Impact of point-of-care Xpert MTB/RIF on tuberculosis treatment initiation: a cluster randomised trial

Richard J Lessells [1,2], Graham S Cooke [3], Nuala McGrath [2,4], Mark P Nicol [5,6], Marie-Louise Newell [7], Peter Godfrey-Faussett [1]

[1] Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, UK

[2] Africa Health Research Institute, Somkhele, South Africa

[3] Division of Infectious Diseases, Imperial College London, London, UK

[4] Academic Unit of Primary Care and Population Sciences and Department of Social Statistics and Demography, University of Southampton, Southampton, UK

[5] Division of Medical Microbiology and Institute for Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa

[6] National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa

[7] Human Health and Development & Global Health Research Institute, Faculty of Medicine, University of Southampton, Southampton, UK

Correspondence to:

Dr Richard J Lessells

Department of Clinical Research

London School of Hygiene and Tropical Medicine

Keppel Street

London

WC1E 7HT

UK

+44 (0)20 7636 8636

richard.lessells@lshtm.ac.uk

Author contributions: Conception and design: RJL, GSC, NM, MPN, MLN, PGF; Randomisation and cluster allocation: NM; Acquisition of data: RJL; Preparation of statistical analysis plan and data analysis: RJL, NM; Interpretation of data: RJL, GSC, NM, MPN, MLN, PGF; Drafting the manuscript: RJL; Revising the manuscript for important intellectual input: GSC, NM, MPN, MLN, PGF; Approval of final version of the manuscript: RJL, GSC, NM, MPN, MLN, PGF.

Funding: This work was supported by the Wellcome Trust (grant number 090999/Z/09/Z, www.wellcome.ac.uk). GSC was supported by the BRC of Imperial College NHS Trust and the NIHR POC diagnostics DEC. NM was supported by the Wellcome Trust (grant number WT083495MA). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Running head: Point-of-care Xpert for TB diagnosis

Descriptor: 11.1 Diagnosis of Tuberculosis or Latent Infection

Word count: 3988 words

At a Glance Commentary

Scientific Knowledge on the Subject

Centralised laboratory-based diagnostic systems for tuberculosis are associated with substantial loss to follow-up and delays prior to treatment. Whether decentralised, point-of-care diagnostic systems can reduce loss to follow-up and treatment delay has not been adequately investigated.

What This Study Adds to the Field

This is the first randomised trial to make a direct comparison between point-of-care and laboratory use of a molecular TB diagnostic. The point-of-care strategy shortened the time to appropriate treatment for people with rifampicin-susceptible TB; three-quarters of Xpert-positive/rifampicin-susceptible cases received same-day diagnosis and treatment. Under both strategies, there were delays for people with drug-resistant TB and people with Xpert-negative/culture-positive TB, highlighting the need for more sensitive rapid diagnostics and further strengthening of health and laboratory systems.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

Abstract

Rationale: Point-of-care (POC) diagnostics have potential to reduce pre-treatment loss to follow-up and delays to initiation of appropriate TB treatment.

Objective: To evaluate the effect of a POC diagnostic strategy on initiation of appropriate TB treatment.

Methods: A cluster randomised trial of adults with cough who were HIV positive and/or at high risk of drug-resistant TB. Two-week time blocks were randomised to two strategies (i) Xpert performed at district hospital laboratory (ii) POC Xpert performed at primary health care clinic. All participants provided two sputum specimens: one for Xpert and the other for culture as reference standard. The primary outcome was the proportion of culture-positive pulmonary TB (PTB) cases initiated on appropriate TB treatment within 30 days.

Measurements and Main Results: Between August 22, 2011 and March 1, 2013, 36 two-week blocks were randomised and 1297 individuals were enrolled (646 in the laboratory arm, 651 in the POC arm); 159 (12.4%) had culture-positive PTB. The proportion of culture-positive PTB cases initiated on appropriate TB treatment within 30 days was 76.5% in the laboratory arm and 79·5% in the POC arm (odds ratio 1·13, 95% confidence interval [CI] 0·51-2.53, p = 0·76; risk difference 3.1%, 95% CI -16.2, 10.1). The median time to initiation of appropriate treatment was 7 days (laboratory) vs. 1 day (POC).

Conclusions: POC positioning of Xpert led to more rapid initiation of appropriate TB treatment. Achieving one-stop diagnosis and treatment for all people with TB will require simpler, more sensitive diagnostics and broader strengthening of health systems.

250 words

Keywords: Tuberculosis, drug-resistant tuberculosis, molecular diagnostics, point-of-care systems, clinical trial

Introduction

Tuberculosis (TB) remains one of the most important causes of global mortality, causing around 5000 deaths every day (1). In sub-Saharan Africa, the human immunodeficiency virus (HIV) epidemic and the spread of drug-resistant TB (DR-TB) contributed to a failure to achieve targets for reduction in TB prevalence and mortality in the United Nations Millennium Development Goals (MDG) (2). Timely detection and treatment of adult pulmonary TB cases is important, not only to limit individual morbidity and mortality but also to interrupt transmission. Centralised laboratory-based TB diagnostic systems are associated with substantial loss to follow-up and delays prior to treatment (3, 4). While diagnostics with improved sensitivity for detecting TB disease could have substantial clinical and public health impact, additional benefit might be achieved by positioning diagnostics at more peripheral levels of the health system (5, 6), however, there is little high quality evidence as to whether implementation of diagnostics at the point of care (POC) improves patient-relevant outcomes.

This evidence is important to inform the scale-up of existing technologies and to guide the development of new diagnostics (7, 8). The aim of this trial was to determine whether a diagnostic strategy involving a rapid molecular test positioned at a rural primary health care (PHC) clinic would reduce delays and loss to follow-up prior to TB treatment, compared to a strategy with centralised laboratory testing.

Methods

Trial design

The study was a cluster randomised trial of adults with possible pulmonary TB and DR-TB, evaluating the impact of Xpert MTB/RIF positioning on the initiation of appropriate TB

treatment (9). The unit of randomisation was a time period (two-week block), with each time period randomised either to a strategy with the Xpert MTB/RIF system placed in a centralised sub-district level laboratory (laboratory strategy) or at the clinic (POC strategy). A cluster represented the group of participants enrolled during the two-week block. The unit of observation was the individual participant.

The trial was conducted in Hlabisa health sub-district, uMkhanyakude district, northern KwaZulu-Natal, South Africa; a predominantly rural area with a high burden of TB, DR-TB and HIV. In 2011 the TB notification rate for the sub-district was 1050 per 100 000 and HIV seroprevalence was 29% in the adult population aged 15-49 years (10). HIV and TB services are provided at 17 PHC clinics and one district hospital through decentralised collaborative programmes. Participants were recruited from the largest PHC clinic, situated approximately 55km by road from the district hospital.

The trial was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (ref. BF033/11), the Ethics Committee of the London School of Hygiene and Tropical Medicine (ref. 5926), and the Health Research Committee of the KwaZulu-Natal Department of Health (ref. 084/11). The trial was registered with Current Controlled Trials on 17 June 2011 (ISRCTN 18642314) and with the South African National Clinical Trials Register on 10 July 2011 (DOH-27-0711-3568).

Participants

Adults (≥18 years) with possible pulmonary TB (defined as cough of any duration) were recruited at the clinic if they were HIV positive and/or had a high risk of DR-TB. These two groups were specified based on their high risk for mortality and prioritisation for Xpert

MTB/RIF testing at the time of the study (11). High risk of DR-TB was defined as per World Health Organization (WHO) case finding recommendations and South African national TB guidelines: failure of standard treatment regimen or retreatment regimen, smear non-conversion at month 2 or 3 of standard treatment regimen or retreatment regimen, relapse or return after loss to follow-up, any other previous TB treatment, household exposure to known multidrug-resistant (MDR) or extensively drug-resistant (XDR)-TB case, health care worker, or prison inmate in previous 12 months (12, 13). Individuals were excluded if they had a previous diagnosis of MDR- or XDR-TB, were severely unwell requiring immediate admission to hospital, or were unable to give informed consent. In the event of enrolment of a participant on more than one occasion, only the data from the first enrolment was included in analysis.

Procedures

Potential participants were identified by clinic staff and referred to a research nurse for assessment. Individuals who were eligible for the study were given information about the study in the local language (isiZulu) and consent was indicated by signature or thumbprint. Clinical and demographic information was collected at enrolment by the research nurse. Two spontaneously expectorated sputum specimens were collected at the study site by the research nurse (the first for Xpert MTB/RIF and the second for culture). In both strategies, the nurse instructed participants to wait one hour between producing the first and the second specimen. Under the POC strategy, participants were advised to wait for their result or, if not possible, to return the next day. Under the laboratory strategy, participants were requested to return to the clinic for results after three working days, based on the typical turnaround time for receipt of sputum results at the time of the study.

A four-module GeneXpert system (Cepheid, Sunnyvale, CA) was installed for each two-week time period at either the district hospital laboratory or the clinic according to the randomisation schedule. In the laboratory strategy, both sputum specimens were transported daily to the National Health Laboratory Service (NHLS) laboratory at the district hospital using the routine specimen transport system. Xpert MTB/RIF testing was performed by a laboratory technician and results were returned to the clinic using the routine transport system. For POC blocks, Xpert MTB/RIF was performed on site by the research nurse in a dedicated room (N95 respirator masks were used but no biosafety cabinet). If no valid result was obtained from the first test and there was sufficient sputum-buffer mix remaining, the test was repeated. If there was insufficient sputum-buffer mix or still no valid result, an additional sputum specimen was obtained from the participant at the earliest opportunity.

