 1).

At enrolment and follow-up visits, participants who submitted an Xpert sample were reviewed within one week, and if Xpert-positive, TB treatment was initiated. If Xpert was negative, research staff repeated WHO symptom screen and facilitated the Xpert-negative algorithm for all who were WHO tool positive, which comprised chest radiograph, spot sputum for TB culture, and/or antibiotic trial as clinically appropriate. The Xpert-negative algorithm was also facilitated, because of *a priori* high risk of active TB, for all pre-ART participants with CD4 $< 200 \times 10^6/l$ who submitted sputum for immediate Xpert at enrolment to XPHACTOR.

At the three-month visit all participants had sputum and blood cultured for mycobacteria (Bactec MGIT 960 and 9240 systems). We allowed a broad window period around the three-month XPHACTOR main study final visit, until around six months, to maximise follow-up.

Study investigation results were returned to clinic staff, who were responsible for management decisions. Clinic records were reviewed at the end of the study to ascertain any additional relevant investigations (e.g. Xpert, mycobacterial culture and imaging requested as part of routine clinical care) and/or TB diagnoses.

Repeat Xpert substudy procedures

XPHACTOR participants who were Xpert-negative with i) CD4 count < 200 cells/mm³, or ii) new HIV diagnosis (HTC group) were eligible for this substudy, irrespective of presence of WHO

tool symptoms; these restrictions aimed to minimise unnecessary testing of individuals at lower risk of active TB. If a participant had more than one negative Xpert result during follow-up, only the first episode was included.

At attendance for Xpert result review, eligible participants were asked for an additional spot sputum sample for “repeat” Xpert, which was frozen at -80°C within 24 hours of collection. All stored samples were thawed and tested with Xpert at the end of the study to evaluate the diagnostic yield that could have been achieved if an immediate repeat Xpert had been sent at the Xpert result review visit. We decided *a priori* not to induce sputum for this substudy in order to reflect what would be achievable in routine practice.

Definitions

Repeat Xpert substudy entry and exit dates. Repeat Xpert substudy cohort entry date was defined as the date that the Xpert result review was conducted and sputum was collected for storage. Cohort exit date was defined as the last XPHACTOR study visit date.

TB case definitions. “Confirmed” TB was defined as a positive result on i) Xpert (on sputum sample) or ii) line probe assay (LPA) performed on smear-positive or cultured isolate (GenoType MTBDR *plus*, Hain Lifesciences) or iii) *Mycobacterium tuberculosis (Mtb)* culture, from any sample (including stored sputum and those requested by health care providers) collected within six months of XPHACTOR enrolment. Clinical TB was defined as TB treatment started within six months of enrolment ascertained from clinical records, self or family report, or reported in the context of a separate verbal autopsy sub-study, in the absence of microbiological confirmation. Six months was chosen because TB disease evolves gradually^{9,10}; data from Zimbabwe estimated the mean duration of smear-positivity prior to TB diagnosis amongst HIV-positive adults at 18–33 weeks¹¹.

“Not TB” was defined as absence of criteria for confirmed or clinical TB, and alive at least 3 months (the minimum follow-up period) after enrolment. Participants who did not fulfil the case definitions for TB or “not TB” were deemed to have unclassifiable outcome and excluded from analyses.

Pulmonary and extrapulmonary TB were classified in accordance with WHO definitions¹².

We also summarise the basis on which treatment was initiated for those who started TB treatment.

Radiological definitions. “Probable radiological TB” was defined as presence of any of cavitation, predominantly upper lobe infiltrates, pleural or pericardial effusion, or clear miliary picture on chest radiograph. “Possible radiological TB” was defined as presence of any of lymphadenopathy (hilar or mediastinal), pulmonary nodules or other infiltrates. Participants with “probable” or “possible” radiological TB features, but without bacteriological confirmation, who started TB treatment within six months of substudy enrolment were assigned as having “clinical” TB.

Study outcomes

The co-primary outcomes were a) the proportion of participants with a positive repeat Xpert and b) the proportion of participants fulfilling study TB case definitions.

Statistical methods

Data were analysed using Stata 14 (Stata Corporation, College Station, TX, USA).

We did not undertake formal sample size calculation for this substudy as the sample size was all those eligible from the parent study.

We compared TB diagnoses made by Xpert using the sample stored at substudy enrolment, with all TB diagnoses fulfilling our case definitions during follow-up. We chose this pragmatic comparison because in real life, individuals with smear or Xpert-negative TB have sequential investigation, rather than all tests performed simultaneously. Our research staff facilitated the Xpert-negative algorithm when participants attended for Xpert result review, and therefore investigations are likely to have been initiated faster than in a routine setting. The proportion of TB diagnoses made by Xpert using the stored sputum was compared with TB diagnoses made during follow-up using McNemar’s test.

In a sensitivity analysis restricted to participants who had at least one component of the Xpert-negative algorithm (chest radiograph, sputum for TB culture, or antibiotic trial) within a two-week window of providing the stored repeat Xpert sample, we compared the proportion of TB diagnoses made by Xpert using the stored sputum with TB diagnoses made by the Xpert-negative algorithm using McNemar’s test.

