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**Impact of the Xpert MTB/RIF tuberculosis diagnostic
system in individuals at high risk of mortality in rural
South Africa**

RICHARD JOHN LESSELLS

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Department of Clinical Research

Faculty of Infectious and Tropical Diseases

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

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Declaration

I, Richard John Lessells, declare that the work presented in this thesis is my own.

Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

I designed the cluster randomised trial and wrote the trial protocol, with scientific input from Peter Godfrey-Faussett, Marie-Louise Newell, Graham Cooke, Nuala McGrath and Mark Nicol. I wrote all the standard operating procedures and data collection tools for the trial. Colin Newell and Mbusiseni Ngema assisted with database design. I trained and supervised the study nurses and research assistants and was responsible for the day-to-day conduct of the trial including data collection and management. Ntando Hlophe and Clifford Makhanya (study nurses) recruited participants into the trial and implemented the trial procedures. I wrote the statistical analysis plan with input from Nuala McGrath. I cleaned the data and conducted the data analysis, with statistical support from Nuala McGrath.



Abstract

This thesis investigates the clinical impact of a point-of-care diagnostic strategy for pulmonary tuberculosis (TB) in a setting at the heart of the TB and human immunodeficiency virus (HIV) epidemics in rural KwaZulu-Natal, South Africa. Although the identification and prompt treatment of active pulmonary TB disease remains the cornerstone of global TB control strategies, weak diagnostic systems contribute to substantial delays and default during the diagnostic process. As new diagnostic technologies are developed, evidence is needed around how best to deliver them within health systems in order to maximize their impact.

The impact of positioning of a molecular diagnostic system (Xpert MTB/RIF) was investigated in a cluster randomised trial. Clusters (two-week time periods) were randomised to one of two strategies: centralised laboratory Xpert MTB/RIF testing or point-of-care Xpert MTB/RIF at the clinic. The trial enrolled 1297 adults with symptoms of pulmonary TB who were HIV infected and/or at high risk of drug-resistant TB. There was some evidence that point-of-care placement shortened the time to initiation of treatment but there was no difference in the overall proportion of culture-positive pulmonary TB cases initiated on appropriate anti-TB treatment within 30 days. Overall mortality was lower than anticipated and, although it was higher with the point-of-care strategy, this effect was not maintained after adjusting for the presence of TB disease and CD4+ T-cell count.

Further analysis suggested that the point-of-care strategy increased the proportion of valid Xpert results from the initial sputum specimen, increased the proportion of

individuals receiving test results and allowed same-day treatment initiation for half of all culture-positive cases that tested positive with Xpert. The diagnostic performance of the Xpert MTB/RIF system was comparable under both strategies. However, delays in initiation of treatment for drug-resistant TB cases and for Xpert-negative/culture-positive cases occurred similarly with both strategies, reducing the potential to detect a real impact on outcomes. Although not a primary focus of the study, the results highlighted deficiencies in the performance of sputum culture, which raise questions about its place as the gold standard diagnostic test.

The development of simple, rapid diagnostics suitable for point-of-care use remains important for TB control in high burden settings. The findings will improve understanding of the key requirements for successful diagnostic strategies and the lessons learnt will help to inform future diagnostic clinical trials. Further research is needed to evaluate how different diagnostic strategies might impact on TB transmission in health care facilities and more broadly in the community.

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List of abbreviations

| | |
|---------|---|
| AFB | Acid-fast bacilli |
| aHR | Adjusted hazard ratio |
| aOR | Adjusted odds ratio |
| ART | Antiretroviral therapy |
| cfu | Colony forming units |
| CI | Confidence interval |
| CONSORT | Consolidated Standards for Reporting Trials |
| CRF | Case report form |
| CRT | Cluster randomised trial |
| Ct | Cycle threshold |
| DNA | Deoxyribonucleic acid |
| DoH | Department of Health |
| DR-TB | Drug-resistant TB |
| DSA | Demographic Surveillance Area |
| DST | Drug susceptibility testing |
| E | Ethambutol |
| H | Isoniazid |
| HIV | Human immunodeficiency virus |
| HR | Hazard ratio |
| IQR | Interquartile range |
| KZN | KwaZulu-Natal |
| LAM | Lipoarabinomannan |
| LED | Light-emitting diode |
| LOD | Limit of detection |
| LPA | Line probe assay |
| MDG | Millennium Development Goals |
| MDR-TB | Multidrug-resistant TB |
| MGIT | Mycobacterial Growth Indicator Tube |
| MODS | Microscopic-observation drug susceptibility |
| MTB | <i>Mycobacterium tuberculosis</i> |
| NGO | Non-governmental organisation |

| | |
|--------|--|
| NHLS | National Health Laboratory System |
| NIMART | Nurse Initiated and Managed Antiretroviral Therapy |
| NPV | Negative predictive value |
| NTM | Non-tuberculous mycobacteria |
| OPD | Outpatient department |
| OR | Odds ratio |
| PCR | Polymerase chain reaction |
| PHC | Primary health care |
| POC | Point of care |
| PPV | Positive predictive value |
| R | Rifampicin |
| RCT | Randomised controlled trial |
| RIF | Rifampicin |
| RR | Relative risk |
| RRDR | Rifampicin resistance determining region |
| S | Streptomycin |
| SA | South Africa |
| SMS | Short message service |
| SNC | Smear non-conversion |
| STARD | Standards for the Reporting of Diagnostic accuracy studies |
| TB | Tuberculosis |
| TST | Tuberculin skin test |
| WHO | World Health Organization |
| XDR-TB | Extensively drug-resistant TB |
| Z | Pyrazinamide |
| ZAR | South African Rand |

Chapter 1 Introduction

1.1 Background

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and other closely related *Mycobacterium* species. Effective antimicrobial chemotherapy that can cure most cases of TB disease has been available for over 50 years. Despite this, every day in 2013 an estimated 25 000 people were diagnosed with TB disease and over 4000 people died as a result of TB disease.[1] The World Health Organization (WHO) declared TB a global public health emergency in 1993.[2] Two decades later, tuberculosis remains one of the ten leading causes of death globally.[3]

The WHO approach to TB control is currently framed within the Stop TB Strategy[4,5]; and more broadly within the Millennium Development Goals (MDGs)[6]: by 2015 to reduce the prevalence of TB and mortality due to TB by 50% compared to 1990 levels. The prevalence target has already been met globally and the mortality target is expected to be met in most regions by 2015. The longer-term target is to eliminate TB as a public health problem by 2050 (defined as annual global incidence of less than one case per million population). Whilst this target may not be achieved with the tools available to us today, elimination could be achieved by 2050 with the parallel development and implementation of new diagnostics, vaccines and drugs.[7,8]

Control of the TB epidemic in sub-Saharan Africa, and particularly in South Africa, has been a particular challenge, due to the co-existent human immunodeficiency

virus (HIV) epidemic and the spread of drug-resistant *M. tuberculosis* strains.[9-13] Approximately one quarter of global TB cases occur in Africa and estimated incidence rates remained higher in 2010 than in 1990.[14] Africa is not on track to meet the MDG TB targets, in particular the target to halve TB mortality by 2015; mortality has fallen at an annual average of only 1.5% between 1990 and 2010.[14]

In South Africa, the combined TB and HIV epidemics have had an enormous impact on population health in the last two decades. TB incidence increased progressively from the 1920's until the 1960's; this was then followed by a modest decline up until 1990.[15,16] TB incidence rates more than trebled between 1990 and 2010 in concert with the explosive HIV epidemic leading to some of the highest population rates of TB disease anywhere in the world (Figure 1-1). In South Africa now almost 1% of the population develop TB disease every year and the country is behind only India and the People's Republic of China in terms of the total number of notified TB cases, with 328 896 cases notified in 2013.[1]

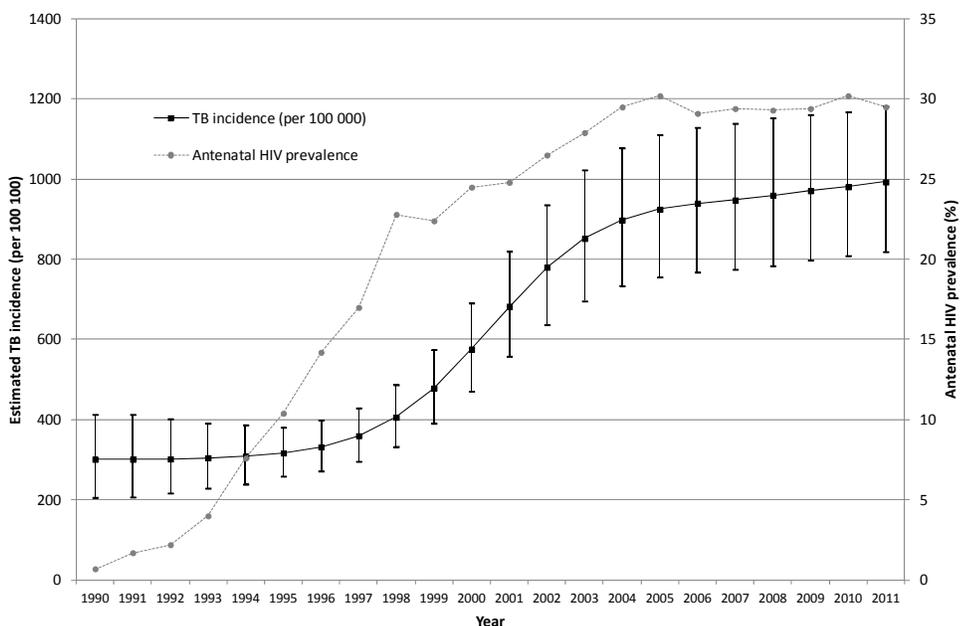


Figure 1-1 Estimated TB incidence and antenatal HIV prevalence in South Africa 1990-2011 [Data sources: WHO Global TB database (<http://www.who.int/tb/country/en/>); South African National HIV and syphilis antenatal sero-prevalence surveys (<http://indicators.hst.org.za/healthstats/13/data>)]

The impact of the co-existent epidemics of TB and HIV has been greatest in KwaZulu-Natal province. This predominantly rural province has the highest HIV prevalence (antenatal prevalence 37.4% in 2011) and the highest TB notification rate (1120 per 100 000 in 2011) in the country. In one rural community in northern KwaZulu-Natal, HIV and TB were estimated together to be responsible for 60.1% of all adult deaths between 2000 and 2010.[17] KwaZulu-Natal is also the epicentre of the drug-resistant TB epidemic, with the emergence of multidrug-resistant (MDR) strains from the 1990s onwards[18,19]; and the later emergence of extensively drug-resistant (XDR) strains, most notably associated with an explosive nosocomial outbreak centred on a single rural district hospital.[20-22]

1.2 TB diagnosis

Given the lack of an effective TB vaccine and the lack of efficacy of population-level prevention strategies,[23,24] TB control at present relies primarily on the identification and treatment of individuals with active TB disease. As *M. tuberculosis* is transmitted primarily by cases with active pulmonary disease, detection of pulmonary TB is the priority for TB control programmes. Early case detection and initiation of appropriate anti-TB therapy is necessary to reduce TB-related mortality and to reduce infectivity in order to interrupt transmission. Evidence from transmission studies suggests that appropriate anti-TB treatment rapidly renders most individuals with pulmonary TB non-infectious within a few days, in both drug-sensitive and drug-resistant disease.[25,26]

Definitive diagnosis of active TB disease requires the culture of *M. tuberculosis* in a clinical sample from the site of disease. As *M. tuberculosis* is a slow-growing organism, culture-based methods are time-consuming and they generally require complex laboratory infrastructure. As a proxy, direct observation of *M. tuberculosis* organisms in sputum by microscopy (sputum smear microscopy) remains the primary diagnostic method in most of the world. However, this diagnostic method has been poorly equipped to control the current TB epidemic in sub-Saharan Africa given its poor sensitivity, particularly in HIV co-infection, and inability to detect drug resistance.[27] Sputum smear microscopy has other limitations, particularly the inability to discriminate between *M. tuberculosis* and non-tuberculous mycobacteria and the inability to determine the viability of observed organisms.

1.3 Positioning of diagnostics

In general, in low- and middle-income countries, people are investigated and treated for TB at primary health care facilities with no supporting on-site laboratory infrastructure. The need for specimens to be sent to centralised laboratories and for patients to return to the facility contributes to delays and default during the diagnostic process, an issue that will be discussed in more detail in chapter 2. The need for simple diagnostic technologies for TB suitable for use at the point of care has been recognised for some time.[28-38] Particularly in the context of the decentralisation and integration of HIV and TB care,[39,40] and even now drug-resistant TB management,[41] South African primary health care services need to be supported by appropriate diagnostic systems.

In the field of HIV, decentralisation of care has been supported by the deployment of simple, rapid point-of-care tests for diagnosis.[30] Furthermore, technologies for point-of-care CD4+ T-cell and HIV RNA monitoring are now being developed and implemented, and already there is some evidence of positive impact.[34,42,43] However, challenges in realising the potential of point-of-care technologies have also been uncovered, for example with a study of point-of-care CD4+ T-cell testing where only 30% of participants received a test on the day of enrolment.[43]

Whilst the benefits of point-of-care strategies might seem clear, there is a need for evidence of impact in different settings, not only to inform implementation of new diagnostics but also to feed back into the development of next generation technologies and systems.

1.4 Project starting points

The development of molecular diagnostics has provided a new opportunity to address some of the deficiencies with systems based on smear microscopy and culture-based diagnostics. Molecular diagnostics are based on the detection of the genetic material of a pathogen (in this case the deoxyribonucleic acid (DNA) of *M. tuberculosis*). Molecular diagnostics for TB have advantages over sputum smear microscopy; in particular improved sensitivity, the ability to differentiate between *M. tuberculosis* and non-tuberculous mycobacteria, and the potential to identify genetic mutations associated with drug resistance. One particular test, the Xpert MTB/RIF assay, was developed with near-patient use in mind.[44-46] This provided the opportunity to evaluate the impact of point-of-care positioning on the diagnosis and treatment of TB.

The framework for the evaluation of diagnostic tests is much less rigorous than for vaccines or drugs.[47-49] In general, evidence of diagnostic test accuracy in the laboratory is sufficient for marketing approval and evidence of impact on patient-relevant outcomes is often not generated. However, diagnostics are different from vaccines and drugs as they do not alter prognosis by themselves but rely on interpretation and appropriate action to be taken based on the test result. Diagnostic tests therefore do not function in isolation but within broader health systems and therefore evaluation of the real world impact requires evidence from well-designed clinical studies with patient-relevant outcomes. Determining the impact of a point-of-care diagnostic strategy has implications for the future development of

diagnostics, not only for tuberculosis but for other infectious diseases of global importance.

1.5 Aims and objectives

The overall aim was to evaluate the impact of a point-of-care diagnostic strategy, using the Xpert MTB/RIF assay, in a rural setting with high levels of TB drug resistance and HIV infection.

The specific objectives were:

- to test the hypothesis that timely initiation of appropriate TB treatment would be improved with the point-of-care strategy (Xpert MTB/RIF positioned at the primary health care clinic) compared to the laboratory strategy
- to evaluate the impact of Xpert MTB/RIF positioning on additional clinical outcomes (time to appropriate TB treatment, mortality, hospital admission, time to initiation of antiretroviral therapy)
- to compare the diagnostic accuracy of the Xpert MTB/RIF system in the two positioning strategies

1.6 Outline of thesis

The introduction to the thesis (chapter 1) describes the current state of the TB epidemic globally and, more specifically, in South Africa. The framework and the aims and objectives for the research are outlined.

In chapter 2, a review of published literature relating to the issue of delays and default during the TB diagnostic process is presented, with a focus on studies from Africa. Specific studies exploring delays and default during the diagnostic process for multidrug-resistant TB are also reviewed. The empirical evidence about interventions to reduce delays and default is also reviewed alongside evidence from mathematical models that have explored the potential impact of alternative TB diagnostic strategies.

Chapter 3 contains a review of the literature to date on the Xpert MTB/RIF assay, exploring the diagnostic performance as well as evidence to date of its impact in programmatic settings.

The methodologies for the design and analysis of the cluster randomised trial are outlined in chapter 4, with a detailed description of the study setting.

The main results from the cluster randomised trial are presented in chapter 5, and the results of the diagnostic accuracy evaluation are presented in chapter 6.

Chapter 7 incorporates discussion of the trial results in the context of other published research and highlights the lessons learnt during the study that are relevant for future diagnostic research.

Chapter 2 TB diagnostic delay and default

Timely initiation of TB treatment is important to improve prognosis at an individual level but also for population-level impact through interruption of TB transmission. Existing diagnostic strategies based around sputum smear microscopy and culture, often in centralised laboratories removed from the point of care, have significant limitations.[27] This has been particularly apparent in sub-Saharan Africa, where the high prevalence of HIV infection and emergence of drug-resistant *M. tuberculosis* strains have exposed the deficiencies of these diagnostics.[50] The poor sensitivity of sputum smear microscopy and the time taken for culture and drug susceptibility testing contribute substantially to diagnostic delay and default, which in turn impacts on individual and population health outcomes.[51]

2.1 TB diagnostic delay

2.1.1 Studies of diagnostic delay in sub-Saharan Africa

Several studies have investigated delays in diagnosis of TB, often with disaggregation of total delay into patient delay and health system delay (Figure 2-1). The results of studies conducted in countries of sub-Saharan Africa where both patient delay and health system delay are reported are shown in Table 2-1.[52-79] Almost exclusively, these studies involve retrospective analysis of TB cases on treatment. There is considerable heterogeneity in the delays reported, at least in part reflecting the different case groups, settings, health system structures, and diagnostic modalities available. Despite this, it is notable that over half of the studies (16/28, 57%) report health system delay longer than the patient delay and a similar majority

(16/28, 57%) describe health system delay of at least four weeks. For the studies exploring pulmonary TB, median overall delay was approximately three months and median health system delay was over 30 days.

Whilst it may seem intuitive to separate delay into patient delay and health system delay, they should not be regarded as unrelated as the underlying factors for delay might in some cases be common to both categories. For example, a poor quality health system might inherently lead to health system delays but also give rise to delays in patients accessing care due to the perceived poor quality. Conversely, factors such as poverty and distance to health facility could lead to delays in patients seeking care but then also further delays during the diagnostic process because of continued problems with health care access.

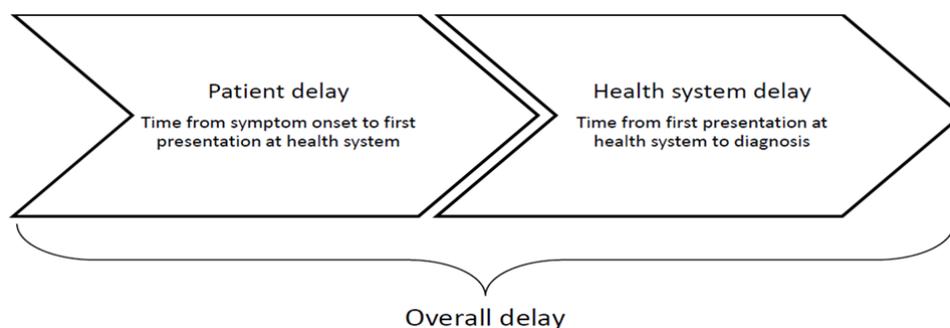


Figure 2-1 Schema for TB diagnostic delay

Another perspective on diagnostic delay is to focus on the number of health facility visits prior to diagnosis and treatment. Relatively few studies have reported on the

number of visits, but those that have reported an average number of visits (median or mean) of between three and four.[52,58,61,68,75] In Malawi, Harries *et al.* defined the maximum number of visits that anyone should make before a TB diagnosis in Malawi as five based on the diagnostic algorithms within national guidelines, yet found that one in three pulmonary TB cases had greater than five visits before diagnosis.[80]

Table 2-1 Studies of TB diagnostic delay from sub-Saharan Africa

| Study | Country | Location | Year | N | Delay | | |
|---|--------------|----------------------------------|--------|-----|-------------------------|------------------------|------------------------|
| | | | | | Overall | Patient | Health system |
| <i>Smear-positive pulmonary TB</i> | | | | | | | |
| Lawn 1998[52] | Ghana | Single teaching hospital | 1995 | 100 | 4 months | 4 weeks | 8 weeks |
| Wandwalo 2000[53] | Tanzania | Urban & rural districts | 1998 | 300 | 136 days | 120 days | 15 days |
| Yimer 2005[54] | Ethiopia | 20 facilities | 2003 | 384 | 80 days | 15 days | 61 days |
| Kiwuwa 2005[55] | Uganda | Single urban referral hospital | 2002 | 231 | 12 weeks | 1 week | 9 weeks |
| Ayuo 2008[56] | Kenya | Referral hospital | 2002-4 | 230 | 44 days | 42 days | 2 days |
| Ngadaya 2009[57] | Tanzania | 16 facilities | 2007 | 226 | 90 days | 62 days | 28 days |
| Sendagire 2010[58] | Uganda | 3 urban PHC clinics | 2007-8 | 242 | 8 weeks | 4 weeks | 4 weeks |
| Lugga 2011[59] | South Sudan | 3 treatment centres | 2007 | 129 | 16 weeks | 4 weeks | 10 weeks |
| Hussen 2012[60] | Ethiopia | 4 facilities | 2011 | 129 | 97 days | 63 days | 34 days |
| Overall* | | | | | 90 days (80-112) | 28 days (28-62) | 34 days (28-61) |
| <i>All pulmonary TB</i> | | | | | | | |
| Steen 1998 [61] | Botswana | Single district | 1993-4 | 212 | 12 weeks | 3 weeks | 5 weeks |
| Pronyk 2001[62] | South Africa | 3 district hospitals | 1999 | 298 | 10 weeks | 4 weeks | 1 week |
| Demissie 2002[63] | Ethiopia | 17 public health centres (urban) | 1998 | 700 | 64 days | 60 days | 6 days |
| Odusanya 2004[64] | Nigeria | Single teaching hospital | 2000-1 | 141 | 14.3 weeks† | 12.3 weeks† | 1.3 weeks† |
| Wondimu 2007[65] | Ethiopia | 13 facilities | 2006 | 197 | 90 days | 28 days | 42 days |
| Ngangro 2012[66] | Chad | 3 hospitals | 2009 | 286 | 57.5 days | 15 days | 36 days |

| Study | Country | Location | Year | N | Delay | | |
|---------------------|--------------|--|---------|-----|------------------------|------------------------|------------------------|
| | | | | | Overall | Patient | Health system |
| Saifodine 2013[67] | Mozambique | 5 clinics | 2009-10 | 622 | 150 days | 61 days | 62 days |
| Ukwaja 2013[68] | Nigeria | 3 rural hospitals | 2011 | 450 | 11 weeks | 8 weeks | 3 weeks |
| Makwakwa 2014 [69] | Malawi | 3 urban TB referral centres | 2011 | 588 | 80 days | 14 days | 59 days |
| Overall* | | | | | 80 days (70-90) | 28 days (21-60) | 35 days (9-42) |
| All forms TB | | | | | | | |
| Lienhardt 2001[70] | The Gambia | 4 facilities (urban & rural) | 1997 | 152 | 8.6 weeks | 0.3 weeks | 8.3 weeks |
| Mesfin 2005[71] | Ethiopia | 16 facilities (hospital/health centre) | 2001-2 | 237 | 99 days | 60 days | 9 days |
| Lorent 2008[72] | Rwanda | Single urban referral hospital | 2006 | 104 | 57 days | 25 days | 28 days |
| Meintjes 2008[73] | South Africa | Single urban referral hospital | 2003 | 104 | 60 days | 14 days | 30 days |
| Verhagen 2010[74] | Tanzania | 1 district hospital | 2008 | 30 | 188 days | 21 days | 26 days |
| Van Wyk 2011[75] | South Africa | 1 urban PHC clinic | 2009 | 210 | 31 days | 8 days | 17 days |
| Belay 2012[76] | Ethiopia | 2 facilities in rural region | 2009-10 | 216 | 70.5 days | 20 days | 33.5 days |
| Lusignani 2013[77] | Angola | 21 facilities | 2008 | 385 | 45 days | 30 days | 7 days |
| Otwombe 2013[78] | South Africa | 3 tertiary hospitals | NS | 891 | 28 days | 28 days | 1 day |
| Yimer 2014[79] | Ethiopia | Single urban referral hospital | 2010 | 201 | 60 days | 21 days | 27 days |
| Overall* | | | | | 60 days (48-68) | 21 days (16-27) | 27 days (11-30) |

PHC, primary health care; PTB, pulmonary tuberculosis

All data for delays from individual studies are medians, unless otherwise stated, and are presented in the time units reported in the study manuscript

* Summary measures of delay for each group of studies are medians (interquartile range)

† Mean

2.1.2 Factors associated with health system delay

The majority of quantitative studies that have assessed factors associated with health system delays have tended to focus on individual socio-demographic and structural health system factors rather than factors specific to diagnostic systems. Amongst the factors consistently associated with longer health system delays are rural (vs. urban) residence, [52,53,60,65,66,79] longer distance to health care facility, [53,54,62,63,66] the first visit at a more peripheral level or outside of the public health system.[54,60,61,68,75-77] In a meta-analysis (which included only two studies), longer distance to health care facility was associated with higher odds of health system delay (unadjusted odds ratio 1.87, 95% CI 1.38-2.53).[81]

Qualitative studies in Zambia and South Africa have highlighted additional factors more specifically related to the diagnostic system.[82,83] The design of the health systems with respect to TB diagnosis, and particularly the need for referrals between different facilities, was identified as a key contributor to diagnostic delays.

Additionally, other characteristics of the health system and health care providers as perceived by individual patients, such as quality of care, waiting times and staff attitudes were recognised as contributing to delays. One of the main contributory factors in diagnostic delay is the cost associated with proceeding through the diagnostic pathway. In urban Zambia and urban Malawi the cost attributable to achieving a TB diagnosis was equivalent respectively to 158% and 248% of mean monthly income.[84,85] The majority of these costs were not direct medical costs (e.g. fees for consultation, diagnostic tests and drugs) but related to lost income, transport to health facilities and food costs during care-seeking episodes.

2.1.3 Impact of diagnostic delay on individual morbidity and mortality

Although it is generally understood that delays in TB diagnosis and treatment initiation lead to poorer individual outcomes, there are actually relatively few empirical data to support this and most retrospective studies of delays suffer from the potential for survival bias when exploring the impact of delays on individual outcomes.

Autopsy studies have consistently demonstrated TB to be the most common cause of death or contributor to death in HIV-infected adults in sub-Saharan Africa.[86-93] In some studies that documented the clinical diagnosis of TB prior to death, around half of those who died from TB were not diagnosed prior to death.[87,92,93] None of these studies specifically documented diagnostic practices prior to death or were able to discriminate patient delays in accessing care from health system and diagnostic delays. Nevertheless, these studies highlight broadly the failure of existing TB diagnostic systems to prevent death in HIV-infected individuals.

In terms of TB disease severity at diagnosis, the evidence of an association with diagnostic delay is quite limited. Again there are clear limitations to retrospective studies and the issue is complicated by the fact that more severe illness may impact on access to care. In rural South Africa, performance status (a measure of general well-being) was worse with increasing overall diagnostic delay, with an increase in median delay from 21 days for fully active individuals (performance status zero) to

90 days for symptomatic individuals unable to carry out work activities or confined to bed or chair (performance status 2-4).[94] In an urban referral centre in Uganda, lung cavitation and sputum smear grade were both associated with diagnostic delay, although the strength of the associations was not reported.[55] In Ghana, conversely, delay of three months or less vs. more than three months was not associated with any difference in the number of radiographic zones involved in pulmonary disease.[52]

In The Gambia, the duration of delay was associated with mortality after treatment initiation, with a five-fold increased relative risk for mortality with delay >8 weeks compared to ≤ 8 weeks, although the number of deaths overall was small ($n = 9$) and the confidence intervals wide (relative risk 5, 95% CI 1.1-24).[70] In that study, the vast majority of the overall delay was reported to be attributable to health system delays as opposed to patient delay. In Limpopo, South Africa, overall delay was associated with mortality (for each month of treatment delay, odds ratio (OR) 1.64, 95% CI 1.23-2.18).[94] This study did not disaggregate delays into patient and health system delays. In the Western Cape province of South Africa, health system delay ≥ 30 days was associated with higher mortality compared to delay <30 days (12% vs. 2%, $p < 0.001$).[73] All of these studies only captured deaths after the initiation of TB treatment and were not designed to identify other deaths during the diagnostic process and so may underestimate the impact of diagnostic delays.

2.1.4 Impact of diagnostic delay on TB transmission

In terms of the impact of diagnostic delay on TB transmission, there are no studies from sub-Saharan Africa specifically exploring this issue. However, there are data addressing this from the United States, China and Yemen.[95-97] These studies used

tuberculin skin test (TST) positivity as a measure of TB infection in contacts of pulmonary TB cases. This has the limitation in this context that TST positivity cannot discriminate between recent and remote exposure to *M. tuberculosis* and so may overestimate the extent of transmission from cases.

In a study of 310 contacts of 54 US-born culture-positive pulmonary TB cases, TST positivity (≥ 5 mm) was associated with overall delay in the index case (TST⁺ 24% with delay < 90 days vs. 40% with delay ≥ 90 days). After adjustment for smear positivity and radiological extent of disease in cases, this association was maintained (for delay ≥ 90 days vs. < 90 days, adjusted odds ratio (aOR) 2.34, 95% CI 1.07 – 5.12). In a parallel group in the same study, there was no association between delays for foreign-born cases and TST positivity in their close contacts, which could be explained by higher levels of baseline TST positivity (indicating latent TB infection) in foreign-born contacts.[95] In a second study from a rural region of southern China, TST positivity (≥ 10 mm) in 1360 household contacts of 393 smear-positive pulmonary TB cases was compared to TST positivity in 308 household contacts of 90 controls without active TB disease. TST positivity was higher in the contacts of TB cases (23.3% vs. 9.7%). With the control contacts as a reference (and after adjustment for age, radiological evidence of cavitation, and sleeping site), aOR for TST positivity was 0.61 (95% CI 0.20-1.87) for delay ≤ 30 days, 1.86 (95% CI 1.20-2.89) for delay 30-60 days, 2.37 (95% CI 1.56-4.11) for delay 60-90 days, and 2.27 (95% CI 1.46-3.63) for delay > 90 days.[96] Conversely, in a study of 505 smear-positive pulmonary TB cases and their household contacts in Yemen, there was no association between delay in TB diagnosis and contact TST positivity (55.0% for delay < 60 days vs. 56.6% for delay ≥ 60 days). This study had significant limitations,

in that contacts from only 18% of the cases were enrolled ($n = 266$), and a further 10% of the contacts enrolled were lost to follow-up between administering and reading the skin test. Also, the relatively high overall TST positivity in a high burden area and low numbers of young children (usually the best indicators of recent transmission) might have limited the ability to detect differences in recent transmission.

In terms of TB transmission within health care facilities, again there are few empirical data on the role of delayed diagnosis. In one study in 17 hospitals in Canada, crude rates of TST conversion among health care workers were higher in hospitals where diagnostic delay was common (defined as more than half of TB patients undiagnosed within 24 hours or more than 30% of TB patients treated after one week or more). However, this association was not maintained after adjustment for overall TB admission rates.[98]

2.1.5 Impact of diagnostic delay on initiation of antiretroviral therapy for HIV-infected individuals

It is now well established that outcomes are improved with early vs. delayed initiation of antiretroviral therapy during anti-tuberculosis treatment for HIV-infected individuals with active TB disease.[99-102] Delays in TB diagnostic processes during the pre-ART period, regardless of the presence or absence of TB disease, could potentially lead to the delayed initiation of antiretroviral therapy and expose individuals to risks of other opportunistic infections and death. A mathematical model suggested that a six-week delay in ART initiation, independent of any TB-associated factors, would increase mortality by around 20%.[103] There

are few data from routine programmatic settings that describe the impact of TB diagnostic delays on ART initiation. One pilot programme in South Africa exploring rapid initiation of ART in pregnancy found that although most women (118/130, 91%) initiated ART on the day that treatment eligibility was determined, waiting for TB culture results was the most common reason for delayed ART initiation (5/12, 42% of those with delay of one week or more).[104]

2.2 TB diagnostic default

One of the major limitations of studies exploring TB diagnostic delay is that they tend to retrospectively evaluate delays for TB cases that start treatment. This has the potential to miss additional drop-out during the diagnostic process, which might also contribute to adverse outcomes and ongoing transmission.