Sputum specimens for culture were forwarded from the clinic via the routine specimen transport system to the district hospital laboratory and onwards the following day to the provincial reference laboratory in Durban. Mycobacterial growth indicator tubes (MGIT) were inoculated and incubated at 37°C for up to six weeks. Positive cultures were identified as *M. tuberculosis* complex using routine tests. The Genotype MTBDR*plus* assay was performed indirectly on culture isolates to identify mutations associated with rifampicin and isoniazid resistance. For isolates demonstrating rifampicin and/or isoniazid resistance, phenotypic drug susceptibility testing (DST) for rifampicin, isoniazid, ofloxacin, and kanamycin was performed.

Clinical management followed standardised diagnostic and treatment algorithms (see Figures E1-E2 in the online data supplement). The research nurse worked in parallel with the TB nurses at the clinic but in a separate room; the research nurse coordinated further

management for trial participants following routine clinic practice (see further details in online data supplement). X-ray facilities were not available at the clinic, only at the district hospital. A medical officer was present at the clinic one day per week but any adults that required further evaluation for TB were referred to the district hospital. Throughout the study period, all participants with rifampicin-resistant TB were admitted to the district hospital and referred to the provincial DR-TB unit in Durban for initiation of DR-TB treatment (further details are provided in the online data supplement) (14).

Clinic review for outcome evaluation was scheduled two months after the enrolment visit. The research nurse collected information regarding initiation of TB treatment, antiretroviral therapy (ART) and hospitalisation. Outcome evaluation was not blinded to randomisation group. If the participant did not attend clinic for follow-up evaluation, information was obtained by telephone or from clinic registers.

Outcomes

The primary outcome was the proportion of culture-positive PTB cases initiated on appropriate TB treatment within 30 days of enrolment. Appropriate treatment was defined according to the results of genotypic and phenotypic tests on the culture isolate (see Table 1 in the online data supplement). Secondary outcomes were: time to initiation of appropriate TB treatment for culture-positive pulmonary TB cases; time to initiation of appropriate DR-TB treatment (for rifampicin-resistant TB cases); all-cause mortality at 60 days; proportion of participants with at least one hospital admission within 60 days; and time to initiation of ART for HIV-positive participants.

Sample size

The study was designed to detect a 10 percentage point increase in the proportion of culture-positive PTB cases initiated on appropriate treatment within 30 days (from 85% in the laboratory arm). Sample size was calculated with the equation of Hayes and Bennett, using the coefficient of variation (κ) (15). With κ=0·05 and a cluster size of 12 culture-positive cases we needed 16 clusters and 188 culture-positive TB cases in each arm to detect this difference with 95% confidence and 80% power. We assumed 10% of individual participants would be lost to follow-up, so we needed 208 culture-positive TB cases in each arm. Based on the assumption that 25% of adults with possible pulmonary TB would have a positive culture, the study was initially planned to enrol 1664 participants.

Randomisation

The allocation schedule for random assignment of two-week blocks was computer generated, using random permuted blocks of eight. Due to extension of the trial, an extra four blocks were randomised. Allocation for each clinic block was placed into sequentially numbered, opaque, sealed envelopes; the envelope was opened on the Friday before the start of a new two-week block and the allocated strategy for the next time block was communicated to study staff.

Statistical methods

Analysis of baseline characteristics was performed to characterise the trial population and to identify any baseline imbalances between the study arms. All analyses were individual-level intention-to-treat analyses which took account of within-cluster correlation. The primary analysis excluded TB cases on treatment at the time of enrolment with a *M. tuberculosis* culture isolate susceptible to rifampicin and isoniazid, as appropriate treatment for these cases would involve continuation of the same drug regimen. Regression modelling using

generalized estimating equations (GEE) with a binomial distribution function and a logit link was applied, specifying an exchangeable working correlation matrix. Any important individual-level characteristics that were unbalanced between arms were considered in the model as covariates. For the secondary outcomes with binary variables, GEE models were also fitted with a binomial distribution function and a logit link. For the secondary outcomes with time-to-event measures, Cox proportional hazard models were used with the shared frailty option to account for clustering by time block. All times were measured from the enrolment date. The proportional hazards assumption was examined graphically using the log-log plot and using the score test based on scaled Schoenfeld residuals (16). Time-to-event data were also plotted as Kaplan-Meier survival curves and the two groups were compared using the log rank test. For the Kaplan-Meier analysis, deaths were censored at 60 days (17). All analyses were performed using Stata version 13.1 (Stata Corporation, College Station, TX).

Results

Between 22 August 2011 and 1 March 2013, 36 two-week blocks were randomised to one of the two diagnostic strategies (Figure 1). In July 2012, following the identification of a shortfall in the enrolment of culture-positive cases, the Trial Steering Committee recommended measures to optimise recruitment and to maximise the yield from sputum cultures. Despite implementation of these measures, enrolment remained below target but due to time and logistical constraints the enrolment phase could not be extended beyond March 2013. With the numbers recruited, the power of the study to detect a 10% difference in the primary endpoint was 55%.

A total of 1526 individuals were screened and 1297 enrolled in the trial (Figure 1). Data from sixteen participants were excluded from all analyses due to duplicate enrolment (n = 14) or incorrect criteria for TB drug resistance risk (n = 2), giving 1281 individuals for analysis (mean 36 per cluster, range 19-56). Altogether, 1185 (92·5%) were HIV positive and 577 (45·0%) had documented risk of DR-TB. The baseline characteristics of the individual participants were well balanced (Table 1).

Overall, 1235 participants (96·4%) submitted two sputum specimens. The proportion of initial specimens from which no Xpert MTB/RIF result was obtained was higher with the laboratory strategy than the POC strategy (7·8% vs. 1·1%, $p < 0.001$), mostly due to specimen leakage in transit (Table 2). The overall proportion of participants with a culture positive for *M. tuberculosis* was 12·9% (159/1235); this was higher in the POC arm than the laboratory arm (14·8% vs. 11·0%, $p = 0·06$) (see Table 2 in the online data supplement). Thirty-two (20·1%) *M. tuberculosis* isolates were rifampicin resistant (see Tables E3-E4 in the online data supplement). Almost one in four specimens (281/1235, 22·8%) did not yield a valid culture result: 133 (10·8%) specimens leaked in transit, 103 (8·3%) cultures were contaminated, and 46 (3·7%) had no documented result. Participants with and without a valid culture result had similar baseline characteristics (see Table E5 in the online data supplement).

Outcomes were evaluated for all 159 culture-positive cases a median of 90 days (IQR 72-153) post-enrolment. Three culture-positive cases were excluded from the primary analysis as they were on TB treatment at enrolment and the *M. tuberculosis* culture isolate was susceptible to rifampicin and isoniazid. The population for analysis therefore included 156 culture-positive PTB cases (68 in laboratory arm; 88 in POC arm). The baseline

characteristics of the culture-positive cases were well balanced (Table 3). The proportion of culture-positive PTB cases initiated on appropriate TB treatment within 30 days of enrolment was 76·5% (52/68) with the laboratory strategy and 79·5% (70/88) with the POC strategy (odds ratio (OR) 1·13, 95% confidence interval (CI) 0·51-2·53, p = 0·76; risk difference 3.1%, 95% CI -16.2, 10.1). The estimated value of the coefficient of variation (κ) was 0·11.

For Xpert-positive/culture-positive cases, 51/57 (89·5%, 95% CI 78·9-95·1) in the laboratory arm and 65/68 (95·6%, 95% CI 87·8-98·5) in the POC arm started appropriate TB treatment within 30 days (Table 4). The majority of Xpert-negative/culture-positive cases did not start appropriate treatment within 30 days (see further details in the online data supplement). Overall, 215 participants started TB treatment within 60 days, 154 (71·6%) on the basis of a positive Xpert result, 14 (6·5%) on the basis of a positive culture, and 45 on clinical or radiological grounds (3.5% of all enrolled or 20.9% of those who started treatment). For two participants the basis for starting treatment was not known. Seven (15·6%) of those cases treated empirically had a subsequent positive culture.

For the analysis of time to appropriate treatment, 156 culture-positive TB cases contributed 2413 days follow-up (median 5·5 days, IQR 1·0-22·5). In the Cox regression model for time to appropriate TB treatment, the proportional hazards assumption was not met. Time to appropriate TB treatment was plotted as Kaplan-Meier survival curves (Figure 2). Six participants (all in the POC arm) died prior to initiation of appropriate TB treatment. The estimated median time to appropriate treatment was 7 days (95% CI 6-10) under the laboratory strategy and 1 day (95% CI 1-2) under the POC strategy. Under the POC strategy, 34 cases commenced appropriate treatment on the day of enrolment (50·0% of Xpert-

positive/culture-positive cases; 75.6% of rifampicin-susceptible Xpert-positive/culture-positive cases).

Thirty-two rifampicin-resistant cases contributed 976 days follow-up (median 23·5 days, IQR 14·5-56·0). In the Cox regression model for time to appropriate TB treatment, the proportional hazards assumption was not met. Two cases died before the initiation of appropriate treatment. Kaplan-Meier curves for time to appropriate TB treatment by arm for the rifampicin-resistant cases are shown in Figure 3. The estimated median time to treatment was 27 days (95% CI 22-51) under the laboratory strategy and 17 days (95% CI 10-60) in the POC arm.