Ethical approval

The study was approved by the ethics committees at the University of the Witwatersrand (approval # M120343), University of Cape Town (approval # 106/2012), and the London School of Hygiene & Tropical Medicine (approval # 6165). All consenting participants gave written consent or, witnessed verbal consent if unable to read or write. All ethics committees approved the consent form. Principles expressed in the Declaration of Helsinki were followed in the conduct of this research.

Results

Between September 2012 and March 2014, 235/410 (57.3%) potentially eligible participants were able to provide a sputum sample, stored for testing at study completion with Xpert (Figure 1). Eight participants with “unclassifiable” outcome were excluded, leaving 227 participants for analysis.

Participant characteristics

Characteristics of the 227 substudy participants and comparison with the 175 excluded because they were unable to produce sputum are presented in Table 1. The majority of participants were female (63%), median age was 37 years (interquartile range [IQR] 31,44), median CD4 count was 100 cells/mm³ (IQR 51,147), and 26% had previously been treated for TB. 78/227 (34%) of participants reported a TB symptom, most often cough

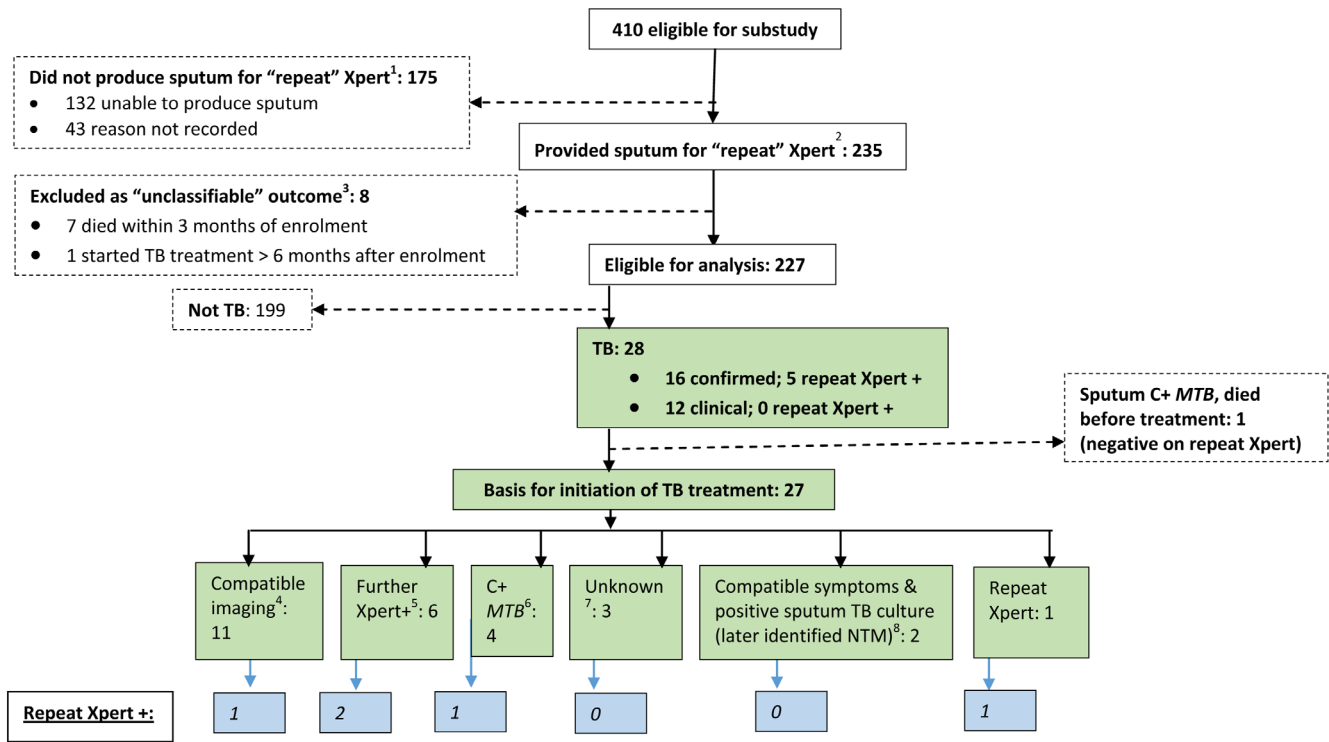


Figure 1. Flow chart of repeat Xpert substudy participants, basis for initiation of TB treatment, and yield from repeat Xpert MTB/RIF. C+ = culture-positive; CXR = chest radiograph; MTB = mycobacterium tuberculosis; NTM = non-tuberculous mycobacteria; USS = ultrasound scan; Xpert+ = sputum Xpert MTB/RIF positive. ¹ 15/175 who were excluded because they did not produce sputum fulfilled case definitions for TB (clinical TB [9/15], confirmed TB [6/15]) and a further 3/175 had unclassifiable outcome ² For 22/235 participants who provided more than one “repeat” sample (all Xpert-negative) only the result of the first sample and data from the associated review visit were used in the analysis ³ All had negative “repeat” Xpert. ⁴ CXR features compatible with “Probable TB” (pleural effusion [4], cavitation and infiltrates [2], miliary TB [1] who had positive repeat Xpert); CXR features compatible with “Possible TB” (2); USS features compatible with TB (Pericardial effusion [1]; abdominal TB [1]). ⁵ Xpert undertaken during study follow-up as part of routine clinical care or for research purposes in accordance with XPHACTOR study algorithm (Supplementary material, Figure 1). ⁶ Samples collected during study follow-up as part of routine clinical care or for research purposes (sputum Mtb culture-positive [3] of whom one had positive repeat Xpert, blood Mtb culture-positive [1]). ⁷ Two identified as having started TB treatment at verbal autopsy. One had *M. xenopi* identified in sputum culture prior to commencement of empiric TB treatment. ⁸ Started on basis of compatible symptoms and positive sputum culture later identified as *M. avium* (1) and *M. intracellulare* (1); both had improvement in symptoms after treatment was initiated.