The need for submission of multiple sputum specimens to maximise the diagnostic yield of smear microscopy creates the potential for default during the sputum submission process. International guidelines, until recently, recommended that TB suspects provide sputum specimens for smear microscopy on two consecutive days. Patient drop-out during this diagnostic process, i.e. failure to return on the second day has been reported to be around 7% in studies from Malawi and South Africa,[105,106] but much higher at 42% in a study from urban Uganda.[107] In the most comprehensive study, at primary health care facilities in Uganda, where completion of sputum examination was defined as having at least one positive or at least two negative sputum smears, 22% of suspects defaulted prior to completion.[108] Only one study has explicitly described the proportion of TB suspects that collected results of smear microscopy. In that study from a central

urban hospital in Malawi, 26% (119/453) did not collect their result; the proportion was not significantly different for smear-positive and smear-negative participants (29% vs. 26%).[105]

Another way to explore default is to look at the proportion of patients with a confirmed diagnosis who do not start treatment, often termed primary default. The 14 studies that have addressed this issue for smear-positive pulmonary TB in sub-Saharan Africa are summarised in Table 2-2.[105,106,108-119] Most of these studies were also included in a systematic review and meta-analyses first published in November 2013, during the preparation of this thesis. [120] The highest rates of primary default were reported in two studies located in central urban hospitals (59% in Malawi and 38% in Ghana).[105,116] In the other studies, the proportions were lower but there was still substantial heterogeneity, with primary default ranging from 5 to 27%. The pooled proportion for all studies, determined as a random effects weighted proportion, was 18.3% (95% CI 12.6-23.9). In one South African study that reported separately for smear-positive and culture-positive cases, default was substantially higher amongst the smear-negative, culture-positive cases (11% smear-positive cases vs. 34% smear-negative, culture-positive cases).[119] One additional study reported overall default of 20.6% (95% CI 16.4-25.5) for bacteriologically-confirmed cases (smear-positive and smear-negative, culture-positive cases) but did not report disaggregated data for the smear-negative/culture-positive cases.[121]

Interpretation of the results from most of these quantitative studies is subject to the limitation that results were based on linkage of records in laboratory registers and treatment registers at the same facility and that precise outcomes for all suspects

could not be ascertained. However, in the few studies where tracing of defaulters was attempted, relatively few people were documented to be receiving treatment (23% of those traced in a large, multisite study and 16% of those traced in a smaller study, both in Malawi).[110,112] Substantial proportions of the unregistered smear-positive cases were discovered to have died: in four studies, 35%, 44%, 63% and 79% of those with outcomes ascertained had died.[109,110,112,119] One study from South Africa which used qualitative methods to explore reasons for primary

Table 2-2 Studies of TB diagnostic default from sub-Saharan Africa (smear-positive pulmonary TB cases)

| Study | Country | Location | Year | N | Proportion not registered on treatment (95% CI) |
|----------------------|----------------|--------------------------------|-------------|----------|--|
| Kemp 1996[105] | Malawi | Single urban hospital | NS | 69 | 59.4 (47.6-70.2) |
| Glynn 1998[109] | Malawi | Single rural district | 1986-1994 | 682 | 5.9 (4.3-7.9) |
| Nyirenda 1998[110] | Malawi | 41 hospitals | 1997 | 3482 | 14.4 (13.3-15.6) |
| Creek 2000[111] | Botswana | 15 facilities, single city | 1997 | 127 | 14.9 (9.7-22.3) |
| Squire 2005[112] | Malawi | 32 facilities, single district | 2000 | 157 | 14.6 (9.9-21.1) |
| Dembele 2006[113] | Burkina Faso | 6 districts | 2001 | 31 | 22.9 (4.5-29.5) |
| Botha 2008a[106] | South Africa | 13 facilities, single district | 2004-5 | 367 | 17.4 (13.8-21.7) |
| Botha 2008b[119] | South Africa | 11 facilities, single province | 2005 | 227 | 10.6 (7.2-15.3) |
| Chadambuka 2011[114] | Zimbabwe | 2 districts | 2005 | 112 | 26.8 (19.4-35.7) |
| Davis 2011[108] | Uganda | 5 primary health care clinics | 2009 | 81 | 23.5 (15.5-33.8) |
| Uchenna 2012[115] | Nigeria | 20 facilities, 5 states | 2009 | 323 | 16.9 (13.0-21.2) |
| Afutu 2012[116] | Ghana | Single urban hospital | 2009 | 84 | 38.1 (28.4-48.8) |
| Bristow 2013[117] | South Africa | 24 facilities, single province | 2009 | 794 | 11.7 (9.7-14.1) |
| Claassens 2013[118] | South Africa | 122 facilities, 5 provinces | 2009 | 122* | 25* |

CI, confidence interval; NS, not stated

* Number of facilities (number of individuals not stated); proportion defined as mean across all facilities

default in those that were alive but not on treatment found that health system factors were responsible in the majority of cases: this included results not being available and incorrect information being given at the clinic.[119] Similarly, in Malawi participants and families of those that had died reported delays in receiving results, false reassurance after negative test results, and unaffordable costs of hospital attendance. [112]

2.3 Delay and default during diagnosis of drug-resistant TB

With traditional culture-based diagnostic modalities, lengthy health system delays in diagnosis of drug-resistant tuberculosis are common. As shown in Figure 2-2, most research in this area has been conducted in South Africa, where most studies have documented delays of between 10 and 16 weeks from sputum collection to initiation of MDR-TB treatment.[46,122-131] In the national referral hospital in Tanzania between 2009 and 2011, the time from sputum collection to initiation of MDR-TB treatment was even longer at almost nine months, although this was during the early phase of setting up a national treatment programme.[132]

The one South African site that has reported shorter delays and indeed has documented a trend of reducing delays has been the non-governmental organisation (NGO)-supported community-based programme in Khayelitsha, South Africa. In this programme, the time between sputum collection and initiation of MDR-TB/XDR-TB treatment reduced from a median of 54 days in 2008 to 27 days in 2011.[131] This programme utilised the line probe assay on culture isolates for detection of drug resistance throughout this period so the shorter delays may relate more to improved access to treatment and other structural health system factors as the community-

based treatment programme expanded.[131] Further evidence of the benefit of decentralisation of drug-resistant TB management was shown in two studies from KwaZulu-Natal where delays were significantly shorter in decentralised compared to centralised treatment strategies (84 days vs 106 days and 72 days vs. 93 days).[122,124]

Given these long delays with drug-resistant TB and with the knowledge that, in one observational study from KwaZulu-Natal,[126] the median survival from sputum collection for MDR-TB cases was 60 days, it is not surprising that there is substantial attrition prior to treatment (Table 2-3). Over an eight year period (2002-2010) in one district of Western Cape Province, South Africa, 34% (256/747) of laboratory-confirmed MDR-TB cases did not start appropriate drug-resistant TB treatment.[133] Similarly in 2011 in Gauteng province, South Africa, 37% of the laboratory-confirmed MDR-TB cases did not start treatment: 12% died and 25% were lost to follow-up prior to the initiation of appropriate treatment.[134] The NGO-supported programme in the Khayelitsha township of Cape Town again has reported better results, with only 14% of laboratory-confirmed MDR/XDR-TB cases between 2008 and 2011 not starting drug-resistant TB treatment, largely due to death (8% overall).[131]

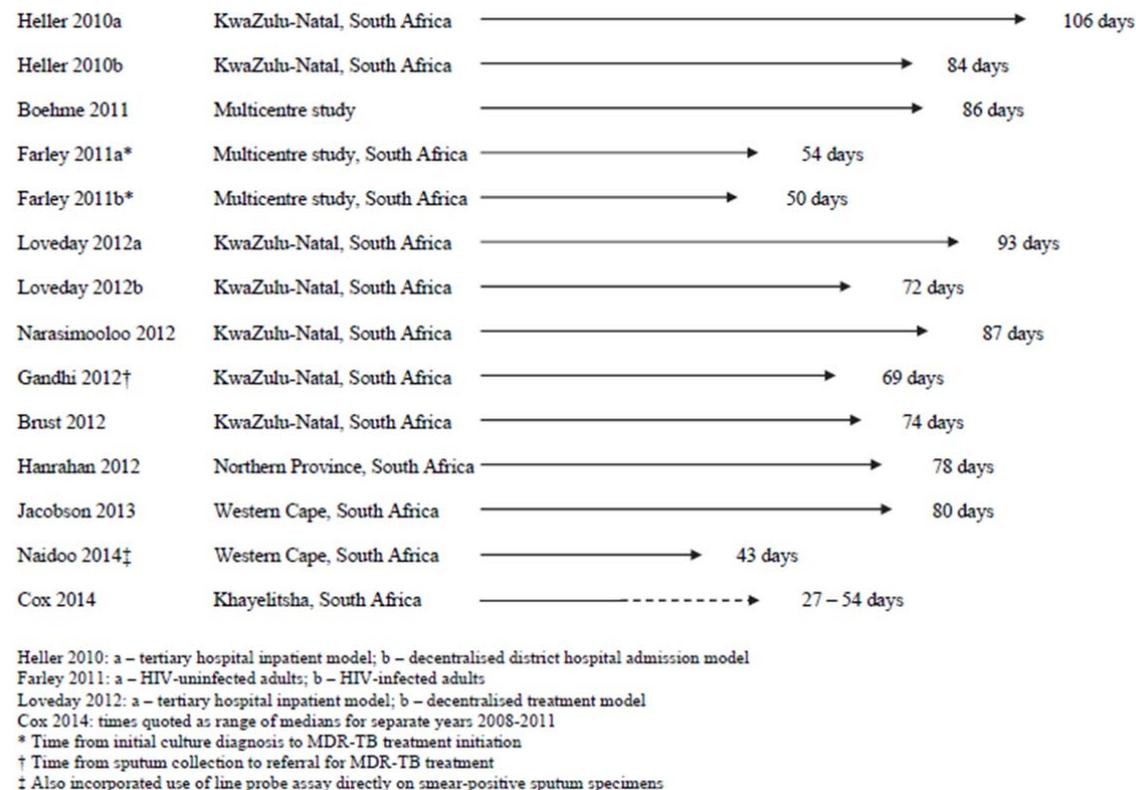


Figure 2-2 Diagnostic delay for multidrug-resistant tuberculosis (studies from sub-Saharan Africa with culture-based diagnostics). All times are from sputum collection to MDR-TB treatment initiation unless otherwise stated

The impact of treatment delays on treatment outcomes for drug-resistant TB has not been well studied. Globally, treatment outcomes for MDR-TB are poor: meta-analyses have estimated overall treatment success (defined as cure or treatment completion) of 54-62%. [135-137] In South Africa, treatment outcomes seem to be quite similar across different programmes, with treatment success in 44-49% of MDR-TB cases. [123,131,133,138,139] The only other study from sub-Saharan Africa to report final treatment outcomes for MDR-TB cases was an NGO-supported integrated TB/HIV programme in Lesotho, which demonstrated treatment success in 62%. [140] Although studies have not formally explored the impact of pre-treatment delay on MDR-TB outcomes, there is evidence from Africa and other settings that sputum smear positivity increases the risk of mortality, [141,142] and that lung cavitation increases the risk of treatment failure. [142]

2.4 Diagnostic strategies to reduce delays and default

2.4.1 Alternative sputum collection strategies for microscopy

To address the issue of patient drop-out during the diagnostic process, alternative strategies for sputum sampling have been explored. ‘Spot’ specimens are collected when a patient is at a health care facility whereas ‘morning’ specimens are collected in the early morning, often at home, on the basis that they tend to have a higher yield than specimens collected at other times. [143] A large cluster randomised trial conducted in Ethiopia, Nigeria, Nepal and Yemen compared the standard two-day ‘spot-morning-spot’ scheme for collecting three specimens to an alternative strategy

of ‘spot-spot-morning’ (referred to as frontloading).[144] Sensitivity and specificity against the reference standard of solid culture were comparable under the two schemes. Diagnostic default was reduced by frontloading - a greater proportion of suspects provided the first two samples in the frontloading scheme than in the standard scheme (97.6% vs. 94.2%, $p < 0.01$).

Separate analyses have also been conducted on single-country data from the same cluster randomised trial. For 243 suspects in Ethiopia, two sputum specimens collected on the first day had a similar yield (for diagnosis based on at least one positive smear) to two specimens collected on consecutive days (94.2% vs. 98.1%, $p > 0.5$).[145] For 224 suspects in Nigeria, two sputum specimens collected on the first day had similar sensitivity to the standard ‘spot-morning-spot’ approach (56.4% of culture-positives vs. 57.7%, $p > 0.5$).[146] These results were collated in a meta-analysis and informed a change in WHO policy to recommend collection of two spot specimens from the same day.[147,148]

A more recent study has taken this single-day approach further and compared the yield from two smears done from a single sputum specimen to two smears from two samples collected in a ‘spot-morning’ approach.[149] The sensitivity using standard light microscopy (with culture as the reference standard) was 55% from the single specimen and 56% from the two specimens. The use of light-emitting diode (LED) microscopy further improved the sensitivity of both approaches but there remained no significant difference between the one specimen and two specimen approaches (61% vs. 64%).

Table 2-3 Pre-treatment default for multidrug-resistant TB cases diagnosed through culture-based methods

| Study | Country | Location | Year | N | Proportion not registered on treatment (95% CI) | Comments |
|--------------------|----------------|-----------------|-------------|----------|--|---|
| Shean 2008 [133] | South Africa | Western Cape | 1992-2002 | 747 | 34.3 (31.0-37.8) | 144 (19%) died/defaulted prior to treatment |
| Ebonwu 2013 [134] | South Africa | Gauteng | 2011 | 942 | 37.0 (34.0-40.2) | 109 (12%) died prior to treatment |
| Naidoo 2014* [130] | South Africa | Western Cape | 2008-2011 | 414 | 9.4 (7.0-12.6) | |
| Cox 2014 [131] | South Africa | Khayelitsha | 2008-2011 | 874 | 13.7 (11.6-16.2) | 73 (8%) died prior to treatment |

CI, confidence interval

* Also incorporated line probe assay testing directly on smear-positive sputum specimens

These approaches to simplify the diagnostic process have the potential to reduce default during the diagnostic process. However, there is no empirical evidence that such strategies lead to more TB cases initiating treatment in a timely fashion. A simple decision analysis model suggested that same-day sputum collection on its own would have relatively limited impact on treatment initiation, whereas the addition of same-day testing and result provision could potentially have a greater impact, through reduction in diagnostic default.[150] Despite the fact that sputum microscopy can be performed at peripheral levels of the health system and, at least in theory, can be performed on the same day as sputum collection, there have been no studies exploring the impact of same-day point-of-care microscopy.

2.4.2 Point-of-care diagnostics

In high HIV prevalence settings, chest X-ray is commonly used for diagnosis of smear-negative pulmonary tuberculosis. The impact of a digital X-ray service placed at an urban health centre in Zambia was evaluated with a ‘before-after’ study design using routine notification data.[151] Prior to the introduction of the point-of-care digital X-ray service, smear-negative patients suspected of having TB would be referred to a tertiary referral centre for chest X-ray. In the period after the introduction of the digital X-ray service at the health centre, there was a reduction in treatment delay (defined as treatment initiation ≥ 7 days from sputum submission) for all TB patients from 18.2% to 13.2% (adjusted odds ratio 0.6, 95% CI 0.4-0.9), without any increase in the proportion of X-rays performed. Although there are limitations to diagnosis based on chest X-rays, in that microbiological confirmation

is not obtained, this provided some evidence of the potential impact of point-of-care diagnostic strategies.

2.4.3 Traditional culture-based techniques

Mycobacterial culture is considered the gold standard for the laboratory diagnosis of active *M. tuberculosis* disease. The introduction of culture into diagnostic algorithms has the potential to increase the diagnostic yield compared to microscopy alone, especially in HIV-infected individuals.[152-155] However, evidence for the programmatic impact of culture-based technologies is relatively weak.

Nested within the TB/HIV in Rio (THRio) study of isoniazid preventive therapy, one study investigated the impact of culture within a screening algorithm in 217 HIV-infected adults.[156] There were 33 cultures positive for *M. tuberculosis* and an additional 17 positive for non-tuberculous mycobacteria (NTM). Of the smear-negative, culture-positive cases, 74% (17/23) started TB treatment but more than one-third of these (6/17) had started treatment on clinical grounds before the availability of the culture result. There were no details on precise timings of treatment in this study. A comparable number of individuals with a negative culture (16/167) were commenced on treatment on clinical grounds.[156] These cases might reflect inappropriate treatment of people without TB disease, although it is recognised that some cases of active pulmonary TB will have culture-negative disease and this study did not seek to determine the appropriateness of treatment in these cases.[157,158]

In a retrospective study of 150 sputum culture-positive and 150 culture-negative individuals from a tertiary hospital in southern India, the turnaround time for solid culture (Lowenstein-Jensen media) was 55 days and turnaround time for phenotypic drug susceptibility testing (DST) was 109 days.[159] Only two cases initiated treatment on the basis of the culture result; the majority of the culture-positive cases had already been commenced on treatment prior to availability of the culture result. Furthermore, although 25% (30/119) of those with DST results had evidence of first-line anti-TB drug resistance, only four individuals had their treatment altered on the basis of the DST result.

The one randomised controlled trial (RCT) to compare a diagnostic strategy of sputum smear microscopy against an intensified strategy of sputum smear microscopy plus liquid culture was performed in Tanzania.[160] This was a small study which in retrospect was underpowered, with only 47 patients analysed for the primary outcome of correct treatment at 8-week follow-up. There were also complex methodologies to evaluate the primary endpoint, as the presence or absence of TB disease incorporated not only microbiological results but also independent clinical assessment. Correct treatment was more likely at eight weeks with the intensified strategy than with standard strategy, although this did not reach statistical significance (100% vs. 88%, $p = 0.14$). All TB cases in both arms were on appropriate treatment; the difference was due to three participants without TB disease being on TB treatment at eight weeks in the standard arm. The small numbers and the fact that 36% of participants died or were lost to follow-up prior to eight weeks limit any major conclusions from the data.

The available evidence would therefore suggest that, although culture-based techniques improve diagnostic yield for tuberculosis, the real world impact of this on TB treatment may be limited, especially where treatment based on clinical and radiological grounds is common. Given the delays inherent with culture-based techniques, it is unsurprising that these do not reduce delays and default prior to treatment. Indeed there is some evidence (discussed in section 2.2) that default prior to treatment is higher for cases positive only on culture, suggesting that as the separation in time from specimen collection to result expands, the risk of default increases. It is plausible that the main programmatic impact of culture-based methods has been more restricted to enabling the detection and appropriate treatment of drug-resistant TB disease.

2.4.4 Microscopic-observation drug susceptibility (MODS) assay

The microscopic-observation drug susceptibility (MODS) assay is a low-cost and rapid culture method which involves direct observation by microscopy of *M. tuberculosis* growth in liquid culture medium and allows for direct drug susceptibility testing by observation of the growth of the organism in the presence of certain anti-TB drugs.[161,162] Numerous studies have demonstrated good diagnostic accuracy in a variety of different settings and have consistently shown that results can be obtained in significantly shorter time than with conventional culture and DST methods.[162-175] In a meta-analysis of nine studies, the mean turnaround time for MODS was 9.9 days.[176] One large study in Peru reported an initial time to detection of *M. tuberculosis* of seven days by MODS compared to 13 days for liquid culture and 26 days for solid culture. Moreover, the overall time to detection of drug resistance was also seven days by MODS compared to 22 days by

the automated liquid culture system (MB/BacT) and 68 days by indirect DST on solid culture media (proportion method).[163] In a study from an area with high levels of drug-resistant TB in KwaZulu-Natal, time to diagnosis of MDR-TB was seven days by MODS compared to 70 days by liquid or solid culture with indirect DST.[174] Neither of these studies investigated the impact on actual diagnosis and treatment of TB and MDR-TB as results from the MODS assay were not used for patient management.

In 2011, the World Health Organization recommended the use of MODS but only in specific conditions, namely in reference laboratories under strict laboratory protocols, and this was seen as an interim solution while capacity for liquid culture and genotypic methods expanded. [177] The limited approval was also based on the fact that conventional culture/DST would still be required to detect additional resistance (i.e. XDR-TB) and that biosafety level 2 facilities would be required, thus generally requiring use in centralised laboratories. The approval of MODS was largely on the basis of the diagnostic accuracy data and again there were limited data regarding impact on patient-relevant outcomes. One study in Peru did explore the impact of MODS, by determining outcomes for patients in a programme where physicians had access to MODS testing as part of routine patient care in addition to conventional culture methods.[178] In this study, 63 individuals had a positive MODS culture, and 14 (22%) of those had MDR-TB. MODS provided the first confirmation of TB diagnosis in 44 of 58 cases (76%) with complete clinical information and the first drug susceptibility information in 48 cases (83%). Although the MODS result should have led to a change in patient management in 24 cases, the appropriate change happened in only 16 of those cases (67%). Furthermore, lengthy

times to initiation of treatment (42 days from the positive MODS result) and to change of treatment in drug-resistant cases (48 days) offset any potential benefit from the rapid diagnosis. This illustrates the fact that theoretical benefits of diagnostics may not translate into positive impact when implemented in real world TB programmes.

2.4.5 Line probe assays

Line probe assays (LPA) are molecular diagnostic tests for the rapid detection of drug resistance that can be performed on smear-positive specimens or on culture isolates of *M. tuberculosis*. Line probe assays were recommended for implementation by the World Health Organization in 2008 on the basis of studies showing good diagnostic accuracy, comparable to culture-based phenotypic DST.[179] Several studies from different settings have since supported the evidence of good diagnostic performance.[180-190] Again, however, there are relatively few data describing the impact on patient-relevant outcomes.

One pre- and post-implementation study in Delhi, India compared the use of solid culture and phenotypic DST ($n = 51$) to the LPA (Genotype MTBDR*plus* assay), used on smear-positive and culture-positive samples ($n = 83$).[191] In this study, the proportion of cases diagnosed with MDR-TB that initiated treatment increased from 61% with culture/DST to 88% with the use of LPA. For those that commenced MDR-TB treatment, the median time from sputum collection to initiation of treatment was reduced from 157 days with solid culture & DST to 38 days with LPA ($p < 0.001$), predominantly due to a reduction in total laboratory time from 107 days to 5 days ($p < 0.0001$). With the use of LPA, the laboratory time only accounted for

13% of the overall time to treatment initiation and delays in submission of samples, reporting results, and initiating treatment became relatively more important.

In a similar quasi-experimental pre- and post-LPA implementation study in Tbilisi, Georgia, 72 smear-positive MDR-TB cases diagnosed using culture & phenotypic DST were compared with 80 cases diagnosed using the Genotype MTBDR*plus* assay directly on sputum.[192] The group diagnosed after the introduction of LPA had a shorter time to commencing MDR-TB treatment (18.2 vs. 83.9 days, $p < 0.01$), shorter time spent on a drug-susceptible TB ward (10.0 vs. 58.3 days, $p < 0.01$), and a lower likelihood of receiving a first-line drug regimen, which could lead to amplified drug resistance (78% vs. 99%, $p < 0.01$). There was also some evidence that treatment outcomes were improved following the implementation of the LPA, with a higher proportion of cases achieving sputum culture conversion after 24 weeks of treatment (86% vs. 63%, $p = 0.01$). The association between LPA use and sputum culture conversion was maintained after adjustment for age, prior history of TB and the presence of ofloxacin resistance (adjusted hazard ratio [aHR] 4.24, 95% CI 2.7-6.8).

In a study of 42 MDR-TB cases managed at a single centre in the United Kingdom over a 22-year period, the use of a line probe assay (INNO-LiPA) directly on smear-positive sputum specimens, compared to conventional culture/DST, shortened the time to diagnosis of MDR-TB from 51 days to 9 days.[193] Another small study of MDR-TB suspects in Latvia, comparing outcomes after implementation of the INNO-LiPA assay ($n = 23$) to a historical cohort using liquid culture/DST ($n = 48$), found that the time to treatment initiation for rifampicin-resistant cases was shorter

with implementation of the LPA (median 14 days vs. 40 days).[194] There was no difference between the two groups in final treatment outcomes (cure achieved in 52% in the LPA group vs. 60% in the liquid culture group), although the small number would have limited the power to detect any difference.

In one before-after study from Northern Province in South Africa, although the introduction of the MTBDR*plus* assay shortened the laboratory turnaround time from a median of 52 days to 26 days, there was a fairly modest reduction in the overall time from sputum collection to MDR-TB treatment initiation (median 78 days pre-implementation (IQR 52-93) vs. 62 days post-implementation (IQR 32-86), $p = 0.05$).[128] In a retrospective study from Western Cape, South Africa, also exploring outcomes prior to and following implementation of the Genotype MTBDR*plus* in routine practice, there was a somewhat greater reduction from median 80 days (IQR 62-100) to 55 days (IQR 38-78).[129]

Collectively these studies highlighted that, especially with drug-resistant tuberculosis, other health system factors contribute substantially to the delays and that the impact of new diagnostics may depend to a great extent on the wider functioning of the health system.[195]

2.5 Potential impact of new tuberculosis diagnostics

It was postulated a few years ago that a test more sensitive than sputum microscopy for TB would be the diagnostic intervention which would alleviate the greatest burden of infectious disease in the developing world.[196] More specifically, one

mathematical model based on the four WHO regions with the highest number of TB deaths annually (Africa, Eastern Mediterranean, Southeast Asia and Western Pacific) suggested that a new rapid diagnostic test with 100% sensitivity and 100% specificity could prevent 359 000 TB deaths annually (approximately one quarter of all TB deaths), relative to smear microscopy, if implemented within existing health care infrastructure.[197] If the test was universally accessible through new delivery channels, requiring no infrastructure, then the impact could be even greater with 625 000 deaths prevented annually (equivalent to 36% of all TB deaths based on 2004 data). With this model, the impact of loss to follow-up within the diagnostic process was particularly marked, with a reduction in the lives saved by almost half with 20% loss to follow-up (failure to provide all samples or to return for results) under the universal access scenario.

Other models have derived fairly consistent estimates of mortality reductions of 17-23% from a more sensitive rapid TB diagnostic, despite exploring different epidemics and different timescales.[198-200] In one model the estimated reduction in mortality achievable by implementation of a new diagnostic test was equivalent in magnitude to that expected from a novel vaccine or an optimised 2-month treatment regimen for active disease.[198] One model based on the TB epidemic in Tanzania demonstrated that the impact of Xpert MTB/RIF on TB incidence would be greater if there was a reduction in diagnostic delays and default and if it improved access to care.[201] Another model has highlighted that improved diagnostic sensitivity in itself has the most significant impact on diagnostic delays, suggesting that a 10% increase in test sensitivity could lead to a 3-5 day reduction in diagnostic delay.[51]

2.6 Summary

Delays in tuberculosis diagnosis are common in Africa and health system delays contribute as much if not more than patient delays, although this differs considerably depending on the setting and the diagnostic systems in place. Furthermore, a substantial proportion of individuals (around one in five) diagnosed with TB do not start treatment, although the reasons for this have not been fully elucidated. Although historically there has been a tendency to consider patient-related factors responsible for delays and default, the evidence suggests a major role for health system factors.[202,203] The problems of delays and default are inherent in diagnostic systems reliant on tests with poor sensitivity that are removed from the point at which patients access care. The problems of delay and default are magnified in the context of drug-resistant TB disease due to laboratory delays with culture-based diagnostics. Although there is limited direct evidence of the consequences of delay and default, it is highly plausible that they impact adversely on individual outcomes and contribute to ongoing TB transmission.

Whilst diagnostic technologies and strategies have been developed with the potential to address the problems of delay and default, there is actually little empirical evidence of real world impact. This is at least partly due to the paucity of high quality diagnostic research studies. Mathematic modelling has highlighted that whilst a diagnostic test with better sensitivity than smear microscopy could have a significant impact on TB mortality and TB incidence, the impact would be greatest if used within a strategy whereby access to care was good and delays and default were minimised.

Chapter 3 Xpert MTB/RIF assay

3.1 Overview

The development of molecular tools, in particular the GeneXpert system and Xpert MTB/RIF assay, offers a new opportunity to tackle some of the problems associated with smear microscopy and culture-based diagnostic systems. It is based on a semi-quantitative in-vitro polymerase chain reaction (PCR) and uses molecular beacon technology for the detection of *M. tuberculosis* complex and rifampicin resistance (through detection of mutations in *rpoB* gene).[204-210] It is an automated, closed cartridge system with results available within two hours. A key feature of the assay is the low bioaerosol infection risk which suggests that the test could be used outside the normal laboratory setting and without bio-safety facilities.[211] Further details of the system are given in Appendix A.

3.2 Analytic performance

Analytic studies were performed using the Xpert MTB/RIF assay on twenty sputum samples spiked with known quantities of *M. tuberculosis*. [44] The limit of detection (LOD) based on these studies was 131 colony forming units (cfu)/ml (95% CI 106.2-176.4). This represents a LOD almost comparable to culture (10-100 cfu/ml) and significantly more sensitive than sputum smear microscopy (~10 000 cfu/ml).[212-214] Additional studies with similarly spiked sputum samples found that the assay detected *M. tuberculosis* in all samples containing 10^3 - 10^7 cfu/ml and in five of six samples containing 100 cfu/ml.[215] The ability to detect genetically different *M. tuberculosis* strains has been tested and the assay detected all 79 distinct strains selected for testing.[44] Specificity was investigated using twenty different non-

tuberculous mycobacterial (NTM) species and 89 other respiratory tract pathogens (bacteria, fungi and viruses). No significant cross-reaction was seen with the NTM species or with other respiratory tract pathogens.[44,215]

3.3 Diagnostic accuracy for detection of *Mycobacterium tuberculosis*

The diagnostic accuracy of the Xpert MTB/RIF assay has now been reported from a number of different studies in a variety of different settings. The performance of a single Xpert MTB/RIF assay for the detection of *M. tuberculosis* in sputum and other respiratory samples (such as fluid from bronchoalveolar lavage) is summarised in Tables 3-1 & 3-2 and Figures 3-1 to 3-4.[44-46,216-248] Excluded from these summaries are studies involving only paediatric populations[249-255]; studies involving only non-respiratory specimens (or where results for respiratory and non-respiratory specimens were not reported separately)[256-269]; studies where specimens were pre-screened by smear microscopy or another PCR method[270,271]; and studies where culture was not the reference standard or where there was no formal reference standard.[272,273] In 27 prospective studies utilising fresh collected sputum samples and where full results were presented, the pooled sensitivity of a single Xpert compared to a reference standard of *M. tuberculosis* culture (calculated as a random effects weighted proportion) was 87% (95% CI 86-88) and specificity was 98% (95% CI 98-98).[45,46,216-240] There was substantial heterogeneity in sensitivity between studies, partly reflecting the different study populations and the different specifications for the culture reference standard. The pooled sensitivity for smear-positive specimens (from 19 studies) was 98% (95% CI 97-98) and for smear-negative specimens (from 20 studies) was 70% (95% CI 67-73).

Table 3-1 Diagnostic accuracy of a single Xpert MTB/RIF test for detection of *M. tuberculosis* in fresh sputum or other respiratory specimens from prospectively enrolled TB suspects

| Study | Location | Specimens | N | Reference standard | Sensitivity | | | Specificity |
|---------------------|--------------|--------------------------------------|------|-----------------------------------|--------------------|-------------------|-------------------|---------------------|
| | | | | | Overall | Smear positive | Smear negative | |
| Boehme 2010[45] | Multicentre | Sputum | 1341 | Liquid (x2) or solid (x2) culture | 92.2 (675/732) | 98.2 (551/561) | 72.5 (124/171) | 99.2 (604/609) |
| Boehme 2011[46] | Multicentre | Sputum | 3909 | Culture‡ | 90.3 (933/1033) | 98.3 (637/648) | 76.9 (296/385) | 99.0 (2846/2876) |
| Bowles 2011[216] | Netherlands | Respiratory specimens | 88 | Liquid culture | 93.8 (60/64) | 100 (40/40) | 83.3 (20/24) | 95.8 (23/24) |
| Ioannidis 2011[217] | Greece | Respiratory specimens | 66 | Liquid or solid culture | 90.6 (29/32) | - | - | 94.1 (32/34) |
| Lawn 2011[218]* | South Africa | Sputum (including induced specimens) | 445 | Liquid culture | 73.3 (55/75) | 100 (21/21) | 63.0 (34/54) | 99.2 (367/370) |
| Malbruny 2011[219] | France | Sputum | 18 | Liquid or solid culture | 100 (5/5) | 100 (4/4) | 100 (1/1) | 100 (13/13) |
| Scott 2011[220] | South Africa | Sputum | 177 | Liquid culture | 86 | - | - | 97 |
| Teo 2011[221] | Singapore | Respiratory specimens | 122 | Liquid or solid culture | 90.3 (56/62) | 100 (43/43) | 68.4 (13/19) | 91.7 (55/60) |
| Al-Ateah 2012[222] | Saudi Arabia | Respiratory specimens | 172 | Liquid or solid culture | 95.5 (42/44) | - | - | 100 (128/128) |
| Balcells 2012[223]* | Chile | Sputum | 160 | Liquid or solid culture | 91.7 (11/12) | 100 (8/8) | 75.0 (3/4) | 99.3 (147/148) |
| Barnard 2012[224] | South Africa | Sputum | 68 | Liquid culture | 71.2 (37/52) | 90.5 (19/21) | 58.1 (18/31) | 100 (16/16) |

| Study | Location | Specimens | N | Reference standard | Sensitivity | | | Specificity |
|-----------------------|--------------|-----------------------|-----|-------------------------|-------------------|-------------------|-----------------|-------------------|
| | | | | | Overall | Smear positive | Smear negative | |
| Carriquiry 2012[225]* | Peru | Sputum | 131 | Liquid or solid culture | 97.8 (44/45) | 100 (31/31) | 92.9 (13/14) | 97.7 (84/86) |
| Ntinginya 2012[226]† | Tanzania | Sputum | 33 | Liquid or solid culture | 100 (5/5) | 100 (3/3) | 100 (2/2) | 100 (28/28) |
| O'Grady 2012[227] | Zambia | Sputum | 643 | Liquid culture | 86.1 (173/201) | 96.2 (102/106) | 74.7 (71/95) | 95.7 (420/442) |
| Yoon 2012[228] | Uganda | Sputum | 436 | Liquid or solid culture | 78.9 (187/237) | 98.1 (153/156) | 42.0 (34/81) | 95.5 (190/199) |
| Al-Darraj 2013[229] | Malaysia | Sputum | 125 | Liquid culture | 53.3 (8/15) | - | - | 100 (110/110) |
| Bates 2013[230] | Zambia | Sputum | 94 | Liquid culture | 80.8 (21/26) | NR | NR | 97.1 (66/68) |
| Bunsow 2013[231] | Spain | Respiratory specimens | 290 | Liquid or solid culture | 97.1 (67/69) | 100 (60/60) | 71.4 (5/7) | 98.6 (218/221) |
| Hanrahan 2013[232] | South Africa | Sputum | 551 | Liquid culture | 65.6 (42/64) | - | - | 99.6 (487/489) |
| Kurbatova 2013[233] | Russia | Sputum | 226 | Liquid or solid culture | 95.3 (102/107) | 100 (91/91) | 68.8 (11/16) | 86.0 (104/121) |
| Kwak 2013[234] | South Korea | Sputum | 661 | Liquid or solid culture | 79.5 (124/156) | 88.9 (56/63) | 73.1 (68/93) | 100§ (505/505) |
| Park 2013[235] | South Korea | Respiratory specimens | 320 | Liquid or solid culture | 67.9 (19/28) | 66.7 (10/15) | 69.2 (9/13) | 97.9 (286/292) |
| Van Rie 2013[236] | South Africa | Sputum | 158 | Liquid culture | 66.7 (10/15) | 100 (3/3) | 58.3 (7/12) | 99.3 (142/143) |

| Study | Location | Specimens | N | Reference standard | Sensitivity | | | Specificity |
|---------------------|-------------|-----------------------|-----|-------------------------|-------------------|----------------|-----------------|-------------------|
| | | | | | Overall | Smear positive | Smear negative | |
| Walusimbi 2013[237] | Uganda | Sputum | 369 | Liquid culture | 48.8 (21/43) | - | 48.8 (21/43) | 95.1 (310/326) |
| Zmak 2013[238] | Croatia | Respiratory specimens | 118 | Liquid or solid culture | 85.7 (6/7) | 100 (3/3) | 75.0 (3/4) | 100 (111/111) |
| Sohn 2014[239] | Canada | Induced sputum | 457 | Liquid culture | 45.8 (11/24) | 85.7 (6/7) | 29.4 (5/17) | 99.8 (432/433) |
| Theron 2014[240] | Multicentre | Fresh sputum | 729 | Liquid culture | 83.2 (154/185) | - | - | 95.1 (517/544) |

* HIV-infected TB suspects only

† Household contacts of index cases with smear-positive pulmonary TB; not clear if all symptomatic

‡ Number and type of culture (liquid/solid) varied according to centre

§ 20 Xpert positive/culture negative cases excluded from diagnostic accuracy estimations (due to clinical and radiological features of TB)

|| Only smear-negative suspects included in study

Table 3-2 Diagnostic accuracy of a single Xpert MTB/RIF for the detection of *M. tuberculosis* in stored sputum or other respiratory specimens

| Study | Location | Samples | N | Reference standard | Sensitivity | | | Specificity |
|----------------------|--------------|--|------|-------------------------|-------------------|-----------------|-----------------|--------------------|
| | | | | | Overall | Smear positive | Smear negative | |
| Helb 2010[44] | Vietnam | Frozen sputum | 107 | Liquid or solid culture | 81.7 (67/82) | 100 (29/29) | 72 (38/53) | 100 (25/25) |
| Armand 2011[241] | France | Frozen respiratory specimens | 117 | Liquid or solid culture | 78.6 (44/56) | 100 (28/28) | 57.1 (16/28) | 100 (10/10) |
| Marlowe 2011[242] | US | Fresh and frozen respiratory specimens | 216 | Culture* | 89.2 (116/130) | 97.7 (85/87) | 72.1 (31/43) | 95.3 (82/86) |
| Miller 2011[243] | US | Frozen respiratory specimens | 89 | Liquid or solid culture | 93.1 (27/29) | 100 (24/24) | 60.0 (3/5) | 96.7 (58/60) |
| Moure 2011[244] | Spain | Frozen respiratory specimens | 105 | Liquid or solid culture | 78.2 (61/78) | - | - | 100 (27/27) |
| Rachow 2011[245] | Tanzania | Frozen sputum | 259 | Liquid or solid culture | 88.4 (61/69) | 98.0 (50/51) | 61.1 (11/18) | 95.8 (182/190) |
| Theron 2011[246] | South Africa | Frozen sputum | 480 | Liquid culture | 78.7 (111/141) | 94.7 (89/94) | 46.8 (22/47) | 94.4 (320/339) |
| Zeka 2011[247] | Turkey | Frozen respiratory specimens | 253 | Liquid or solid culture | 86.2 (50/58) | 100 (27/27) | 74.2 (23/31) | 99.4 (194/195) |
| Antonienka 2013[248] | Germany | Frozen respiratory specimens | 121 | Liquid or solid culture | 74.6 (50/67) | 94.1 (16/17) | 68.0 (34/50) | 96.1 (51/53) |
| Theron 2014[240] | Multicentre | Frozen sputum | 1388 | Liquid culture | 83.2 (292/351) | - | - | 91.8 (952/1037) |