For the analyses involving all participants with possible TB or DR-TB, 28·3% (362/1281) had no post-enrolment follow-up (28·0% for laboratory arm vs. 28·5% for POC arm). Participants with no post-enrolment follow-up were less likely to be on ART and had marginally higher CD4+ cell counts at enrolment, but were otherwise similar to those whose outcome was evaluated (see Table E6 in the online data supplement). Figure E3 in the online data supplement shows the outcomes at day 60 for all trial participants. Overall, 24 (2·6%) participants died within 60 days of enrolment, a greater proportion in the POC arm (3·5%, 95% CI 2·2-5·6) compared to the laboratory arm (1·7%, 95% CI 0·9-3·4): OR 2·33, 95% CI 1·13-4·80 (p = 0·022); risk difference -1.8%, 95% CI -3.8, 0.3. After adjustment for baseline CD4+ T-cell count and culture result, this difference did not reach statistical significance (aOR 1·92, 95% CI 0·89-4·16 (p = 0·096). A similar proportion of participants in the two arms were admitted to hospital within 60 days of enrolment (2.0% in laboratory arm vs. 3.1% in POC arm): OR 1·60, 95% CI 0·68-3·77 (p = 0·286); risk difference -1.1%, 95% CI -3.1, 0.9. The estimated median time to ART initiation for HIV-positive participants eligible for

but not yet receiving ART was 24·1 days (95% CI 22·1-32·1) in the laboratory arm and 20·1 days (95% CI 17·1-22·1) in the POC arm. There was no evidence that time to ART initiation was different according to Xpert placement (HR 1·22, 95% CI 0·91-1·64, p = 0·184).

An exploratory *post hoc* analysis was performed to explore the effect of POC positioning on treatment initiation at different time thresholds (2 days, 5 days, and 14 days from enrolment). The proportion of culture-positive cases that had initiated appropriate treatment was significantly greater in the POC arm at all three time points (Table 5).

Discussion

This is the first randomised trial to evaluate the effect of providing point-of-care molecular diagnostics for adults with possible pulmonary TB and DR-TB in a rural primary health care setting. Our data complement those from randomised trials that have compared Xpert to smear microscopy in similar southern African settings (18-20); and other non-randomised studies that have explored the impact of decentralised Xpert testing (21-23).

Point-of-care placement shortened the time to initiation of appropriate TB treatment and enabled same-day diagnosis and treatment for half of the Xpert-positive/culture-positive cases. With POC placement, almost all Xpert-positive, rifampicin-susceptible cases started treatment within the national target of two days (24). The failure to achieve same-day treatment for all people with a positive Xpert was partly explained by people choosing not to wait for same-day results and restricted Xpert operating hours. Other studies have also shown that POC implementation does not automatically translate to same-day treatment and

collectively this evidence highlights the need for innovation in both technology and health systems to enable same-day treatment for all TB cases (21-23, 25).

Although the proportion of individuals initiating appropriate treatment within 30 days was higher in the POC arm, this did not reach statistical significance. Our ability to detect a difference between the two strategies at 30 days was limited by low statistical power. The power of the study was reduced primarily by the lower than expected proportion of participants with culture positive tuberculosis, as well as slightly higher than expected between-cluster variability. Our *post hoc* analysis showed a significant difference in proportions of individuals starting treatment (at days two, five, and 14), suggesting that the POC strategy did have the intended effect in facilitating earlier treatment. We selected the threshold of 30 days on the basis of our considered opinion of what would be seen to be of clinical and public health relevance, but with the benefit of hindsight this was probably not the ideal endpoint. Many different study designs and outcomes have been used in TB diagnostic research and this is an area that would certainly benefit from more consensus (26).

Pre-treatment loss to follow-up was lower than expected under the laboratory strategy. Of the Xpert-positive rifampicin-susceptible cases in the laboratory arm, only two (4%) did not start any treatment within 30 days. This suggests that the routine measures to recall those who tested positive and who did not initially return functioned well during the trial. It is possible that this was partly due to the Hawthorne effect (27), or that the study personnel helped to improve the routine systems. However, analysis of routine laboratory and programme data has shown that pre-treatment loss to follow-up has reduced in this area in the last few years, and is lower than other published data from South Africa (19): in 2014 in seven clinics in the same area (including the study clinic), pre-treatment loss to follow-up for people with

positive Xpert (rifampicin susceptible) was 5% (unpublished data). Under the standard-of-care strategy, the laboratory and specimen transport systems worked better than we had expected. This suggests that while the results may be generalizable within South Africa, POC systems may have greater impact in settings where logistics and laboratory systems preclude the prompt return of results.

Whether the shorter time to treatment initiation and fewer pre-treatment clinic visits observed in this study could result in public health benefit, in terms of reducing transmission, is a question that remains to be tested. Given that the median reported duration of cough was two weeks, shortening the time to appropriate TB treatment by six days could have an important effect on the overall infectious time. A reduction in time to appropriate treatment is of particular importance for drug-resistant TB, yet rifampicin resistance was the main reason for treatment delay in both arms. During preparation for the trial, it was anticipated that the district hospital would become a fully decentralised DR-TB treatment site (28). However, this did not happen according to anticipated timelines and throughout the study period people with DR-TB had to be referred to the provincial DR-TB unit in Durban (~250km) for treatment initiation. The delay between referral and the initial visit at the provincial DR-TB unit was the main component of the overall delay to DR-TB treatment initiation. Nevertheless, the time to initiation of DR-TB treatment for both strategies was comparable to other programmes in South Africa (29, 30); but longer than the median time of seven days achieved by one decentralised DR-TB programme in Cape Town (31). These data emphasise how novel molecular diagnostics may have greatest impact when access to treatment is not limited.

Cost-effectiveness analyses have suggested that POC placement of Xpert MTB/RIF at current prices would need to produce substantial clinical benefits to offset the increased costs associated with primary health care clinic deployment in South Africa, although these analysed only health system costs without consideration of patient costs (32). In that analysis, the increased costs were related to the need for more instruments and staff, and to the decreased operational efficiency at clinic level. Although economic analysis is beyond the scope of this paper, our findings to some extent support this notion as even in this busy primary health care clinic, the system was never operating at full capacity. However, the nurse was easily trained to use the system and was able to do this amongst other duties. The big difference for POC deployment would therefore be the capital expenditure costs for instruments, which would be depreciated over the next few years. Given that the benefits could be greater with further decentralisation of DR-TB care or in settings with weaker laboratory systems, we still need better understanding of the cost drivers to inform diagnostic systems in different settings. With respect to patient costs, the shorter time to treatment and fewer clinic visits, particularly same-day treatment, could have particular benefit in rural communities such as this, where one in two people with TB incur catastrophic costs (33, 34).

With new technologies being developed that may be more convenient for decentralised use (35), this study provides rare real-world evidence of the benefits and limitations of point-of-care diagnostics. The findings from this cluster randomised trial suggest that strengthening of the diagnostic cascade to get all TB cases on treatment in a timely fashion will require a combination of technological advances (simpler, more sensitive diagnostics better suited for point-of-care use (35, 36)) allied with broader strengthening of health systems to limit treatment delays, especially for drug-resistant TB (37). There remains a need to push for the

development of simple diagnostic technologies suitable for true POC use and affordable for widespread use (7, 8, 38).

Acknowledgements

We thank the community members who participated in the study and the Community Advisory Board of the Africa Centre for Health and Population Studies. We thank the study team (N Hlophe, C Makhanya, T Mtshali, P Dlamini, and M Mabaso) and the health care workers at the study site for care of the study participants. We thank the Department of Health and National Health Laboratory Service for Hlabisa sub-district, uMkhanyakude district and KwaZulu-Natal province for allowing us to work at the study site and for supporting the research. We acknowledge Cepheid and the Foundation for Innovative New Diagnostics for the concessionary pricing of Xpert MTB/RIF cartridges. We thank the independent members of the Trial Steering Committee (D Moore, K Fielding, and M Moosa) for their support and scientific guidance. We acknowledge the support of the Wellcome Trust Bloomsbury Centre for Global Health Research.

References

1. World Health Organization. Global tuberculosis report 2016. 2016. Available from: http://apps.who.int/iris/bitstream/10665/250441/1/9789241565394-eng.pdf?ua=1.

2. United Nations Development Programme UNECfA, African Union and African Development Bank Group,. MDG Report 2013: Assessing progress in Africa toward the Millennium Development Goals. Geneva; 2013.

3. MacPherson P, Houben RM, Glynn JR, Corbett EL, Kranzer K. Pre-treatment loss to follow-up in tuberculosis patients in low- and lower-middle-income countries and high-burden countries: a systematic review and meta-analysis. *Bull World Health Organ* 2014; 92: 126-138.

4. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC Public Health* 2008; 8: 15.

5. Lin HH, Dowdy D, Dye C, Murray M, Cohen T. The impact of new tuberculosis diagnostics on transmission: why context matters. *Bull World Health Organ* 2012; 90: 739-747A.

6. Dowdy DW, Davis JL, den Boon S, Walter ND, Katamba A, Cattamanchi A. Population-level impact of same-day microscopy and Xpert MTB/RIF for tuberculosis diagnosis in Africa. *PLoS One* 2013; 8: e70485.

7. Batz HG, Cooke GS, Reid SD. Towards lab-free tuberculosis diagnosis. Geneva/New York: Medecins Sans Frontieres/Stop TB Partnership TB/HIV Working Group/Treatment Action Group; 2011.

8. Howitt P, Darzi A, Yang GZ, Ashrafian H, Atun R, Barlow J, Blakemore A, Bull AM, Car J, Conteh L, Cooke GS, Ford N, Gregson SA, Kerr K, King D, Kulendran M, Malkin RA, Majeed A, Matlin S, Merrifield R, Penfold HA, Reid SD, Smith PC, Stevens MM, Templeton MR, Vincent C, Wilson E. Technologies for global health. *Lancet* 2012; 380: 507-535.

9. Lessells RJ, Cooke GS, McGrath N, Nicol MP, Newell ML, Godfrey-Faussett P. Impact of a novel molecular TB diagnostic system in patients at high risk of TB mortality in rural South Africa (Uchwepheshe): study protocol for a cluster randomised trial. *Trials* 2013; 14: 170.

10. Zaidi J, Grapsa E, Tanser F, Newell ML, Barnighausen T. Dramatic increase in HIV prevalence after scale-up of antiretroviral treatment. *AIDS* 2013; 27: 2301-2305.

11. World Health Organization. Policy statement: automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Geneva: World Health Organization; 2011.

12. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis: emergency update 2008. Geneva: World Health Organization; 2008.

13. Department of Health RoSA. National tuberculosis management guidelines. Pretoria; 2009.

14. Department of Health RoSA. Management of drug-resistant tuberculosis: Policy guidelines. Pretoria: Department of Health; 2011.

15. Hayes RJ, Bennett S. Simple sample size calculation for cluster-randomized trials. *Int J Epidemiol* 1999; 28: 319-326.

16. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 1994; 81: 515-526.

17. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *Journal of the American Statistical Association* 1999; 94: 496-509.

18. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, Bara W, Mungofa S, Pai M, Hoelscher M, Dowdy D, Pym A, Mwaba P, Mason P, Peter J, Dheda K, team T-N. 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: 424-435.

19. Churchyard GJ, Stevens WS, Mametja LD, McCarthy KM, Chihota V, Nicol MP, Erasmus LK, Ndjeka NO, Mvusi L, Vassall A, Sinanovic E, Cox HS, Dye C, Grant AD, Fielding KL. Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF. *The Lancet Global health* 2015; 3: e450-457.

20. Cox HS, Mbhele S, Mohess N, Whitelaw A, Muller O, Zemanay W, Little F, Azevedo V, Simpson J, Boehme CC, Nicol MP. Impact of Xpert MTB/RIF for TB diagnosis in a primary care clinic with high TB and HIV prevalence in South Africa: a pragmatic randomised trial. *PLoS Med* 2014; 11: e1001760.

21. Hanrahan CF, Clouse K, Bassett J, Mutunga L, Selibas K, Stevens W, Scott L, Sanne I, Van Rie A. The patient impact of point-of-care vs. laboratory placement of Xpert((R)) MTB/RIF. *Int J Tuberc Lung Dis* 2015; 19: 811-816.

22. Van Den Handel T, Hampton KH, Sanne I, Stevens W, Crous R, Van Rie A. The impact of Xpert((R)) MTB/RIF in sparsely populated rural settings. *Int J Tuberc Lung Dis* 2015; 19: 392-398.

23. Schumacher SG, Thangakunam B, Denkinger CM, Oliver AA, Shakti KB, Qin ZZ, Michael JS, Luo R, Pai M, Christopher DJ. Impact of point-of-care implementation of Xpert(R) MTB/RIF: product vs. process innovation. *Int J Tuberc Lung Dis* 2015; 19: 1084-1090.

24. Department of Health RoSA. National tuberculosis management guidelines. Pretoria, South Africa; 2014.

25. Muyoyeta M, Moyo M, Kasese N, Ndhlovu M, Milimo D, Mwanza W, Kapata N, Schaap A, Godfrey Faussett P, Ayles H. Implementation research to inform the use of Xpert MTB/RIF in primary health care facilities in high TB and HIV settings in resource constrained settings. *PLoS One* 2015; 10: e0126376.

26. Schumacher SG, Sohn H, Qin ZZ, Gore G, Davis JL, Denkinger CM, Pai M. Impact of Molecular Diagnostics for Tuberculosis on Patient-Important Outcomes: A Systematic Review of Study Methodologies. *PLoS One* 2016; 11: e0151073.

27. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. *J Clin Epidemiol* 2014; 67: 267-277.

28. Department of Health RoSA. Multi-drug resistant tuberculosis. A policy framework on decentralised and deinstitutionalised management for South Africa. Pretoria, South Africa: Department of Health; 2011.

29. Dlamini-Mvelase NR, Werner L, Phili R, Cele LP, Mlisana KP. Effects of introducing Xpert MTB/RIF test on multi-drug resistant tuberculosis diagnosis in KwaZulu-Natal South Africa. *BMC Infect Dis* 2014; 14: 442.

30. Naidoo P, du Toit E, Dunbar R, Lombard C, Caldwell J, Detjen A, Squire SB, Enarson DA, Beyers N. A comparison of multidrug-resistant tuberculosis treatment commencement times in MDRTBPlus line probe assay and Xpert(R) MTB/RIF-based algorithms in a routine operational setting in Cape Town. *PLoS One* 2014; 9: e103328.

31. Cox HS, Daniels JF, Muller O, Nicol MP, Cox V, van Cutsem G, Moyo S, De Azevedo V, Hughes J. Impact of decentralized care and the Xpert MTB/RIF test on rifampicin-resistant tuberculosis treatment initiation in Khayelitsha, South Africa. *Open Forum Infect Dis* 2015; 2: ofv014.

32. Schnippel K, Meyer-Rath G, Long L, MacLeod W, Sanne I, Stevens WS, Rosen S. Scaling up Xpert MTB/RIF technology: the costs of laboratory- vs. clinic-based roll-out in South Africa. *Trop Med Int Health* 2012; 17: 1142-1151.

33. Cleary S, Birch S, Chimbindi N, Silal S, McIntyre D. Investigating the affordability of key health services in South Africa. *Soc Sci Med* 2013; 80: 37-46.

34. Foster N, Vassall A, Cleary S, Cunnama L, Churchyard G, Sinanovic E. The economic burden of TB diagnosis and treatment in South Africa. *Soc Sci Med* 2015; 130: 42-50.

35. Cepheid. World's Most Portable Molecular Diagnostics System Unveiled at AACC. 2015 Jul 28 26 Nov 2015]. Available from: http://ir.cepheid.com/releasedetail.cfm?releaseid=924108.

36. World Health Organization. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017.

37. Wilson D, Howell V, Toppozini C, Dong K, Clark M, Hurtado R. Against all odds: diagnosing tuberculosis in South Africa. *J Infect Dis* 2011; 204 Suppl 4: S1102-1109.

38. Jani IV, Peter TF. How point-of-care testing could drive innovation in global health. *N Engl J Med* 2013; 368: 2319-2324.

Figure legends

Figure 1 Study flow diagram for clusters and individual participants

Figure 2 Kaplan-Meier curves for time to initiation of appropriate TB treatment before death for culture-positive cases

Figure 3 Kaplan-Meier curves for time to initiation of appropriate TB treatment before death for culture-positive rifampicin-resistant cases

Table 1 Baseline demographic and clinical characteristics for participants with possible TB or drug-resistant TB

Variable		**Laboratory (n = 640)**	**Point-of-care (n = 641)**
Sex	Female (n, %)	393 (61·4)	422 (65·8)
Age (years)	Median (IQR)	36 (30-43)	36 (28-45)
Body mass index (kg/m^2)	Median (IQR)	22·6 (20·2-26·5)	22·9 (20·1-27·0)
Current symptoms	Cough only (n, %)	157 (24·5)	147 (22·9)
	Weight loss (n, %)	332 (51·9)	335 (52·3)
	Fever (n, %)	269 (42·0)	256 (40·0)
	Night sweats (n, %)	295 (46·2)	298 (46·7)
Duration of cough (weeks)*	Median (IQR)	2 (1-4)	3 (1-4)
Current IPT use	Yes (n, %)	8 (1·3)	11 (1·7)
Risk of drug resistance	None (n, %)	351 (54·8)	353 (55·1)
	Treatment failure (n, %)	4 (0·6)	7 (1·1)
	Smear non-conversion (n, %)	18 (2·8)	21 (3·3)
	Previous TB treatment (n, %)	253 (39·5)	247 (38·5)
	Household contact (n, %)	22 (3·4)	15 (2·3)
	Health care worker (n, %)	12 (1·9)	9 (1·4)
	Prison last 12 months (n, %)	7 (1·1)	10 (1·6)
HIV status	Positive (n, %)	589 (92·0)	596 (93·0)
	Negative (n, %)	39 (6·1)	39 (6·1)
	Never tested (n, %)	6 (0·9)	3 (0·5)
	Not disclosed (n, %)	5 (0·8)	3 (0·5)
	Missing (n, %)	1 (0·2)	0
Antiretroviral therapy†	Current (n, %)	238 (40·4)	222 (37·3)
CD4+ cell count (cells/µL)†‡	Median (IQR)	280 (147-455)	247 (119-415)
	≤50 (n, %)	41 (6·4)	66 (10·3)

51-200 (n, %)	152 (23·8)	150 (23·4)
201-350 (n, %)	149 (23·3)	158 (24·6)
351-500 (n, %)	85 (13·3)	81 (12·6)
>500 (n, %)	108 (16·9)	92 (14·4)
Missing (n, %)	54 (8·4)	49 (7·6)

* Cough duration missing for 11 participants (laboratory, *n* = 3; point-of-care, *n* = 8)
† Proportions are of HIV-positive participants
‡ CD4+ cell count up to 18 months prior to or 30 days after enrolment

Table 2 Results from Xpert MTB/RIF tests

Xpert MTB/RIF result	**Laboratory (n = 619)**	**Point-of-care (n = 616)**
First sputum specimen		
MTB DETECTED	98 (15·8)	108 (17·5)
Rif Resistance NOT DETECTED	*82 (13·2)*	*91 (14·8)*
Rif Resistance DETECTED	*16 (2·6)*	*17 (2·8)*
MTB NOT DETECTED	473 (76·4)	501 (81·3)
INVALID	6 (1·0)	4 (0·6)
ERROR	5 (0·8)	2 (0·3)
Not processed (specimen leaked)	37 (6·0)	1 (0·2)
All sputum specimens		
MTB DETECTED	105 (17·0)	108 (17·5)
Rif Resistance NOT DETECTED	*87 (14·1)*	*91 (14·8)*
Rif Resistance DETECTED	*18 (2·9)*	*17 (2·8)*
MTB NOT DETECTED	505 (81·6)	502 (81·5)
INVALID	1 (0·2)	3 (0·5)
ERROR	1 (0·2)	2 (0·3)
Not processed (specimen leaked)	7 (1·1)	1 (0·2)

40/48 participants in laboratory arm with no valid result from the first sputum specimen submitted a second specimen after a median of 5 days (IQR 4-8); all but one of the repeat specimens yielded a valid result. One participant in point-of-care arm with an invalid result on the first sputum specimen submitted a second specimen on the same day which yielded a valid result; six other participants in the point-of-care arm with no valid result from the first sputum specimen did not submit a second specimen.