(23%, 52/227) or weight loss (19%, 43/227) (Table 1). Amongst the remaining 149/227 (66%) of participants who reported no WHO-tool symptoms at attendance for Xpert result, sputum was collected for repeat Xpert due to *a priori* high risk of active TB because newly-diagnosed HIV-positive (42); pre-ART with CD4 count <200 cells/mm³ (42); CD4 count <100 cells/mm³ (33); on ART with CD4 count 100–199 cells/mm³ (17); BMI <18.5 kg/m² or weight loss ≥10%, (15). Enrolment to the repeat Xpert study was at median 7 days (IQR 7,8) from collection of the initial sputum sample for Xpert.

Tuberculosis diagnoses

12% (28/227) of substudy participants fulfilled case definitions for TB, of which 16 were confirmed and 12 were clinical (Table 2). One participant died before TB treatment could be commenced, and for one, the treatment start date was unknown. The remaining 26 started TB treatment at a median 49 days (IQR 0,108) after substudy entry. The range for time from substudy entry to earliest of positive TB investigation (including

chest radiograph) or date TB treatment was started (amongst all fulfilling our case definitions for TB) was 0–118 days. 24% (19/78) participants who were WHO tool positive when the sample for stored repeat Xpert was collected fulfilled TB case definitions (confirmed 11, clinical 8).

Basis for commencement of TB treatment (n=27). Thirteen participants started treatment based on positive mycobacteriology. Amongst these, eleven started treatment because of a bacteriologically-confirmed TB result (Xpert [7, of which one was the stored repeat Xpert sample]; *Mtb* isolated from sputum [3] or blood [1]). A further two participants started treatment because of compatible symptoms and positive sputum mycobacterial culture (later identified as non-tuberculous mycobacteria [NTM]) with symptomatic improvement on standard TB treatment (Figure 1).

Eleven participants started TB treatment because of compatible imaging. Nine had compatible chest radiographs, of whom

Table 1. Characteristics of substudy participants (n=227) vs. eligible non-productive of sputum (n=175).

Characteristic	Study participants (N=227)	Did not provide sputum for "repeat" Xpert (N=175)
	N (%)	N (%)
Demographics		
Age, years - Median (IQR)	37 (31-44) (N=226)	36 (30-43)
Female	144 (63.4%)	109 (62.3%)
Black African	222 (97.8%)	175 (100%)
Participant category		
On ART	99 (43.6%)	67 (38.3%)
Pre-ART	75 (33.0%)	60 (34.3%)
HTC	53 (23.4%)	48 (27.4%)
HIV/TB history		
Previous TB treatment	59 (26.0%)	37 (21.1%)
Ever had IPT	18 (7.9%)	6 (3.4%)
Ever had CPT	122 (53.7%)	84 (48.0%)
BMI/CD4 when immediate Xpert was requested		
BMI, kg/m ² - Median (IQR)	23.3 (20.1-27.4) (N=226)	23.4 (20.1-28.1)
CD4 ¹ , cells/mm ⁴ - Median (IQR)	100 (51-147) (N=188)	113 (56-169) (N=148)
WHO tool symptoms when sample for "repeat" Xpert was requested		
WHO-positive	78 (34.4%)	44 (25.1%)
Cough	52 (22.9%)	23 (13.1%)
Weight loss	43 (18.9%)	32 (18.3%)
Night sweats	16 (7.0%)	9 (5.1%)
Fever	7 (3.1%)	2 (1.1%)
TB diagnoses over 6 months follow-up		
Total	28 (12.3%)	18 (10.3%)
Confirmed TB	16 (7.1%)	7 (4.0%)
Clinical TB	12 (5.3%)	11 (6.3%)
Follow-up		
Time from XPHACTOR enrolment to 3-month¹ study visit, days - Median (IQR)	84 (84,95) (N=220)	86 (84,106) (N=169)

IPT= Isoniazid preventive therapy; BMI = body mass index; CPT= Cotrimoxazole preventive therapy; HTC= Enrolled from HIV testing and counselling service; WHO positive = self-report of any of current cough, fever, night sweats or unintentional weight loss.