* Exact culture method not reported

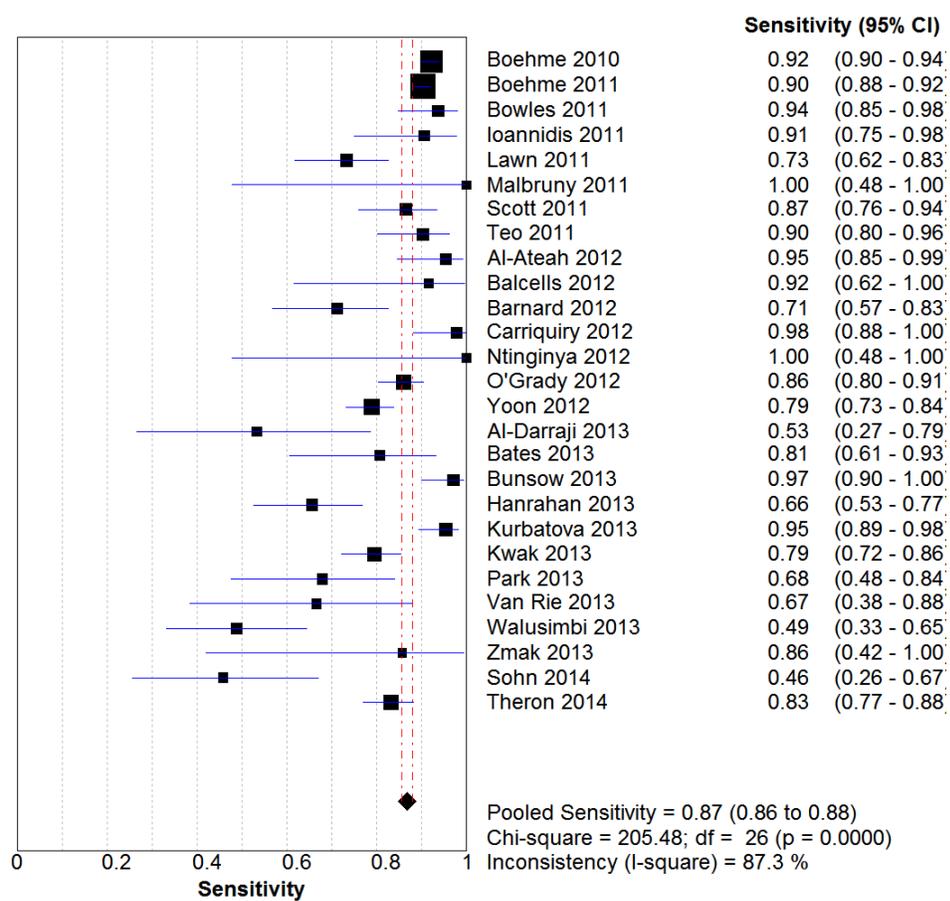


Figure 3-1 Sensitivity of a single Xpert MTB/RIF for detection of *M. tuberculosis* in prospectively enrolled TB suspects with culture as a reference

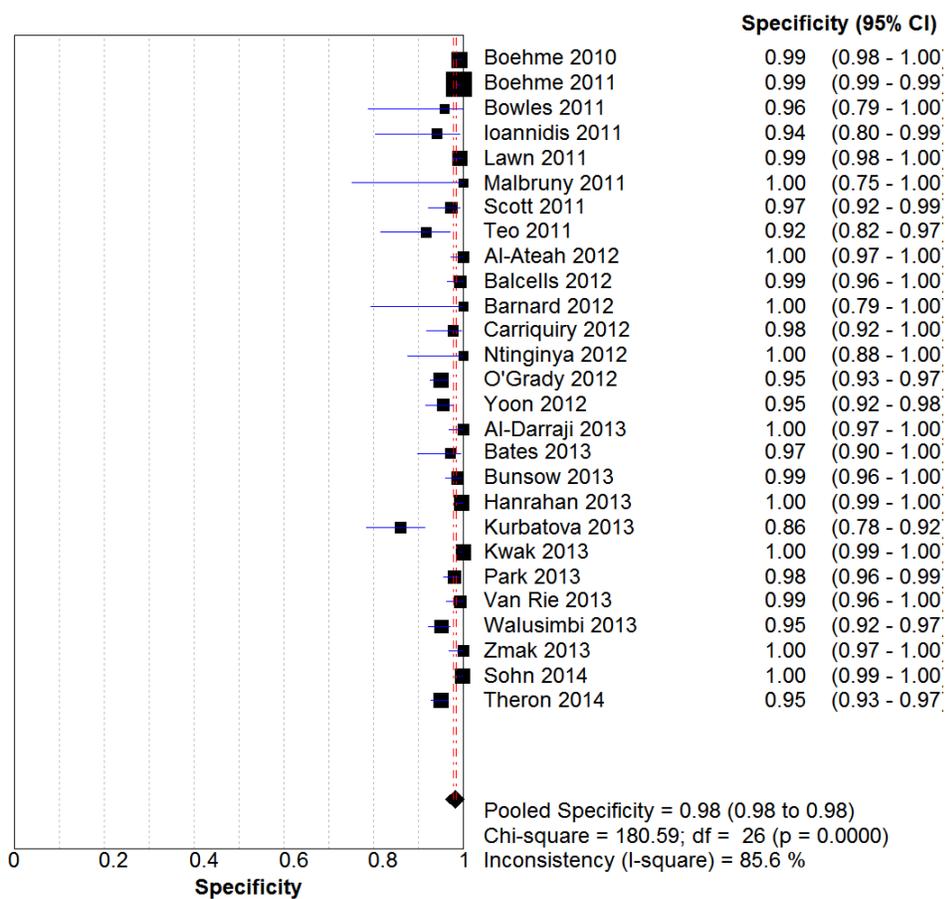


Figure 3-2 Specificity of a single Xpert MTB/RIF for detection of *M. tuberculosis* in prospectively enrolled TB suspects with culture as a reference

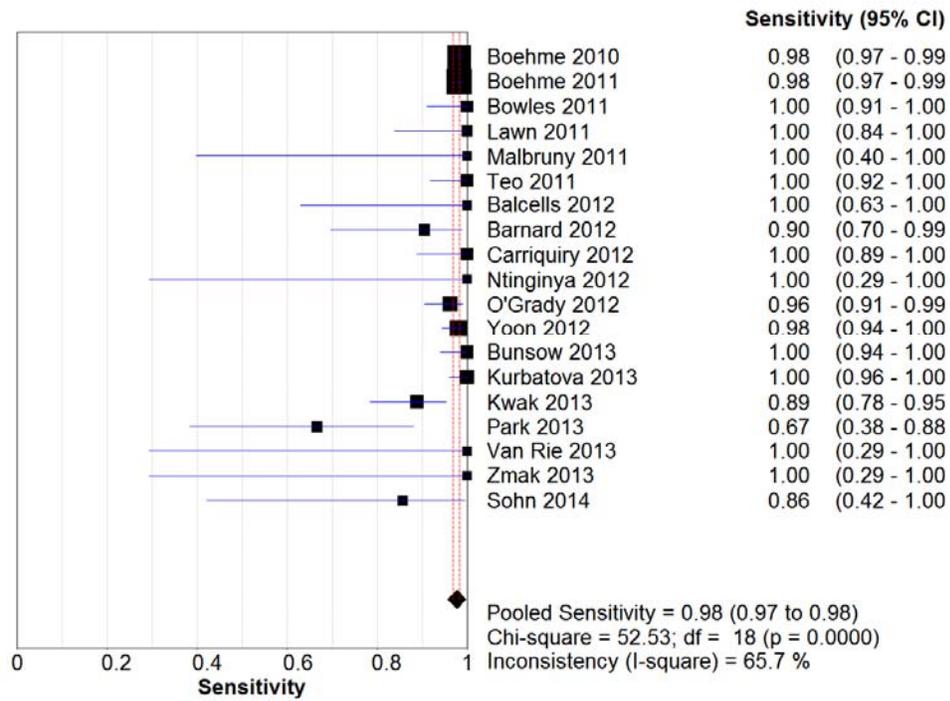


Figure 3-3 Sensitivity of a single Xpert MTB/RIF for detection of smear-positive *M. tuberculosis* in prospectively enrolled TB suspects with culture as a reference

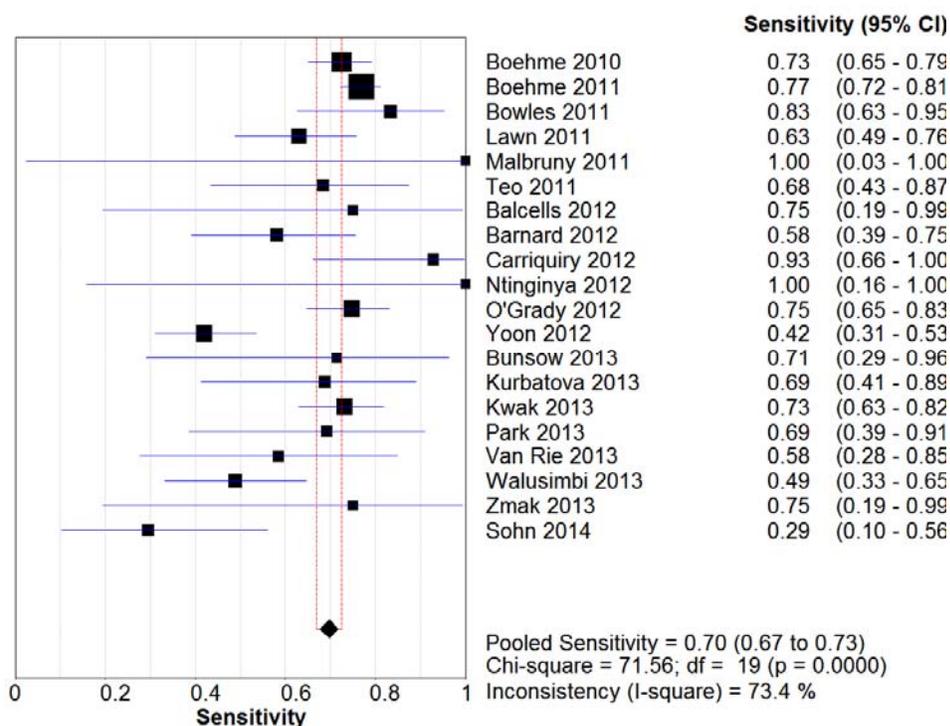


Figure 3-4 Sensitivity of a single Xpert MTB/RIF for detection of smear-negative *M. tuberculosis* in prospectively enrolled TB suspects with culture as a reference

The sensitivity and diagnostic yield of sputum smear microscopy are reduced in HIV-infected individuals.[274,275] Whether HIV infection also impacts on the performance of the Xpert MTB/RIF assay has been explored in eight studies (Table 3-3).[45,46,220,227,228,230,240,246] There is some evidence from these studies of reduced sensitivity for detection of *M. tuberculosis* in HIV infection[45,46,230,240,246]; although two studies showed no difference in sensitivity,[220,228] and one study amongst hospital inpatients in Zambia demonstrated a modest increase in sensitivity amongst HIV-infected

participants.[227] There was also some evidence to suggest a small reduction in specificity amongst HIV-infected subjects.[220,228,230,240,246] There is conflicting evidence around the effect of CD4+ T-cell count on the sensitivity of Xpert MTB/RIF. Whilst two prospective studies reported higher sensitivity with lower CD4+ T-cell count,[218,276] one study using archived sputum specimens reported reduced sensitivity in those individuals with low CD4+ T-cell count (<200 cells/ μ l vs. \geq 200 cells/ μ l).[246] However, none of these diagnostic studies was powered specifically to detect differences in Xpert MTB/RIF performance by CD4+ T-cell count.

3.4 Diagnostic accuracy for the detection of rifampicin resistance

The Xpert MTB/RIF assay detects the presence of the common mutations in the *rpoB* gene that are found in >95% of rifampicin-resistant *M. tuberculosis* strains.[277] The performance of a single Xpert MTB/RIF test performed on fresh sputum samples for the detection of rifampicin resistance compared to a reference standard of phenotypic drug susceptibility testing (DST) and/or line probe assay (LPA) is shown in Table 3-4 and Figures 3-5 & 3-6. Across 15 studies, the pooled sensitivity of a single Xpert for the detection of rifampicin resistance was 93% (95% CI 90-95) and specificity was 98% (95% CI 97-98). It should be noted that the majority of studies, other than the two large multicentre studies and two other studies specifically in drug-resistant TB suspects, included fewer than 20 rifampicin-resistant cases.

The specificity of the assay with regards to rifampicin resistance has been of concern since the introduction of the Xpert assay.[278] The specificity documented in the

multicentre validation study of 98.1% was with a reference standard of phenotypic DST.[45] However, after taking *rpoB* gene sequencing results into account, specificity was reported as 100%, suggesting that the test had correctly identified the presence of *rpoB* mutations but that in some cases these were not associated with phenotypic rifampicin resistance. In the subsequent demonstration study, concerns about false-positive resistance results led to change in the software definitions during the study: resistance was defined on the basis of a difference in cycle threshold (ΔC_t) between two probes of greater than 3.5 cycles and this was changed to 5 cycles. As a result, specificity improved from 96.2% (779/810) to 98.3% (796/810), although sensitivity was reduced as a result from 96.8% (242/250) to 94.4% (236/250). Subsequently, additional cartridge and software changes were made to improve the accuracy of the assay and new Xpert kits (version G4) were introduced in 2012.[279,280]

In the one study from India showing very low sensitivity for the detection of rifampicin resistance (64.4%) using the most recent Xpert MTB/RIF assay (version G4), the 21 discordant specimens (Xpert rifampicin susceptible/LPA rifampicin resistant) were examined further by phenotypic DST and genome sequencing.[281] In 20 specimens phenotypic DST confirmed rifampicin resistance, and in most of those (18/20) sequencing of the *rpoB* gene identified characteristic mutations associated with rifampicin resistance (L533P and S531L). It is not clear whether the false negative Xpert results were due to strain diversity and altered probe binding, technical assay issues, or possibly mixed populations of susceptible and resistant *M. tuberculosis* bacilli. It is noteworthy that the majority of study participants were on failing re-treatment regimens at the time of enrolment, raising the strong possibility

of mixed populations with evolving resistance *in vivo*. It has been documented that the assay only reliably detects the L533P mutation if 100% of the DNA population carries the mutation.[215] The problem of false negative Xpert rifampicin results for strains carrying the L533P mutation has also been reported elsewhere.[282]

In two other studies, specimens showing discordance between Xpert result and phenotypic DST (Xpert resistant/phenotypic DST susceptible) were also examined by *rpoB* gene sequencing. In the one discordant case in the clinical validation study and in nine of 15 discordant cases in the multicentre validation study, common mutations associated with rifampicin resistance were identified through *rpoB* sequencing.

It is now well documented that various genotypic and phenotypic DST methods can give discordant results for rifampicin resistance.[282-290] It is also documented that adverse clinical outcomes can occur with *M. tuberculosis* strains harbouring *rpoB* mutations but with phenotypic susceptibility to rifampicin.[284,290] The diagnostic accuracy of Xpert MTB/RIF for rifampicin resistance therefore needs to be interpreted in the context of the uncertainty over the reference standard and the true clinical significance of genotypic and phenotypic resistance. As molecular methods replace culture-based methods, there is a need for research to better understand how genotypic and phenotypic resistance predict treatment outcomes, for not only rifampicin but also other established and novel anti-TB drugs.[291]

Table 3-3 Diagnostic accuracy of Xpert MTB/RIF for detection of *M. tuberculosis* according to HIV infection status

| Study | N* | Sensitivity | | Specificity | |
|-------------------|----------|-------------------|-------------------|-------------------|-------------------|
| | | HIV-infected | HIV-uninfected | HIV-infected | HIV-uninfected |
| Boehme 2010[45] | 392/584 | 93.9 | 98.4 | - | - |
| Boehme 2011[46] | 602/1088 | 82.4 (173/210) | 90.7 (304/335) | 99.2 (389/392) | 99.3 (748/753) |
| Scott 2011[220] | 124/26 | 84 | 83 | 96 | 100 |
| Theron 2011[246] | 130/286 | 69.6 (32/46) | 82.9 (68/82) | 91.7 (77/84) | 95.6 (195/204) |
| O'Grady 2012[227] | 408/196 | 88.2 (142/161) | 74.3 (26/35) | 95.1 (235/247) | 96.3 (155/161) |
| Yoon 2012[228] | 328/107 | 78.7 (144/183) | 79.6 (43/54) | 94.5 (137/145) | 100 (53/53) |
| Bates 2013[230] | 62/22 | 80.1 (17/21) | 100 (3/3) | 95.1 (39/41) | 100 (19/19) |
| Theron 2014a[240] | 449/272 | 78.2 (97/124) | 93.3 (56/60) | 93.5 (304/325) | 97.2 (206/212) |
| Theron 2014b[240] | 835/537 | 78.9 (186/236) | 92.1 (105/114) | 89.3 (535/599) | 95.0 (402/423) |

* Numbers are HIV-infected then HIV-uninfected participants

Table 3-4 Diagnostic accuracy of a single Xpert MTB/RIF for the detection of rifampicin resistance

| Study | Location | Samples | N | Reference standard | Sensitivity | Specificity |
|----------------------|-----------------|--|------|----------------------|--------------------|--------------------|
| Helb 2010[44] | Uganda | Frozen sputum | 64 | Phenotypic DST | 100 (9/9) | 98.2 (54/55) |
| Boehme 2010[45] | Multicentre | Fresh sputum | 720 | Phenotypic DST | 97.6 (200/205) | 98.1 (505/515) |
| Boehme 2011[46] | Multicentre | Fresh sputum | 1060 | Phenotypic DST ± LPA | 94.4* (236/250) | 98.3* (796/810) |
| Bowles 2011[216] | The Netherlands | Fresh and frozen respiratory specimens | 40 | Phenotypic DST | 100 (8/8) | 100 (32/32) |
| Ioannidis 2011[217] | Greece | Fresh respiratory specimens | 32 | Phenotypic DST ± LPA | 75.0 (3/4) | 100 (28/28) |
| Lawn 2011[218] | South Africa | Fresh sputum | 55 | Phenotypic DST | 100 (4/4) | 94.1 (48/51) |
| Scott 2011[220] | South Africa | Fresh sputum | 16 | Phenotypic DST | 100 (4/4) | 66.7 (8/12)† |
| Barnard 2012 [224] | South Africa | Fresh sputum | 36 | LPA | 100 (3/3) | 100 (33/33) |
| Carriquiry 2012[225] | Peru | Fresh sputum | 39 | Phenotypic DST | 100 (6/6) | 90.9 (30/33) |
| O'Grady 2012[227] | Zambia | Fresh sputum | 96 | Phenotypic DST | 81.3 (13/16) | 97.5 (78/80) |
| Antonienka 2013[248] | Germany | Frozen respiratory specimens | 50 | Phenotypic DST | 100 (2/2) | 100 (48/48) |

| Study | Location | Samples | N | Reference standard | Sensitivity | Specificity |
|---------------------|-----------------|-----------------------------|----------|---------------------------|--------------------|--------------------|
| Kurbatova 2013[233] | Russia | Fresh sputum | 100 | Phenotypic DST | 98.2 (55/56) | 100 (17/17) |
| Kwak 2013[234] | South Korea | Fresh sputum | 99 | Phenotypic DST | 88.9 (8/9) | 100 (90/90) |
| Park 2013[235] | South Korea | Fresh respiratory specimens | 19 | Phenotypic DST | 100 (2/2) | 100 (17/17) |
| Rufai 2014[281] | India | Fresh sputum | 137 | LPA ± phenotypic DST | 64.4 (38/59) | 94.9 (74/78) |

DST, drug susceptibility testing; LPA, line probe assay

* After *post hoc* change to software definitions for rifampicin resistance

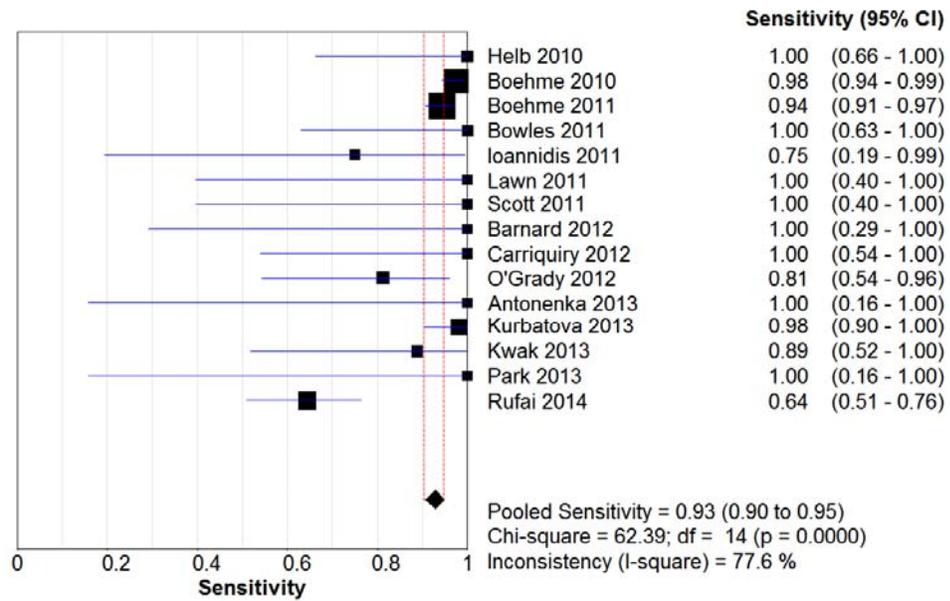


Figure 3-5 Sensitivity of a single Xpert for detection of rifampicin resistance with phenotypic DST and/or line probe assay as a reference

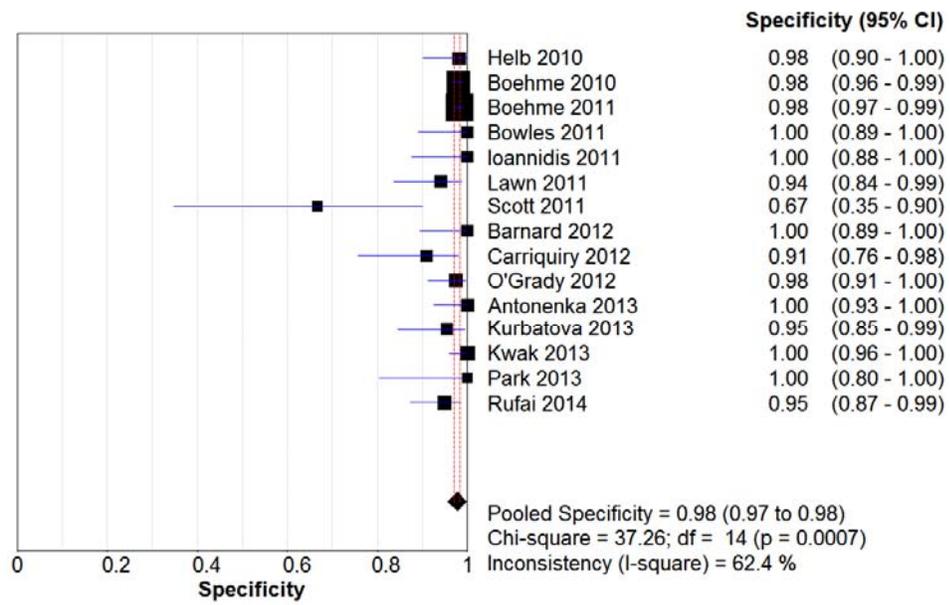


Figure 3-6 Specificity of a single Xpert for detection of rifampicin resistance with phenotypic DST and/or line probe assay as a reference

3.5 Xpert MTB/RIF assay failure

Studies of diagnostic accuracy tend to evaluate only subjects that have valid results from both the index test and reference test. As a result, estimates of diagnostic accuracy tend not to be affected by test failures. The Xpert MTB/RIF assay incorporates internal quality control mechanisms and the test can fail in three ways: ‘error’, indicating a failure in the probe check control or a system component failure; ‘invalid’, indicating that the sample processing control has failed, either due to incorrect sample processing or PCR inhibition; or ‘no result’, usually signifying power failure or other termination of test. The frequency of test failures could potentially impact on the impact of Xpert MTB/RIF in routine implementation.

Table 3-5 shows the studies where the proportion of tests that failed to give a valid result has been reported. The reporting is not consistent, in that it is not always explicit whether repeat tests were allowed with the remaining sample/buffer mix. In the 15 studies summarised, the proportion with no valid results ranged from 0.6% to 13.5%. [45,46,217,218,220,221,224,225,230,233,237,239,276,292,293] The highest proportions were reported in two large studies of routine Xpert implementation, [292,293] as well as a study from a large tertiary hospital in Canada. [239] In the study of Xpert implementation at decentralised microscopy centres in India, there was heterogeneity across the 18 sites, with the proportion of failed tests ranging from 3.9% to 14.2%. [293] Similarly in the multi-country TB REACH implementation project, the proportion of failed tests ranged from 5.9% to 16.3% across the different sites. [292] In both of these large scale implementation projects, the majority of test failures (60-65%) were due to errors. Across all studies, the majority of specimens that underwent repeat testing yielded a valid result. The

observed heterogeneity in similar settings and the validity of repeat tests might suggest that operator-dependent factors were the most important contributors to the indeterminate results, although assay-related factors may also play a part.

3.6 Impact of Xpert MTB/RIF on patient-relevant outcomes

The most robust evidence around the impact of Xpert MTB/RIF comes from two clinical trials comparing Xpert to smear microscopy. The TB-NEAT study was a multicentre randomised control trial in South Africa, Zimbabwe, Zambia and Tanzania.[240] A total of 1502 individuals with symptoms suggestive of pulmonary TB disease at primary health care (PHC) facilities were randomly allocated to receive sputum smear microscopy (in all but one site performed on site at the PHC facility) or Xpert MTB/RIF (in all cases performed on site). Sputum culture was the reference standard for TB diagnosis in both arms. The trial was primarily designed to detect a difference in TB-related morbidity in culture-positive cases who had initiated TB treatment. Morbidity was measured using the TBscore, a scale based on the presence of TB symptoms and signs where a higher score on the scale 0-13 indicates more morbidity [294]; and the Karnofsky performance status scale, a general indicator of wellbeing and functional status where 0 indicates death and 100 indicates normal health with no complaints and no evidence of disease.[295,296]

Table 3-5 Studies reporting frequency of Xpert MTB/RIF tests with no valid results

| Study | Country | Location/context | Number of specimens tested | Number with no valid result | Proportion with no valid result (95% CI) |
|----------------------|----------------|----------------------------------|-----------------------------------|------------------------------------|---|
| Boehme 2010[45] | Multi-country | Multicentre validation study | 5190 | 192 | 3.7 (3.2-4.3) |
| Boehme 2011[46] | Multi-country | Multicentre demonstration study | 5321 | 126 | 2.4 (2.0-2.8) |
| Ioannidis 2011[217] | Greece | National reference laboratory | 121 | 2 | 1.7 (0.5-5.8) |
| Lawn 2011[218] | South Africa | Centralised hospital laboratory | 908 | 5 | 0.6 (0.2-1.3) |
| Scott 2011[220] | South Africa | Centralised hospital laboratory | 205 | 12 | 5.9 (3.4-10.0) |
| Teo 2011[221] | Singapore | Centralised hospital laboratory | 131 | 9 | 6.9 (3.7-12.5) |
| Barnard 2012[224] | South Africa | Centralised hospital laboratory | 282 | 7 | 2.5 (1.2-5.0) |
| Carriquiry 2012[225] | Peru | Centralised hospital laboratory | 134 | 2 | 1.5 (0.4-5.3) |
| Bates 2013[230] | Zambia | Centralised hospital laboratory | 94 | 3 | 3.2 (1.1-9.0) |
| Kurbatova 2013[233] | Russia | Centralised hospital laboratory | 238 | 8 | 3.4 (1.7-6.5) |
| Walusimbi 2013[237] | Uganda | Centralised hospital laboratory | 430 | 19 | 4.4 (2.9-6.8) |
| Sohn 2013[239] | Canada | Centralised hospital laboratory | 502 | 44 | 8.8 (6.6-11.6) |
| Balcha 2014[276] | Ethiopia | Centralised hospital laboratory | 1536 | 95 | 6.2 (5.1-7.5) |
| Cresswell 2014[292] | Multi-country | Various | 47 973 | 5107 | 10.7 (10.4-10.9) |
| Raizada 2014[293] | India | Decentralised microscopy centres | 40 035 | 2878 | 7.2 (6.9-7.5) |

There was no difference between the microscopy and Xpert groups in the TBscore at two months (median score 2 for both groups, $p = 0.85$) or six months (median score 1 for both groups, $p = 0.35$). Similarly there was no difference in the Karnofsky performance status score at two months (median score 80 for microscopy group vs. 90 for Xpert group, $p = 0.23$) or six months (median score 100 for both groups, $p = 0.85$). Xpert detected a greater proportion of culture-positive cases than microscopy (83% vs. 50%) and a greater proportion of patients subsequently confirmed as culture positive started TB treatment on the same day of presentation in the Xpert arm (66% vs. 44%, $p < 0.001$). In addition, a greater proportion of culture-positive cases had started treatment by day 56 in the Xpert arm (92% vs. 85%, $p = 0.03$), although the proportion of all participants on treatment by day 56 was similar in the two groups (43% in the Xpert group vs. 42% in the microscopy group). This finding reflected high rates of empirical treatment prior to or in the absence of microbiological confirmation of TB disease and this may have contributed to the lack of difference between the groups in TB-related morbidity.[240]

The second trial was a pragmatic, cluster randomised trial nested within the national roll-out of Xpert MTB/RIF in South Africa.[297] Twenty clusters (each cluster consisted of two PHC clinics and an off-site laboratory) were allocated to Xpert or microscopy. Analysis included 4656 individuals investigated for TB. There was no difference in the primary outcome of mortality at 6 months: 3.9% in Xpert group vs. 5.0% in microscopy group, risk ratio 0.86 (95% CI 0.56-1.28). There was a modest increase in diagnostic yield from the initial sputum specimen (9.2% with Xpert vs. 7.8% with microscopy) but the proportion of those who tested positive who were not

on treatment by day 28 was similar in both groups (17.0% in Xpert group vs. 14.9% in microscopy group).[297]

Another randomised controlled trial in Zimbabwe focused on the impact of Xpert for TB screening prior to ART. In this trial at a single large urban HIV treatment centre, 424 HIV-infected adults eligible for ART (CD4+ T-cell count ≤ 350 cells/ μ l), were randomised to TB screening by fluorescence microscopy (FM) or Xpert MTB/RIF, regardless of the presence or absence of symptoms suggestive of TB.[298] The primary endpoint was a composite endpoint of the proportion that developed incident TB or died within three months of enrolment. There was no evidence of any difference in this composite endpoint: 12% with FM vs. 9% with Xpert (difference -3%, 95% CI -9%-4%, $p = 0.39$). Xpert did not significantly increase the initial diagnostic yield (7% of participants had a positive test with FM vs. 9% with Xpert, $p = 0.29$). Of all TB cases diagnosed at baseline, the majority had negative index tests and a diagnosis based on clinical and radiographic features (69% with FM and 54% with Xpert) and this might again have affected the ability to detect a difference in outcomes between the two diagnostic strategies.[298]

In the large Xpert MTB/RIF demonstration study, the system was positioned within primary health care facilities in six diverse settings.[46] The overall median time to receipt of Xpert results by clinicians was 1 day (IQR 0-2) and only 0.6% (9/1438) was lost or unreported. This contrasted with median time to receipt of culture results of 58 days (IQR 42-62), with 16.7% (848/5089) lost or unreported. Xpert shortened the time to treatment for culture-confirmed pulmonary TB cases: the median time to treatment during the Xpert implementation phase compared to the validation phase

(where treatment was based on smear and culture results) was 2 days vs. 4 days for smear-positive cases and 5 days vs. 56 days for smear-negative cases.[46]

A before-after study compared Xpert MTB/RIF to the baseline diagnostic strategy of smear microscopy and culture in adults admitted to a tertiary hospital in Kampala, Uganda.[228] For culture-confirmed pulmonary TB cases, the median time to treatment was shorter under the Xpert strategy (median 0 days, IQR 0-2) than under the baseline smear microscopy and culture strategy (median 1 day, IQR 0-26). The ability to show only a slight difference was partly explained by the fact that around 70% of culture-positive TB cases were smear positive and that turnaround of smear microscopy results was rapid. There was no difference in mortality at two months amongst all TB suspects (17% in baseline strategy vs. 17% in Xpert strategy) or amongst culture-confirmed TB cases (17% vs. 14%).[228]

A series of studies have reported on the point-of-care implementation of Xpert MTB/RIF at a large primary health care clinic in Johannesburg, South Africa.[232,236,299] These studies demonstrated the feasibility of same-day treatment initiation, with over 80% commencing treatment on the same day and 96% of all Xpert-positive cases starting treatment. These studies also highlighted some human resource and operational challenges to implementing within a clinic environment, specifically that more staff were required and processes took longer than anticipated.[299] It was noteworthy that of the drug-susceptible cases diagnosed by Xpert, only 48% had a successful outcome (cure or completion) and the proportion who defaulted during treatment was particularly high at 23%, although there was no context given as to default rates prior to the implementation of

Xpert.[232] This does, however, raise the question as to whether rapid diagnosis and treatment initiation, particularly if earlier in disease progression when people are less symptomatic, could lead to higher default rates after the initiation of treatment. This is something that needs to be closely monitored in routine programmes as Xpert is introduced.

In terms of the impact specifically for MDR-TB, one before-after study in ten primary health care facilities in Cape Town, South Africa compared outcomes with diagnostic algorithms incorporating either LPA ($n = 414$) or Xpert ($n = 127$).[130] There was no significant difference in pre-treatment default for MDR-TB cases (9% with LPA-based algorithm vs. 6% with Xpert-based algorithm) but the median time from sputum collection to initiation of MDR-TB treatment was reduced from 43 days with the LPA-based algorithm to 17 days with the Xpert-based algorithm. In this study, there were differences in the algorithms other than the diagnostic test used and the individuals entering the diagnostic algorithm were not directly comparable across the two time periods. There was also the possibility that other changes in the health system occurred between the two time periods, although it was notable again that the laboratory turnaround time with Xpert (less than 24 hours) only comprised a small proportion of the overall time to treatment initiation.

The first district in South Africa to achieve full coverage with Xpert MTB/RIF was the rural Sisonke district in KwaZulu-Natal. This was linked to a decentralised MDR-TB treatment model. Between October 2011 and October 2012, a total of 21 650 Xpert tests were performed and 1409 Xpert-positive cases were diagnosed, 140 (9.9%) of which were rifampicin resistant. 40% of rifampicin-susceptible cases

started treatment within two days and 67% within five days. 36% of rifampicin-resistant cases started treatment within two days and 54% within five days.[300]

Some of the limitations of placement of Xpert in centralised laboratories were highlighted in a study of 403 HIV-infected symptomatic TB suspects from an urban hospital in Durban. All participants had specimens sent for smear microscopy, culture and Xpert MTB/RIF. The total diagnostic time (time from sputum collection to receipt of results by a clinician) was longer for Xpert than for smear microscopy (median 6.4 days vs. 3.3 days, $p < 0.001$). Of the 86 cases with a positive Xpert, only 32 (37.2%) started treatment on the basis of the Xpert result, with most starting on the basis of an earlier positive smear or on clinical grounds.[301] Amongst the limitations of this study was the fact that this was performed during the first phase of Xpert roll-out within the National Health Laboratory Service (NHLS) in KwaZulu-Natal and that smear microscopy was conversely performed in a private-sector laboratory with results electronically reported to the study site.

3.7 Summary

Xpert MTB/RIF is a molecular diagnostic test which can accurately identify *M. tuberculosis* directly from sputum specimens and which can detect the presence of mutations conferring rifampicin resistance. Xpert MTB/RIF is more sensitive than smear microscopy, although sensitivity is somewhat reduced in HIV infection. Assay failure does occur and seems to be more common in the setting of routine implementation than in earlier demonstration studies. Although Xpert has an increased diagnostic yield compared to smear microscopy, the magnitude of this in most clinical studies has been fairly modest and so far the evidence suggests that this

does not translate to significant impact on the mortality and morbidity of TB cases or of all those undergoing investigation. Use of Xpert outside existing laboratory structures as a point-of-care tool at primary health care level has been shown to be feasible but whether or not point-of-care placement impacts on patient-relevant outcomes has not yet been established.

Chapter 4 Trial methodology

4.1 Aims and objectives

The aim of the cluster-randomised trial was to evaluate the impact of point-of-care placement of Xpert MTB/RIF in a rural primary health care system with high levels of TB drug resistance and HIV infection. The primary objective was to test the hypothesis that timely initiation of appropriate TB treatment for culture-confirmed cases is improved with placement of the diagnostic system at the point of care (primary health care clinic) compared to placement at the district hospital laboratory.