Table 3 Baseline demographic and clinical characteristics for culture-positive TB cases included in primary analysis

Variable		**Laboratory (n = 69)**	**Point-of-care (n = 88)**
Sex	Female (n, %)	32 (47·1)	53 (60·2)
Age (years)	Median (IQR)	34 (28-41)	33 (27-41)
Body mass index (kg/m^2)	Median (IQR)	20·5 (18·2-22·0)	21·0 (18·6-25·0)
Current symptoms	Cough only (n, %)	9 (13·2)	10 (11·4)
	Weight loss (n, %)	53 (77·9)	67 (76·1)
	Fever (n, %)	28 (41·2)	34 (38·6)
	Night sweats (n, %)	39 (57·4)	50 (56·8)
Duration of cough (weeks)	Median (IQR)	3 (1-6)	3 (2-4)
Current IPT use	Yes (n, %)	1 (1·5)	1 (1·1)
Risk of drug resistance	None (n, %)	33 (48·5)	52 (59·1)
	Treatment failure (n, %)	1 (1·5)	2 (2·3)
	Smear non-conversion (n, %)	3 (4·4)	3 (3·4)
	Previous TB treatment (n, %)	30 (44·1)	31 (35·2)
	Household contact (n, %)	6 (8·8)	4 (4·6)
	Health care worker (n, %)	-	-
	Prison last 12 months (n, %)	1 (1·5)	2 (2·3)
HIV status	Positive (n, %)	64 (94·1)	87 (98·9)
	Negative (n, %)	3 (4·4)	1 (1·1)
	Never tested (n, %)	1 (1·5)	-
	Not disclosed (n, %)	-	-
	Missing (n, %)	-	-
Antiretroviral therapy*	Current (n, %)	19 (29·7)	31 (35·6)
CD4+ cell count (cells/µL)*†	Median (IQR)	219 (98-371)	203 (99-328)
	≤50 (n, %)	6 (8·8)	10 (11·4)

51-200 (n, %)	21 (30·9)	29 (33·0)
201-350 (n, %)	14 (20·6)	24 (27·3)
351-500 (n, %)	12 (17·6)	8 (9·1)
>500 (n, %)	7 (10·3)	9 (10·2)
Missing (n, %)	8 (11·8)	8 (9·1)

* Proportions are of HIV-positive participants
† CD4+ cell count up to 18 months prior to or 30 days after enrolment

Table 4 Proportion of culture-positive pulmonary TB cases who started appropriate TB treatment within 30 days, according to Xpert MTB/RIF result

	Laboratory	**Point-of-care**
30 days		
Xpert positive	51/57 (89·5%)	65/68 (95·6%)
Xpert positive – rifampicin susceptible	*42/45* (93·3%)*	*55/56† (98·2%)*
Xpert positive – rifampicin resistant	*9/12‡ (75·0%)*	*10/12 (83·3%)*
Xpert negative	0/10	5/20§ (25·0%)
Xpert no result	1/1‖ (100%)	-
Total	52/68 (76·5%)	70/88 (79·6%)
60 days		
Xpert positive	53/57 (93.0%)	65/68 (95.6%)
Xpert positive – rifampicin susceptible	*42/45 (93.3%)*	*55/56 (98.2%)*
Xpert positive – rifampicin resistant	*11/12 (91.7%)*	*10/12 (83.3%)*
Xpert negative	4/10 (40.0%)	11/20 (55.0%)
Xpert no result	1/1 (100%)	-
Total	58/68 (85.3%)	76/88 (86.4%)

* One participant with multidrug resistance on culture isolate but rifampicin susceptibility on Xpert initiated inappropriate TB treatment after 3 days (switched to appropriate treatment beyond 30 days)
† One participant with multidrug resistance on culture isolate but rifampicin susceptibility on Xpert initiated inappropriate TB treatment on the day of enrolment (switched to appropriate treatment beyond 30 days)
‡ One participant with isoniazid monoresistance on culture isolate but rifampicin resistance on Xpert initiated inappropriate TB treatment after 15 days
§ Four participants with negative Xpert started treatment on basis of chest X-ray (after 1, 8, 11, and 14 days respectively); one participant started treatment on basis of positive culture (after 30 days)
‖ One participant with no Xpert result (specimen leaked) started treatment on basis of clinical features (after 1 day)

Table 5 Exploratory analyses with different time thresholds for initiation of appropriate anti-TB treatment

Time threshold	Laboratory arm (n = 68)		Point-of-care arm (n = 88)		Odds ratio (95% CI)	p value
	n	% (95% CI)	n	% (95% CI)		
2 days	5	7.4 (2.4-16.3)	53	60.2 (49.2-70.5)	17.1 (5.3-55.1)	<0.001
5 days	22	32.4 (21.5-44.8)	56	63.6 (52.7-73.6)	3.6 (1.8-7.3)	<0.001
14 days	42	61.8 (49.2-73.3)	66	75.0 (64.6-83.6)	1.9 (1.0-3.6)	0.057

CI, confidence interval

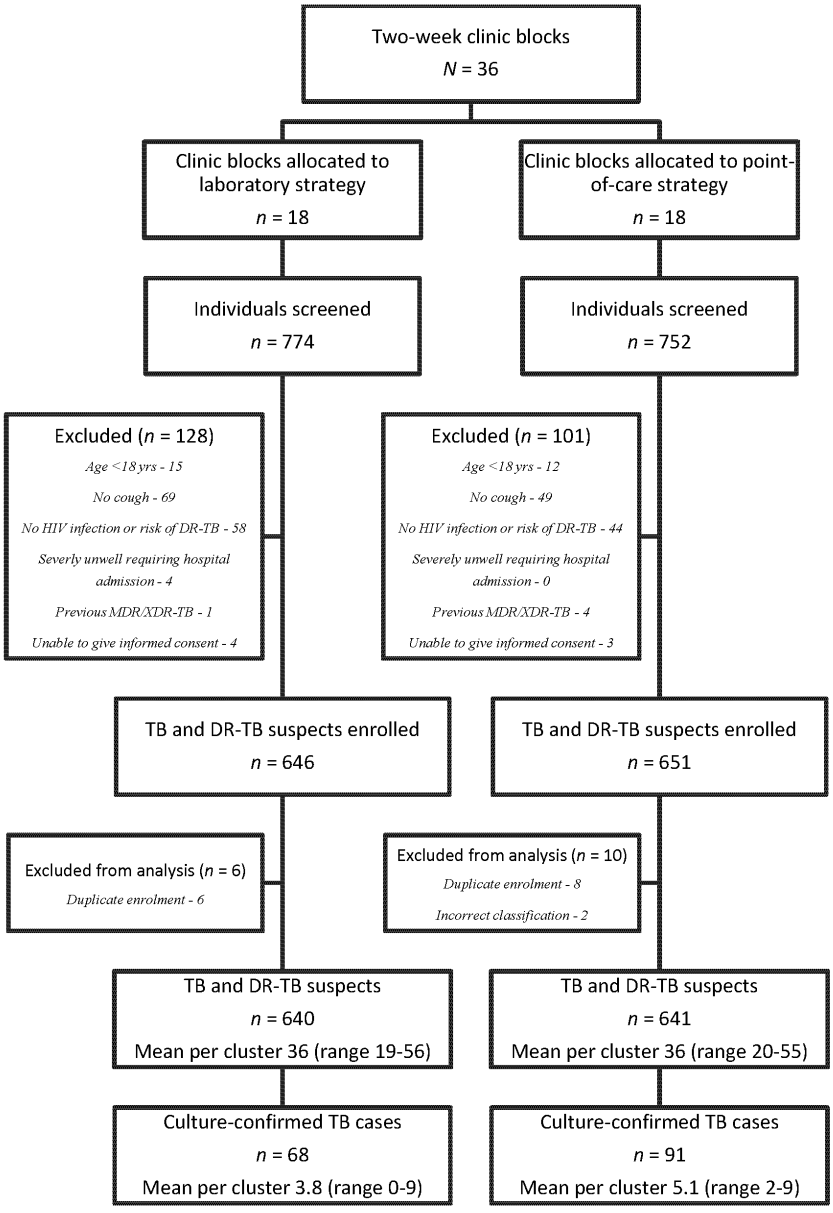


Figure 1 Study flow diagram for clusters and individual participants

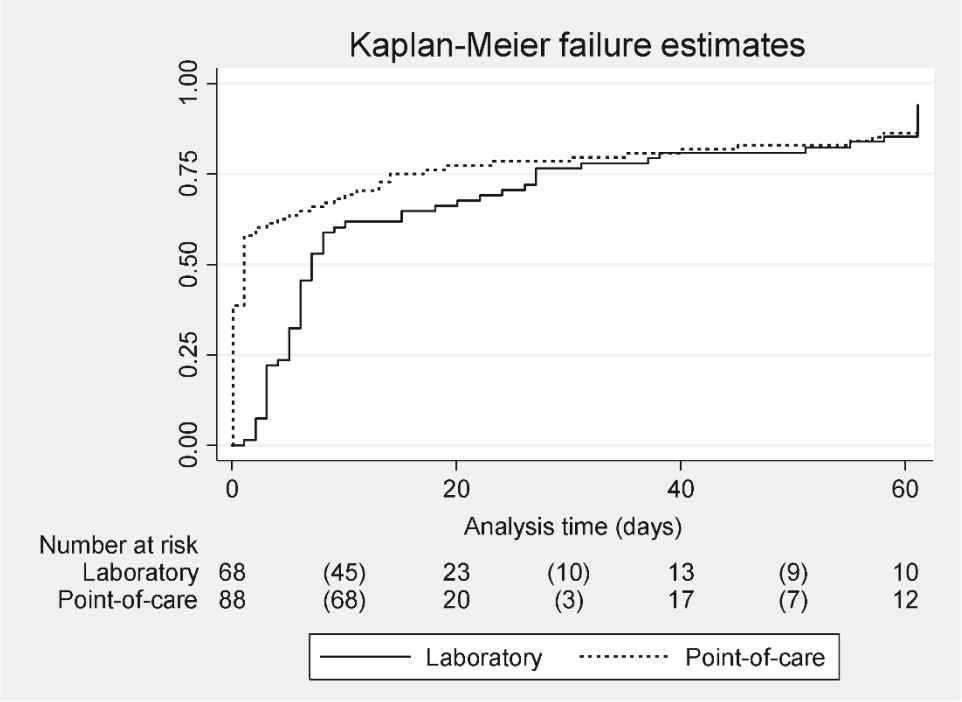


Figure 2 Kaplan-Meier curves for time to initiation of appropriate TB treatment before death for culture-positive cases