¹ Most recent clinic CD4 cell count when participant attended for Xpert result review. CD4 available for 188/227 participants enrolled (99/99 on ART, 75/75 pre-ART, 14/54 HTC); and 148/175 who did not provide sputum for repeat Xpert" (67/67 on ART, 60/60 pre-ART, 21/48 HTC)

Table 2. Basis for TB diagnoses in repeat Xpert substudy (N=28).

Characteristic	Participants diagnosed with TB N=28 N (%)
Case definition	
Bacteriologically confirmed TB:	16 (57%)
Sputum Xpert positive ¹	6 (21%)
Sputum <i>Mtb</i> culture positive	4 (14%)
Sputum both Xpert and <i>Mtb</i> culture-positive	4 (14%)
Blood <i>Mtb</i> culture-positive	1 (4%)
Pleural fluid cultured isolate LPA-positive	1 (4%)
Clinical TB:	12 (43%)
Site of TB	
Pulmonary TB only	18 (64%)
Extrapulmonary TB only ²	5 (18%)
Both pulmonary and extrapulmonary TB ³	2 (7%)
Not recorded	3 (11%)

LPA = line probe assay

¹ Includes two participants for whom bacteriological confirmation was provided by stored sputum which was Xpert-positive; one of whom started treatment based on this result, and the other had already started TB treatment because of compatible chest radiograph (miliary TB).

² Pleural effusion (3); positive mycobacterial blood culture (1); pericardial effusion (1)

³ Compatible abdominal ultrasound and sputum *Mtb* culture-positive (1), pleural effusion and sputum Xpert positive (1)

four were subsequently bacteriologically confirmed (Xpert 2, pleural fluid cultured isolate LPA-positive 1, positive *Mtb* sputum culture 1). Two participants started treatment based on ultrasound scans, one compatible with abdominal TB (subsequently confirmed by positive *Mtb* from sputum culture); and the other showing pericardial effusion. The basis for starting TB treatment was not clear for the remaining three participants (Figure 1).

Diagnoses made by repeat Xpert on stored sputum samples. 5/227 (2.2%) had a positive repeat Xpert. The sensitivity of repeat Xpert was 31.3% (5/16; 95% CI 11.0-58.7%) for the primary outcome of confirmed TB and 18% (5/28, 95% CI: 6.1%-36.9%) for the secondary outcome of confirmed / clinical TB combined (Figure 2). In a matched analysis the odds of TB diagnosis was much greater by other modalities during follow-up than by the repeat Xpert, odds ratio 24.0 (95% CI: 3.9-986.9; $p < 0.0001$, McNemar's test). Amongst the five participants with positive repeat Xpert, three were in the pre-ART group, and two in the on ART group. We were unable to undertake multivariable analysis to look at independent predictors of positive repeat Xpert on the stored sample because only five were positive.

In a sensitivity analysis restricted to 123 participants who had at least one component of the Xpert-negative algorithm within a two-week window of providing the stored repeat Xpert sample, 23 participants fulfilled our TB case definitions (13/23 confirmed, 10/23 clinical). The stored sputum sample was positive by Xpert for four participants (sensitivity of repeat Xpert for confirmed and clinical TB combined 17% [4/23]; for sputum culture-confirmed TB 20% [1/5]). Ten participants started TB treatment because of evaluation by the Xpert-negative algorithm (four confirmed, six clinical), of whom two also had positive stored repeat Xpert. Eleven other participants fulfilled TB case definitions during study follow-up (eight confirmed, three clinical). We did a matched analysis, classifying as "not TB" for the purpose of this analysis, 11 participants who fulfilled our TB case definitions but were not identified by either the Xpert-negative algorithm or stored repeat Xpert. The odds of TB diagnosis by the Xpert-negative algorithm was greater than by repeat Xpert, but did not attain statistical significance, odds ratio 4.0 (95% CI: 0.8-38.7; $p = 0.11$, McNemar's test).

The participant who started TB treatment solely based on the stored repeat Xpert sample was in the pre-ART group with a CD4 cell count of 113 cells/mm³ at substudy enrolment,