Secondary objectives included:

- To evaluate the impact of Xpert MTB/RIF positioning on additional clinical outcomes (time to appropriate TB treatment, time to appropriate drug-resistant TB treatment, time to ART initiation, all-cause mortality, and hospital admission)
- To compare the diagnostic accuracy of Xpert MTB/RIF (sensitivity and specificity) under different positioning strategies.

4.2 Trial setting

The trial was conducted in Hlabisa health sub-district, uMkhanyakude district, northern KwaZulu-Natal province, South Africa and co-ordinated at the Africa Centre for Health and Population Studies (Figure 4-1). The Africa Centre is a Wellcome Trust-funded research institution affiliated to the University of KwaZulu-Natal (www.africacentre.com). The Africa Centre carries out research on population and health issues affecting a rural community with a high burden of HIV and TB.

Population-based demographic and health surveys take place within the Demographic Surveillance Area, an area of 438 km² with a population of approximately 85 000 people.[302] The centre also supports the local Department of Health to deliver integrated HIV & TB treatment and care through the primary health care (PHC) system in Hlabisa health sub-district (an area of 1430 km² with a population of approximately 228 000).

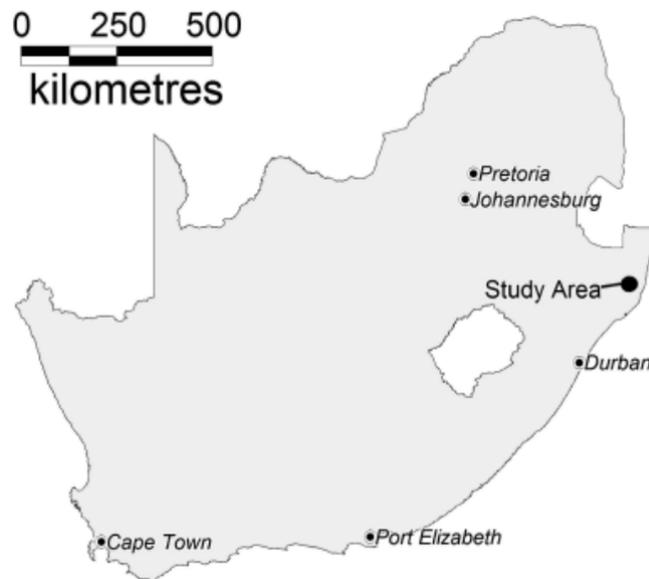


Figure 4-1 Map showing location of Hlabisa sub-district within South Africa

KwaZulu-Natal province, South Africa can be considered to be the epicentre of the combined TB/HIV epidemic. Hlabisa health sub-district is a prime example of a rural district with a huge burden of disease and mortality attributable to HIV and TB. The majority of the community live in scattered homesteads that are not concentrated

into villages or compounds. The district (uMkhanyakude) is one of the most deprived in South Africa, with 43% unemployment and only 13% of the population having access to piped water inside dwellings.[303]

A population-based HIV survey has demonstrated extremely high HIV seroprevalence in the area - overall prevalence 29% in the adult population (aged 15-49 years) in 2011.[304] TB notification rates rose significantly in Hlabisa health sub-district from the early 1990's in association with the increase in HIV seroprevalence and peaked in 2008 at over 1700 per 100 000, at which point 76% of TB cases were co-infected with HIV.[305] Since then there has been evidence of a decline in TB notifications (largely related to a decline in smear-negative pulmonary TB) and in 2011 the notification rate was 1050 per 100 000 with a smear-positive pulmonary TB notification rate of 390 per 100 000. Despite the decline, this remains amongst the highest TB notification rates in the world, rivalled only by the neighbouring Kingdom of Swaziland. HIV and TB were estimated together to be responsible for 60% (7539/12 539) of all deaths in adults (age ≥ 15 years) between 2000 and 2010 in the Africa Centre Demographic Surveillance Area. Although there was evidence of a decline since 2003 in HIV/TB-related mortality, in 2010 HIV/TB continued to exceed all other causes of death combined for males aged 25-59 years and females aged 15-54 years.[17]

TB has for a long time placed a substantial burden on the district hospital, and latterly on the primary health care system.[306-312] In 2011, TB was responsible for one in six inpatient episodes and case fatality rate for episodes involving TB (20.6 deaths per 100 episodes) was more than double that for hospital episodes unrelated

to TB.[309] In HIV-infected individuals initiating ART at PHC clinics between 2004 and 2008, 20% of males and 16% of females were receiving treatment for TB at the time of ART initiation.[312] In a cohort study nested within the larger HIV treatment and care programme, for adults who started ART between March 2010 and December 2011, TB was responsible for one in three deaths in the first year of ART.[311] The heavy burden of TB at district hospital and PHC level highlights the potential for TB transmission within the health system, which is of particular concern in the context of inadequate infection control in South African hospitals and clinics.[313-320]

The emergence of drug-resistant TB in Hlabisa was described in the 1990s.[18] The epidemic of drug-resistant TB has latterly been characterised by some of the highest population rates of MDR-TB anywhere in the world (overall 57 per 100,000 for uMkhanyakude district in 2007, not disaggregated for new and previously treated cases) with only isolated cases of extensively drug-resistant TB (XDR-TB).[19] More recently with the introduction of Xpert, uMkhanyakude district has been shown to have the highest rate of rifampicin-resistant TB in the country (16.2% of all positive Xpert tests, again not disaggregated for new and previously treated cases, demonstrated rifampicin resistance).[303] Both of these estimates were not disaggregated for new cases and previously treated cases. The delivery of MDR-TB treatment was partly decentralised in 2008, which allowed earlier initiation of treatment than in the previous hospital-based system with some evidence of improved clinical outcomes on treatment.[122] However, almost 50% of laboratory-confirmed cases had died or were untraceable by the time the culture/DST results were obtained.[122]

HIV and TB treatment and care are delivered at each of the 17 PHC clinics (including one situated at the district hospital) through decentralised collaborative programmes (Figure 4-2).[321] Previous work in Hlabisa sub-district has revealed that TB patients have a mean travel time of two hours to and from the PHC clinic and a mean travel cost of ZAR 23 (approximately equivalent to £1.30).[322] This trial recruited patients from the largest PHC clinic (KwaMsane clinic), situated in a small urban township in the south of the sub-district. KwaMsane clinic investigates around 1800 people for TB each year and treats approximately 400 TB cases. KwaMsane clinic is situated approximately 55km by road from the district hospital.

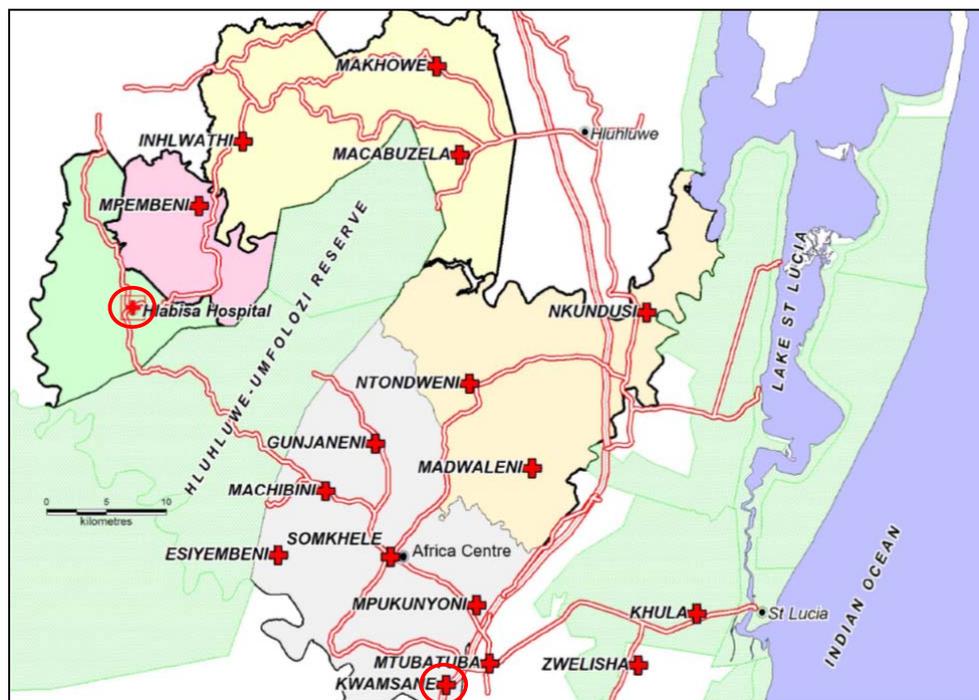


Figure 4-2 Map of Hlabisa sub-district showing location of primary health care clinics. Circles signify district hospital and KwaMsane clinic. Grey shaded area represents the Africa Centre Demographic Surveillance Area

4.3 Trial design

The study was a cluster randomised trial of adult pulmonary TB and drug-resistant TB (DR-TB) suspects evaluating the impact of Xpert MTB/RIF positioning on clinical outcomes. The unit of randomisation was a time block (two-week period) – time periods were randomly allocated to placement of the Xpert MTB/RIF diagnostic system at either centralised sub-district level (district hospital laboratory) or at primary health care clinic level (point-of-care). A cluster therefore represented all individuals enrolled during a two-week period. Randomisation was done in permuted blocks of eight for clusters 1-32 and four for clusters 33-36 (due to the extension of the trial, see section). The unit of observation for all analyses was the individual participant. The trial schema is shown in Figure 4-3.

The trial was designed as a pragmatic trial, in order to evaluate the effectiveness of a point-of-care diagnostic strategy under real-world conditions. Few trials are purely explanatory (exploring efficacy of an intervention) or pragmatic (exploring effectiveness) and this distinction is often conceptualised as a continuous spectrum rather than a dichotomy. The pragmatic-explanatory continuum indicator summary (PRECIS) tool can help to determine where on the continuum a trial might lie.[323-325] This tool is based on ten key domains that determine the extent to which a trial is pragmatic or explanatory. Although this tool was not utilised in the design of the trial, it was used retrospectively to illustrate the pragmatism of the trial (Appendix B).

In many domains, the trial design was more towards the pragmatic end of the spectrum. Certainly the inclusion of all eligible individuals requiring investigation for pulmonary TB, or specifically drug-resistant TB, with minimal exclusion criteria was reflective of real-world practice. Also the follow-up schedule was reflective of the routine programme, with no additional visits specifically for the study, other than for outcome ascertainment.

One of the key aspects of the trial that limited pragmatism was the fact that specific study personnel (nurse and research assistant) were placed at the primary health care clinic for the purposes of the study, and programme staff were not involved in directly implementing the trial procedures. This decision was taken because it was felt that the time taken for the individual informed consent process and data collection would infringe too much on the routine programme operations. Also, the study nurse followed standardised algorithms and management protocols with little room for flexibility, but that also reflected how the TB control programme operates in this setting. Another main limitation of the pragmatism was the fact that a single GeneXpert system was moved between the two locations (primary health care clinic and hospital laboratory) to comply with the randomisation schedule. This was due to the fact that resources only allowed purchase of a single system and that the system was only operational in one location at any one point, but nevertheless this was poorly representative of what would happen in a routine programme setting. Lastly, the need for continuous oversight of the trial meant that adherence of the study nurse to the trial protocol and standard operating procedures (SOPs) was closely monitored, probably more so than would be the case in a routine programme setting. However, this is unavoidable within the ethical and regulatory framework of clinical

trials, where the proper conduct of the trial and protection of trial participants are paramount.

In summary, therefore, the trial was designed and implemented to be as pragmatic as possible, but failed to meet the criteria for a fully pragmatic approach.

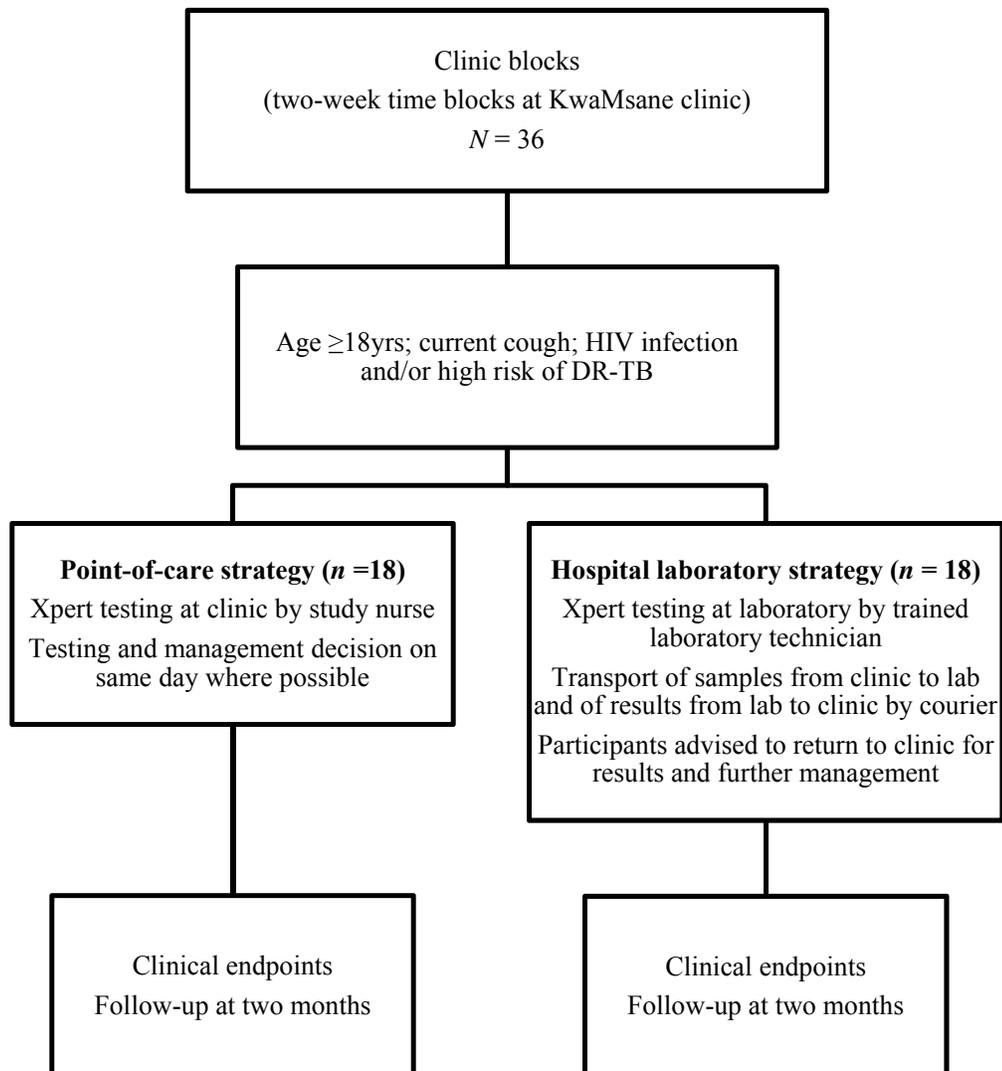


Figure 4-3 Trial schema

4.4 Trial outcomes

4.4.1 Primary outcome

The primary outcome measure was the proportion of culture-confirmed pulmonary TB cases initiated on appropriate anti-TB treatment within 30 days of enrolment

4.4.2 Secondary outcomes

The clinical secondary outcome measures were separated into those among culture-confirmed TB cases and those among TB and DR-TB suspects:

Culture-confirmed pulmonary TB cases

- Time to initiation of appropriate TB treatment (days) for culture-confirmed pulmonary TB cases
- Time to initiation of appropriate DR-TB treatment for pulmonary rifampicin-resistant TB cases confirmed by culture and either line probe assay (LPA) or phenotypic drug susceptibility testing (DST)

TB and MDR-TB suspects

- All-cause mortality in TB and DR-TB suspects at 60 days
- Proportion of TB suspects with at least one hospital admission within 60 days
- Time to initiation of antiretroviral therapy (ART) for HIV-infected TB and DR-TB suspects not yet receiving but eligible for ART

4.5 Trial population

Adult pulmonary TB or drug-resistant pulmonary TB suspects were recruited at KwaMsane clinic (the largest PHC clinic in Hlabisa sub-district). Only individuals that were HIV-infected and/or had a high risk of drug-resistant TB were included, due to their high risk of mortality and prioritisation for Xpert MTB/RIF testing at the time of study design, according to WHO recommendations.[326]

Inclusion criteria:

- Adult (≥ 18 years old)
- Current pulmonary TB symptoms (defined as cough of any duration)
- Confirmed HIV infection and/or high risk for drug-resistant TB (adapted from South African national guidelines and WHO guidelines for programmatic management of MDR-TB)[327,328]:
 - Failure of standard treatment or retreatment regimen
 - Smear non-conversion at month 2 or 3 of standard treatment regimen or retreatment regimen
 - Relapse or return after default
 - Any other previous TB treatment (at any time point)
 - Household exposure to known MDR/XDR-TB case
 - Health care workers
 - Prison inmate in previous 12 months

Exclusion criteria:

- Severely unwell requiring immediate admission to hospital
- Previous MDR/XDR-TB diagnosis or treatment (documented or self-reported)
- Suspicion of extra-pulmonary TB only (absence of cough)
- Unable to give informed consent

4.6 Randomisation

The allocation schedule for random assignment of two-week time blocks was computer generated, using random permuted blocks of eight for blocks 1-32 and four for blocks 33-36. The computer-generated sequence for blocks 1-32 was produced by an independent biostatistician prior to the start of the trial; the sequence for blocks 33-36 was produced in December 2012. Allocation for each clinic block was placed into sequentially numbered, opaque, sealed envelopes by the biostatistician; the envelope was opened on the Friday before the start of a new two-week block by the principal investigator and the allocated strategy for the next time block was communicated to study staff.

4.7 Intervention

The GeneXpert system is a 4-site, fully automated instrument integrating real-time amplification and detection of pathogens (Appendix A). The Xpert MTB/RIF assay employs a hemi-nested PCR of the *rpoB* core region of *Mycobacterium tuberculosis* (81 base pair region, also called rifampicin resistance-determining region (RRDR)). It uses five molecular beacons (different colours), each hybridising to a different

target segment within *rpoB* - binding does not occur if the sequence differs from wild type by even a single nucleotide substitution. The assay incorporates an internal control (*Bacillus globigii* spores). A positive test indicates two or more probes positive within two cycles of each other. Rifampicin resistance is reported if there is failure of one or more *rpoB* specific beacon to hybridise properly. A negative test indicates *B. globigii* signal but no *M. tuberculosis* signal. The assay produces a result of 'invalid' if there is neither a *B. globigii* nor a *M. tuberculosis* signal, indicating that the sample processing control has failed, either due to incorrect sample processing or PCR inhibition. An 'error' result indicates a failure in the probe check control or a system component failure. Finally, 'no result' usually signifies power failure or some other termination of test.

The Xpert MTB/RIF assay was incorporated into diagnostic algorithms adapted from the WHO standardised diagnostic algorithms for HIV-infected individuals with suspected TB and individuals at high risk of drug-resistant TB (Figures 4-4 and 4-5).[329]

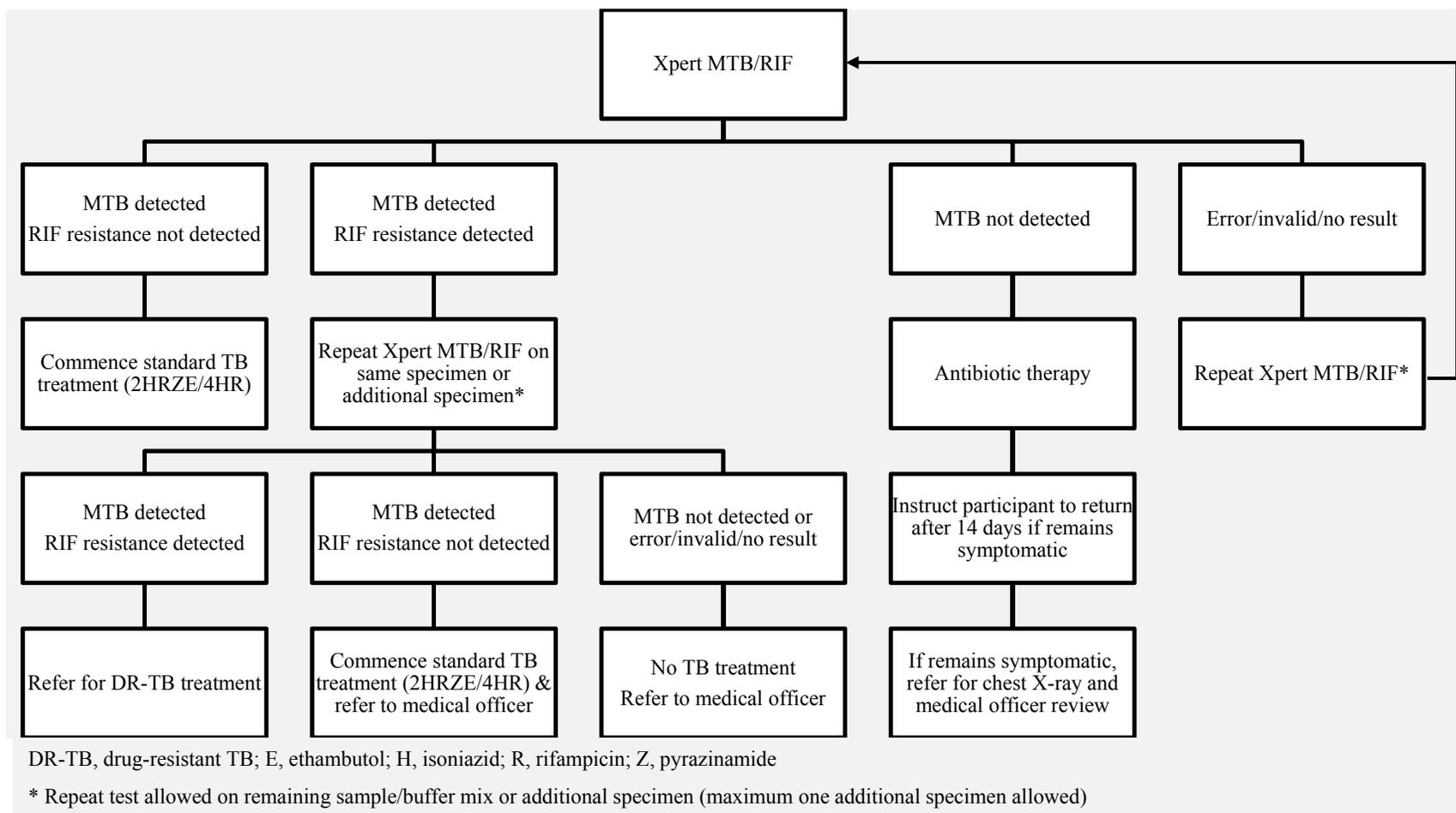


Figure 4-4 Algorithm for management of Xpert MTB/RIF results for participants not currently on TB treatment

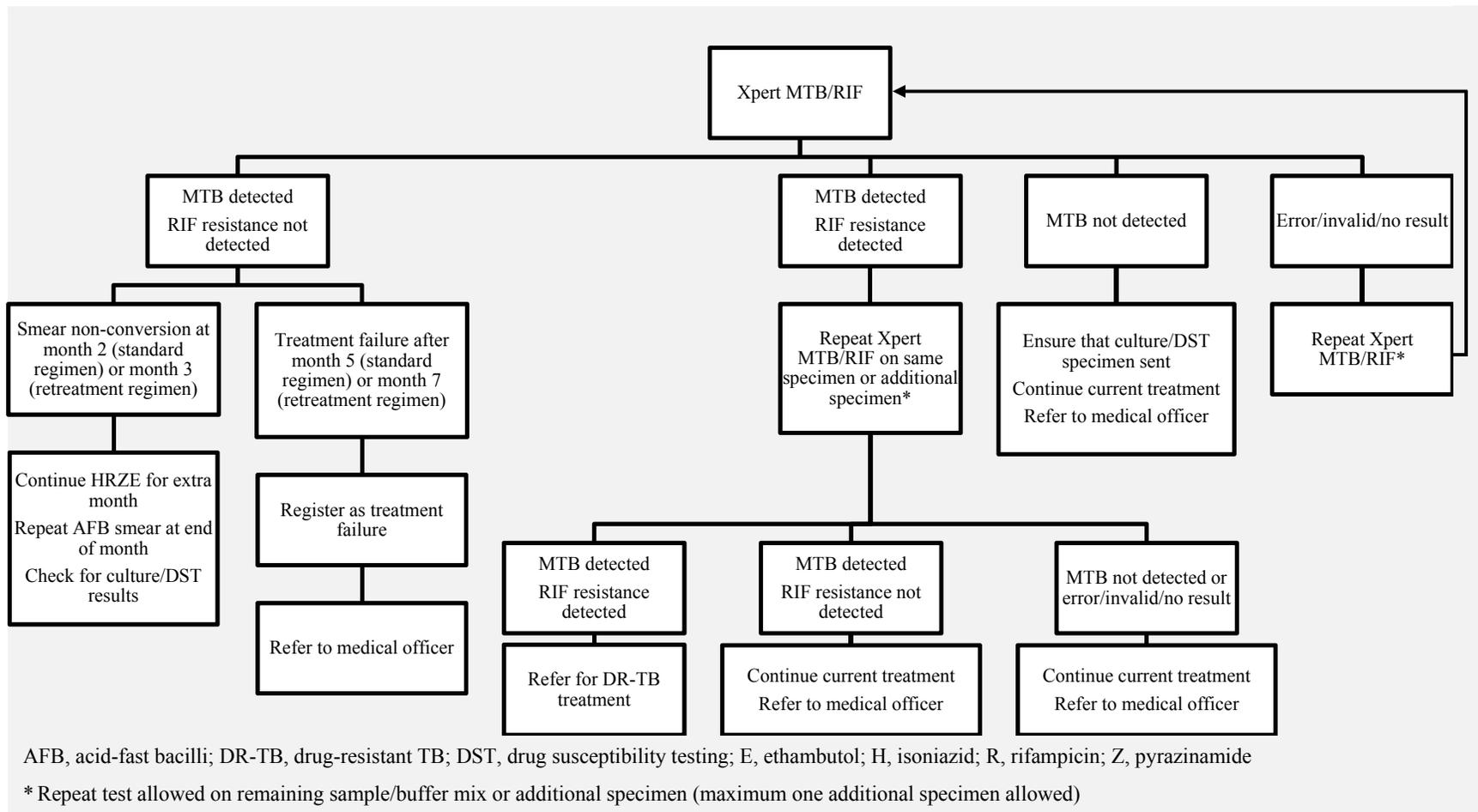


Figure 4-5 Algorithm for management of Xpert MTB/RIF results for participants currently on TB treatment

4.8 Procedures

4.8.1 Identification of participants

Potential participants were identified by health care workers at the single PHC clinic. All subjects who presented to any area of the clinic with symptoms suggestive of pulmonary TB (cough \pm fever/night sweats/weight loss) were given basic information about the trial and referred to the study nurse, who was situated at the clinic and who worked alongside the clinic TB nurses. The study enrolled participants between the hours of 0800 and 1630 on weekdays (Monday to Friday). The specific areas where subjects were identified were the TB clinic, HIV clinic, HIV counselling and testing services, antenatal care, and general primary health care services. A cough triage system was in operation throughout the study period, whereby all PHC attendees were asked about the presence of cough on arrival at the clinic. Individuals reporting cough were directed towards the TB area within the clinic. Throughout the study period, all HIV-infected adults attending for treatment or care were also screened for TB symptoms at every clinic visit in accordance with national guidelines.[330,331]

4.8.2 Informed consent

The study nurse checked the eligibility criteria and completed the form entitled 'Eligibility criteria' (Appendix C). Subjects that met the eligibility criteria were provided information (verbal and written) about the study in isiZulu and/or English (Appendix D) and those that were willing to participate were taken through the informed consent process and were asked to sign the consent form. Agreement of

participants who were illiterate was indicated by thumbprint on the consent form and a literate witness signed on their behalf (Appendix E).

4.8.3 Baseline evaluation

The study nurse collected basic demographic information on the 'Enrolment form' (Appendix F); this included phone numbers (for the participant and for a nominated second contact) and physical address for the purposes of contacting for outcome evaluation. Information about current symptoms, previous TB history, HIV status, CD4+ T-cell count, viral load and antiretroviral therapy use was entered into the baseline case report form (CRF) (Appendix G)

4.8.4 Sputum specimen collection

Spontaneously expectorated sputum specimens were collected at the study clinic (two spot specimens per patient). Sputum collection occurred outside. Each sputum specimen was collected into a sterile, wide-mouthed specimen container with a tightly fitting screw top. Simple instructions on how to submit a good quality sputum specimen were given to each participant with the aid of a pictorial card with instructions in isiZulu, based on instructions shown previously to increase case detection.[332] Every effort was taken to prevent contamination of the exterior of the specimen container. Each specimen container was placed into an individual disposable watertight sealed plastic bag.

The nurse instructed participants to wait one hour between producing the first and the second sputum specimen. The first sputum specimen was used for Xpert MTB/RIF testing and the second specimen for culture, line probe assay (LPA) ±

drug susceptibility testing (DST). Whilst awaiting testing or transport, sputum samples were stored at the clinic in a cooler box surrounded by frozen ice packs.

After the first three months of the study, when it became clear that leakage of specimen containers during transit was a significant problem, each specimen container was sealed with plastic paraffin film before transport to the district hospital laboratory. This was performed for the specimens due for Xpert MTB/RIF testing and those sent for culture and DST.

Sputum induction was not utilised for the study, even for those unable to expectorate, as it was not in use anywhere in the sub-district prior to or during the study.

4.8.5 Sputum specimen testing

A four-module GeneXpert machine with desktop computer was installed for each two-week time period at either the district hospital laboratory or the primary health care clinic according to the randomisation schedule. There was only one GeneXpert system for the trial and it was moved between the two locations when required, according to the randomisation. At both locations, Xpert MTB/RIF testing followed the manufacturer's instructions.[333] Prior to 28 May 2012, Xpert version G3 cartridges were used; from 28 May 2012 onwards, Xpert version G4 cartridges were used.[279] The change in cartridges was based on what was supplied by the distributor at the time of cartridge restocking.

For laboratory clusters, both sputum specimens were transported daily to the National Health Laboratory Service (NHLS) laboratory at the district hospital using the existing routine courier 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. 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 smear microscopy results at the clinic prior to the study.

For point-of-care clusters, Xpert MTB/RIF was performed on site by the trained study nurse in a dedicated room within a park home (a prefabricated multi-room modular unit located outside the main clinic building). N95 respirator masks were used but no biosafety cabinet. Under the point-of-care strategy, participants were advised to wait for their result the same day. If they were unable or unwilling to wait at the clinic, they were advised to return the following day or any subsequent day.

The Xpert MTB/RIF assay can generate three types of results other than positive and negative: error, invalid and no result. These results are associated with different problems with the sample and with the assay. In the event of any of these results, and if sufficient sample/buffer mix remained, a repeat Xpert MTB/RIF test was performed. If insufficient sample remained or if there was still no definitive result, a second sputum specimen was collected at the earliest convenience (usually when the patient collected the initial result). A second sputum specimen was also collected in the event of leakage of the initial specimen during transport from clinic to laboratory.

Second sputum specimens were tested under whichever strategy was operating at the time that the second specimen was collected.

A repeat Xpert test was also recommended in the protocol in the event of a result demonstrating the presence of rifampicin-resistant *M. tuberculosis*. The repeat test could be performed with remaining sample/buffer mix from the original specimen or with a fresh specimen collected when the participant collected their result. This was included because of the concern about suboptimal specificity and the potential for false-positive rifampicin resistance results at the time of study design, so as to gather data on the reliability of repeat Xpert tests in differentiating true positive and false positive results.[46,278,334]

Specimens for culture and drug susceptibility testing (DST) were forwarded via the routine motorised transport system to Hlabisa laboratory and then onwards to the provincial NHLS laboratory at Inkosi Albert Luthuli Hospital in Durban (approximately 260km by road). Mycobacterial growth indicator tubes (MGIT) were inoculated and incubated for up to 6 weeks. Identification of *M. tuberculosis* was confirmed from all positive cultures using niacin and nitrate and/or Rapid MPT64 antigen assay (Standard Diagnostics, Inc. (SD), Yongin, Korea). The Genotype MTBDR*plus* assay (Hain Lifescience, Nehren, Germany) was performed on culture positive isolates to identify mutations associated with rifampicin and isoniazid resistance. Phenotypic DST for key first-line and second-line drugs (rifampicin, isoniazid, ofloxacin, and kanamycin) was performed using the 1% proportion method on Middlebrook 7H10 agar plates, only for isolates with rifampicin and/or

isoniazid resistance on LPA. Standard drug concentrations were used: 1 µg/ml rifampicin, 0.2 µg/ml isoniazid, 5 µg/ml kanamycin and 2 µg/ml ofloxacin.

4.8.6 HIV testing

All participants with unknown or negative HIV status were counselled and offered rapid HIV testing prior to enrolment. This mirrored routine clinical practice at the clinic, where all TB suspects are offered HIV counselling and testing before sputum collection. All HIV-infected participants were referred for CD4+ T-cell count and HIV care and treatment as per routine clinic procedures.

4.8.7 Additional investigations

Other investigations (e.g. chest X-ray) and referrals were ordered at the discretion of the study nurse and other clinic staff and were according to national guidelines and local protocols. I supported the clinic by holding a weekly clinic as a medical officer, to which complex patients or suspects requiring further investigation could be referred. Additional samples were collected at the discretion of the medical officer if there were features suggestive of extrapulmonary TB (e.g. lymph node aspirate, pleural aspirate), but these specimens were submitted for laboratory testing as per routine protocols and did not undergo Xpert MTB/RIF testing. Otherwise, during the trial there were one to two medical officer visits per week, one specifically for initiation of antiretroviral therapy and the other for primary health care management (e.g. hypertension, diabetes, epilepsy). There were no X-ray facilities at the PHC clinic so a request for chest X-ray required referral to the district hospital. At the district hospital, a daily ‘cough clinic’ operated in concert with but physically separated from the main outpatient department where TB suspects with negative sputum microscopy or Xpert MTB/RIF results could be assessed by a medical

officer with a chest X-ray.[335] There was a private X-ray facility in the nearest town (~6km distance), where a chest X-ray cost ZAR 150 (approximately equivalent to £10). Participants were also free to access other public and private health practitioners and facilities. Only the public sector is allowed to stock and distribute anti-TB treatment in South Africa; it is not generally available in the private sector in South Africa. After diagnosing someone with TB, private doctors are meant to immediately refer the individual to a public health facility.

4.8.8 Clinical management

Clinical decisions were made on the basis of the Xpert MTB/RIF results and, where appropriate, other clinical and laboratory information. The standard treatment regimen (isoniazid [H], rifampicin [R], pyrazinamide [Z] and ethambutol [E]) could be initiated by the study nurse or TB nurse. All patients diagnosed with DR-TB were first seen by the medical officer 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 were booked by the medical officer after reviewing the patient. Generally, patients were admitted to the TB inpatient ward at Hlabisa Hospital two to three days before their scheduled appointment, travelled to and from Durban on an outpatient basis with Department of Health transport, and then stayed on the TB ward at Hlabisa Hospital for at least one month for supervision of treatment and monitoring for toxicity. Following the first month and if patients were clinically stable, treatment continued at home (injectable agents were given at the nearest PHC clinic or by mobile injection teams) 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 generally admitted to King Dinuzulu Hospital for specialist inpatient management.

Management of suspects who tested negative by Xpert MTB/RIF followed existing protocols for smear-negative TB suspects. Antibiotics were prescribed (amoxicillin 500mg tds for five days or erythromycin 500mg qds for five days) and patients were advised to return if symptoms had not improved after 14 days. Patients who remained symptomatic following this course of antibiotics could be referred to the district hospital for chest X-ray and review by a medical officer.

4.9 Outcome evaluation

To ascertain the primary and secondary outcomes, all participants enrolled in the trial were invited to attend for clinic review with the study nurse two months after the enrolment visit; a specific date was given at the end of the enrolment visit although participants were told that they could attend any time from that date onwards. Participants who attended for follow-up were reimbursed with a ZAR 50 food voucher (approximately equivalent to £3.30). Participants were also asked to consent to telephone follow-up and/or home visit in case clinic visit was not possible. A message was sent to their designated phone via short message service (SMS) to remind the participant if they did not attend on the given day.