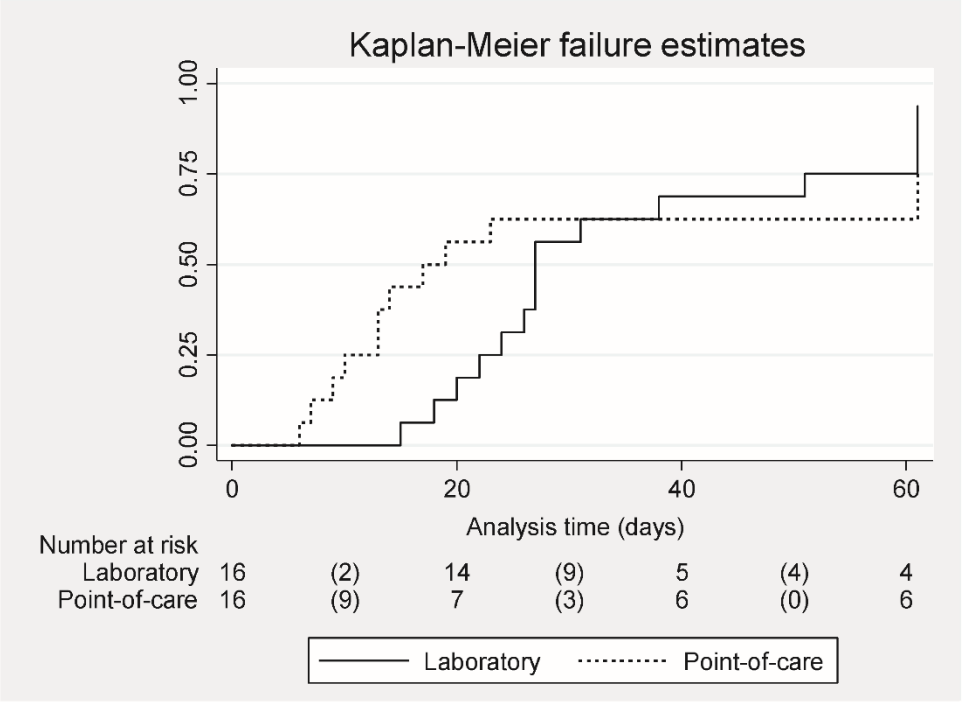


Figure 3 Kaplan-Meier curves for time to initiation of appropriate TB treatment before death for culture-positive rifampicin-resistant cases

Impact of point-of-care Xpert MTB/RIF on tuberculosis treatment initiation: a cluster randomised trial

Richard J Lessells, Graham S Cooke, Nuala McGrath, Mark P Nicol, Marie-Louise Newell, Peter Godfrey-Faussett

Online data supplement

Methods

Clinical management of participants

The Xpert MTB/RIF assay was incorporated into diagnostic algorithms adapted from the WHO standardised diagnostic algorithms for HIV-infected individuals with possible TB and individuals at high risk of drug-resistant TB (Figures E1 & E2) (E1).

The research nurse worked alongside the TB nurses at the primary health care clinic at all times. The research nurse was located in a separate room in a parkhome adjoining the TB department. The research nurse was responsible for sputum specimen collection and delivery of specimens to the courier. When participants did not return as scheduled to receive their Xpert results, the research nurse was responsible for contacting participants to encourage them to return. This procedure was aligned with the routine clinic systems at the time of the study. Results were not communicated to participants by short message service (SMS). Referrals for further diagnostic evaluation at the district hospital (e.g. chest X-ray or medical officer review) were organised by the research nurse in close collaboration with the TB nurses.

Pathways for referral and treatment of drug-resistant TB cases

All participants diagnosed with drug-resistant TB (DR-TB) were seen by a medical officer at the clinic or at the district hospital and then referred to the provincial DR-TB centre, King Dinuzulu Hospital (formerly King George V Hospital) in Durban for specialist assessment and treatment initiation. Appointments at King Dinuzulu Hospital were booked by the medical officer after reviewing the participant. Generally, people with DR-TB were admitted to the TB inpatient ward at Hlabisa Hospital two to three days before their scheduled appointment, travelled to Durban on

an outpatient basis, and then stayed at Hlabisa at least one month for supervision of treatment and monitoring for toxicity under the satellite model (E2). Following the first month, if patients were clinically stable, treatment continued at home (injectable agents were given at the nearest PHC clinic or by a mobile injection team) and patients made monthly visits to King Dinuzulu Hospital for follow-up and pharmacy refill. Patients with XDR-TB or complicated MDR-TB (pregnant females, renal failure, or liver failure) were admitted to King Dinuzulu Hospital for specialist inpatient management.

Results

Culture and drug susceptibility test (DST) results

The results of the Mycobacterial Growth Indicator Tube (MGIT) culture are shown in Table E2. The overall yield from culture was lower than that from Xpert MTB/RIF with 12.9% (159/1235) of participants having a culture positive for *M. tuberculosis*, compared to 16.7% (206/1235) having a positive Xpert from the initial sputum specimen. Considering only results of specimens that were processed and where a result could be identified, 15.0% (159/1057) of cultures were positive for *M. tuberculosis*.

Of the 159 culture isolates identified as *M. tuberculosis*, 32 (20.1%) were rifampicin resistant by line probe assay (LPA) and/or phenotypic drug susceptibility testing (DST) (Table E3). Phenotypic drug susceptibility testing was performed if the LPA detected isoniazid and/or rifampicin resistance or if LPA results were indeterminate. For the purposes of analysis, rifampicin resistance was defined as an isolate with rifampicin resistance on either or both tests (LPA and phenotypic DST). Twenty-eight isolates were resistant to rifampicin by LPA and thirty were resistant to rifampicin by phenotypic DST. Concordance between the two methods was good (Table E4).

The characteristics of the participants with an evaluable culture result, defined as positive for *M. tuberculosis*, positive for NTM, positive with no definitive identification or negative, were compared with the characteristics of those without an evaluable result. The two groups were broadly comparable, except that those without an evaluable result were less likely to be HIV

positive, and those that were HIV positive had lower CD4+ T-cell counts and were somewhat less likely to be on ART (Table E5).

Initiation of inappropriate treatment

Three participants initiated anti-TB treatment within 30 days that was defined as inappropriate according to the drug susceptibility pattern (two in the laboratory arm and one in the point-of-care arm). In all three cases, this was due to discordant rifampicin resistance results: in two cases defined as MDR-TB by LPA and phenotypic DST, Xpert was reported as rifampicin susceptible and standard first-line anti-TB treatment was started; in the third case defined as isoniazid mono-resistant by LPA and phenotypic DST (rifampicin susceptible on both LPA and phenotypic DST), Xpert detected rifampicin resistance and MDR-TB treatment was initiated.

Outcomes for Xpert negative/culture positive cases

The majority (26/31, 83.9%) of Xpert negative/culture positive cases did not initiate appropriate TB treatment within 30 days. Approximately half (16/31, 51.6%) did not initiate appropriate treatment within 60 days.

In the laboratory strategy, none of 11 Xpert negative/culture positive cases started appropriate TB treatment within 30 days. Of the eight cases with fully susceptible TB, five (62.5%) started appropriate TB treatment on the basis of the positive culture (after 37, 55, 58, 86 and 92 days) and three were not recorded as having started treatment. Of the three cases with rifampicin-resistant TB (all with MDR-TB), all three started drug-resistant TB treatment (after 51, 92, and

125 days) respectively; one of those cases had initially commenced standard first-line TB treatment on the basis of the preliminary positive culture result before DST results.

In the POC strategy, 5/20 (25.0%) of Xpert negative/culture positive cases commenced appropriate TB treatment within 30 days, four on the basis of chest X-ray and one on the basis of the positive culture (all five had fully susceptible TB). Of the remaining 12 cases with fully susceptible TB, two commenced treatment on the basis of chest X-ray (after 45 and 58 days), four commenced treatment on the basis of the positive culture (after 35, 40, 55, and 57 days), three participants died, and three were not recorded as having started TB treatment. Of the three cases with rifampicin-resistant TB (all with MDR-TB), one commenced drug-resistant TB treatment (after 125 days), one died after commencing standard first-line TB treatment (on the basis of the positive culture results before DST results), and one was not recorded as having started any TB treatment.