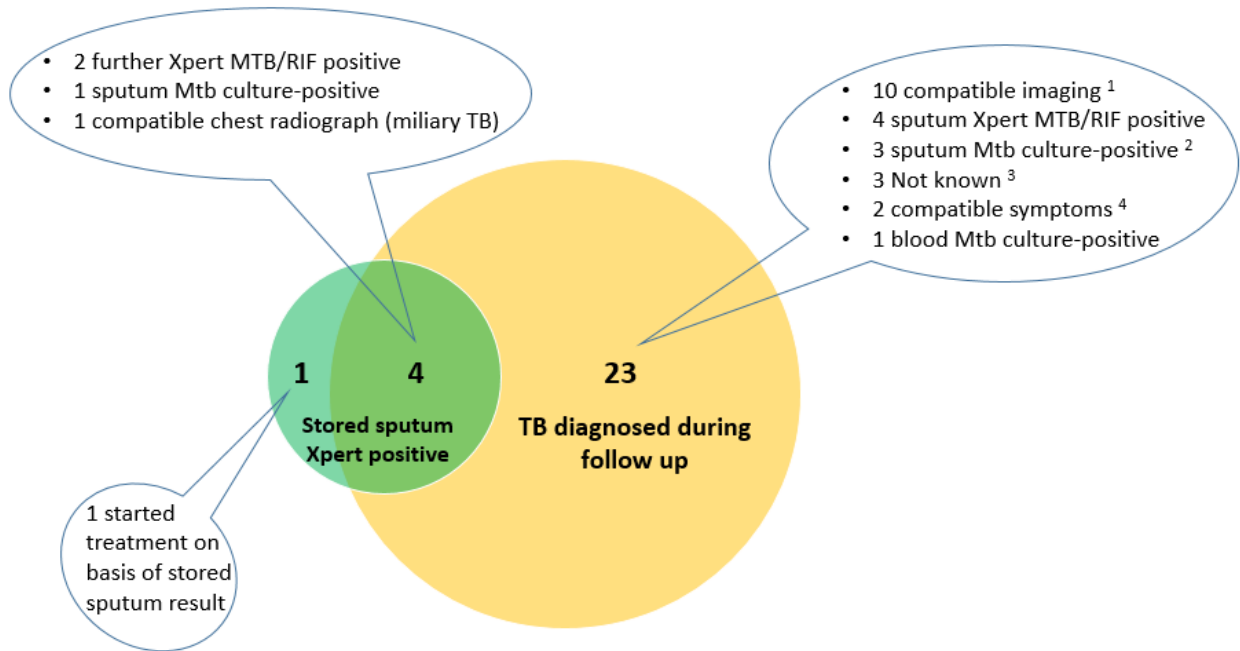


Figure 2. Number of participants diagnosed with TB by “repeat” Xpert vs. number diagnosed during follow-up (N=28). CXR = chest radiograph; USS = ultrasound scan ¹ CXR features compatible with “Probable TB” (pleural effusion [4], cavitation and infiltrates [2]); CXR features compatible with “Possible TB” (2); USS features compatible with TB (Pericardial effusion [1]; abdominal TB [1]) ² One participant died before treatment commenced ³ Two identified as having started TB treatment at verbal autopsy. One had *M. xenopi* identified in sputum culture prior to commencement of empiric TB treatment. ⁴ Started on basis of compatible symptoms and positive sputum culture later identified as *M. avium* (1) and *M. intracellulare* (1); both had improvement in symptoms after treatment was initiated.

had no previous history of TB treatment, and had a five week history of cough and fever when the initial sputum sample for Xpert was collected. The sputum culture, the only component of the Xpert-negative algorithm arranged at the Xpert review visit, was contaminated. This participant initiated ART on the day of entry to the substudy, was WHO-tool negative at all subsequent study visits, and had negative sputum and blood for mycobacterial culture at the 3-month visit. The remaining four participants with positive stored repeat Xpert sample started TB treatment before the stored sample was processed, based on further evaluation during follow-up: sputum Xpert-positive (2, one with rifampicin resistance); sputum *Mtb* culture-positive (1); and compatible chest radiograph (1).

Investigations performed for participants during substudy follow-up

Figure 2 in the supplementary material summarises all investigations undertaken for TB during substudy follow-up, aside from 3-month visit mycobacterial cultures and Xpert on stored sputum samples. As part of routine care or facilitated by research staff for the Xpert-negative algorithm, 97/227 (43%) had a chest radiograph (38/97 [39%] fulfilled criteria for radiological TB), and 100/227 (44%) had mycobacterial culture on sputum (3/100 [3%] *Mtb* positive). 34/227 (15%) of participants were prescribed an antibiotic trial at the Xpert result review, and 14/21 (67%) of those reviewed reported resolution of symptoms.

89 participants submitted sputum specimens for Xpert as part of routine care or because they fulfilled XPHACTOR algorithm criteria at monthly follow-up visits, for whom 6/89 (7%) were positive. An additional four participants had positive Xpert, of which three were stored sputum samples for repeat Xpert (bacterial confirmation provided solely by stored sample [2], also positive *Mtb* sputum culture [1]), and one was collected after the 3-month visit (Table 2).

The mycobacterial cultures performed routinely at the 3-month visit yielded *Mtb* isolates in 2% (5/219) of sputum and 0/220 blood samples.

Discussion

Among HIV-positive individuals at high risk of active TB, with a negative sputum Xpert result, very few TB diagnoses would have been made in this study by immediately repeating Xpert. We limited our study to HIV-positive individuals at highest risk of active TB, i.e. those who were WHO tool positive with CD4<200x10⁶/l, or pre-ART with CD4<200x10⁶/l, or newly diagnosed, of whom some were asymptomatic, to minimise unnecessary testing of individuals at lower risk of active TB. The low yield from repeat Xpert in those with negative initial Xpert is likely due to paucibacillary or subclinical disease which is more likely in asymptomatic individuals, or extrapulmonary disease. These TB diagnoses may be better identified by alternative diagnostic modalities, such as chest

radiography. A South African study of patients with sputum screened for TB by Xpert and mycobacterial culture prior to ART initiation, using a gold standard of culture-confirmed TB (N=85), found that those who were Xpert-negative had higher CD4 cell counts and lower viral loads than those who were Xpert-positive¹³. We did not have enough positive stored Xpert results to undertake a similar analysis.