The study nurse was responsible for collecting the data relating to the primary and secondary outcomes at time of follow-up. Data was collected on the 'Follow-up Case Report Form' (Appendix H), in particular regarding TB treatment initiation and/or changes, hospital admissions, HIV testing, CD4+ T-cell count testing and ART

initiation (where appropriate). In the event that no contact was made with the patient or with the named contact persons, information was collated from clinic TB files and registers and the operational database for the HIV programme – permission to use this data was included in the informed consent process.

4.10 Sample size calculation

The baseline assumptions for the primary outcome analysis were that:

- Kwamsane clinic screened approximately 150 new TB suspects per month, 120 of whom would meet eligibility criteria
- An estimated 25% of TB and DR-TB suspects would have a positive MGIT culture
- The sensitivity of a single Xpert MTB/RIF test compared to reference standard of single MGIT culture would be approximately 75% [218,246]
- An estimated 10% of Xpert MTB/RIF positive cases in the hospital laboratory arm would not return for or receive result as indicated
- An estimated 50-70% of Xpert MTB/RIF negative cases would be diagnosed by other means (e.g. chest X-ray) prior to 30 days but that this proportion would be lower in the laboratory arm

The study was designed to detect a 10 percentage point increase in the proportion of culture-confirmed 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).[336] With $\kappa=0.05$ and a mean cluster size of 12, it was estimated that 16 clusters and 188 culture-positive TB cases were needed in each arm to detect this difference with 95% confidence and 80%

power. It was assumed that 10% of individual participants would be lost to follow-up at time of outcome evaluation (60 days), so 208 culture-positive TB cases were needed in each arm. Based on the assumption that 25% of TB suspects would have a positive culture, this required enrolment of 1664 TB suspects, which equated to ~85% of eligible suspects at KwaMsane clinic over the planned time period of the study (August 2011-December 2012).

Although the original sample size was 32 clusters, the final sample size was 36 clusters, as enrolment into the trial was extended due to the lower than expected culture positivity rate (see p114).

In terms of the key secondary endpoints, all-cause mortality was measured in all participants, regardless of TB status. The sample size of 16 clusters per arm and 60 participants per cluster gave approximately 80% power to detect a 33% change in mortality from a baseline of 12% in the district hospital arm with 95% confidence. In the absence of local data on mortality amongst people investigated for TB, the assumption for baseline mortality was based on previously published data reporting mortality in TB suspects,[73,337] and local data on mortality amongst TB and MDR-TB cases.[122]

The coefficient of variation (κ) was small, but with time blocks as the unit of randomisation there was expected to be minimal variation between clusters. This value of κ corresponded to a range of proportions appropriately treated in the laboratory arm of 77-94% for individual clusters. There was no reliable data from previous cluster randomised trials to inform the value of κ . There have been few

published trials where the unit of randomisation is a time block rather than a geographical or organisational cluster. The trials that have been published did not use consistent methods for sample size calculation - some adjusted appropriately for cluster variation [338-340]; others arbitrarily inflated the sample size from that for an individual RCT [341]; and one based the sample size on the numbers available to participate.[342]

4.11 Statistical analysis

4.11.1 Participants and baseline characteristics

A trial profile of participants was prepared as per the Consolidated Standards of Reporting Trials (CONSORT) guidelines, including the extension to cluster randomised trials.[343-345] The baseline demographic and clinical characteristics were presented in a table according to study arm.

4.11.2 Baseline analysis

Comparison of baseline characteristics was performed to characterise the study population and to identify baseline imbalances occurring due to chance between the study arms. No test of statistical significance was performed and confidence intervals and *p* values were not reported. The purpose of the comparison was to determine whether any baseline covariates needed to be adjusted for in the final analyses. The baseline analysis was performed for all participants and then separately for culture-positive cases eligible for the primary outcome analysis.

4.11.3 Primary analysis

4.11.3.1 Overview

The primary analysis was the comparison of the proportion of culture-confirmed TB cases commenced on appropriate anti-TB treatment within 30 days of enrolment (binary outcome).

4.11.3.2 Population for analysis

This analysis was performed on an intention-to-treat basis. All individuals were analysed on the basis of the group to which they were randomised, regardless of the circumstances under which they received the intervention. The analysis included individuals with a positive culture (using the Mycobacterial growth indicator tube (MGIT) system) identified as *Mycobacterium tuberculosis*, but excluded cases on TB treatment at the time of enrolment (smear non-converters or failures still on treatment) that had a positive culture isolate susceptible to rifampicin and isoniazid. These participants were excluded because the appropriate management would involve continuation of the same treatment and therefore participants would reach the primary endpoint by default, without any specific action having been taken.

4.11.3.3 Definitions for primary endpoint

The definition of appropriate anti-TB treatment was based on the drug susceptibility pattern with reference to standard treatment guidelines (Table 4.1). Rifampicin and isoniazid resistance were defined on the basis of documented resistance with either phenotypic DST or LPA. The concordance between phenotypic DST and LPA results was described.

In practical terms, rifampicin mono-resistance was treated with a standardised MDR-TB regimen (with the addition of isoniazid) whereas isoniazid mono-resistance was treated with standard first-line regimen for 6-9 months.[346] Although streptomycin was included as part of the standard re-treatment regimen in national guidelines at the time of study,[327] there was already a process of withdrawing its use even before the introduction of Xpert MTB/RIF. Therefore, the use of streptomycin in re-treatment cases susceptible to rifampicin was not required to meet the definition of appropriate treatment.

4.11.3.4 Statistical methods

This binary outcome was analysed at an individual level, accounting for within-cluster correlation. Regression modelling using generalised estimating equations with a binomial distribution function and a logit link was applied, specifying an exchangeable working correlation matrix. The odds ratio was reported with 95% confidence intervals and a p value from the Wald test. Where a major imbalance existed in any of the baseline individual-level covariates and the covariate could plausibly influence the outcome, a supplementary analysis was performed for the effects of the individual-level covariates. This was considered supportive to the primary analysis.

4.11.3.5 Analysing for effect modification of primary outcome

To determine whether the effect of Xpert MTB/RIF positioning differed for the subgroup of people at high risk of drug resistance, an interaction term was incorporated into the model to examine the evidence for effect modification. The

estimated difference in intervention effect between those with high risk of drug resistance versus those without risk of drug resistance was reported with 95% confidence intervals and a p value from the Wald test for the interaction.

4.11.3.6 Missing data

A complete case analysis was conducted (i.e. only including cases with post-baseline follow-up). There was no imputation for missing outcome data. Incomplete cases were described and their baseline characteristics were compared with those of the complete cases to determine the extent of any difference.

Table 4-1 Definitions of appropriate anti-TB regimen for primary and secondary endpoints

| Case definition | Appropriate initial anti-TB drug regimen |
|---|--|
| <i>M. tuberculosis</i> susceptible to rifampicin and isoniazid | Isoniazid + rifampicin + pyrazinamide + ethambutol ± streptomycin* |
| <i>M. tuberculosis</i> with mono-resistance to isoniazid | Isoniazid + rifampicin + pyrazinamide + ethambutol ± streptomycin* |
| <i>M. tuberculosis</i> with mono-resistance to rifampicin | Standardised second-line regimen ^l ± isoniazid |
| Multidrug-resistant <i>M. tuberculosis</i> (MDR-TB) [†] | Standardised second-line regimen ^l |
| Extensively drug-resistant <i>M. tuberculosis</i> (XDR-TB) [‡] | Standardised XDR-TB regimen [¶] |
| <i>M. tuberculosis</i> with unknown drug susceptibility [§] | Isoniazid + rifampicin + pyrazinamide + ethambutol |

* At the time of the study, streptomycin was included as part of standard re-treatment regimen in national guidelines, although in practice was rarely prescribed

[†] MDR-TB defined as resistance to rifampicin and isoniazid

[‡] XDR-TB defined as MDR plus resistance to ofloxacin and kanamycin

[§] Drug susceptibility test not performed or unsuccessful

^l Standardised regimen according to national guidelines (kanamycin/amikacin + fluoroquinolone + ethionamide + cycloserine/terizidone ± pyrazinamide ± ethambutol)[346]

[¶] Standardised regimen according to national guidelines (capreomycin + fluoroquinolone + ethionamide + cycloserine/terizidone + PAS + clofazimine)[346]

4.11.3.7 Participants investigated under alternative strategy

There were instances where individuals randomised to laboratory or clinic blocks were actually investigated under the alternative diagnostic strategy, either due to delay in specimen submission or a requirement for additional specimens. There were also instances where individuals underwent testing under both strategies, in the event of repeat testing occurring in a different time block. These occurrences were reported in the results section but all analyses were performed on an intention-to-treat basis.

4.11.4 Secondary analyses

4.11.4.1 Overview

The secondary analyses of clinical outcomes included binary data and time-to-event data. The secondary outcomes are summarised in Table 4.2 and described below.

4.11.4.2 Time to initiation of appropriate TB treatment for culture-confirmed pulmonary TB cases

The population for this analysis was as per the primary outcome, i.e. individuals with a positive MGIT culture identified as *Mycobacterium tuberculosis*, excluding cases on TB treatment at the time of enrolment (smear non-converters or failures still on treatment) with a positive culture isolate susceptible to rifampicin and isoniazid.

Time was measured in days from date of enrolment to date of treatment commencement. Follow-up time was right censored at time of death for participants who died prior to appropriate treatment and at 60 days for those who had not initiated appropriate anti-TB treatment. The definition of appropriate TB treatment was as per the primary outcome (Table 4.1). A complete case analysis was conducted, so individuals with no post-baseline follow-up were excluded. This time-to-event outcome was measured at an individual level, accounting for within-cluster

correlation. Cause-specific Cox proportional hazards regression models were fitted with the shared frailty option to account for the cluster randomisation. Hazard ratios were presented with 95% confidence intervals. The proportional hazards assumption was examined graphically using the log-log plot and also using the score test based on scaled Schoenfeld residuals.[347]

4.11.4.3 Time to initiation of appropriate DR-TB treatment for pulmonary rifampicin-resistant TB cases confirmed by culture and either line probe assay (LPA) or phenotypic drug susceptibility testing (DST)

The population for this analysis was individuals with a positive MGIT identified as *Mycobacterium tuberculosis* and resistance to rifampicin ± isoniazid identified either on line probe assay or phenotypic drug susceptibility testing (rifampicin mono-resistance or multidrug resistance (MDR)). Time was measured from date of enrolment to date of commencement of appropriate drug-resistant TB treatment. Follow-up time was censored at time of death for participants who died prior to appropriate DR-TB treatment and at 60 days for those who had not initiated appropriate DR-TB treatment. A complete case analysis was conducted, so individuals with no post-outcome follow-up were excluded. This time-to-event outcome was measured at an individual level, accounting for within-cluster correlation. Cox proportional hazard models were fitted with the shared frailty option to account for the cluster randomisation. Hazard ratios were presented with 95% confidence intervals. The proportional hazards assumption was examined graphically using the log-log plot and also using the score test based on scaled Schoenfeld residuals.[347]

Table 4-2 List of secondary outcomes with populations for analysis and exclusions

| Secondary outcome | Population for analysis | Exclusions from analysis |
|---|--|--|
| Time to initiation of appropriate TB treatment for culture-confirmed pulmonary TB cases | Individuals with a positive culture growth identified as <i>Mycobacterium tuberculosis</i> | Individuals with missing outcome data Individuals on TB treatment at enrolment with a culture isolate susceptible to rifampicin and isoniazid |
| Time to initiation of appropriate DR-TB treatment for rifampicin-resistant TB cases confirmed by culture and either LPA or phenotypic DST | Individuals with rifampicin-resistant TB confirmed by culture and either LPA or phenotypic DST | Individuals with missing outcome data |
| All-cause mortality in TB and DR-TB suspects at 60 days | All individuals enrolled | Individuals with missing outcome data |
| Proportion of TB suspects and DR-TB suspects with at least one hospital admission within 60 days | All individuals enrolled | Individuals with missing outcome data |
| Time to initiation of antiretroviral therapy (ART) - for HIV-infected TB and DR-TB suspects not yet receiving but eligible for ART | HIV-infected individuals not receiving but eligible for ART based on CD4+ T-cell count ≤ 350 cells/ μ l, DR-TB or any TB after 1 Jun 2012 | Individuals with missing outcome data HIV-infected individuals with unknown ART eligibility HIV-infected individuals previously exposed to ART (except for prevention of mother-to-child transmission) |

ART, antiretroviral therapy; DR-TB, drug-resistant TB; DST, drug susceptibility testing; LPA, line probe assay

4.11.4.4 All-cause mortality in TB and DR-TB suspects at 60 days

The population for this analysis was all enrolled individuals (i.e. all TB suspects and DR-TB suspects). A complete case analysis was conducted, so individuals with no post-baseline follow-up were excluded. Participants lost to follow-up were described and compared with those with known outcomes to determine the extent of any differences. Deaths recorded on the follow-up CRF or in programme registers were included for the endpoint. No specific information was collected about cause of death. This binary outcome was measured and analysed at an individual level, and the analysis accounted for within-cluster correlation. Regression modelling using generalised estimating equations with a binomial distribution function and a logit link were applied, specifying an exchangeable working correlation matrix. The odds ratio with 95% confidence intervals was reported with a p value from the Wald test.

4.11.4.5 Proportion of TB suspects and DR-TB suspects with at least one hospital admission within 60 days

The population for this analysis was all enrolled individuals (i.e. all TB suspects and DR-TB suspects). A complete case analysis was conducted, so individuals with no post-baseline follow-up were excluded. As the follow-up CRF did not contain information on dates of hospital admission and follow-up often occurred beyond 60 days, it was necessary to cross-validate admissions with the district hospital information system. This outcome therefore only incorporated admissions to Hlabisa Hospital. This binary outcome was measured and analysed at an individual level, and the analysis accounted for within-cluster correlation. Regression modelling using generalised estimating equations with a binomial distribution function and a logit

link was applied, specifying an exchangeable working correlation matrix. The odds ratio with 95% confidence intervals was reported with a p value from the Wald test.

4.11.4.6 Time to initiation of antiretroviral therapy (ART) - for HIV-infected TB suspects and DR-TB suspects not yet receiving but eligible for ART

The population for this analysis was all individuals with documented HIV infection at enrolment who were antiretroviral therapy (ART) naïve and eligible for ART.

Eligibility for ART was defined by CD4+ T-cell count ≤ 350 cells/ μ l (prior to or on the date of enrolment), rifampicin-resistant TB disease or any TB disease after 1 June 2012. This was the date of implementation of the modified eligibility criterion recommending ART for all HIV-infected individuals with active TB disease. The presence of rifampicin-resistant TB disease or any TB disease after 1 June 2012 for the purposes of this analysis was based on the final Xpert MTB/RIF result, as this would in routine practice determine eligibility for ART.

In the protocol and the statistical analysis plan, WHO clinical stage 4 disease was also included in the definition for ART eligibility. However, this was dropped for final analysis as information on clinical stage was not collected during the trial and was captured in the HIV programme database only when individuals started ART.

Time was measured from date of enrolment to date of ART initiation. The date of ART initiation was based on documented date on follow-up CRF or, if the date was not recorded, from the HIV programme database. Follow-up time was censored at time of death for any participants that died prior to ART initiation. This time-to-event outcome was measured at an individual level, accounting for within-cluster correlation. Cox proportional hazard models were fitted with the shared frailty option

to account for the cluster randomisation. Hazard ratios were presented with 95% confidence intervals. The proportional hazards assumption was examined graphically using the log-log plot and also using the score test based on scaled Schoenfeld residuals.[347]

4.12 Economic evaluation and assessment of operational feasibility of point-of-care Xpert

The trial protocol laid out a framework for an economic evaluation to explore the cost-effectiveness of point-of-care Xpert placement (Appendix Q). The intention was to perform a nested sub-study to collect information on patient and household costs incurred during the diagnostic process. Unfortunately, due to staffing and time constraints this was not possible. Similarly, an evaluation of the operational feasibility of point-of-care Xpert was planned, with certain key indicators to be assessed throughout the trial (Appendix Q). Due to staffing constraints and workload, information was collected on some, but not all, of these indicators thus giving an incomplete assessment of operational feasibility.

4.13 Ethics approval and trial registration

The trial was approved by the Ethics Committee of the London School of Hygiene and Tropical Medicine, reference 5926 (Appendix I), the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, reference BF033/11 (Appendix J), the and the Health Research Committee of the KwaZulu-Natal Department of Health, reference 084/11 (Appendix K). Approval for the study was also obtained from Hlabisa Hospital (Appendix L) and the Africa Centre for Health

& Population Studies Community Advisory Board (Appendix M). The trial was registered with Current Controlled Trials on 17 June 2011 [ISRCTN 18642314] (Appendix N) and with the South African National Clinical Trials Register on 10 July 2011 [DOH-27-0711-3568] (Appendix O).

4.14 Trial oversight

The principal investigator was responsible for the conduct of the study, including study design, implementation, data collection, and data analysis. He was also responsible for co-ordination of local staff, liaison with Department of Health and National Health Laboratory Service, liaison with other relevant bodies, and protection of human subjects.

The Trial Management Committee consisted of all the Investigators and was responsible for the overall management of the trial and decisions about continuation of the trial. Regular teleconferences were held throughout the trial.

The Trial Steering Committee consisted of all the members of the Trial Management Committee plus three independent members (Professor David Moore [London School of Hygiene & Tropical Medicine], Professor Yunus Moosa [University of KwaZulu-Natal], and Dr Katherine Fielding [London School of Hygiene & Tropical Medicine]) and a representative from the funder (Wellcome Trust). The Steering Committee provided supervision for the trial and provided advice through the independent chairperson. The Steering Committee met six-monthly by teleconference.

Chapter 5 Trial results

5.1 Participant flow

A total of 36 two-week time blocks between 22 August 2011 and 01 March 2013 were randomised to one of the two strategies for Xpert MTB/RIF positioning. In July 2012, following the identification of a shortfall in the enrolment of culture-positive cases, the Trial Steering Committee recommended measures to enhance recruitment and to maximise the yield from sputum cultures. Despite implementation of these measures, enrolment remained below target. A decision was taken to extend the trial beyond the originally planned 32 clusters to maximise recruitment but the recruitment phase was not able to be extended beyond March 2013 due to time and financial constraints.

1526 individuals were screened and 1297 (85.0%) were enrolled in the study. There were a further 16 exclusions (14 duplicate enrolments and two who were later found not to have met the eligibility criteria) leaving 1281 participants for analysis. Figure 5.1 shows the flow of clusters and participants throughout the study. The reasons for exclusion are summarised in Table 5-1.

The distribution of participants between the different groups (HIV-infected and risk of drug-resistant TB) was comparable across the trial arms (Table 5-2). Overall, more than 90% of participants were HIV-infected and just under half had one or more risk factors for drug-resistant TB.

Table 5-1 Reasons for individual exclusion

| Reason | Laboratory | Point-of-care |
|--|------------|---------------|
| Exclusion from study* | 128 | 101 |
| Age <18 years | 15 | 12 |
| No cough | 69 | 49 |
| No documented HIV infection and no risk of drug resistance | 58 | 44 |
| Severely unwell requiring hospital admission | 4 | 0 |
| Previous MDR/XDR-TB | 1 | 4 |
| Suspicion of EPTB only | 7 | 6 |
| Unable to give informed consent | 4 | 3 |
| Exclusion from analysis | 6 | 10 |
| Duplicate enrolment† | 6 | 8 |
| On TB treatment at enrolment and not meeting correct criteria for risk of drug resistance‡ | 0 | 2 |

* Individuals could have more than one reason for exclusion

† For individuals enrolled twice, second enrolment excluded from analysis

‡ On TB treatment without evidence of smear non-conversion or treatment failure: one individual presented with cough during treatment for TB meningitis, another individual presented with persistent cough three weeks into standard first-line treatment for smear-positive pulmonary TB

Table 5-2 Distribution of participants between risk groups

| Risk group | Laboratory (<i>n</i> = 640) | Point-of-care (<i>n</i> = 641) |
|--|---------------------------------|------------------------------------|
| HIV-infected only | 351 (54.8) | 353 (55.1) |
| Risk of drug-resistant TB only | 51 (8.0) | 45 (7.0) |
| HIV-infected & risk of drug-resistant TB | 238 (37.2) | 243 (37.9) |

There was significant variability in cluster size in terms of both TB and DR-TB suspects and culture-positive TB cases. The number of suspects per cluster ranged between 19 and 56 and the number of culture-positive cases per cluster ranged between zero and nine. The mean cluster size was well balanced across the two arms

in terms of suspects but the point-of-care arm had more culture-positive TB cases (91 cases, mean 5.1 per cluster) than the laboratory arm (68 cases, mean 3.8 per cluster).

5.2 Baseline analysis

The baseline demographic and clinical characteristics of the individual participants are shown in Table 5-3. The majority of suspects (76.3%) had at least one other symptom suggestive of TB (fever, night sweats or weight loss) besides the cardinal symptom of cough. The median duration of cough for all suspects was two weeks (interquartile range (IQR) 1-4). Almost 40% of suspects had at least one prior episode of TB. Of those, 390 (78.0%) had one previous TB episode, 81 (16.2%) had two previous TB episodes, and 29 (5.8%) had three or more previous TB episodes. The latest TB episode was within five years of enrolment for most participants with any previous TB ($n = 306$, 61.2%).

The two arms were well balanced for most of the baseline characteristics. There was some evidence of imbalance for the CD4+ T-cell count in HIV-infected participants, with a higher median CD4+ T-cell count (280 cells/ μ l vs. 247 cells/ μ l) and lower proportion with CD4+ T-cell count <50 cells/ μ l (6.4% vs. 10.3%) in the laboratory arm. The proportion of HIV-infected participants on ART was comparable for the two groups. Most of the difference in CD4+ T-cell counts was attributable to those on ART (median CD4+T-cell count 327 cells/ μ l in laboratory arm vs. 293 cells/ μ l in point-of-care arm). CD4+ T-cell counts were similar across the arms for those not on ART (235 cells/ μ l in laboratory arm vs. 231 cells/ μ l in point-of-care arm).

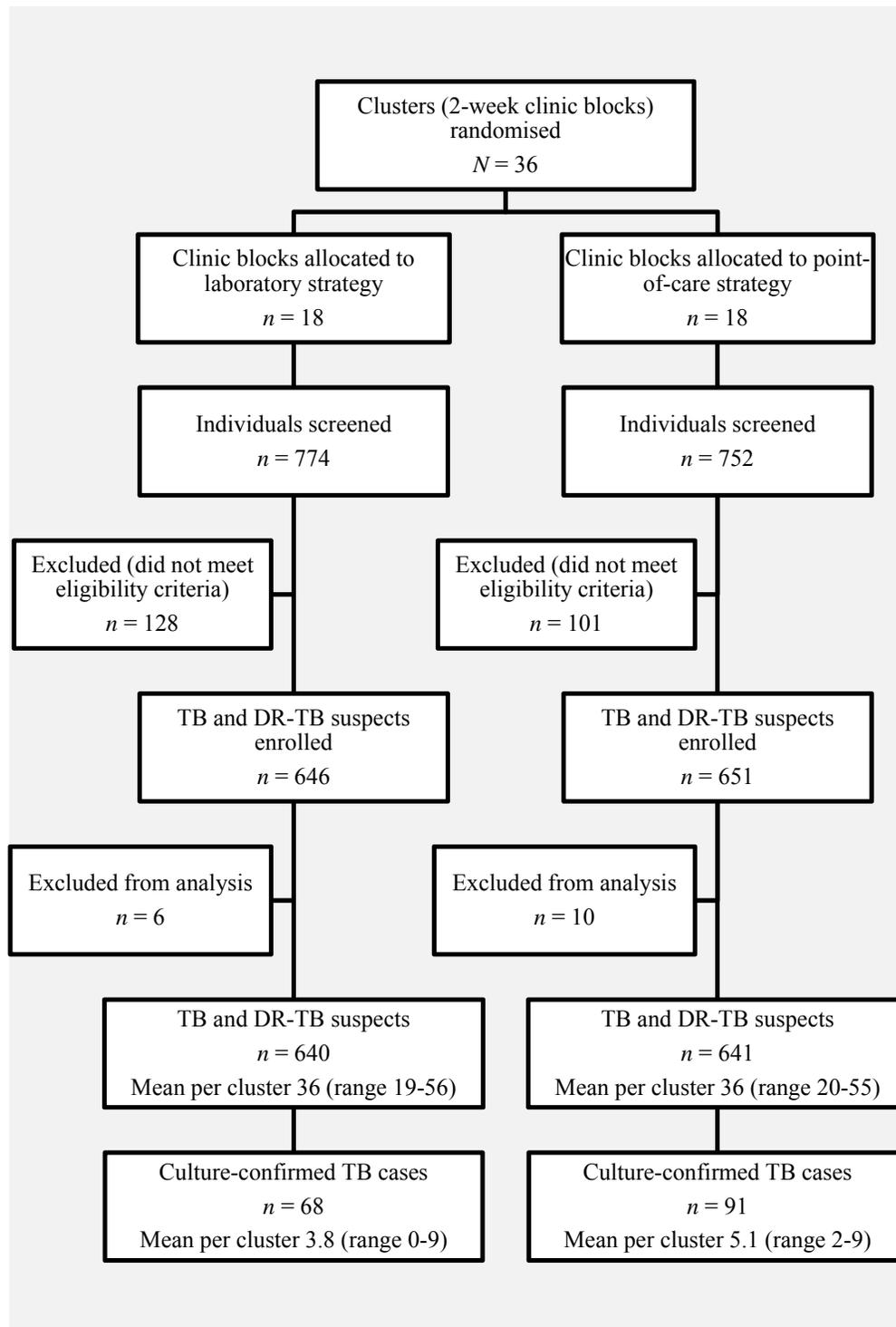


Figure 5-1 Trial profile

Table 5-3 Baseline characteristics of individual participants

| Variable | | Laboratory (<i>n</i> = 640) | Point-of-care (<i>n</i> = 641) |
|--------------------------------------|---------------------------------------|---------------------------------|------------------------------------|
| Sex | Female (<i>n</i> , %) | 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 (<i>n</i> , %) | 157 (24.5) | 147 (22.9) |
| | Weight loss (<i>n</i> , %) | 332 (51.9) | 335 (52.3) |
| | Fever (<i>n</i> , %) | 269 (42.0) | 256 (40.0) |
| | Night sweats (<i>n</i> , %) | 295 (46.2) | 298 (46.7) |
| Duration of cough (weeks)* | Median (IQR) | 2 (1-4) | 3 (1-4) |
| Current IPT use | Yes (<i>n</i> , %) | 8 (1.3) | 11 (1.7) |
| Risk of drug resistance | None (<i>n</i> , %) | 351 (54.8) | 353 (55.1) |
| | Treatment failure (<i>n</i> , %) | 4 (0.6) | 7 (1.1) |
| | Smear non-conversion (<i>n</i> , %) | 18 (2.8) | 21 (3.3) |
| | Previous TB treatment (<i>n</i> , %) | 253 (39.5) | 247 (38.5) |
| | Household contact (<i>n</i> , %) | 22 (3.4) | 15 (2.3) |
| | Health care worker (<i>n</i> , %) | 12 (1.9) | 9 (1.4) |
| | Prison last 12 months (<i>n</i> , %) | 7 (1.1) | 10 (1.6) |
| HIV infection | Positive (<i>n</i> , %) | 589 (92.0) | 596 (93.0) |
| | Negative (<i>n</i> , %) | 39 (6.1) | 39 (6.1) |
| | Never tested (<i>n</i> , %) | 6 (0.9) | 3 (0.5) |
| | Not disclosed (<i>n</i> , %) | 5 (0.8) | 3 (0.5) |
| | Missing (<i>n</i> , %) | 1 (0.2) | 0 |
| Antiretroviral therapy† | Current (<i>n</i> , %) | 238 (40.4) | 222 (37.3) |
| CD4+ T-cell count (cells/μl)†‡ | Median (IQR) | 280 (147-455) | 247 (119-415) |
| | ≤50 (<i>n</i> , %) | 41 (6.4) | 66 (10.3) |
| | 51-200 (<i>n</i> , %) | 152 (23.8) | 150 (23.4) |
| | 201-350 (<i>n</i> , %) | 149 (23.3) | 158 (24.6) |
| | 351-500 (<i>n</i> , %) | 85 (13.3) | 81 (12.6) |
| | >500 (<i>n</i> , %) | 108 (16.9) | 92 (14.4) |
| | Missing (<i>n</i> , %) | 54 (8.4) | 49 (7.6) |

IPT, isoniazid preventive therapy; IQR, interquartile range

* Cough duration missing for 11 participants (laboratory, *n* = 3; point-of-care, *n* = 8)

† Proportions are of HIV-infected participants

‡ CD4+ T-cell count closest to enrolment date (up to 18 months prior to or 30 days after enrolment)

5.3 Diagnostic process

5.3.1 Sputum specimen submission

A total of 1235 suspects (96.4%) submitted two spontaneously expectorated initial sputum specimens. Of these, 1197 (96.9%) submitted their initial two sputum specimens on the day of enrolment, 26 (2.1%) submitted specimens the following day, and 12 (1.0%) submitted specimens on a later date. The proportion of suspects that submitted specimens did not differ by trial arm (laboratory 96.7% vs. point-of-care 96.1%). There was also no evidence that the distribution of timing of specimen submission differed across the trial arms (Table 5-4). There were four occurrences of participants submitting the initial two sputum specimens during a different time block (cluster) and two of these crossed trial arms (one suspect was enrolled during laboratory block and submitted sputum specimens during point-of-care block; one suspect was enrolled during point-of-care block and submitted sputum specimens during laboratory block).

5.3.2 Xpert MTB/RIF

The results of Xpert MTB/RIF testing are shown in Table 5-5. Initial Xpert MTB/RIF tests yielded a valid result (positive or negative) in 553/619 (89.3%) in the laboratory strategy, compared to 596/616 (96.8%) in the POC strategy ($p < 0.001$). Allowing for repeat Xpert MTB/RIF tests using the remaining sputum/sample treatment reagent mix, a valid result was obtained in 571/619 (92.2%) in the laboratory strategy and 609/616 (98.9%) in the POC strategy ($p < 0.001$). The most obvious difference between the trial arms was with respect to the specimens not processed (in all cases due to specimen leakage during transit): 6.0% (37/619) of

initial specimens in the laboratory arm were not processed vs. 0.2% (1/616) in the point-of-care arm ($p < 0.001$).

Including only results from the initial specimen, 9.9% (61/619) in the laboratory arm and 3.4% (21/616) in the point-of-care arm did not collect their Xpert result within 60 days ($p < 0.001$).

Of the 48 participants in the laboratory strategy without a valid result from the initial sputum specimen, 40 submitted a second specimen after a median of 5 days (IQR 4-8); all but one of the repeat specimens yielded a valid Xpert result (seven positive and 32 negative). Of the seven participants in the POC strategy without a valid result from the initial sputum specimen, only one submitted a second specimen which produced a negative Xpert result. Overall, allowing for a maximum of two sputum specimens, a valid result was obtained for 610/619 (98.5%) in the laboratory strategy and 610/616 (99.0%) in the POC strategy ($p = 0.441$).

When considering results of all Xpert tests performed, around one in six participants had a positive Xpert test (positive for detection of *M. tuberculosis*) and, of those, around one in six had rifampicin resistance detected. There was no evidence of any significant differences in Xpert results between trial arms. Overall, defining indeterminate Xpert results as any of 'error', 'invalid', or 'no result', 3.9% (49/1235) of first Xpert tests gave an indeterminate result, and this was slightly higher in the laboratory arm than in the point-of-care arm (4.7% vs. 3.1%, $p = 0.146$). After allowing for repeat Xpert tests on the first sputum specimen, 1.4% (17/1235) yielded

an indeterminate result, with minimal difference between trial arms (laboratory 1.8% vs. point-of-care 0.9%, $p = 0.226$).

Table 5-4 Sputum submission and processing

| | Laboratory (n = 640) | Point-of-care (n = 641) |
|--------------------------------------|---------------------------------|------------------------------------|
| <i>Sputum submission</i> | | |
| Submitted initial sputum specimens | 619 | 616 |
| Day of enrolment | 602 (97.3) | 595 (96.6) |
| One day after enrolment | 10 (1.6) | 16 (2.6) |
| Two or more days after enrolment | 7 (1.1) | 5 (0.8) |
| Submitted additional sputum specimen | 40 (6.5) | 1 (0.2) |
| <i>Xpert testing</i> | | |
| First sputum specimen tested | 582 (94.0) | 615 (99.8) |
| Result from first sputum specimen | 571 (92.2) | 609 (98.9) |
| Result from second sputum specimen | 39 (6.1) | 1 (0.2) |
| Definitive Xpert result | 610 (98.5) | 610 (99.0) |

Table 5-5 Xpert MTB/RIF results

| Xpert MTB/RIF result | Laboratory (n = 619) | Point-of-care (n = 616) |
|-------------------------------|---------------------------------|------------------------------------|
| <i>First Xpert test</i> | | |
| MTB DETECTED | 97 (15.7) | 107 (17.4) |
| Rif Resistance NOT DETECTED | 81 (13.1) | 90 (14.6) |
| Rif Resistance DETECTED | 16 (2.6) | 17 (2.8) |
| MTB NOT DETECTED | 456 (73.7) | 489 (79.4) |
| ERROR | 9 (1.5) | 10 (1.6) |
| INVALID | 16 (2.6) | 9 (1.5) |
| NO RESULT | 4 (0.6) | 0 |
| Not processed* | 37 (6.0) | 1 (0.2) |
| <i>First sputum specimen†</i> | | |
| 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) |
| ERROR | 6 (1.0) | 4 (0.6) |
| INVALID | 5 (0.8) | 2 (0.3) |
| NO RESULT | 0 | 0 |
| Not processed* | 37 (6.0) | 1 (0.2) |
| <i>All Xpert tests‡</i> | | |
| 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) |
| ERROR | 1 (0.2) | 3 (0.5) |
| INVALID | 1 (0.2) | 2 (0.3) |
| NO RESULT | 0 | 0 |
| Not processed ^a | 7 (1.1) | 1 (0.2) |

* Not processed was in all cases due to specimen leakage during transport

† This allowed for repeat testing using residual specimen/buffer mix

‡ A maximum of two sputum specimens could be submitted for Xpert MTB/RIF testing

Only 18/33 (54.5%) of those with an initial positive Xpert with rifampicin resistance detected submitted a second sputum specimen for Xpert testing as recommended in the protocol. In all but one instance, the second Xpert confirmed the initial result (positive with rifampicin resistance detected). In the one exception, an error was reported for the second specimen. The relatively low level of adherence to this aspect of the protocol may have been because repeat Xpert testing for rifampicin-resistant cases was not included in the national TB guidelines and national Xpert algorithm, which were implemented around the time that the trial started, and possibly also that the nurses gained confidence in the accuracy of Xpert as the trial progressed.

5.3.3 Culture and drug susceptibility testing (DST) results

A total of 1235 participants submitted a sputum specimen for culture and drug susceptibility testing (DST) according to the protocol. The results of the Mycobacterial Growth Indicator Tube (MGIT) culture are shown in Table 5-6. The most striking finding was that the overall yield from culture was actually 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. Non-tuberculous mycobacteria were isolated in only 13 cases (1.1% of all suspects or 7.5% of positive cultures) and one case had a positive culture without definitive identification. The lower yield from culture was at least partly explained by the substantial attrition during the laboratory processes. Overall 11.5% (142/1235) of specimens were not processed (in the majority because of specimen leakage in transport), 8.3% (103/1235) of cultures were contaminated, and in 2.9% (36/1235) no culture result was returned to the clinic nor could be identified in the laboratory information system. 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*.