Table E1 Definitions of appropriate TB treatment for primary and secondary endpoints

Case definition*	Appropriate initial anti-TB drug regimen
M. tuberculosis susceptible to rifampicin and isoniazid	Isoniazid + rifampicin + pyrazinamide + ethambutol ± streptomycin
M. tuberculosis with mono-resistance to isoniazid	Isoniazid + rifampicin + pyrazinamide + ethambutol ± streptomycin
M. tuberculosis with mono-resistance to rifampicin	Standardised second-line regimen‡ (kanamycin/amikacin + fluoroquinolone + ethionamide + cycloserine/terizidone ± pyrazinamide ± ethambutol) ± isoniazid
Multidrug-resistant *M. tuberculosis* (MDR-TB): resistance to rifampicin and isoniazid	Standardised second-line regimen‡ (kanamycin/amikacin + fluoroquinolone + ethionamide + cycloserine/terizidone ± pyrazinamide ± ethambutol)
Extensively drug-resistant *M. tuberculosis* (XDR-TB): MDR plus resistance to ofloxacin and kanamycin	Standardised XDR-TB regimen‡ (capreomycin + fluoroquinolone + ethionamide + cycloserine/terizidone + PAS + clofazimine)
M. tuberculosis with unknown drug susceptibility†	Isoniazid + rifampicin + pyrazinamide + ethambutol

PAS, para-aminosalicylic acid
* Case definition based on results of MGIT culture + line probe assay + phenotypic DST
† Drug susceptibility test not performed or unsuccessful
‡ According to national treatment guidelines (E3)

Table E2 Results of Mycobacterial Growth Indicator Tube (MGIT) culture

Result	**Laboratory (*n* = 619)**	**Point-of-care (*n* = 616)**
Positive (*M. tuberculosis*)	68 (11.0)	91 (14.8)
Positive (non-tuberculous mycobacteria)	7 (1.1)	6 (1.0)
Positive (no identification)	1 (0.2)	-
Negative	402 (64.9)	379 (61.5)
Contaminated	56 (9.0)	47 (7.6)
Not processed	71 (11.5)	71 (11.5)
Specimen leaked in transit	*63(10.2)*	*70 (11.4)*
*Incorrect details**	*2 (0.3)*	*0*
Processed for smear microscopy†	*6 (1.0)*	*1 (0.2)*
No result	14 (2.3)	22 (3.6)

* Participant details on laboratory form and specimen container did not match
† Specimen processed for smear microscopy in error instead of culture

Table E3 Results of drug susceptibility testing (combined from line probe assay and phenotypic drug susceptibility testing)

Result	Laboratory (*n* = 68)	Point-of-care (*n* = 91)
Rifampicin and isoniazid susceptible	51 (75.0)	72 (79.1)
Isoniazid susceptible, rifampicin indeterminate*	-	1 (1.1)
Rifampicin mono resistance	2 (2.9)	1 (1.1)
Isoniazid mono resistance	1 (1.5)	2 (2.2)
Rifampicin + isoniazid resistance	14 (20.6)	15 (16.5)

* Line probe assay reported as isoniazid susceptible, rifampicin inconclusive; phenotypic DST unsuccessful

Table E4 Concordance between line probe assay and phenotypic DST for rifampicin & isoniazid

Line probe assay result	Phenotypic DST result					
	Rifampicin			Isoniazid		
	Susceptible	Resistant	Inconclusive	Susceptible	Resistant	Inconclusive
Susceptible	3	1	0	3	2	0
Resistant	2	26	0	0	29	0
Inconclusive	1	3	1	1	1	0

Table E5 Characteristics of participants with and without an evaluable culture result

Variable		Evaluable culture result (n = 953)	No evaluable culture result (n = 282)
Sex	Female (n, %)	619 (65.0)	169 (59.9)
Age (years)	Median (IQR)	36 (29-44)	36 (30-44)
Body mass index (kg/m^2)	Median (IQR)	22.7 (20.2-26.8)	22.9 (20.1-26.5)
Current symptoms	Cough only (n, %)	218 (22.9)	67 (23.8)
	Weight loss (n, %)	493 (51.7)	153 (54.3)
	Fever (n, %)	395 (41.5)	116 (41.1)
	Night sweats (n, %)	442 (46.5)	137 (48.9)
Current TB treatment	Yes (n, %)	37 (3.9)	11 (3.9)
Previous TB treatment	Yes (n, %)	368 (38.6)	121 (42.9)
Current IPT use	Yes (n, %)	15 (1.6)	1 (0.4)
Risk of drug resistance	Yes (n, %)	426 (44.7)	139 (49.3)
HIV infection	Yes (n, %)	891 (93.6)	251 (89.0)
Antiretroviral therapy*	Current (n, %)	363/891 (40.7)	85/251 (33.9)
CD4+ T-cell count (cells/µl)*	Median (IQR)	277 (140-449)	238 (114-396)

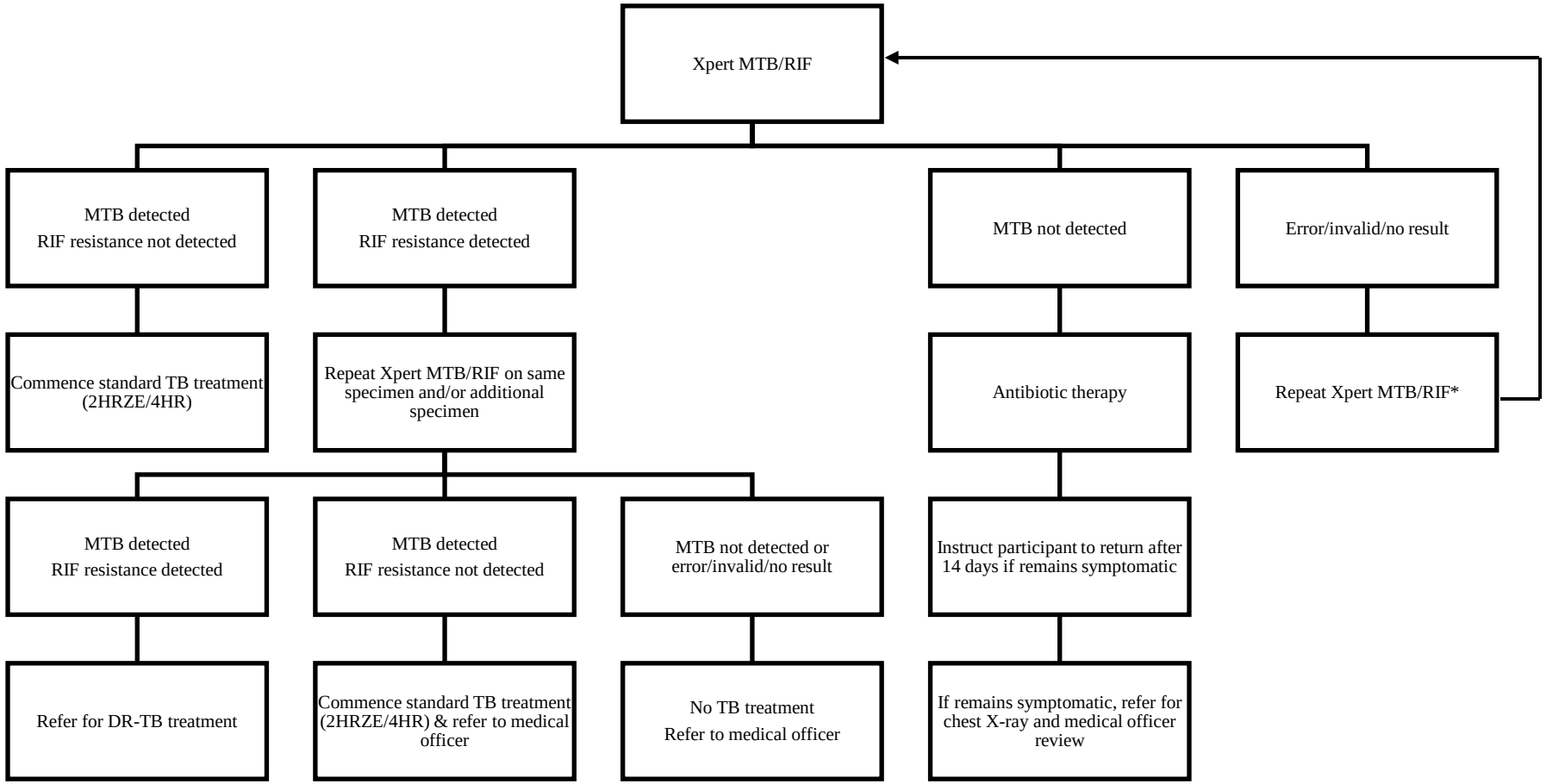
IPT, isoniazid preventive therapy; IQR, interquartile range
* For HIV-infected participants only

Table E6 Comparison of baseline characteristics for participants with outcome evaluated vs. those lost to follow-up

Variable		Outcome evaluated (*n* = 919)	Lost to follow-up (*n* = 362)
Sex	Female (*n*, %)	593 (64.5)	222 (61.3)
Age (years)	Median (IQR)	36 (29-44)	36 (28-45)
Body mass index (kg/m^2)	Median (IQR)	22.7 (20.1-26.7)	22.7 (20.3-26.9)
Current symptoms	Cough only (*n*, %)	218 (23.7)	86 (23.8)
	Weight loss (*n*, %)	475 (51.7)	192 (53.0)
	Fever (*n*, %)	357 (38.9)	168 (46.4)
	Night sweats (*n*, %)	430 (46.9)	163 (45.2)
Current IPT use	Yes (*n*, %)	15 (1.6)	4 (1.1)
Risk of drug resistance	None (*n*, %)	494 (53.8)	210 (58.0)
	Treatment failure (*n*, %)	10 (1.1)	1 (0.3)
	Smear non-conversion (*n*, %)	25 (2.7)	12 (3.3)
	Previous TB treatment (*n*, %)	373 (40.6)	127 (35.1)
	Household contact (*n*, %)	28 (3.1)	9 (2.5)
	Health care worker (*n*, %)	17 (1.9)	4 (1.1)
	Prison last 12 months (*n*, %)	9 (1.0)	8 (2.2)
HIV infection	Positive (*n*, %)	856 (93.1)	329 (90.9)
	Negative (*n*, %)	52 (5.7)	26 (7.2)
	Never tested (*n*, %)	5 (0.5)	4 (1.1)
	Not disclosed (*n*, %)	6 (0.7)	2 (0.6)
	Missing (*n*, %)	0	1 (0.3)
Antiretroviral therapy*	Current (*n*, %)	364 (42.5)	96 (29.2)
CD4+ T-cell count (cells/μL)*	Median (IQR)	256 (134-428)	286 (141-446)
	≤50 (*n*, %)	80 (9.4)	27 (8.2)
	51-200 (*n*, %)	225 (26.3)	77 (23.4)
	201-350 (*n*, %)	223 (26.1)	84 (25.5)
	351-500 (*n*, %)	117 (13.7)	49 (14.9)
	>500 (*n*, %)	144 (16.8)	56 (17.0)
	Missing	67 (7.8)	26 (10.9)
HIV RNA (copies/ml)†	<400 (*n*, %)	206 (81.8)	60 (82.2)
	<40 (*n*, %)	190 (75.4)	54 (74.0)