Our study illustrates the realities of implementing the test negative algorithm in HIV-positive individuals. Despite research staff facilitating the algorithm, less than half (100/227) of participants produced sputum for mycobacterial culture during follow-up (vs. 100% assumed by Schnippel)⁷. We found sensitivity of the repeat Xpert was only 18% (5/28) for all TB or 31% (5/16) for bacteriologically-confirmed TB vs. 79% assumed by Schnippel^{6,7}. Data from South Africa demonstrate poor adherence in routine care settings to TB diagnostic algorithms amongst HIV-positive individuals with initial negative Xpert test^{14,15}. The aforementioned model^{6,7} assumes 1% of patients with TB symptoms start TB treatment based on a clinical diagnosis, but we found this to be far greater; and the model does not consider extrapulmonary TB (one-fifth of our participants diagnosed with TB had only extrapulmonary disease). An economic evaluation of repeat sputum Xpert vs. the Xpert-negative algorithm for HIV-positive individuals using assumptions that are more realistic is needed.

Evaluation of the 2007 WHO algorithm for smear-negative TB (comprising chest radiograph, single sputum for mycobacterial culture, and antibiotic trial), in HIV-positive individuals being investigated for TB in Cambodia¹⁶, against a gold standard of culture-confirmed TB based on multiple specimens, demonstrated sensitivity of 60%¹⁶. Sensitivity of this algorithm is imperfect, and there is a risk of overtreatment when only clinical-radiological features are used to start TB treatment. 40% (11/27) of our study participants who started TB treatment did so because of compatible imaging, of whom almost half were subsequently bacteriologically confirmed, highlighting its value to support rapid initiation of TB treatment. Our findings are in accord with data from the XTEND trial, which found that compatible chest radiograph was the main reason for initiating empiric TB treatment in a cohort of patients investigated for TB in primary care in South Africa, amongst whom microbiological confirmation was subsequently obtained for 13%¹⁷. South African national guidelines now recommend chest radiography for all individuals with symptoms suggestive of TB who cannot produce a sputum sample, but limited access to radiography facilities may limit implementation⁴. Amongst our study participants who provided sputum for mycobacterial culture prior to their 3-month visit there was a low yield of *Mtb* (3/100 [3%]), and the yield from further Xpert during follow-up was 7% (6/89), representing just over half (9/16) of all confirmed TB diagnoses. Our findings highlight the need for more sensitive diagnostic tests, and for repeating TB investigation using all available modalities, in HIV-positive individuals with initial negative sputum test result who remain symptomatic or have advanced immunosuppression.

Our study has some limitations. In the parent XPHACTOR study, Xpert testing was prioritised in people with BMI<18.5kg/m² or CD4<100, those newly diagnosed HIV-positive or pre-ART with CD4<200, as well as those with TB symptoms⁸. Thus the population in this substudy did not all have classic “TB symptoms” at the time of collection of either initial or repeat sputum samples for testing with Xpert. These individuals may have had paucibacillary or subclinical disease which would not have been detected by Xpert. However, TB prevalence in our substudy population was high, and we anticipate our results to be relevant at least to these high-risk groups. Our results are also relevant to efforts to improve TB case finding among asymptomatic HIV-positive people^{18,19}. We froze all our raw sputum samples within 24 hours of collection, and all were thawed and tested within 6 months of collection, in line with other studies²⁰. We assumed that all participants starting TB treatment or with a sample which was bacteriologically confirmed collected within six months of enrolment were likely to have had active TB at enrolment, regardless of whether it was diagnosable using sputum based tests at the time of enrolment. In fact, our study participants who started TB treatment commenced within a median of seven weeks from collection of the “repeat” Xpert sample. Some sputum samples for mycobacterial culture and chest radiographs were taken at an interval after participants returned for their initial Xpert test result, reflecting real-life investigation practice; we cannot be certain of the same result if they had been performed at the same time as sample collection for repeat Xpert. However, our findings suggest that following the Xpert-negative algorithm is more likely to lead to TB diagnosis than immediate repeat Xpert test.

Strengths of our study include systematic evaluation of participants and longitudinal follow-up which minimised the number of TB diagnoses missed, and the pragmatic nature of the study which reflected as far as possible real-life conditions, albeit with optimised implementation of TB diagnostic algorithms.

Conclusions

Amongst ambulatory HIV-positive individuals at high risk of active TB, if an initial Xpert is negative, the Xpert-negative pathway should be implemented and there should be a low threshold for investigating those who remain at high risk using all clinically appropriate diagnostic modalities. In addition, those for whom no TB diagnosis is made must be made aware of the importance of returning for review if symptoms persist or recur. Our findings do not support sending an immediate repeat Xpert and highlight the need for more sensitive diagnostic tests capable of detecting pulmonary and extrapulmonary TB.