Table 5-6 Results of Mycobacterial Growth Indicator Tube (MGIT) culture

| Result | Laboratory (n = 619) | Point-of-care (n = 616) |
|--|---------------------------------|------------------------------------|
| Positive (<i>M. tuberculosis</i>) | 68 (11.0) | 91 (14.8) |
| Positive (NTM) | 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) |
| <i>Specimen leaked</i> | 63(10.2) | 70 (11.4) |
| <i>Incorrect details*</i> | 2 (0.3) | 0 |
| <i>Processed for smear microscopy†</i> | 6 (1.0) | 1 (0.2) |
| No result | 14 (2.3) | 22 (3.6) |

NTM, non-tuberculous mycobacteria

* Participant details on laboratory form and specimen container did not match

† Specimen processed in error for smear microscopy instead of culture

The baseline 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 ($n = 953$), were compared with the characteristics of those without an evaluable result ($n = 282$). Those with an evaluable culture result were more likely to be HIV infected (93.6% vs. 89.0%, $p = 0.010$), more likely to be on ART if HIV-infected (40.7% vs. 33.9%, $p = 0.049$) and have higher baseline CD4+ T-cell count (median 277 cells/ μ l vs. 238 cells/ μ l, $p = 0.053$), but otherwise their

characteristics were similar to those of the participants without an evaluable result (Table 5-7).

Table 5-7 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

* Proportions are of HIV-infected participants

The results of drug susceptibility testing collated from line probe assay (LPA) and phenotypic DST are illustrated in Table 5-8. Concordance between line probe assay and phenotypic DST for rifampicin and isoniazid is presented in Table 5-9.

Overall, 20.1% (32/159) of *M. tuberculosis* isolates were rifampicin resistant, the majority of which (29/32, 90.6%) were multidrug resistant. Isoniazid mono-

resistance was rare, detected in only 1.9% (3/159) of *M. tuberculosis* isolates.

Overall, rifampicin resistance was present in 9.7% (9/93) of culture-positive cases with no previous exposure to anti-TB treatment and 25.9% (14/54) of previously treated cases (Table 5-10).

Table 5-8 Results of drug susceptibility testing (combined from line probe assay and phenotypic DST)

| 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 (MDR) | 14 (20.6) | 15 (16.5) |

Phenotypic DST was performed if line probe assay detected isoniazid and/or rifampicin resistance and if line probe assay results were indeterminate

MDR, multidrug resistance

* LPA reported as isoniazid susceptible, rifampicin inconclusive (phenotypic DST unsuccessful)

Table 5-9 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 |

Figures in bold were defined as resistant for the purposes of analysis

Table 5-10 Rifampicin resistance according to history of TB treatment and trial arm

| | Laboratory (n = 68) | Point-of-care (n = 91) | Total |
|---|--------------------------------|-----------------------------------|----------------|
| New cases | 3/37 (8.1%) | 6/56 (10.7%) | 9/93 (9.7%) |
| <i>With documented risk of drug resistance*</i> | 1/4 (25.0%) | 1/4 (25.0%) | 2/8 (25.0%) |
| <i>Without documented risk of drug resistance</i> | 2/33 (6.1%) | 5/52 (9.6%) | 7/85 (8.2%) |
| Previously treated cases | 9/27 (33.3%) | 5/27 (18.5%) | 14/54 (25.9%) |
| Cases on treatment at enrolment† | 4/4 (100%) | 5/8 (62.5%) | 9/12 (75.0%) |
| All cases | 16/69 (23.2%) | 16/91 (17.6%) | 32/160 (20.0%) |

* Two previously untreated rifampicin-resistant cases had household exposure to known drug-resistant cases

† Includes participants with treatment failure and sputum smear non-conversion

5.3.4 Imbalance in culture positivity between trial arms

The imbalance in culture positivity between the trials arms was unexpected given the trial design: as noted above, despite similar numbers of suspects, the point-of-care arm had a higher proportion of culture-positive TB cases (Table 5-11). The difference in proportion between the arms persisted after allowance for unevaluable culture results.

Table 5-11 Proportion of participants with positive culture by trial arm

| | Laboratory | Point-of-care |
|---|-------------------|----------------------|
| All participants | 68/640 (10.6) | 91/641 (14.2) |
| Participants who submitted sputum | 68/619 (11.0) | 91/616 (14.8) |
| Participants with evaluable culture result* | 68/478 (14.2) | 91/475 (19.2) |

* Excluding contaminated cultures, those not processed, and those without valid result

There were no significant differences between the arms in presenting symptoms or duration of cough (Table 5-3). There was no difference in the proportion of participants that had already presented at least once previously to a health care facility with cough (34.1% in the laboratory arm vs. 36.4% in the point-of-care arm). The lower median CD4+ T-cell count (247 cells/ μ l vs. 280 cells/ μ l) and the higher proportion with CD4+ T-cell count <50 cells/ μ l (10.3% vs. 6.4%) in the point-of-care arm could suggest some bias with sicker patients being enrolled during point-of-care clusters, although as noted previously the difference between the arms in median CD4+ T-cell count was largely due to participants on ART and not untreated individuals.

There was an uneven distribution of culture-positive cases across clusters (Figure 5-2). There was some evidence of seasonality in the number of suspects enrolled, with more suspects enrolled during clusters in winter months (June-August). There was a difference in culture positivity between arms in the first eight clusters (7.5% in laboratory arm vs. 17.5% in POC arm), which could suggest differential enrolment of participants more likely to have TB into point-of-care clusters during the early phase of the trial. It could also be that there was a pool of cases that were undiagnosed prior to the start of the trial and that these cases were more likely to present during point-of-care blocks. Removing the first eight blocks, the overall proportion with a positive culture was more balanced between the two arms (12.3% in laboratory arm vs. 13.8% in POC arm).

There was also evidence that the difference in culture positivity between arms was more marked for participants enrolling in the second week of a cluster block (Table

5-12), although there were no significant differences in culture yield across the arms depending on the day of the week enrolled (Table 5-13). The difference by week could suggest that community members or staff learnt when the diagnostic system was in operation at the clinic and that people strongly suspected to have TB were as a result more likely to present or be enrolled during the second week of a cluster.

Table 5-12 Culture positivity by enrolment week of block

| Week | Laboratory | Point-of-care |
|-------------|-------------------|----------------------|
| 1 | 29/281 (10.3) | 36/288 (12.5) |
| 2 | 39/320 (12.2) | 55/306 (18.0) |

Table 5-13 Culture positivity by enrolment day (of either week during each block)

| Day | Laboratory | Point-of-care |
|------------|-------------------|----------------------|
| Monday | 18/125 (14.4) | 22/134 (16.4) |
| Tuesday | 20/141 (14.2) | 24/133 (18.1) |
| Wednesday | 12/121 (9.9) | 17/96 (15.0) |
| Thursday | 14/166 (8.4) | 19/145 (11.6) |
| Friday | 4/48 (8.3) | 9/50 (18.0) |

One aspect that could not be explored was whether there was differential adherence to the recommended one hour gap between collection of the two sputum specimens, as data on precise timing of specimen collection was not recorded. Lower adherence to this time gap in the laboratory arm, if participants were not waiting at the clinic, could plausibly have affected the mycobacterial yield in the second sputum specimens, which were systematically submitted for culture.

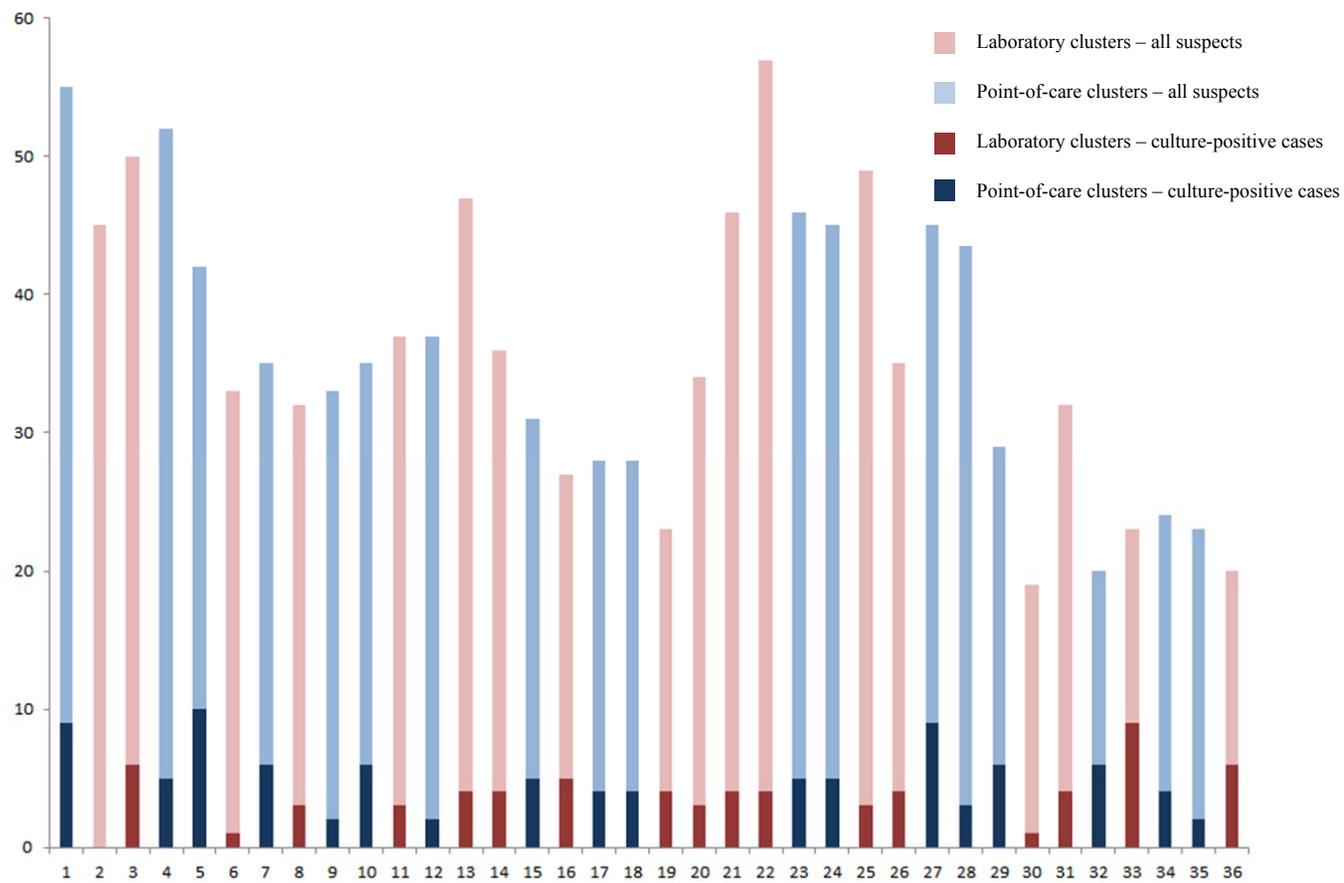


Figure 5-2 Frequency distribution of suspects and culture-positive cases by cluster

In summary, there was some evidence to suggest that there may have been some positive selection bias of people more likely to have TB into the point-of-care arm, although this could not be proven.

5.4 Concordance between Xpert MTB/RIF and culture results

The concordance between Xpert MTB/RIF result and MGIT culture result for all participants is displayed in Table 5-14. Only six of those that submitted sputum specimens (0.5%) had no result from either Xpert or culture. Of the 39 participants with positive Xpert and negative culture, 19 (48.7%) were on TB treatment at enrolment. In the majority of these cases (17/19), Xpert detected rifampicin susceptible *M. tuberculosis* and so may have detected non-viable bacilli.

A further six of the remaining 20 participants with positive Xpert and negative culture had at least one previous episode of TB and in three of these cases the most recent episode of TB treatment had occurred within the year prior to enrolment (two were enrolled due to treatment failure) again raising the possibility that non-viable bacilli might have been detected by the Xpert assay. Nevertheless, the majority of those not on treatment with discordant Xpert positive/culture negative results had not previously received treatment and so the reason for the discordant results was not clear. Whilst the possibility of false positive Xpert detection of *M. tuberculosis* cannot be discounted, it is also plausible that differences between the two sputum specimens or technical issues with the cultures might have given rise to these discordant results.

Table 5-14 Overall concordance between Xpert MTB/RIF and culture results, all participants

| | | MGIT culture result | | | Total |
|---------------|-----------|---------------------|----------|------------|-------|
| | | Positive | Negative | No result* | |
| Xpert result† | Positive | 128 | 39 | 46 | 213 |
| | Negative | 30 | 734 | 243 | 1007 |
| | No result | 1 | 8 | 6 | 15 |
| | Total | 159 | 781 | 295 | 1235 |

* No result for MGIT culture included contaminated cultures and cultures positive for non-tuberculous mycobacteria (as in these cases the presence or absence of *M. tuberculosis* cannot be definitively ascertained)

† Xpert result defined as result of first valid test (on initial specimen or repeat specimen)

5.5 Outcome data

Outcome data were obtained for 919 (71.7%) participants and the proportion was not different between trial arms: laboratory arm ($n = 461$, 72.0%) vs. point-of-care arm ($n = 458$, 71.5%). Outcome ascertainment was prioritised for culture-positive cases and so follow-up data was available for all cases (159/159).

The sources of outcome data for suspects and cases are shown in Table 5-15.

Overall, 90.0% (827/919) of all outcome data came from contact with the participant; 81.5% (749/919) came from a participant follow-up visit at the clinic. These proportions were lower for cases: 76.7% (122/159) of outcome data for cases came from contact with the participant; 65.4% (104/159) came from a participant follow-up visit at the clinic. The proportions were lower because, where outcome data had not been collected from the participant or designated contact, information was collated from the clinic TB and HIV registers and this was done predominantly for culture-positive cases.

The median time to follow-up for all participants with outcome data ascertained from participant or nominated contact ($n = 878$) was 92 days (IQR 72-156). The time to follow-up was somewhat shorter for the 443 participants in the laboratory arm (86 days, IQR 71-153) than for the 435 participants in the point-of-care arm (105 days, IQR 73-160).

Table 5-15 Source of information for outcome data

| Source of data | Laboratory | Point-of-care |
|--------------------------------|------------|---------------|
| All participants | 461 | 458 |
| Participant clinic visit | 389 (84.4) | 360 (78.6) |
| Participant phone call | 32 (6.9) | 46 (10.0) |
| Nominated contact clinic visit | 5 (1.1) | 6 (1.3) |
| Nominated contact phone call | 10 (2.2) | 19 (4.2) |
| Home visit | 7 (1.5) | 4 (0.9) |
| Clinic registers | 17 (3.7) | 18 (3.9) |
| Other* | 0 | 2 (0.4) |
| Missing | 1 (0.2) | 3 (0.7) |
| Culture-positive cases | 68 | 91 |
| Participant clinic visit | 52 (76.5) | 52 (57.1) |
| Participant phone call | 4 (5.9) | 14 (15.4) |
| Nominated contact clinic visit | 0 | 0 |
| Nominated contact phone call | 0 | 7 (7.7) |
| Home visit | 0 | 0 |
| Clinic registers | 12 (17.6) | 15 (16.5) |
| Other* | 0 | 2 (2.2) |
| Missing | 0 | 1 (1.1) |

* Other included two reports of deaths: from TB tracing team ($n = 1$) and hospital staff ($n = 1$)

5.6 Primary outcome

The primary outcome measure was the proportion of culture-confirmed pulmonary TB cases initiated on appropriate anti-TB treatment within 30 days of enrolment. For this analysis, three cases were excluded from the point-of-care arm 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-confirmed pulmonary TB cases (68 in laboratory arm; 88 in point-of-care arm). The baseline characteristics of the culture-positive cases were balanced, except for a higher proportion of females and a higher proportion with risk of TB drug resistance in the point-of-care arm (Table 5-16). The median time to outcome evaluation was 80 days (IQR 72-133) in the laboratory arm and 100 days (IQR 73-160) in the point-of-care arm.

The proportion of cases initiated on appropriate anti-TB treatment within 30 days of enrolment was 76.5% (52/68) in the laboratory arm and 79.5% (70/88) in the point-of-care arm. In the primary analysis using generalised estimating equations with a binomial distribution function and a logit link, and allowing for within-cluster correlation, the odds ratio (OR) for initiating appropriate anti-TB treatment within 30 days for the point-of-care arm compared to the laboratory arm was 1.13 (95% CI 0.51-2.53, $p = 0.76$) (Table 5-17). The estimated value of the coefficient of variation (κ) was 0.11.

As there was an imbalance in sex and baseline risk of drug resistance and these could plausibly affect the likelihood of starting appropriate anti-TB treatment within 30

days, the analysis was repeated, as specified *a priori*. This made no significant difference to the results (Table 5-17).

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 5-18). Overall, 215 participants started TB treatment within 60 days, 154 (71.6%) on the basis of a positive Xpert result and 14 (6.5%) on the basis of a positive culture. 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. Forty-five participants started treatment on clinical or radiological grounds, of whom only 7 (15.6%) had a subsequent positive culture identified as *M. tuberculosis* (Table 5-19).

The majority (25/30, 83.3%) of Xpert negative/culture positive cases did not initiate appropriate TB treatment within 30 days and 50.0% (15/30) did not initiate appropriate treatment within 60 days. In the laboratory strategy, none of ten Xpert negative/culture positive cases started appropriate TB treatment within 30 days. Of the seven 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

two 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); one of those cases had initially commenced first-line anti-TB treatment on the basis of the initial 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 started drug-resistant TB treatment (after 125 days), one died after commencing first-line anti-TB treatment (on the basis of the positive culture results before DST results), and one was not recorded as having started any TB treatment.

In summary, there was no evidence of an effect of Xpert positioning on the proportion of culture-positive pulmonary TB cases initiated on appropriate anti-TB treatment within 30 days.

Table 5-16 Baseline demographic and clinical characteristics for culture-confirmed pulmonary TB cases included in primary analysis

| Variable | | Laboratory (n = 68) | 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 infection | Positive (n, %) | 64 (94.1) |
| | 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+ T-cell count (cells/ μ l)*† | Median (IQR) | 219 (98-371) | 203 (99-328) |
| | \leq 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-infected participants

† CD4+ T-cell count up to 18 months prior to or 30 days after enrolment

Table 5-17 Proportion of culture-confirmed pulmonary TB cases initiated on appropriate anti-TB treatment within 30 days, by trial arm

| | | Proportion initiated on appropriate anti-TB treatment within 30 days | Unadjusted analysis | | Adjusted analysis* | |
|---------------|----|---|----------------------------|-----------------------|-------------------------------------|-----------------------|
| | | % (95% CI) | Odds ratio (95% CI) | <i>p</i> value | Adjusted odds ratio (95% CI) | <i>p</i> value |
| Laboratory | 68 | 76.5 (64.6 – 85.9) | | 1 | | 1 |
| Point-of-care | 88 | 79.5 (70.9 – 88.1) | 1.13 (0.51-2.53) | 0.76 | 1.14 (0.50-2.57) | 0.76 |

CI, confidence interval

* Adjusted for sex and baseline risk of TB drug resistance

Table 5-18 Proportion of culture-confirmed pulmonary TB cases who started appropriate TB treatment within 30 and 60 days, according to Xpert MTB/RIF result

| Xpert result | Laboratory | Point-of-care |
|--|-------------------|----------------------|
| 30 days | | |
| Xpert positive | 51/57 (89.5%) | 65/68 (95.6%) |
| <i>Xpert positive – rifampicin sensitive</i> | 42/45 (93.3%) | 55/56 (98.2%) |
| <i>Xpert positive – rifampicin resistant</i> | 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%) |
| <i>Xpert positive – rifampicin sensitive</i> | 42/45 (93.3%) | 55/56 (98.2%) |
| <i>Xpert positive – rifampicin resistant</i> | 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%) |

* Four subjects with negative Xpert started treatment on basis of chest X-ray (after 1, 8, 11, and 14 days); one subject started treatment on basis of positive culture (after 30 days)

† One subject with no Xpert result (specimen leaked) started treatment on basis of clinical features (after 1 day)

Table 5-19 Basis of TB diagnosis for participants who started TB treatment within 60 days

| Basis of diagnosis | Laboratory | Point-of-care |
|---------------------------|-------------------|----------------------|
| Sputum Xpert | 73 | 81 |
| Sputum culture | 7 | 7 |
| Chest X-ray* | 14 | 26 |
| Clinical features | 2† | 3‡ |
| Not known | 1 | 1 |
| Total | 97 | 118 |

Of the 45 participants who started treatment based on chest X-ray or clinical features, one had a positive Xpert, 40 had a negative Xpert, and 4 had no valid Xpert result

* Included 1 case of pleural TB in point-of-care arm; all others notified as pulmonary TB

† Included 1 pulmonary, 1 lymph node

‡ Included 2 pulmonary, 1 lymph node

5.7 Secondary outcomes

5.7.1 Time to initiation of appropriate anti-TB treatment for culture-confirmed pulmonary TB cases

The population for the analysis of time to appropriate anti-TB treatment was the same as for the primary analysis: 156 culture-confirmed pulmonary TB cases (68 in laboratory arm; 88 in point-of-care arm) contributed 2413 days follow-up (median 5.5 days, IQR 1.0-22.5). Follow-up time was censored at the earlier of appropriate TB treatment initiation or 60 days. Six participants (all in the point-of-care arm) died prior to the initiation of appropriate anti-TB treatment (median time to death 27 days, IQR 17-35).

In the Cox regression model, the proportional hazards assumption was not met ($p = 0.012$). An attempt was made to split the survival analysis at different time-points (seven and 14 days) but the proportional hazards assumption was still violated.

Time to appropriate anti-TB treatment for the two groups was plotted as Kaplan-Meier survival curves (Figure 5-3). Deaths were censored at 60 days.[348] The time to appropriate anti-TB treatment data for the two groups were compared using the log rank test ($p = 0.026$). The estimated median time to appropriate treatment was 7 days (95% CI 6-10) in the laboratory arm and 1 day (95% CI 1-2) in the point-of-care arm.

Under the POC strategy, 34 cases commenced appropriate treatment on the day of enrolment, all on the basis of a positive Xpert without evidence of rifampicin resistance. This represented 50.0% (34/68) of Xpert-positive/culture-positive cases, or 38.6% (34/88) of all culture-positive cases eligible for the primary analysis. The distribution of time to appropriate anti-TB treatment is shown in Figures 5-4 and 5-5.

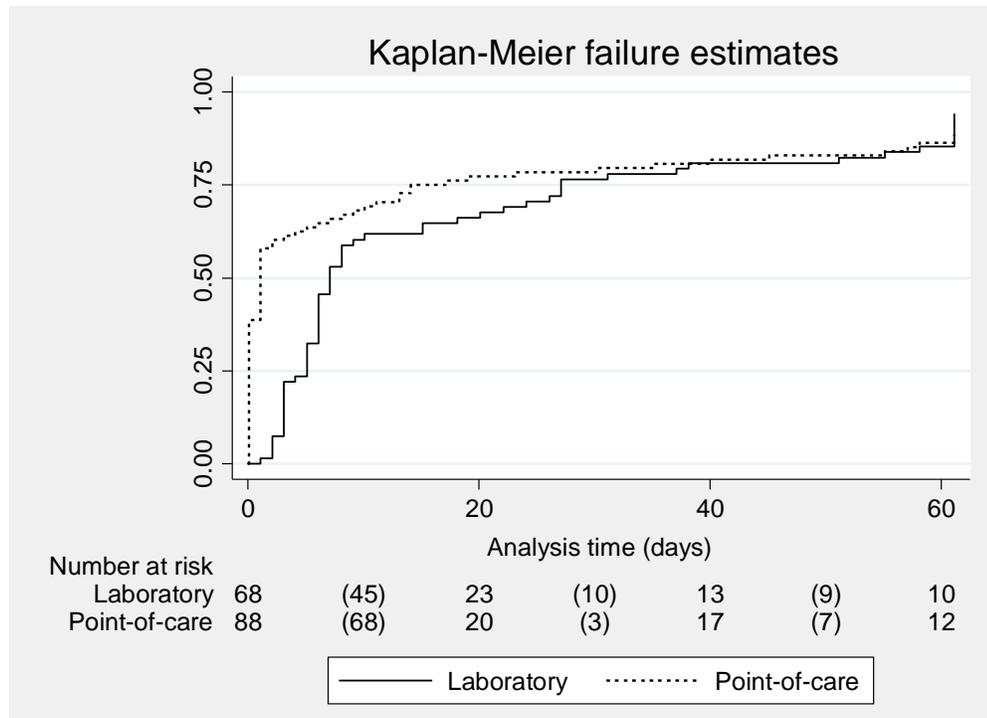


Figure 5-3 Kaplan-Meier curves for initiation of appropriate anti-TB treatment before death for culture-confirmed TB cases

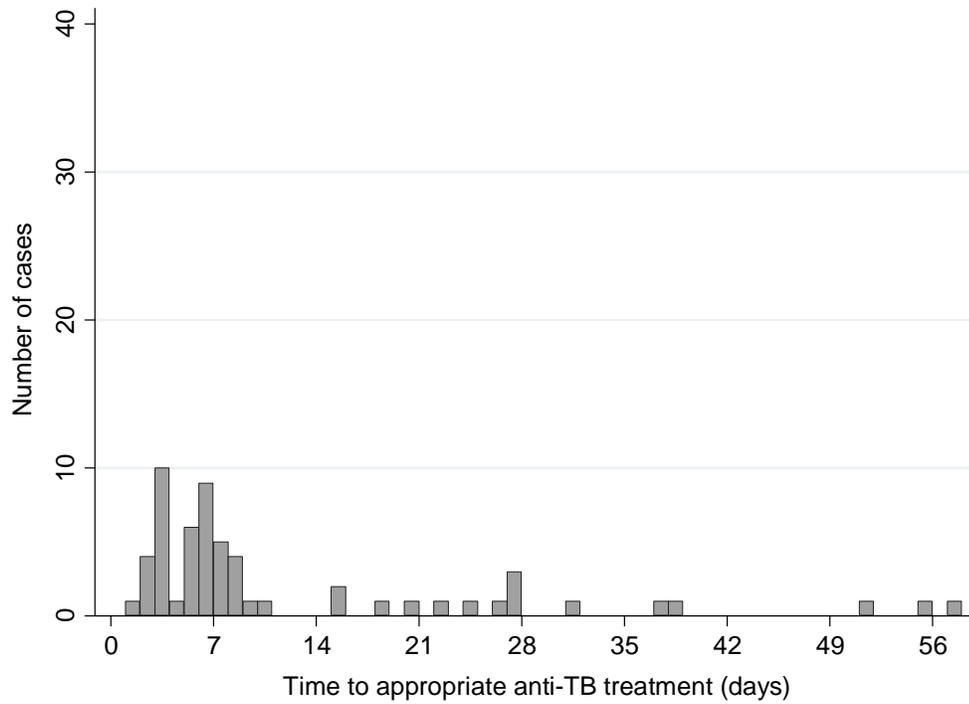


Figure 5-4 Time to appropriate anti-TB treatment for culture-positive cases, laboratory arm ($n = 58$)

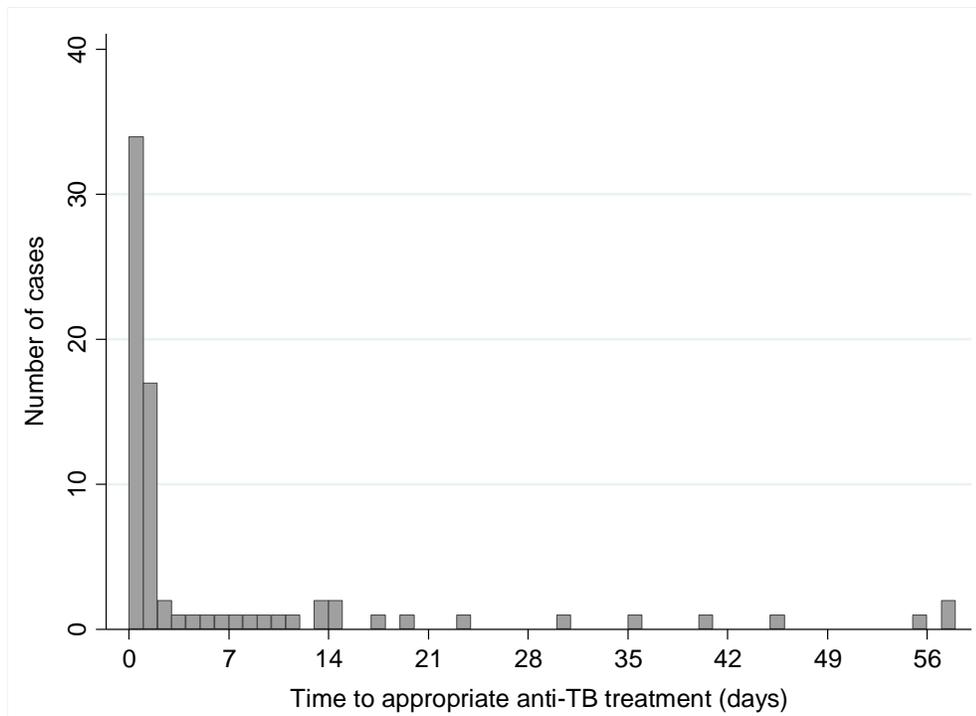


Figure 5-5 Time to appropriate anti-TB treatment for culture-positive cases, point-of-care arm ($n = 76$)

The number of clinic visits made before commencing appropriate anti-TB treatment was calculated for the culture-positive, rifampicin-susceptible cases that did not commence treatment ($n = 43$ laboratory arm, $n = 60$ point-of-care arm). These were the cases that would be expected to be detected by Xpert and to commence anti-TB treatment at the clinic without referral. The results are displayed in Figure 5-6.

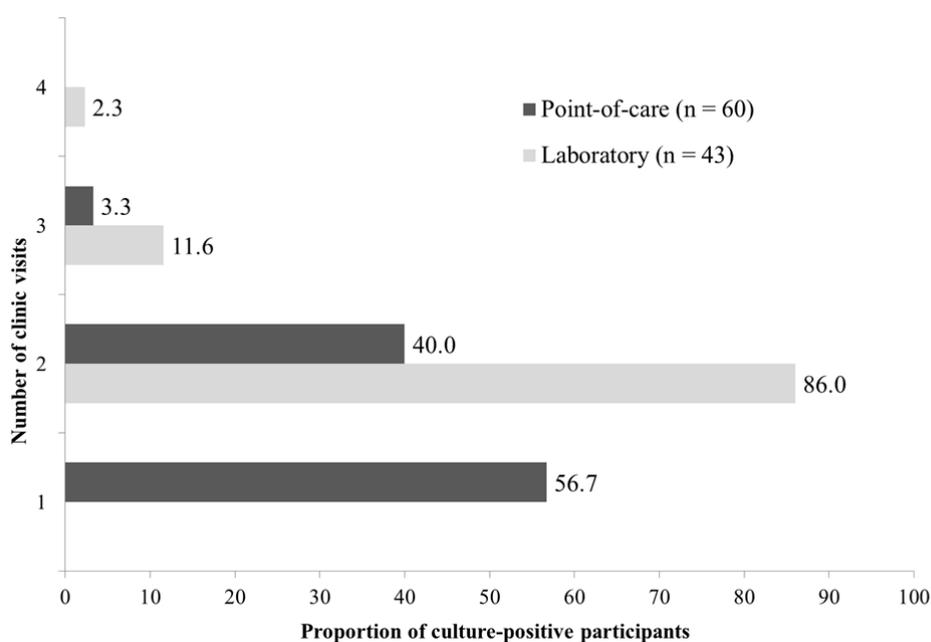


Figure 5-6 Number of clinic visits required to commence appropriate anti-TB treatment (for culture-positive, rifampicin-susceptible cases)

5.7.2 Time to initiation of appropriate DR-TB treatment for pulmonary rifampicin-resistant cases confirmed by culture and drug susceptibility testing

A total of 32 rifampicin-resistant cases (16 in laboratory arm, 16 in point-of-care arm) contributed 976 days follow-up (median 23.5 days, IQR 14.5-56.0). Two cases

died before the initiation of appropriate anti-TB treatment (both in the point-of-care arm).

In the Cox regression model, the proportional hazards assumption was not met ($p = 0.014$). Time to appropriate drug-resistant TB treatment for the two groups was plotted as Kaplan-Meier survival curves (Figure 5-4). Deaths were censored at 60 days.[348] The time to appropriate drug-resistant TB treatment data for the two groups were compared using the log rank test ($p = 0.467$). The estimated median time to treatment initiation was 27 days (95% CI 22-51) in the laboratory arm and 17 days (95% CI 10-60) in the point-of-care arm.

Five rifampicin-resistant cases did not initiate appropriate treatment – two Xpert-positive cases died after referral to the provincial drug-resistant TB unit but before starting drug-resistant TB treatment (14 and 17 days after enrolment); one Xpert-negative, culture-positive case died before formal diagnosis and before referral to the provincial drug-resistant TB unit (66 days after enrolment); one declined referral to the provincial drug-resistant TB unit and disengaged from clinical care; and one was referred and seen at the provincial drug-resistant TB unit but was not commenced on treatment(due to negative Xpert and normal chest X-ray this participant was placed on a monitoring programme with continued evaluation of symptoms and periodic repeat sputum cultures).

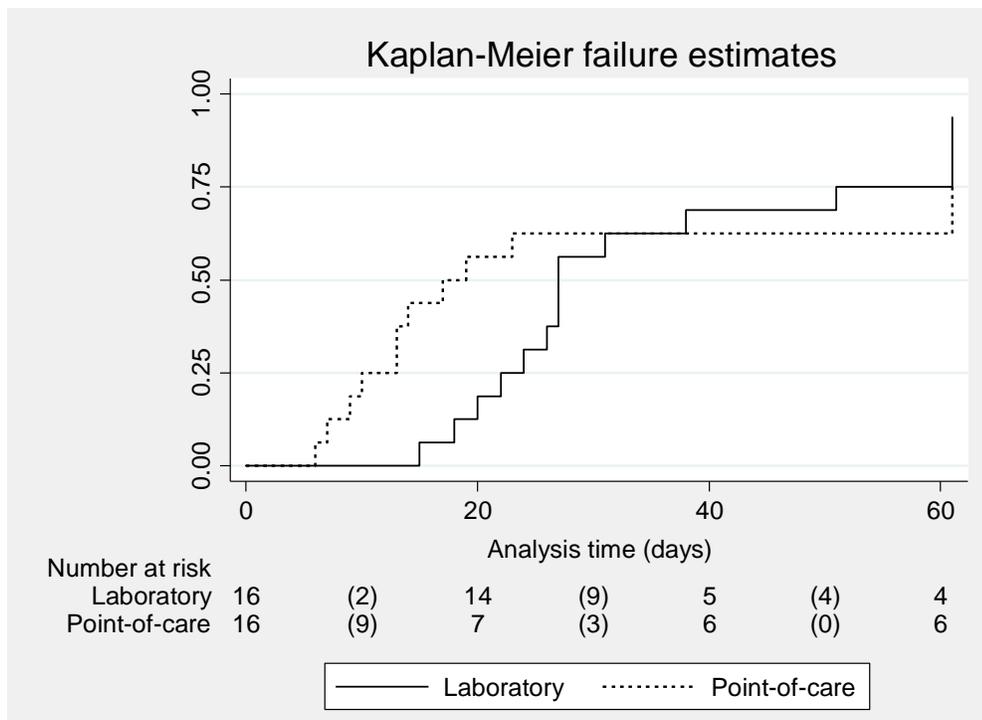


Figure 5-7 Kaplan-Meier curves for time to initiation of appropriate drug-resistant TB treatment before death for rifampicin-resistant cases

5.7.3 All-cause mortality in TB and DR-TB suspects at 60 days

A total of 919 TB and DR-TB suspects with valid follow-up data were included in this analysis. The proportion with follow-up data was similar in the laboratory arm (461/640, 72.0%) and the point-of-care arm (458/919, 71.5%). Table 5-20 compares the characteristics of those included in the analysis with those of the 362 participants lost to follow-up and with unknown outcomes. The proportion of HIV-infected participants on ART was noticeably higher in those included in the analysis (42.5% vs. 29.2%, $p < 0.001$), perhaps because participants on ART were more likely to attend for study follow-up as they were attending regularly for routine care.

Otherwise there were no significant differences between those with known outcomes and those lost to follow-up.

Overall, 24 (2.6%) participants died within 60 days of enrolment; more participants died in the point-of-care arm ($n = 16$, 3.5%) than in the laboratory arm ($n = 8$, 1.7%): OR 2.33, 95% CI 1.13-4.80, $p = 0.022$. This association was no longer significant after adjustment for baseline CD4+ T-cell count and culture result (Table 5-21).