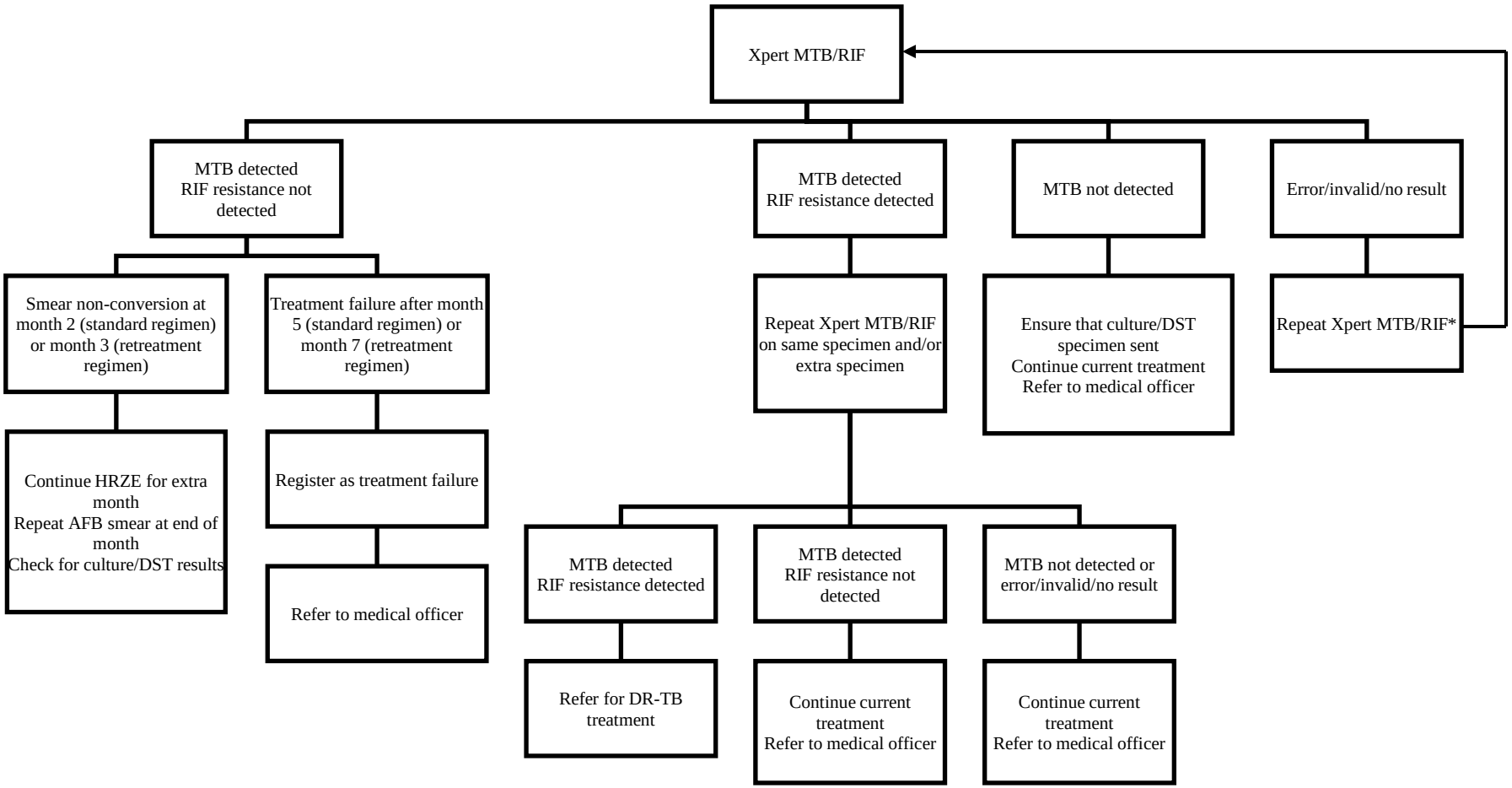
* Proportions are of HIV-infected participants
† Proportions are of HIV-infected participants on ART for more than three months

Figure E1 Clinical management algorithm according to Xpert MTB/RIF results for participants not currently on TB treatment



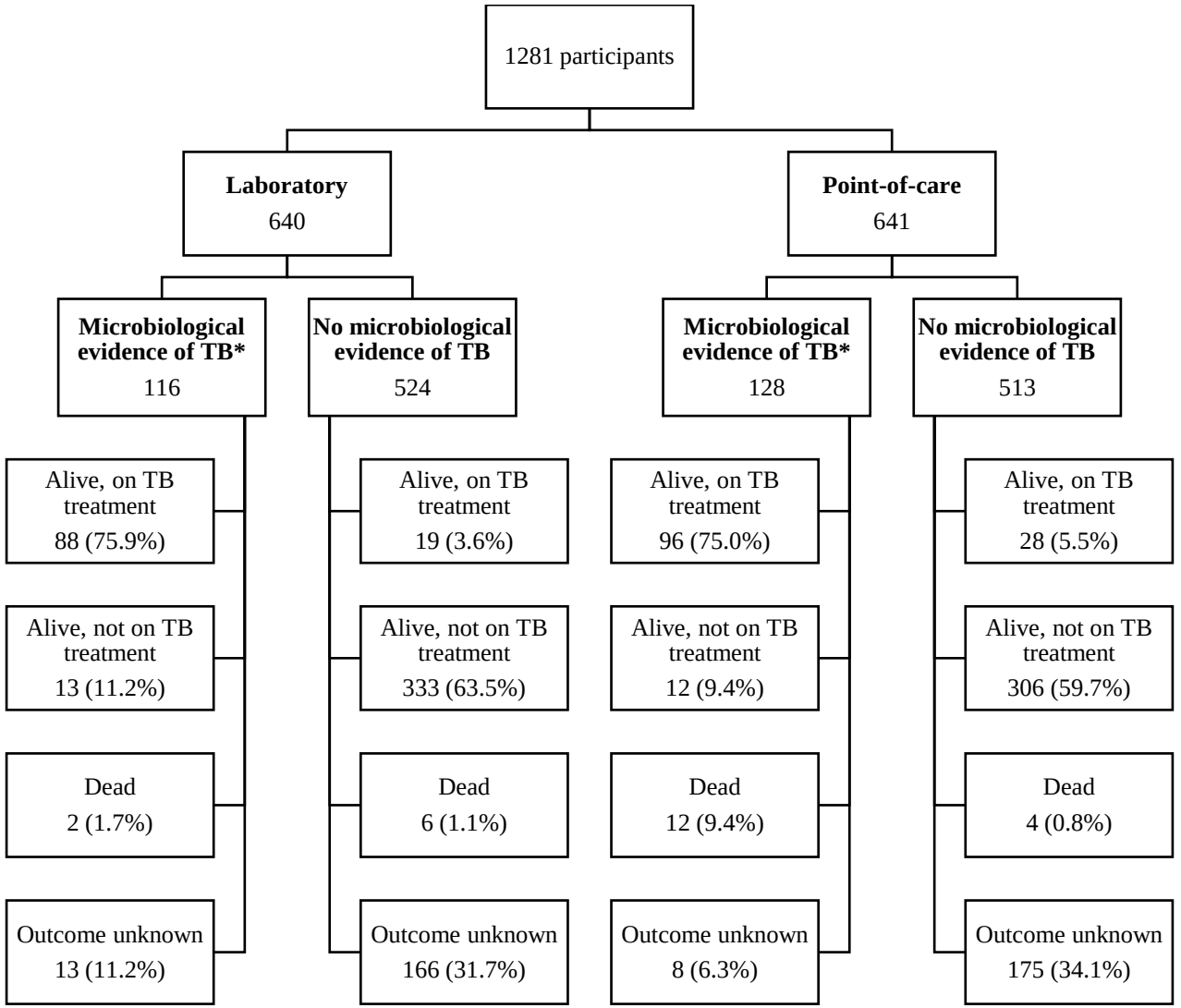
* Repeat test allowed on same specimen or additional specimen

Figure E2 Clinical management algorithm according to Xpert MTB/RIF results for participants currently on TB treatment



* Repeat test allowed on same specimen or additional specimen

Figure E3 Final outcomes at day 60 for all participants, according to study arm and evidence of TB



* Microbiological evidence of TB included positive M. tuberculosis culture and/or positive Xpert MTB/RIF

References

E1. World Health Organization. Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational 'How-to'; practical considerations. Geneva; 2011.

E2. Department of Health RoSA. Multi-drug resistant tuberculosis. A policy framework on decentralised and deinstitutionalised management for South Africa. Pretoria, South Africa: Department of Health; 2011.

E3. Department of Health RoSA. Management of drug-resistant tuberculosis: Policy guidelines. Pretoria: Department of Health; 2011.