Data availability

The XPHACTOR “Investigating TB if initial Xpert is negative” dataset, which includes data underlying this substudy, has been uploaded to the LSHTM Data Compass repository: <https://doi.org/10.17037/DATA.28421>.

The reader will need to request the dataset from LSHTM (request access is provided within the data record) with a brief

summary of how the dataset will be utilised. On request, a data sharing agreement will be made available which will first need to be signed, prior to provision of the dataset. This enables LSHTM to confirm that the reader is using the data for HIV or TB-related research, which is required because study participants consented to use of their data for HIV or TB-related research only.

The data is shared under a Data Sharing Agreement license (see above).

The study team wish to avoid unnecessary barriers to access and will seek to respond to data requests as quickly as possible.

Acknowledgements

We thank the study participants; the nursing and medical staff of Chris Hani Baragwanath and Mamelodi hospitals, Ramakokoni and Jabulani Dumane community health clinics, South Africa; the staff of National Health Laboratory Services, South Africa; and the staff of Aurum Institute for their essential contributions to this study.

Supplementary material

Supplementary material: XPHACTOR study flow, procedures and algorithm.

[Click here to access the data.](#)

References

- World Health Organization: **Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update.** WHO Guidelines Approved by the Guidelines Review Committee. Geneva 2013. [PubMed Abstract](#)
- Samb B, Sow PS, Kony S, *et al.*: **Risk factors for negative sputum acid-fast bacilli smears in pulmonary tuberculosis: results from Dakar, Senegal, a city with low HIV seroprevalence.** *Int J Tuberc Lung Dis.* 1999; **3**(4): 330–6. [PubMed Abstract](#)
- Steingart KR, Schiller I, Horne DJ, *et al.*: **Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults.** *Cochrane Database Syst Rev.* 2014; **2014**(1): CD009593. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Department of Health - Republic of South Africa: **National Tuberculosis Management Guidelines 2014.** [2nd April 2015]. [Reference Source](#)
- Lawn SD, Brooks SV, Kranzer K, *et al.*: **Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study.** *PLoS Med.* 2011; **8**(7): e1001067. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Meyer-Rath G, Schnippel K, Long L, *et al.*: **The impact and cost of scaling up GeneXpert MTB/RIF in South Africa.** *PLoS One.* 2012; **7**(5): e36966. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Schnippel K, Meyer-Rath G, Long L, *et al.*: **Diagnosing Xpert MTB/RIF negative TB: impact and cost of alternative algorithms for South Africa.** *S Afr Med J.* 2013; **103**(2): 101–6. [PubMed Abstract](#) | [Publisher Full Text](#)
- Hanifa Y, Fielding KL, Chihota VN, *et al.*: **A clinical scoring system to prioritise investigation for tuberculosis among adults attending HIV clinics in South Africa.** *PLoS One.* 2017; **12**(8): e0181519. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dheda K, Barry CE 3rd, Maartens G: **Tuberculosis.** *Lancet.* 2016; **387**(10024): 1211–26. [PubMed Abstract](#) | [Publisher Full Text](#)
- Cobelens F, Kik S, Esmail H, *et al.*: **From latent to patent: rethinking prediction of tuberculosis.** *Lancet Respir Med.* 2017; **5**(4): 243–4. [PubMed Abstract](#) | [Publisher Full Text](#)
- Corbett EL, Bandason T, Cheung YB, *et al.*: **Prevalent infectious tuberculosis in Harare, Zimbabwe: burden, risk factors and implications for control.** *Int J Tuberc Lung Dis.* 2009; **13**(10): 1231–7. [PubMed Abstract](#) | [Free Full Text](#)
- World Health Organization: **Definitions and reporting framework for tuberculosis – 2013 revision.** (updated December 2014) 2013; [20th April 2015]. [Reference Source](#)
- Lawn SD, Kerkhoff AD, Vogt M, *et al.*: **Characteristics and early outcomes of patients with Xpert MTB/RIF-negative pulmonary tuberculosis diagnosed during screening before antiretroviral therapy.** *Clin Infect Dis.* 2012; **54**(8): 1071–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- McCarthy KM, Grant AD, Chihota V, *et al.*: **Implementation and Operational Research: What Happens After a Negative Test for Tuberculosis? Evaluating Adherence to TB Diagnostic Algorithms in South African Primary Health Clinics.** *J Acquir Immune Defic Syndr.* 2016; **71**(5): e119–26. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Naidoo P, Dunbar R, Lombard C, *et al.*: **Comparing Tuberculosis Diagnostic Yield in Smear/Culture and Xpert® MTB/RIF-Based Algorithms Using a Non-Randomised Stepped-Wedge Design.** *PLoS One.* 2016; **11**(3): e0150487. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Koole O, Thai S, Khun KE, *et al.*: **Evaluation of the 2007 WHO guideline to improve the diagnosis of tuberculosis in ambulatory HIV-positive adults.** *PLoS One.* 2011; **6**(4): e18502. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- McCarthy K, Fielding K, Churchyard GJ, *et al.*: **Empiric tuberculosis treatment in South African primary health care facilities - for whom, where, when and why: Implications for the development of tuberculosis diagnostic tests.** *PLoS One.* 2018; **13**(1): e0191608. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hamada Y, Lujan J, Schenkel K, *et al.*: **Sensitivity and specificity of WHO's recommended four-symptom screening rule for tuberculosis in people living with HIV: a systematic review and meta-analysis.** *Lancet HIV.* 2018; **5**(9): e515–e523. [PubMed Abstract](#) | [Publisher Full Text](#)
- World Health Organization: **WHO consolidated guidelines on tuberculosis Module 2: Screening – Systematic screening for tuberculosis disease.** 2021; (accessed May 15th, 2021). [Reference Source](#)
- Theron G, Zijenah L, Chanda D, *et al.*: **Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial.** *Lancet.* 2014; **383**(9915): 424–35. [PubMed Abstract](#) | [Publisher Full Text](#)
- Hanifa Y, Fielding K, Grant AD, *et al.*: **Data for: "The utility of repeat Xpert MTB/RIF testing to diagnose tuberculosis in HIV-positive adults with initial negative result".** [Online]. *London School of Hygiene & Tropical Medicine, London, United Kingdom.* 2016. <http://www.doi.org/10.17037/DATA.284>