Overall, 0.9% (7/781) of those with a negative culture and 6.3% (10/159) of those with a positive culture died within 60 days. Of those with a positive culture, 5.5% (7/128) of those with a positive Xpert and 10.0% (3/30) of those with a negative Xpert died within 60 days.

A summary of the clinical characteristics of the 24 participants who died within 60 days is presented in Table 5-22. Most were HIV-infected ($n = 22$, 91.6%) and, of those, the majority were not yet on ART ($n = 16$, 72.7% of HIV-infected) and had a CD4+ T-cell count <200 cells/ μ l at enrolment ($n = 14$, 63.6%). Seven of the 16 not yet on ART were diagnosed with HIV in the 30 days prior to enrolment (three of those diagnosed on the day of enrolment).

Over half of the participants who died within 60 days ($n = 14$, 58.3%) had microbiological evidence of pulmonary TB disease: seven participants Xpert positive/culture positive; three Xpert negative/culture positive; two Xpert positive/culture negative; and two Xpert positive with no culture result. All three Xpert negative/culture positive cases died before the initiation of TB treatment; all were HIV-infected not yet on ART and with low CD4+ T-cell counts (15 cells/ μ l, 90

cells/ μ l and 274 cells/ μ l) and only one started ART. Four of the 14 TB cases had rifampicin resistance detected by Xpert (two confirmed by culture and LPA/DST) and all four died before appropriate DR-TB was commenced (whilst awaiting initial appointment at provincial drug-resistant TB unit). One of the four was on first-line anti-TB treatment at enrolment and continued this up to the time of death but the other three had no anti-TB treatment prior to death.

The temporal trend in deaths within 60 days was somewhat uneven as mortality declined over the course of the trial. 12 deaths (50.0%) occurred in the first 12 clusters; 8 deaths (33.3%) occurred in the middle 12 clusters; and 4 deaths (16.7%) occurred in the last 12 clusters.

Table 5-20 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) |
| | 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/ μ l)* | 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) |

* Proportions are of HIV-infected participants

Table 5-21 Mortality within 60 days of enrolment, by trial arm

| | Mortality | | Unadjusted analysis | | Adjusted analysis* | |
|---------------|-----------|---------------|---------------------|----------------|------------------------------|----------------|
| | <i>N</i> | % (95% CI) | Odds ratio (95% CI) | <i>p</i> value | Adjusted odds ratio (95% CI) | <i>p</i> value |
| Laboratory | 461 | 1.7 (0.9-3.4) | 1 | | 1 | |
| Point-of-care | 458 | 3.5 (2.1-5.6) | 2.33 (1.13-4.80) | 0.022 | 1.92 (0.89-4.16) | 0.096 |

* Adjusted for *M. tuberculosis* culture result and CD4+ T-cell count

Table 5-22 Characteristics of participants who died within 60 days of enrolment

| Cluster | Sex | Age | DR-TB risk | HIV status | On ART | CD4+ T-cell count | Xpert result | Culture result | Notes |
|--------------------------|-----|-----|-------------|------------|--------|-------------------|----------------------------|----------------|---|
| <i>Laboratory arm</i> | | | | | | | | | |
| 2 | M | 32 | Previous TB | Negative | - | - | Negative | No result | |
| 11 | F | 30 | No | Positive | No | 66 | Negative | Negative | |
| 14 | M | 53 | Previous TB | Positive | Yes | 102 | Negative | Contaminated | |
| 20 | M | 43 | No | Positive | No | - | - | - | Did not submit sputum |
| 21 | M | 63 | No | Positive | No | 92 | Negative | Negative | |
| 21 | F | 45 | No | Positive | No | 162 | Positive, RIF ^S | Positive | RIF/INH susceptible; died after initiation of appropriate TB Rx |
| 22 | F | 23 | No | Positive | No | 20 | Positive, RIF ^R | Not processed | Died before initiation of DR-TB Rx |
| 30 | F | 33 | No | Positive | No | 61 | Negative | Negative | |
| <i>Point-of-care arm</i> | | | | | | | | | |
| 1 | M | 28 | Previous TB | Positive | Yes | 67 | Negative | Negative | |
| 1 | F | 29 | No | Positive | Yes | 433 | Negative | Negative | |
| 1 | M | 31 | No | Positive | No | 16 | Positive, RIF ^S | Positive | RIF/INH susceptible; died after initiation of appropriate TB Rx |
| 4 | M | 48 | Previous TB | Positive | No | - | Positive, RIF ^S | No result | Died after initiation of appropriate TB Rx |

| Cluster | Sex | Age | DR-TB risk | HIV status | On ART | CD4+ T-cell count | Xpert result | Culture result | Notes |
|---------|-----|-----|-------------|------------|--------|-------------------|----------------------------|----------------|---|
| 4 | M | 40 | Previous TB | Positive | No | - | Positive, RIF ^S | Positive | RIF/INH susceptible; died after initiation of appropriate TB Rx |
| 5 | M | 26 | No | Positive | No | 17 | Negative | No result | |
| 7 | M | 29 | No | Positive | No | 274 | Negative | Positive | RIF/INH susceptible; died before initiation of TB Rx |
| 9 | M | 48 | No | Positive | Yes | 257 | Negative | Not processed | |
| 10 | F | 21 | No | Positive | Yes | 136 | Positive, RIF ^S | Positive | RIF/INH susceptible; died after initiation of appropriate TB Rx |
| 12 | F | 33 | SNC | Positive | No | 461 | Positive, RIF ^S | Positive | MDR on DST; died before initiation of DR-TB Rx |
| 15 | F | 53 | No | Positive | Yes | 203 | Positive, RIF ^S | Positive | RIF/INH susceptible; died after initiation of appropriate TB Rx |
| 17 | M | 40 | Previous TB | Positive | No | 15 | Negative | Positive | RIF/INH susceptible; died before initiation of TB Rx |
| 23 | F | 20 | No | Positive | No | 52 | Positive, RIF ^R | Positive | MDR on DST; died before initiation of DR-TB Rx |
| 27 | M | 30 | Previous TB | Positive | No | 19 | Positive, RIF ^R | Negative | Died before initiation of DR-TB Rx |
| 29 | M | 37 | SNC | Negative | - | - | Positive, RIF ^S | Negative | Died on treatment (on Rx at initiation) |
| 34 | M | 49 | No | Positive | No | 90 | Negative | Positive | RIF/INH susceptible; died before initiation of TB Rx |

ART, antiretroviral therapy; DR-TB, drug-resistant TB; DST, drug susceptibility testing; INH, isoniazid; MDR, multidrug resistance; ^R, resistant; RIF, rifampicin; Rx, treatment; ^S, sensitive; SNC, smear non-conversion

5.7.4 Proportion of TB suspects and DR-TB suspects with at least one hospital admission within 60 days

The 919 TB and DR-TB suspects with valid follow-up data were included in this analysis. In order to ascertain dates of hospital admission, details of participants who reported hospital admission were cross-validated with the district hospital information system. A similar proportion of participants in the two arms were admitted to hospital within 60 days of enrolment: 1.4% (95% CI 0.5-2.3) in laboratory arm vs. 2.2% (95% CI 1.0-3.3) in point-of-care arm (OR 1.60, 95% CI 0.68-3.77, $p = 0.286$).

5.7.5 Time to initiation of antiretroviral therapy (ART) for HIV-infected TB suspects and DR-TB suspects not yet receiving but eligible for ART

Of the 689 ART-naïve HIV-infected participants, 452 (65.6%) were eligible for ART at enrolment based on CD4+ T-cell count ≤ 350 cells/ μ l prior to or on the day of enrolment, rifampicin-resistant TB or active TB disease following 1 June 2012 (Figure 5-5). 321 of those (71.0% of those eligible) had follow-up data available and contributed 8911 days of follow-up (median 22 days, IQR 11-42). Those with follow-up data were more likely than those without follow-up data to have culture-positive TB disease (23.4% vs. 0) but otherwise there were no significant differences. In particular, the median CD4+ T-cell count was similar for those with follow-up data and those without follow-up data (169 vs. 177 cells/ μ l, $p = 0.943$).

The majority of individuals who were eligible did commence ART within 60 days of enrolment: 115/153 (75.2%) in the laboratory arm and 133/168 (79.2%) in the point-

of-care arm. The time to ART initiation for the two trial arms is shown in Figure 5-6. The estimated median time to ART initiation was 24.1 days (95% CI 22.1-32.1) in the laboratory arm vs. 20.1 days (95% CI 17.1-22.1) in the point-of-care arm. The proportional hazards assumption was met ($p = 0.514$). There was no evidence that time to ART initiation was different in the point-of-care arm than in the laboratory arm (HR 1.22, 95% CI 0.91-1.64, $p = 0.184$).

Overall, there were 83 participants eligible for ART, not on TB treatment at enrolment but with a positive Xpert test and with follow-up data available. Similar proportions in the laboratory arm (29/45, 64.4%) and the point-of-care arm (28/38, 73.7%) had started ART within 60 days of enrolment. The median time to ART initiation for these TB cases was 23 days (IQR 21-34) in the laboratory arm ($n = 29$) vs. 17 days (IQR 14.5-23) in the point-of-care arm ($n = 28$). Similar proportions of these TB cases had started both TB treatment and ART by 30 days (61.9% in laboratory arm vs. 59.1% in POC arm). For those with the most advanced HIV disease (CD4+ T-cell count ≤ 200 cells/ μ l), a somewhat higher proportion in the POC arm (13/16, 81.3%) than in the laboratory arm (11/17, 64.7%) had commenced both TB treatment and ART by 30 days but this difference was not significant ($p = 0.438$).

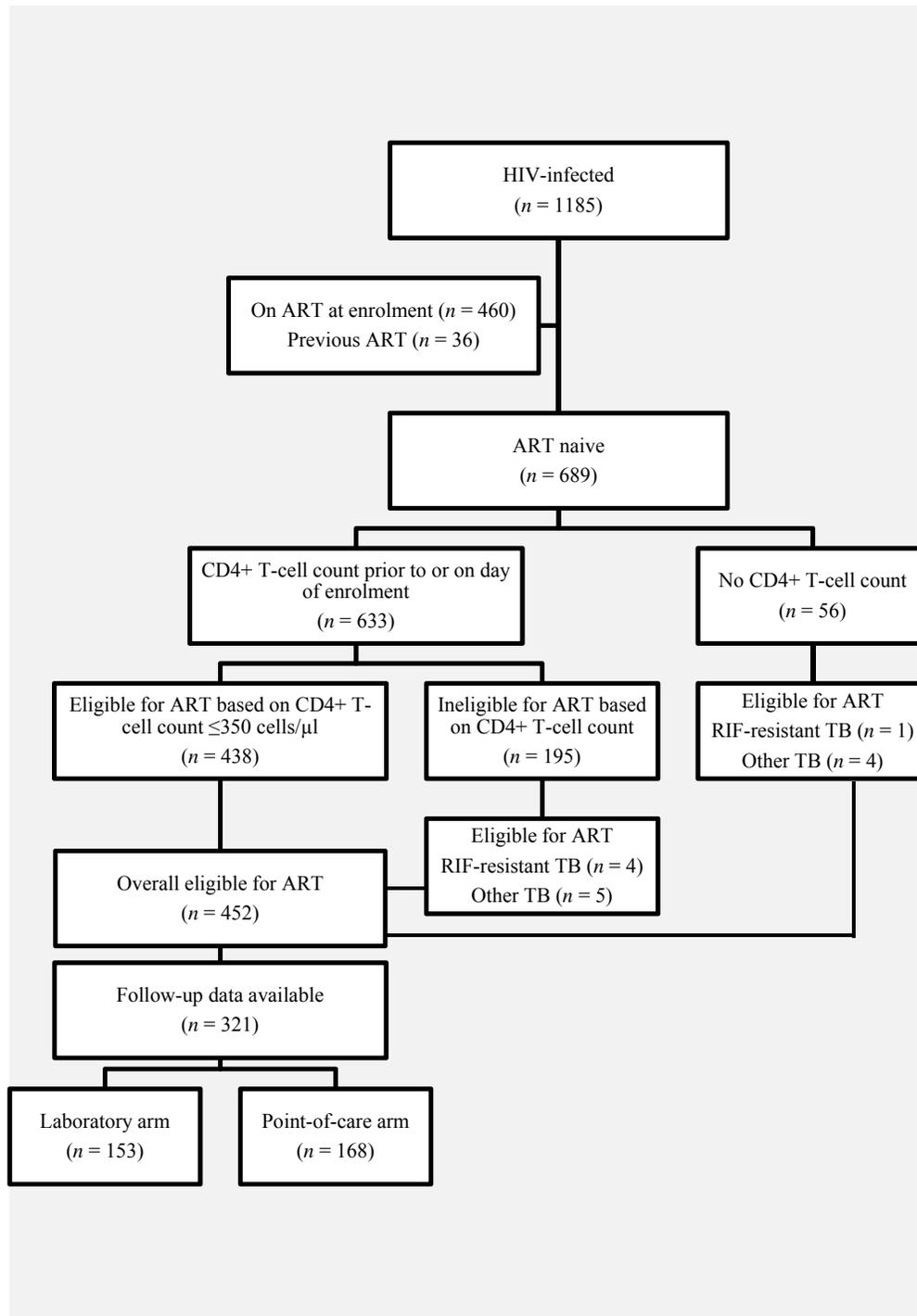


Figure 5-8 Profile of ART-naive individuals eligible for ART and with follow-up data

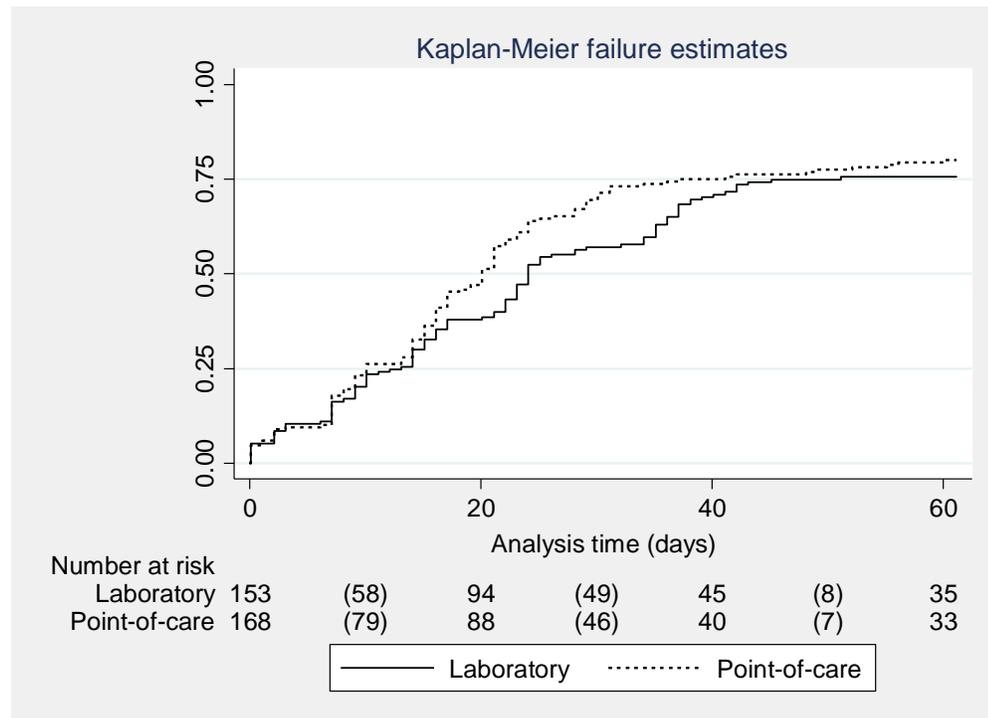


Figure 5-9 Time to antiretroviral therapy (ART) initiation in HIV-infected suspects eligible for ART

5.8 *Post hoc* analysis

5.8.1 Initiation of appropriate anti-TB treatment at different time thresholds

To explore the effect of different time thresholds (5 days, 7 days, and 14 days) for the primary endpoint of appropriate anti-TB treatment, *post hoc* analysis was performed. Regression modelling using generalised estimating equations with a binomial distribution function and a logit link was applied. The odds ratio was reported with 95% confidence intervals and a *p* value from the Wald test.

The results are presented in Table 5-23.

5.8.2 Initiation of appropriate anti-TB treatment according to Xpert MTB/RIF result

Given the problem of significant number of unevaluable sputum cultures, the proportion of participants that initiated appropriate anti-TB treatment was explored using the result of the Xpert MTB/RIF assay as the determinant of the appropriateness of treatment. For this analysis, there were 213 participants with a positive Xpert MTB/RIF test. Similarly to the primary outcomes analysis, participants that were on treatment at enrolment and were identified as having rifampicin-susceptible TB by Xpert ($n = 28$) were excluded as continued treatment would by default be considered appropriate. A further 13 participants with no post-baseline follow-up were excluded, leaving 172 participants (82 in the laboratory arm and 90 in the point-of-care arm).

The proportion of cases initiated on appropriate anti-TB treatment within 30 days of enrolment was 91.5% (75/82) in the laboratory arm and 95.6% (86/90) in the point-of-care arm. In an analysis using generalised estimating equations with a binomial distribution function and a logit link, and allowing for within-cluster correlation, there was no evidence of a difference between the two strategies (OR 2.20, 95% CI 0.75-6.45, $p = 0.15$). This did not change after adjustment for the presence of rifampicin resistance (based on Xpert result). The presence of rifampicin resistance (based on Xpert result) was strongly associated with lower likelihood of initiating appropriate anti-TB treatment within 30 days (OR 0.16, 95% CI 0.05-0.53).

Table 5-23 Results from exploratory analyses with different time thresholds for initiation of appropriate anti-TB treatment

| Time threshold | Laboratory arm | | Point-of-care arm | | Odds ratio (95% CI) | <i>p</i> value |
|----------------|----------------|------------------|-------------------|------------------|---------------------|----------------|
| | <i>n</i> | % (95% CI) | <i>n</i> | % (95% CI) | | |
| 5 days | 22 | 32.4 (21.5-44.8) | 56 | 63.6 (52.7-73.6) | 3.62 (1.81-7.26) | <0.001 |
| 7 days | 36 | 52.9 (40.4-65.2) | 58 | 65.9 (55.0-75.7) | 1.74 (0.94-3.21) | 0.076 |
| 14 days | 42 | 61.8 (49.2-73.3) | 66 | 75.0 (64.6-83.6) | 1.88 (0.98-3.59) | 0.057 |

Chapter 6 Diagnostic performance of Xpert MTB/RIF

Reporting of the diagnostic performance of Xpert MTB/RIF for the detection of *M. tuberculosis* and for the detection of rifampicin resistance was done with reference to the Standards for the reporting of diagnostic accuracy studies (STARD) statement.[349,350]

6.1 Diagnostic performance of Xpert MTB/RIF for detection of *M. tuberculosis*

6.1.1 Methodology

This analysis was based on individual-level data and made no allowance for correlation within clusters. Sensitivity, specificity, positive predictive value and negative predictive value were compared for the two trial arms. Estimation of accuracy of Xpert MTB/RIF for the detection of *M. tuberculosis*, against the reference standard of single MGIT culture, was based on individual participants with paired valid Xpert MTB/RIF and MGIT culture results. Valid Xpert results (the first valid result from either the initial or repeat sputum specimen) included *M. tuberculosis* detected or not detected. Indeterminate Xpert results (invalid, error or no result) were excluded. A valid MGIT culture was defined as positive for *M. tuberculosis* or negative. Contaminated cultures and positive cultures identified as non-tuberculous mycobacteria were excluded, as in these instances the presence or absence of *M. tuberculosis* cannot definitively be ascertained.

This was performed as an intention-to-treat analysis. There were two protocol violations where sputum was tested under the alternative strategy to the randomised allocation but this represented less than 0.2% of the participants. The analysis only included individuals not on TB treatment at the time of enrolment, so as to avoid bias from detection of non-viable bacilli with Xpert in those on TB treatment. Sensitivity, specificity, positive predictive value, and negative predictive value were reported with 95% confidence intervals. To determine whether there was any significant difference between the two arms for each of these estimates, the difference between proportions was calculated with 95% confidence intervals and a two-sample z test was used.

The performance of the two different versions of the Xpert MTB/RIF cartridge (G3 and G4) for the detection of *M. tuberculosis* was compared.

6.1.2 Results

893 participants had paired valid Xpert MTB/RIF and MGIT culture results (Figure 6-1). In this sample, the prevalence of culture-positive TB was 16.4% (95% CI 14.1-18.9). Overall sensitivity was 79.5% (95% CI 72.0-85.7), specificity 97.3% (95% CI 95.9-98.4), positive predictive value 85.3% (95% CI 78.2-90.8), and negative predictive value 96.0% (95% CI 94.4-97.3).

The prevalence of culture positive TB was higher in the point-of-care arm than in the laboratory arm. There was no evidence of any difference in diagnostic accuracy under the two positioning strategies (Table 6-1).

There was no significant difference in performance between the Xpert G3 and G4 cartridge with regards to the detection of *M. tuberculosis*: sensitivity 79.2% (95% CI 68.0-87.8) for G3 vs. 79.7% (95% CI 68.8-88.2) for G4 and specificity 97.6% (95% CI 95.5-98.9) for G3 vs. 97.1% (95% CI 94.8-98.5) for G4.

The Xpert MTB/RIF gives a semi-quantitative result (high, medium, low, very low) as an estimate of the mycobacterial burden in the tested sputum specimen. This result is based on the cycle threshold of the first positive probe. The profile of semi-quantitative results for the 174 participants with a positive Xpert is displayed in Figure 6-2. There was a fairly even distribution between three groups (medium, low, and very low) with fewer specimens yielding a semi-quantitative result of high. As shown in the figure, there was no substantial difference in the distribution between rifampicin-resistant results and rifampicin-sensitive results.

The distribution of semi-quantitative results for culture-positive and culture-negative cases is shown in Figure 6-3. The majority ($n=14$, 70%) of those with a positive Xpert and negative culture had a semi-quantitative result of very low, which could suggest that in these cases Xpert detected a very small quantity of *M. tuberculosis* DNA but there were not enough viable bacilli present to grow in liquid culture.

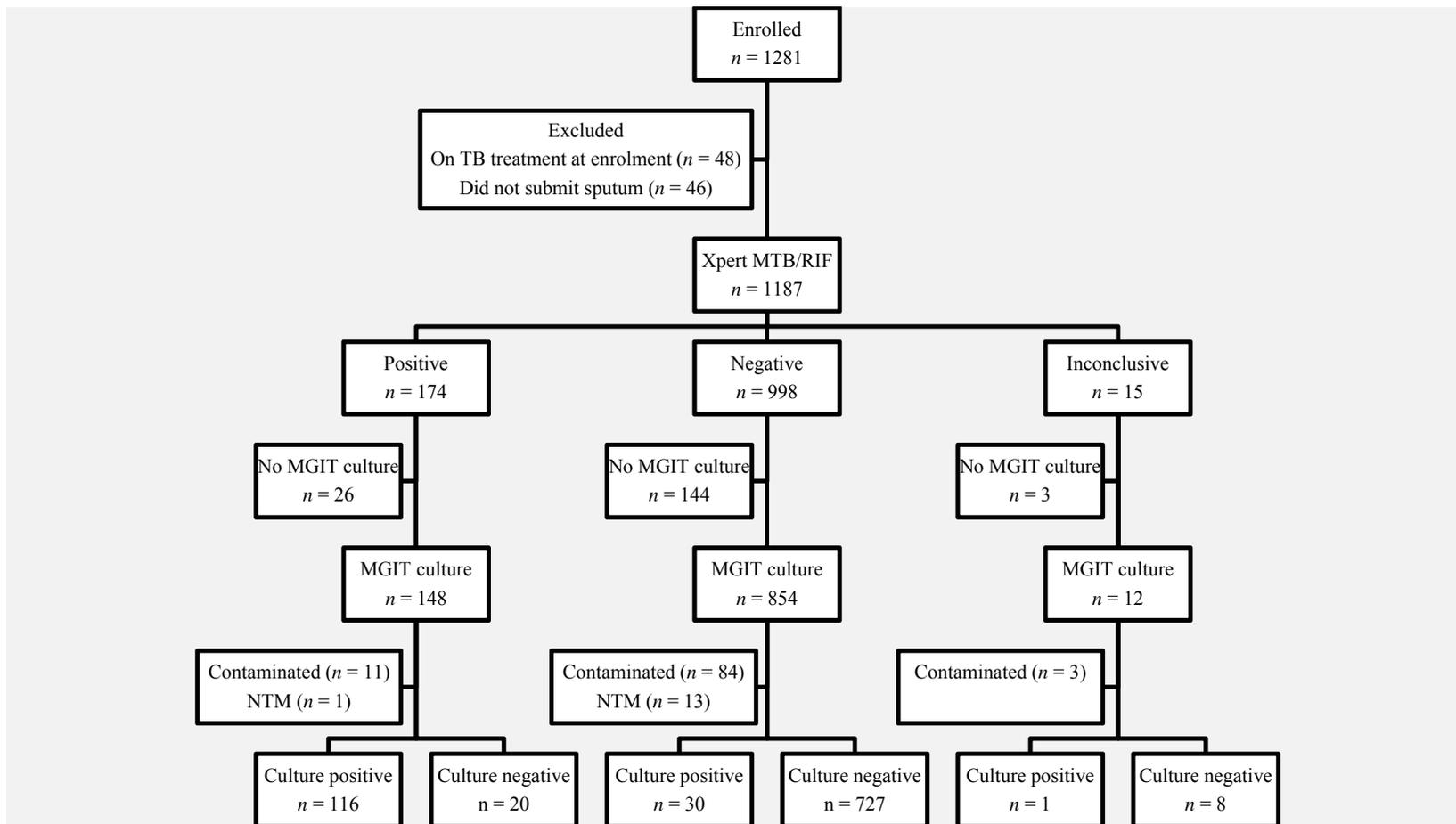


Figure 6-1 Flow diagram for diagnostic accuracy of Xpert MTB/RIF for detection of *M. tuberculosis*

Table 6-1 Diagnostic accuracy of Xpert MTB/RIF for the detection of *M. tuberculosis*, by arm

| | Laboratory (n = 449) % (95% CI) | Point-of-care (n = 444) % (95% CI) | Difference (95% CI) | p value |
|---------------------------------------|--|---|----------------------------|----------------|
| Prevalence culture positive TB | 14.0 (11.1-17.6) | 18.7 (15.3-22.6) | - | - |
| Sensitivity | 84.1 (72.7-92.1) | 75.9 (65.3-84.6) | 8.2 (-4.7-21.1) | 0.223 |
| Specificity | 96.9 (94.6-98.4) | 97.8 (95.7-99.0) | -0.9 (-3.2-1.4) | 0.450 |
| Positive predictive value | 81.5 (70.0-90.1) | 88.7 (79.0-95.0) | -7.2 (-19.2-4.8) | 0.237 |
| Negative predictive value | 97.4 (95.3-98.7) | 94.6 (91.8-96.7) | 2.8 (0-5.5) | 0.052 |

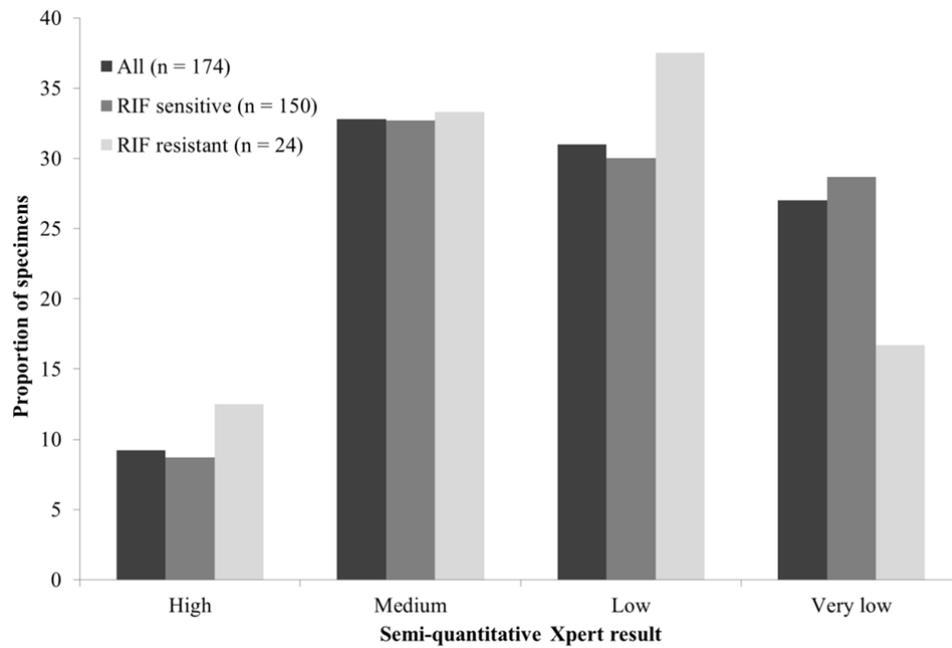


Figure 6-2 Semi-quantitative Xpert results according to rifampicin susceptibility

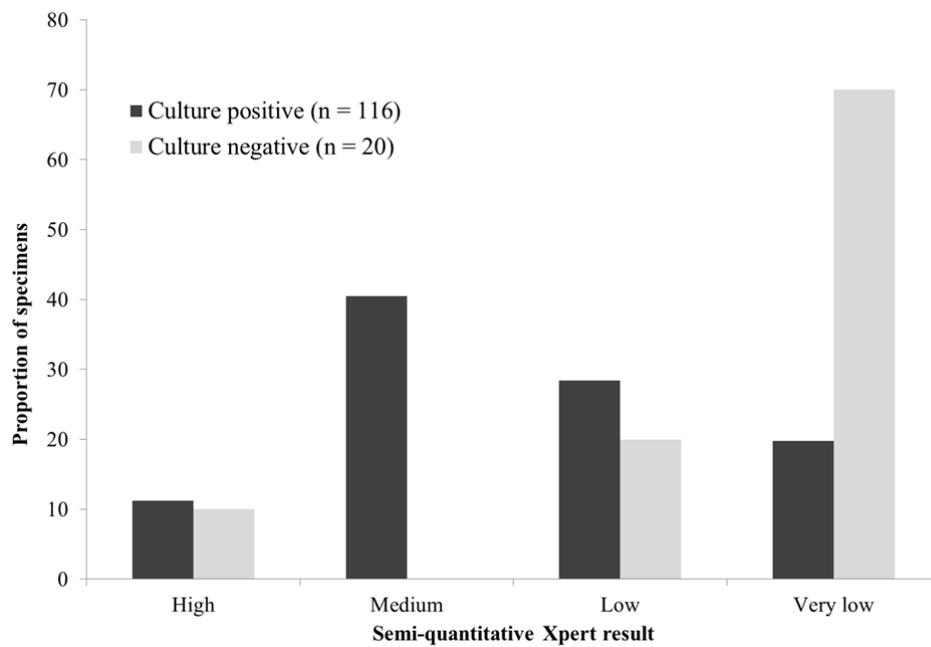


Figure 6-3 Semi-quantitative Xpert results according to culture positivity

In five instances of discordant Xpert positive/culture negative results, rifampicin resistance was also detected by Xpert. Four of these participants had no specific risk factors for DR-TB. Rifampicin-resistant TB was subsequently confirmed by culture and phenotypic DST in two of these cases, prior to starting DR-TB treatment. In a third case, Xpert MTB/RIF on a second sputum specimen gave the same result (*M. tuberculosis* detected and rifampicin resistance detected) although smear microscopy and culture were negative prior to commencing DR-TB treatment. The fourth case had a subsequent positive culture prior to DR-TB treatment but phenotypic DST of the culture isolate detected no resistance to rifampicin (or isoniazid). In the last of these five cases, no further sputum specimens were tested and the participant died before commencing DR-TB treatment (Table 6-2).

Table 6-2 Details of participants with discordant Xpert positive/culture negative results and rifampicin resistance detected by Xpert

| DR-TB risk | Assay | Semi-quantitative result | Probe* | Repeat Xpert | Repeat culture | Notes |
|------------|-------|--------------------------|--------|-----------------------|--|-------|
| No | G3 | Very low | E | Pos, RIF ^R | Neg x 2 | |
| No | G3 | Very low | E | Pos, RIF ^R | Pos MDR x 2 | |
| No | G4 | Very low | B | - | Pos MDR/Neg | |
| Yes | G4 | High | E | - | - | Died |
| No | G4 | Very low | D | - | Pos, RIF ^S INH ^S | |

INH, isoniazid; MDR, multidrug resistance; Neg, negative; Pos, positive; ^R, resistant; RIF, rifampicin; ^S, sensitive

* Specific probe detecting mutation

The sensitivity of Xpert MTB/RIF for the detection of *M. tuberculosis* was somewhat higher at low CD4+ T-cell counts, although specificity was similar at all CD4+ T-cell counts (Table 6-3).

Table 6-3 Diagnostic accuracy of Xpert MTB/RIF for the detection of *M. tuberculosis* by CD4+ T-cell count in HIV-infected participants

| CD4+ T-cell count | | Laboratory | POC | Overall |
|-------------------|-------------|-----------------------------|-----------------------------|-------------------------------|
| ≤50 | Sensitivity | 5/5 (100) [47.8-100] | 8/10 (80.0) [44.4-97.5] | 13/15 (86.7) [59.5-98.3] |
| | Specificity | 18/18 (100) [81.5-100] | 35/39 (89.7) [75.8-97.1] | 53/57 (93.0) [83.0-98.1] |
| 51-200 | Sensitivity | 18/20 (90.0) [68.3-98.8] | 21/27 (77.8) [57.7-91.4] | 39/47 (83.0) [69.2-92.4] |
| | Specificity | 74/79 (93.7) [85.8-97.9] | 70/71 (98.6) [92.4-100] | 144/150 (96.0) [91.5-98.5] |
| 201-350 | Sensitivity | 11/14 (78.6) [49.2-95.3] | 17/22 (77.3) [54.6-92.2] | 28/36 (77.8) [60.8-89.9] |
| | Specificity | 93/94 (98.9) [94.2-100] | 90/91 (98.9) [94.0-100] | 183/185 (98.9) [96.1-99.9] |
| 351-500 | Sensitivity | 9/11 (81.8) [48.2-97.7] | 5/7 (71.4) [29.0-96.3] | 14/18 (77.8) [52.4-93.6] |
| | Specificity | 53/55 (96.4) [87.5-99.6] | 53/53 (100) [93.3-100] | 106/108 (98.1) [93.5-99.8] |
| >500 | Sensitivity | 4/6 (66.7) [22.3-95.7] | 7/9 (77.8) [40.0-97.2] | 11/15 (73.3) [44.9-92.2] |
| | Specificity | 74/77 (96.1) [89.0-99.2] | 58/59 (98.3) [90.9-100] | 132/136 (97.1) [92.6-99.2] |

Figures in parentheses are percentages and figures in square brackets are 95% confidence intervals

6.2 Diagnostic performance of Xpert MTB/RIF for detection of rifampicin resistance

6.2.1 Methodology

This analysis was based on individual-level data and made no allowance for correlation within clusters. The reference standard was the result of line probe assay or phenotypic DST on the culture isolate (an isolate was considered resistant if resistance was identified on either LPA or phenotypic DST, but as shown in Table 5-9 the majority of isolates were resistant on both tests). Estimation of sensitivity and specificity of Xpert MTB/RIF for the detection of rifampicin resistance was based on individual participants with *M. tuberculosis* detected by Xpert MTB/RIF and with a positive MGIT culture and valid drug susceptibility test (LPA or phenotypic DST) result. This included individuals on TB treatment at the time of enrolment (e.g. participants with smear non-conversion or treatment failure). Sensitivity, specificity, positive predictive value, and negative predictive value were reported with 95% confidence intervals. To determine whether there was any significant difference between the two arms for each of these estimates, the difference between proportions was calculated with 95% confidence intervals and a two-sample *z* test was used.

6.2.2 Results

127 participants had paired valid Xpert MTB/RIF and LPA/DST results (Figure 6-4). In this sample, the prevalence of rifampicin resistance was 20.5% (95% CI 14.4-28.3). Overall sensitivity was 88.5% (95% CI 69.8-97.6), specificity 99.0% (95% CI 94.6-100), positive predictive value 95.8% (95% CI 78.9-99.9), and negative

predictive value 97.1% (91.7-99.4). The diagnostic accuracy for the detection of rifampicin resistance was not significantly different between the two different Xpert positioning strategies (Table 6-4).