Open Peer Review

Current Peer Review Status: ? ✓

Version 1

Reviewer Report 01 June 2018

<https://doi.org/10.21956/gatesopenres.13882.r26494>

© 2018 Lim T. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Tow Keang Lim

Division of Respiratory and Critical Care Medicine, National University Hospital, Singapore, Singapore

This study examined the sensitivity of repeating an expectorated sputum specimen for Xpert testing for TB in a group of high risk immuno-compromised patients who had returned negative tests initially. The repeat Xpert test had lower than expected sensitivity (~20%) and contributed very little to treatment timeliness. However the overall incidence of TB was low and it was mostly pauci-bacillary disease. These results may not be applicable to patients with higher disease burden or for induced sputa.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 31 May 2018

<https://doi.org/10.21956/gatesopenres.13882.r26447>

© 2018 Hanrahan C. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Colleen F. Hanrahan

Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

Overall

- This study provides some interesting data on how useful a 2nd Xpert is to follow-up on those initially negative by Xpert. The addition of LAM to this study needs to be clearly and explicitly explained. Currently it reads as a very extraneous add-on.
- There are too many tables/figures for the amount of actual data. I find the presentation of data currently to be excessive and unnecessarily complicated and duplicative.

Introduction:

- Aim is awkwardly stated. Possibly reword with study population at the end, and try to make it more succinct.
- LAM needs to be included as an aim from the start. Otherwise it feels like a very tangential add on.

Methods:

- I would like a formal definition of the primary outcome and any secondary outcomes. They are probably in there, but not explicitly stated enough.
- I think a flow diagram of testing would be helpful to flesh out the methods.
- It's not clear to me where LAM fits in. An Xpert negative pt is re-screened for symptoms, has a chest x-ray and sputum for culture, and/or antibiotic trial is given. But then suddenly under statistical methods, you mention LAM for the first time. LAM is not part of the SA algorithm. We need to know when this was done, and even why it is relevant to this study.

Results:

- Not sure why 1 person who started TB treatment >6 months after enrollment would be excluded? This gets back to my previous point that you need to explicitly state the primary outcome, so we can understand why you would have excluded these 7 individuals.
- I'm confused by figure 1. What I'd like to know is what is the additional yield of Xpert, but I

can't tell that from this figure. The way it looks is that all the people who had confirmed TB were not diagnosed with clinical TB. However, I can't believe that is true. I would prefer to see who was diagnosed how up front (clinical, microbiological) and who wasn't ("not tb"). Then from those boxes, show us how many in each were Xpert positive from the 2nd sputum.

- I'm confused by the section on TB diagnosis. Those diagnosed by Xpert don't appear to be by the 2nd Xpert that was taken and stored, because lower down you detail those individuals. But a 2nd Xpert is not part of the SA algorithm. Something needs to be done to make this clearer.
- Table 2 confuses me for the same reason that figure 1 confuses me.
- If you change figure 1 as suggested above, then you don't need a venn diagram. That diagram conveys very little information.
- Figure 3 is totally confusing because people can be in more than one of the top boxes...I'm not sure what this adds, particularly how it speaks to your primary outcome.

Discussion:

- Although the authors note that their population was not limited to symptomatic individuals as a potential weakness, they do not explicitly state that an asymptomatic population is likely to have paucibacillary or subclinical disease, for which Xpert would not have good sensitivity. The SA algorithm for TB investigation starts with a positive symptom screen, not merely for being HIV positive with a low CD4 despite having no symptoms. So these findings are not necessarily generalizable to actual practice.
- I would also note that the prevalence of clinical diagnosis is not necessarily generalizable. It's unclear the level of care that was given to these participants- was this routine care available in a primary public health clinic, or is this specialized care as part of a research study? These details could help the reader evaluate how generalizable the findings are.

Conclusions:

- The reference to the "Xpert negative pathway" is vague - authors should be specific about what kinds of followup are most useful. Authors should also include something about LAM.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