Sensitivity for the detection of rifampicin resistance differed by Xpert MTB/RIF cartridge (G3 cartridge 75.0%, 95% CI 42.8-94.5 vs. G4 cartridge 100%, 95% CI 76.8-100) although specificity did not (G3 cartridge 100%, 95% CI 93.0-100 vs. G4 cartridge 98.0%, 95% CI 89.4-99.9).

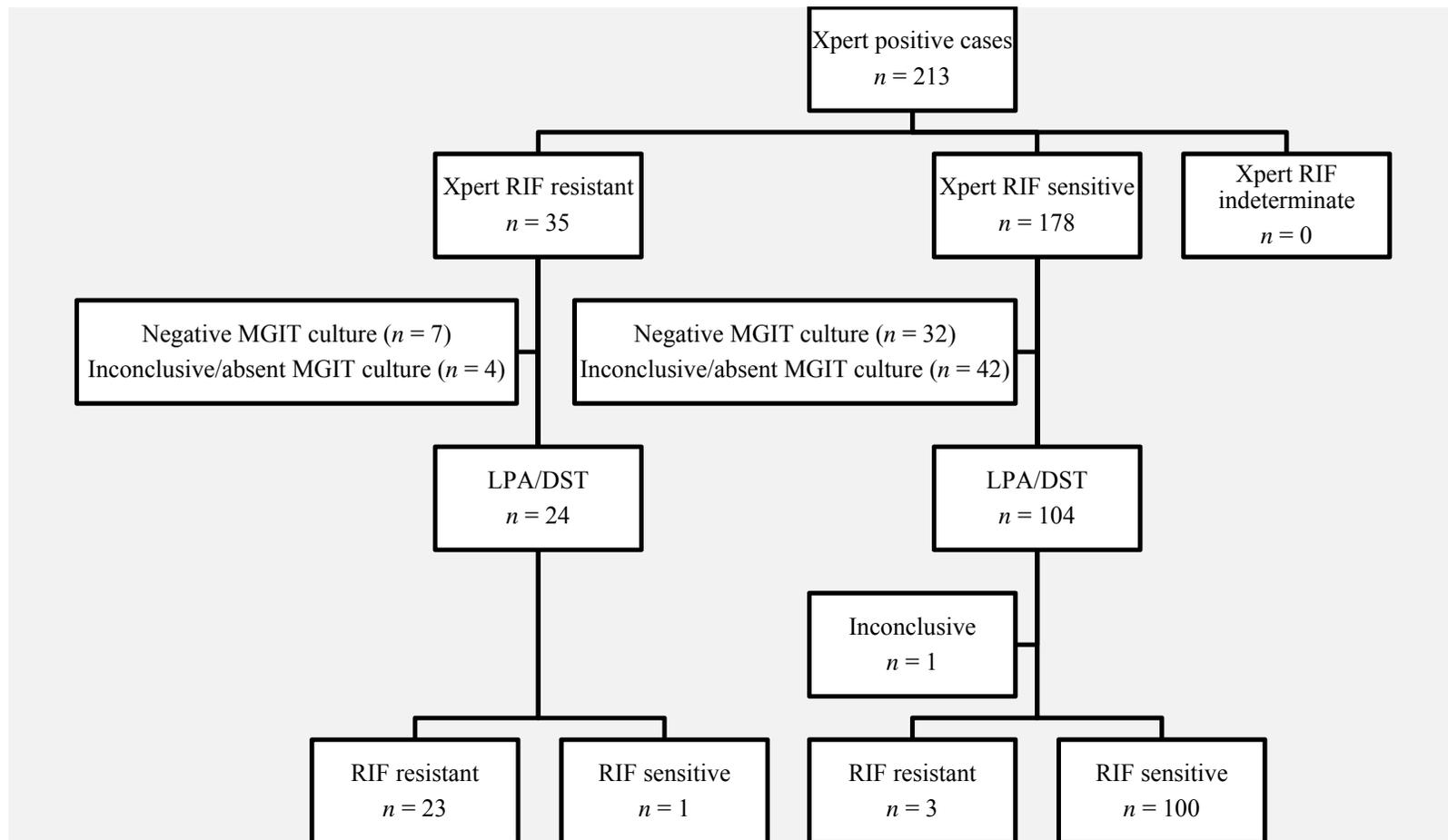


Figure 6-4 Flow diagram for diagnostic accuracy of Xpert MTB/RIF for detection of rifampicin resistance

Table 6-4 Diagnostic accuracy of Xpert MTB/RIF for detection of rifampicin resistance, by arm

| | Laboratory (n = 57) % (95% CI) | Point-of-care (n = 70) % (95% CI) | Difference (95% CI) | p value |
|---|---|--|----------------------------|----------------|
| Prevalence rifampicin resistance | 23.0 (13.0-35.8) | 19.0 (10.0-29.7) | - | - |
| Sensitivity | 84.6 (54.6-98.1) | 92.3 (64.0-99.8) | -7.7 (-32.1-16.7) | 0.539 |
| Specificity | 97.7 (88.0-99.9) | 100 (93.7-100) | -2.3 (-6.7-2.1) | 0.253 |
| Positive predictive value | 91.7 (61.5-99.8) | 100 (73.5-100) | -8.3 (-24.0-7.3) | 0.307 |
| Negative predictive value | 95.6 (84.9-99.5) | 98.3 (90.8-100) | -2.7 (-9.6-4.2) | 0.416 |

There were four participants with discordant results for rifampicin resistance: three with Xpert reported as rifampicin sensitive but LPA/DST on culture isolate reported as rifampicin resistant; and one with Xpert reported as rifampicin resistant but LPA/DST on culture isolate reported as rifampicin sensitive. The details for these participants are summarised in Table 6-5.

All three of those with discordant Xpert rifampicin sensitive/culture rifampicin resistant results had risk factors for the presence of drug-resistant TB. One case had treatment failure of a standard first-line anti-TB regimen (2HRZE/4HR) for isoniazid mono-resistant disease. In this case it was noted that the difference in cycle threshold (ΔC_t) of 3.6 would have given a rifampicin resistant result under the original assay definitions (before the definitions were changed to improve specificity of the assay).[278] In this case the clinical suspicion was that the patient harboured mixed populations of isoniazid mono-resistant and multidrug-resistant bacilli (as the ability of the Xpert assay to detect certain mutations in the presence of mixed populations is poor).[215]

The one case with a discordant Xpert rifampicin resistant/culture rifampicin sensitive result had no specific risk factor for DR-TB. The Xpert assay detected rifampicin resistance due to probe E delay. The MGIT culture performed for the study was positive and line probe assay was reported as rifampicin and isoniazid sensitive. Two cultures sent prior to DR-TB treatment were positive and phenotypic DST for both was reported as resistant to isoniazid and kanamycin but sensitive to rifampicin.

Table 6-5 Details of participants with discordant rifampicin resistance results

| DR-TB risk | Assay | Xpert resistance result | Δ Ct | LPA | Phenotypic DST | Repeat culture* |
|-------------------------|-------|-------------------------|-------------|-----------------------------------|--------------------|-----------------|
| Yes (treatment failure) | G3 | RIF ^S | 3.6 | RIF ^S INH ^R | MDR | MDR |
| Yes (DR-TB contact) | G3 | RIF ^S | 1.2 | RIF ^I INH ^S | MDR | No result |
| Yes (previous TB) | G3 | RIF ^S | 1.6 | No result | MDR | Negative |
| No | G4 | RIF ^R | 4.3† | RIF ^S INH ^R | INH monoresistance | Polyresistant‡ |

Δ Ct, difference in cycle threshold; I, inconclusive; R, resistant; S, sensitive

* Repeat cultures performed subsequently, prior to commencing DR-TB treatment

† Probe E delay

‡ Two cultures positive with resistance to isoniazid and kanamycin but sensitivity to rifampicin on phenotypic DST

6.3 Operational feasibility of point-of-care Xpert

There were incomplete data on operational feasibility, as data collection did not take place for all the indicators outlined in the protocol. Data were collected on power supply, operating temperature for the GeneXpert system, and storage temperature for the Xpert MTB/RIF test kits (temperatures were only reliably collected at the PHC clinic). Data on Xpert indeterminate results were presented in section 5.3.2.

6.3.1 Power supply

There was one instance of interrupted power supply under the laboratory strategy. In this case the power supply was interrupted during a test run and the back-up

generator did not start. In this event, the system produced an output of ‘No result’ for all four tests on that run (see Table 5-5).

Under the point-of-care strategy there were two instances of interrupted power supply. On both occasions, this occurred whilst no tests were running on the GeneXpert system. During these two episodes, the backup generator was started and power was maintained to allow continued operation of the GeneXpert system.

6.3.2 Operating temperature for GeneXpert system

The recommended temperature range for operation of the GeneXpert system is 15-30°C. Daily minimum and maximum temperatures were recorded in the room dedicated to operation of the GeneXpert system. A total of 158 daily readings were recorded (equivalent to 87.8% of days in which the system was operational at the clinic). The median minimum and maximum temperatures were 16°C (IQR 13-15) and 28°C (22-31) respectively. On 51 days (32.3%) the maximum temperature exceeded the upper limit of the recommended range (>30°C).

6.3.3 Storage temperature for Xpert MTB/RIF kits

The recommended temperature range for storage of Xpert MTB/RIF test kits is 2-28°C. Daily minimum and maximum temperatures were recorded in the clinic room where test kits were stored (this was a separate room to that used for operation of the GeneXpert system). A total of 267 daily readings were recorded (equivalent to 74.2% of days during the study period, as cartridges were stocked there throughout the trial). The median minimum and maximum temperatures were 14°C (IQR 13-15)

and 21°C (19-25). On 27 days (10.1%) the maximum temperature exceeded the upper limit of the recommended range (>28°C).

Chapter 7 Discussion

7.1 Main findings

As effective anti-TB treatment exists that in most cases can prevent mortality as well as rapidly reduce infectiousness, the key to reducing deaths and curbing TB transmission at a population level is early detection and initiation of appropriate anti-TB treatment. This was the first study designed to test the hypothesis that timely initiation of appropriate anti-TB treatment would be improved through positioning of a molecular diagnostic system at the place at which patients access care (primary health care clinic) compared to within a centralised laboratory. Although it was not possible to demonstrate clear benefits with respect to the clinical endpoints selected for the study, there were important benefits from point-of-care placement and nurse-performed Xpert in rural primary health care was demonstrated to be possible.

Overall, 77% of culture-positive cases were initiated on appropriate anti-TB treatment within the 30-day threshold. Considering only those in whom Xpert detected *M. tuberculosis*, 92% started appropriate anti-TB treatment within 30 days. The majority of cases that did not start appropriate treatment within 30 days had therefore tested negative with Xpert. This highlights that test sensitivity remains critical for getting the right people onto treatment. Nevertheless, the reduced sensitivity of Xpert compared to liquid culture in the study needs to be contextualised as almost one in four culture specimens gave no valid result and the operational yield of Xpert (under both laboratory or point-of-care strategies) was actually greater than that of liquid culture. This emphasises that culture may be an imperfect gold standard in the setting of real world implementation.

7.1.1 Impact of point-of-care positioning on initiation of anti-TB treatment

There was no significant difference in the proportion of culture-positive cases initiated on appropriate anti-TB treatment within 30 days. Under both diagnostic strategies, around three-quarters of cases started appropriate treatment within the designated timeframe. In both arms, the majority of cases detected by Xpert without evidence of rifampicin resistance commenced treatment in a timely fashion and point-of-care positioning allowed for earlier initiation of treatment for drug-susceptible cases.

The primary endpoint was chosen to evaluate whether the point-of-care strategy could reduce delays and primary default whilst maintaining comparable diagnostic accuracy to the laboratory setting. The assessment of the appropriateness of treatment within the outcome was felt to be important given concerns about the accuracy of Xpert for detection of rifampicin resistance and uncertainty as to whether Xpert would perform as well in the clinic setting. Also, at the time of study design there was relatively little evidence about the performance for detection of rifampicin resistance in settings with very high levels of drug resistance and in patient groups at high risk of drug resistance. In reality, the diagnostic performance in this study was good and there were only three cases where cases started what was deemed to be an inappropriate regimen, on the basis of discordance between the Xpert rifampicin resistance result and phenotypic DST result.

The majority of the culture-positive cases that did not start appropriate treatment by 30 days had tested negative with Xpert. Whilst there was some evidence to suggest that more were diagnosed by other means (predominantly X-ray) in the point-of-care arm, the numbers were small and certainly insufficient to influence the overall outcome. Fewer cases than expected started treatment on an empirical basis within 30 days – only four cases in the point-of-care arm and none in the laboratory arm. This may be because it was still relatively early in the disease course (median duration of cough was only three weeks) and that individuals did not initially seek alternative diagnostic methods after testing negative with Xpert. This confirms the importance of test sensitivity in reducing diagnostic delays, as previously suggested on the basis of mathematic modelling.[51] The developers of Xpert MTB/RIF have recently announced plans to improve sensitivity of the assay with the aim of achieving a limit of detection of approximately 10 cfu/ml, comparable to liquid culture.[351] If this improved sensitivity is realised, the impact on diagnostic delay will be of particular interest.

In the absence of strong evidence to guide the time threshold for the primary endpoint, 30 days was chosen based on our considered opinion as to what would be clinically relevant. Although it is recognised that the reduction in the time to diagnosis is important for TB control,[352] the lack of data on critical time thresholds of importance for influencing individual prognosis and tuberculosis transmission does hamper the selection of appropriate endpoints for diagnostic clinical trials. *Post hoc* analysis suggested that significant differences between the two strategies could have been detected if the threshold for initiation of appropriate anti-TB treatment was much shorter (i.e. five days). However, whilst a shorter

threshold might have provided more power to detect a difference between the two strategies, it is not clear whether such a difference at that time point would have clinical or public health significance.

The sample size calculation included the assumption that 10% of Xpert MTB/RIF positive cases in the laboratory arm would not return for or receive result as indicated but in the trial only 3% in the laboratory arm had not received the result by 30 days. Although around 10% of participants overall in the laboratory arm were not documented to have received their result, this was mostly individuals with negative test results. This suggests either that subjects testing positive were more likely to return for their result or that the routine measures to recall those who tested positive and who did not initially return functioned well during the trial. It is also possible that the trial and the study personnel improved the performance of routine programmatic measures such as this. Data was not available on the levels of primary default at other clinics in the sub-district during the study period, although this could have provided a useful comparison.

Although a greater proportion of participants in the laboratory arm than in the POC arm did not have a valid result from the first sputum specimen (8% vs. 1%), repeat Xpert tests on additional sputum specimens were allowed within the trial protocol. In the laboratory arm, three culture-positive cases without a valid Xpert result from the initial specimen (two leaked during transit, one invalid) had a positive Xpert with the subsequent specimen and started appropriate anti-TB treatment as a result. While the allowance for collection of additional sputum specimens might have limited the

power to detect a difference in the primary outcome between the strategies, it reflected standard practice in a routine TB programme.

The point-of-care strategy provided a shorter time to commencement of appropriate anti-TB treatment. There was a difference in median time to appropriate anti-TB treatment of six days (1 day vs. 7 days). The study was not designed to determine whether this might have an impact on individual morbidity, although this is probably unlikely as disease progression in five days would be expected to be limited. Point-of-care positioning allowed same-day diagnosis and treatment initiation for 34 participants. This represented half of the Xpert-positive/culture-positive cases in the POC arm or just over a third of all culture-positive cases. Most of those who did not receive same-day diagnosis and treatment under the point-of-care strategy commenced treatment the following day. This proportion that received same-day treatment initiation was lower than that in the TB-NEAT study, where 66% of culture-positive cases in the Xpert arm started anti-TB treatment on the day of sputum submission.[240] The proportion with rifampicin resistance was higher than in the TB-NEAT study and these cases all had delays to treatment initiation. The TB-NEAT study predominantly involved large urban health care facilities whereas this study was performed at a rural primary health care clinic. Several factors may influence people's willingness to wait for test results and logistically there were constraints in this study, with testing only performed during daytime working hours. It does suggest, however, that to achieve same-day treatment initiation for all individuals, a reduction in the two-hour turnaround for testing would be highly desirable.

Whether or not the shorter time to treatment could influence transmission is of particular interest. With untreated active TB disease in this context, there are risks of transmission in the community and within healthcare facilities. Nosocomial transmission of TB in South Africa has contributed to the failure of TB control, most notably with the explosive outbreak of extensively drug-resistant TB in Msinga sub-district, KwaZulu-Natal in 2005-6.[20,21] Health care workers in South Africa remain at increased risk of TB infection and disease,[313,353-357] and specifically drug-resistant TB.[358,359] Health care facilities and health care workers remain poorly equipped to effect TB infection control.[313-320,360] In this context, the identification and treatment of individuals with active pulmonary TB disease becomes a priority, as appropriate anti-TB treatment rapidly renders patients non-infectious, with both drug-susceptible and drug-resistant disease.[25,26] As the study included ambulant individuals and cases had a relatively short duration of symptoms, it would be expected that many would be active in the community and visiting congregate settings, including workplaces, church, schools and health care facilities. In this context, any reduction in the time to appropriate treatment could plausibly affect onward transmission.

It was not possible to establish whether the time to appropriate treatment for drug-resistant TB cases was shorter under the point-of-care strategy, primarily as there was insufficient power in this analysis. More generally though, any potential effect from Xpert positioning was offset by health system delays across the board for drug-resistant TB cases. During preparation for the trial, it was anticipated that the local district hospital would become a fully decentralised DR-TB treatment site.[41] However, this did not happen according to anticipated timelines. The hospital

continued to function as a satellite unit, where care was co-ordinated by the provincial specialist DR-TB unit but aspects of care (e.g. initial inpatient treatment) were delivered at the district hospital.[122] During the trial, therefore, DR-TB cases still had to be referred for outpatient consultation to the provincial DR-TB unit in Durban for treatment initiation and the delay between referral and this initial visit was the main component of the overall delay.

Furthermore, four patients with rifampicin resistance detected by Xpert died prior to the initiation of appropriate DR-TB treatment. All had been referred to the provincial DR-TB unit for treatment. Whether or not death would have been prevented with more rapid initiation of drug-resistant TB treatment in these cases is not clear – three of the four had advanced HIV disease (CD4+ T-cell count <100 cells/ μ l) and were not yet on ART. These deaths have to be put into the context of the situation prior to Xpert implementation, where up to 40% of MDR-TB cases died before laboratory diagnosis.[122]

Despite these delays, the time to appropriate DR-TB compares favourably to the situation prior to the implementation of Xpert and the situation more broadly in the province. In the study area, median time from sputum collection to commencement of DR-TB treatment for 50 cases managed under the satellite model in 2008, and diagnosed using culture and phenotypic DST, was 84 days.[122] This was broadly similar to other public sector programmes in South Africa (Figure 2-2).[123-129] The overall median time of 23 days in this study with Xpert is therefore a substantial improvement. Our findings are similar to those from Cape Town, where the median time to MDR-TB treatment commencement under an Xpert-based algorithm was 17

days (95% CI 13-22).[130] In a retrospective study of routine implementation in Durban, 68% of rifampicin-resistant cases successfully traced had commenced treatment within 4 weeks. However, this represented fewer than half of all diagnosed cases, as there was a substantial number that were untraceable.[361] The South African National Strategic Plan 2012-2016 includes the target of five working days from suspicion of drug-resistant TB to starting appropriate treatment.[40] This was not achieved for any of the DR-TB cases in this study. There is some preliminary evidence that this is possible where Xpert has been implemented within a framework of decentralised MDR-TB treatment and care.[300]

In order to facilitate the rapid initiation of treatment for drug-resistant cases, there are initiatives to capacitate nurses to initiate and monitor MDR-TB treatment.[362] These will need to be rapidly scaled up and supported if treatment targets are to be achieved. The potential for nurses to have an expanded role in the diagnosis and treatment of TB, and specifically drug-resistant TB, could empower them, in similar ways to what has been seen with Nurse Initiated and Managed Antiretroviral Therapy (NIMART) in South Africa.[363,364] However, the empowerment that can be produced has to be balanced against the pressures of increased workload and responsibility.[363,364] There was certainly anecdotal evidence during the study that the nurses appreciated the potential for same-day TB diagnosis and treatment and enjoyed the expanded role of performing tests normally confined to the laboratory. However, this requires more formal study with larger numbers of nurses to see whether nurse-delivered diagnostic testing would be sustainable in the long term.

7.1.2 Mortality

Overall mortality amongst all individuals investigated for TB and DR-TB was lower than expected (2.6% died within 60 days of enrolment). In unadjusted analysis, mortality was higher in the POC arm, but following adjustment for the presence of TB disease (*M. tuberculosis* culture positivity) and baseline CD4+ T-cell count, this association was no longer statistically significant. The presence of TB disease was the factor most strongly associated with mortality in this study population (6.3% of those with a positive culture for *M. tuberculosis* died within 60 days vs. 0.9% with a negative culture). This emphasises the importance of detecting and treating TB disease in a timely fashion.

There was no evidence that the difference in mortality between the arms was due to poorer Xpert performance and missed diagnoses of TB and DR-TB under the point-of-care strategy. The observed difference was partly explained by the imbalance in TB disease and CD4+ T-cell count. It is possible that there were other unmeasured clinical differences in participants that contributed to different mortality risks between the two arms. As noted during the exploration of the imbalance in culture positivity between the arms (section 5.3.4), it is possible that the physical presence of Xpert at the clinic promoted access to testing for more unwell individuals. One of the exclusion criteria for the study was being severely unwell and requiring admission to hospital. Four individuals were excluded from the study for this reason during laboratory blocks but none was during point-of-care blocks. It is plausible that the study team enrolled severely unwell individuals during point-of-care blocks because they knew a result would be obtained within two hours.

A limitation of the mortality endpoint was that it represented all-cause mortality and specific causes of death were not sought as part of the study. Although TB and HIV remain the most common causes of death in adults in the study area, other unrelated causes such as injury and trauma are also common in young adults.[365,366]

Although there would be no reason to believe that deaths unrelated to TB and HIV would be systematically different between the two trial arms, the low overall mortality would mean that a small number of deaths could bias the mortality estimates.

Mortality might have been underestimated in this study because of the relatively high rate of loss to follow-up (28% lost to follow-up at two months). It is well documented in HIV programmes that mortality can be high in those lost to follow-up and failure to account for this can lead to underestimation of mortality.[367-369] Having said that, it is also possible that mortality was overestimated given the fact that sputum culture positivity was the factor most strongly associated with mortality and loss to follow-up was biased towards culture-negative cases. Other baseline characteristics were broadly similar for those lost to follow-up and those whose outcomes were known. Although fewer of those lost to follow-up were on ART (29% vs. 43%), CD4+ T-cell counts were similar. Despite the incomplete outcome ascertainment, there was no difference in follow-up between the two arms and therefore the risk of bias was low.

The study was also conducted through a period of rapidly shifting epidemiology of HIV. There had been rapid scale-up of HIV testing, through both the national HIV counselling and testing (HCT) campaign [370] and local initiatives for home and

mobile testing.[371] The trial took place following the change in CD4+ T-cell threshold for ART eligibility in HIV-infected adults to 350 cells/ μ l, which has led to earlier presentation for HIV care and treatment.[372] As a consequence, mortality has reduced not only in those accessing ART but more broadly at a population level.[372-374] The proportion of HIV-infected participants on ART at enrolment was similar to the population-level coverage in the area, suggesting that the study population was broadly representative of those living with HIV in the area.[304,375] The median CD4+ T-cell count of 263 cells/ μ l was lower than the median for HIV-infected individuals in the population (374 cells/ μ l),[376] but this was not unexpected as those with lower CD4+ T-cell counts are more likely to be symptomatic and to be accessing health care. Most deaths in this study still occurred in persons with advanced HIV disease not yet on ART. This highlights the continued challenge to facilitate earlier access to HIV testing and more effective linkage to care and treatment.

It is difficult to directly compare mortality across the clinical trials as study populations are not directly comparable and mortality endpoints might differ. In the XTEND and TB-NEAT studies recruiting individuals at primary health care clinics, overall mortality was measured at six months: 4.4% in XTEND and 8.1% in TB-NEAT.[240,297] The XTEND study was performed in South Africa only, whereas the TB-NEAT study took place in South Africa, Zambia, Zimbabwe and Tanzania and there was some evidence that mortality was not uniform across the study sites.[240] The TB-NEAT study also reported two-month mortality in culture-positive cases that commenced anti-TB treatment of 5.2%, which is comparable to the 6.1% two-month mortality in all culture-positive cases in our study. In the study

of Mupfumi *et al.*, which enrolled only HIV-infected adults initiating ART at a large urban hospital in Zimbabwe, overall mortality was 7.9% at three months.[298] In a prospective study from a single primary health care clinic, overall mortality at six months was very low at 0.8%, although in this study the rate of loss to follow-up was high and it is possible that mortality might have been substantially underestimated.[232]

The relatively low overall mortality underlines the point that amongst individuals with symptoms suggestive of TB, there is a considerable difference between those attending primary health care clinics and hospital inpatients. In the study of hospitalised patients with symptoms suggestive of TB in Uganda where Xpert was used for TB diagnosis, mortality was 17% at two months.[228] In a similar study in a large referral hospital in Uganda of sputum smear-negative TB suspects, mortality at two months was 32%.[377] The study populations in hospital-based studies generally include participants with more advanced TB and HIV disease; in the two Ugandan studies, median CD4+ T-cell count was 54 cells/ μ l and 46 cells/ μ l respectively.[228,377] There is unlikely to be a universal TB diagnostic strategy for use in both ambulatory primary health care attendees and hospitalised patients and trial designs and endpoints need to be specific to the context.

7.1.3 Integrated antiretroviral therapy

One of the postulated benefits of point-of-care Xpert was the potential for more prompt initiation of antiretroviral therapy for eligible HIV-infected patients. It was thought that more rapid identification or exclusion of TB disease might expedite progress through the pre-ART phase and enable earlier initiation of ART. The data did not support this hypothesis, as time to ART initiation was similar under the

point-of-care and laboratory strategies. Similarly for the subset of participants with rifampicin-susceptible TB detected by Xpert, there was no difference in time to ART initiation between study arms and no difference in the proportion that had started both TB treatment and ART by 30 days.

The sub-population for this analysis was a heterogeneous group, as ART-naïve HIV-infected participants were enrolled in the study at different time-points in the pre-ART phase. Participants enrolled on the day of HIV testing, the day of CD4+ T-cell count testing, the day of CD4+ T-cell count result collection, the day of attendance at group counselling sessions prior to ART, the day of planned ART initiation, or any other day prior to ART if presenting specifically for TB investigation. Whilst data was not collected systematically on which of these categories each participant belonged to, there was no reason to believe that the distribution of participants would have varied between the study arms.

7.1.4 Patterns of anti-TB drug resistance

The majority of *M. tuberculosis* isolates in this study were rifampicin and isoniazid susceptible. However, almost one in four isolates had evidence of anti-TB drug resistance, the most common pattern being multidrug resistance (resistance to rifampicin and isoniazid). As the study population preferentially included people at high risk of anti-TB drug resistance, this should not be considered to be representative of population-level resistance patterns. Having said that, our finding that 16% of Xpert-positive specimens had documented rifampicin resistance is very similar to recent district-level data for all individuals tested by Xpert in 2013-2014.[303] This might suggest that the drug resistance patterns are broadly representative of those in individuals being investigated for TB in the local area.

Rifampicin resistance was present in around one in four previously treated culture-positive cases and one in ten cases with no previous exposure to anti-TB drugs. This provides evidence for the sustained transmission of drug-resistant TB in this community and underscores the need to develop effective strategies to interrupt TB transmission.

One of the reasons that the Xpert MTB/RIF assay incorporates testing only for rifampicin resistance is that the presence of rifampicin resistance is considered a good proxy for multidrug resistance. In this study, 90% of rifampicin-resistant strains also had evidence of isoniazid resistance which is similar to provincial-level data.[378] Distinguishing between rifampicin mono-resistance and multidrug resistance may still be important and this highlights the need for further testing with culture and phenotypic DST when rifampicin resistance is detected by Xpert.

Understanding the frequency of isoniazid mono-resistance arguably has more importance for the widespread roll-out of Xpert as a replacement for smear microscopy. There were only three cases of isoniazid mono-resistant TB (2% of culture-positive cases). With Xpert testing, in the absence of LPA or phenotypic DST, isoniazid resistance will not be identified and cases will be treated as having drug-susceptible disease with standard first-line anti-TB drug regimens. It is recognised that outcomes with standard first-line or re-treatment regimens (with the addition of streptomycin) are suboptimal in the presence of isoniazid mono-resistance,[379,380] although the evidence base to inform best practice in this context is weak.[328] Preliminary evidence from mathematical modelling has

suggested that the inability to detect isoniazid resistance may have a limited impact on population-level impact and cost-effectiveness of Xpert.[381,382] Having said that, the prevalence of isoniazid resistance varies quite substantially across regions of the world,[383] and strengthened surveillance of anti-TB drug resistance will be critical as access to Xpert expands into different areas.

No cases of extensively drug-resistant TB (XDR-TB) or even pre-XDR-TB (resistance to either fluoroquinolones or injectable second-line agents, but not both) were observed in this study. XDR-TB has spread throughout KwaZulu-Natal province and clinical cases were seen elsewhere in the sub-district during the course of the study.[22] The recent data from the province where half of the cases identified with rifampicin-resistant TB by Xpert did not have a sputum specimen submitted for confirmatory phenotypic DST with second-line sensitivities is of concern.[361] In this regard, further development of rapid diagnostics capable of expanded resistance testing is important and should be welcomed.[351]

7.1.5 Diagnostic performance of Xpert MTB/RIF

Prior to design of the trial, there was no published evidence about the use of Xpert MTB/RIF at primary health care level. It was therefore unknown whether the diagnostic technology would perform comparatively outside the normal laboratory infrastructure as within. In terms of diagnostic accuracy for the detection of *M. tuberculosis*, sensitivity and specificity were similar under both positioning strategies. Overall sensitivity of a single Xpert MTB/RIF was approximately 80%, which was slightly lower than the pooled sensitivity of 87% from 27 studies (Figure 3-1), but similar to the sensitivity in two prospective studies in South Africa with

predominantly or exclusively HIV-infected subjects.[218,220] This suggests that the diagnostic system can deliver acceptable performance in this rural setting not only under normal laboratory conditions but also within a primary health care clinic when operated by a nurse.

There was evidence that sensitivity was improved with low CD4+ T-cell counts in HIV-infected individuals, consistent with two other published studies.[218,276] However, none of these studies have been powered to specifically address the question of differential sensitivity by CD4+ T-cell count. The significance of this finding in our study is unclear in the absence of smear microscopy results and the lack of knowledge of radiological patterns of disease.

While Xpert only detected four out of five culture-positive cases, the overall positive yield from Xpert actually exceeded that of culture. The positive yield of *M. tuberculosis* from a single sputum specimen for Xpert was 17.5% (216/1235) compared to 13.0% (160/1235) from a single culture. The main explanation for this was the high number of culture specimens that leaked in transit or were contaminated. While the study was not designed to detect differences between Xpert and culture, this illustrates some of the limitations of culture-based diagnostics especially where laboratories are far removed from the point of care. While culture may be considered the gold standard for laboratory detection of *M. tuberculosis*, it may be that it is a poor standard in routine operational settings. This also highlights the challenges for diagnostic evaluation where the reference standard may be inappropriate.[384]

The simultaneous detection of genotypic resistance to rifampicin is one of the key benefits of the Xpert MTB/RIF system. In the early phases of Xpert evaluation and implementation, concerns arose about suboptimal specificity for the detection of rifampicin resistance.[278,334] This study was performed in an area with high levels of drug resistance and specifically included subjects with high risk of drug-resistant TB disease. Overall specificity was somewhat higher than in the large published studies (99.0%) and there was only one false positive rifampicin resistance result. Even this case had documented polyresistance on subsequent phenotypic DST (resistance to isoniazid and kanamycin), which raises the possibility that rifampicin resistance-associated mutations were correctly detected by Xpert. Unfortunately it was not possible to perform sequencing of the *rpoB* gene to determine whether mutations associated with rifampicin resistance were indeed present. There is an increasing body of evidence describing discordant genotypic and phenotypic resistance for rifampicin.[283-290] There is also some evidence that treatment outcomes with standard first-line regimens are poorer where there is genotypic evidence of resistance but phenotypic susceptibility.[284,290] There remains much need of further research to determine whether genotypic or phenotypic resistance better predict treatment outcomes, not only for rifampicin but also for other anti-TB drugs, in order to better inform diagnostic and treatment strategies.

Interestingly, sensitivity for the detection of rifampicin resistance was relatively low (88.5%) and there were three cases where Xpert detected no mutations but phenotypic DST suggested phenotypic rifampicin resistance. It was unfortunate that further laboratory work was not possible to investigate these discordant cases. All three cases had documented risk of drug resistance and two had previously received

anti-TB treatment. Only one case had further confirmation of phenotypic resistance before commencing drug-resistant TB treatment. One plausible explanation for these discordant cases would be the presence of mixed susceptible and resistant bacillary populations in an individual. It has been documented that for certain *rpoB* mutations (e.g. L533P), Xpert will only determine resistance if nearly all of the *M. tuberculosis* bacilli in the sample carry the resistance-conferring mutation.[215,385] The sensitivity of Xpert to detect rifampicin resistance has been shown to be reduced in the presence of confirmed mixed strain infections.[385] Further work is needed to determine the impact of mixed strain infections on Xpert-based diagnosis in different settings, especially as the prevalence of mixed strain infections has been reported to be as high as 30% in some studies.[386] There has been much discussion about the potential harm from false positive rifampicin resistance results, mainly through exposing individuals to potential toxicity from second-line TB drug regimens. However, false negative resistance results could impact more broadly at a population level, by allowing amplification of drug resistance and continued transmission of drug-resistant strains.[47] This is of particular concern in South Africa, where there is evidence of poor compliance to diagnostic algorithms, with only half of those with a positive Xpert demonstrating rifampicin resistance having a specimen submitted for culture and phenotypic DST.[361]

It was noteworthy that these three discordant resistance results (sensitive by Xpert, resistant by phenotypic DST) were obtained with the earlier version of the Xpert MTB/RIF cartridge (G3). As a result, sensitivity for the detection of rifampicin resistance was better with the G4 cartridge than the G3 cartridge (100% vs. 75%). Although changes to the cartridge were primarily to reduce the rate of errors and

improve specificity (with regards to rifampicin resistance), there were some modifications, particularly with probe B, to improve the detection of rifampicin resistance.[279] While the improved accuracy with the G4 cartridge is therefore encouraging, the numbers of rifampicin-resistant cases was small and confirmation of improved sensitivity for the detection of rifampicin resistance is needed from larger studies and routine programmes.

Approximately one in 25 initial Xpert tests produced either an error, invalid result or no result. This proportion was reduced to 1.4% following repeat tests on remaining sample/buffer mix. The proportion of indeterminate results with the first sputum specimen was similar under both strategies (1.8% with laboratory testing, 0.9% with point-of-care testing). This was towards the lower end of the range of assay failure rates reported previously (Table 3-5), even though most studies have been from hospital laboratories. There was no evidence that the failure rate changed over the course of the study or as operators became more familiar with test procedures. There were two nurses employed one after the other during the trial but there was no evidence that the diagnostic performance of Xpert differed between the two nurses.

The nurses performing point-of-care testing and the laboratory technician dedicated to Xpert testing all received the same training in the procedures. However, there remained the possibility that operator performance could impact on Xpert results, especially as the nurse was performing point-of-care testing in between attending to patients. There was also the possibility that temperature or power supply issues would hamper Xpert performance at the primary health care clinic yet this was not observed. This suggests that in this setting, the clinic infrastructure was sufficient to

allow proper functioning of the diagnostic system and that the system is robust enough to operate in the clinic environment.

7.1.6 Need for diagnostics to uncover other causes of respiratory symptoms

This study focused on the diagnosis of pulmonary TB, yet it is notable that even in this high prevalence setting with high levels of HIV co-infection, the majority of individuals presenting with cough did not have pulmonary TB. Other forms of TB that can present with cough, such as pleural, pericardial and intrathoracic lymph node TB, were surprisingly infrequent in this study population. Although outcomes for those without pulmonary TB were generally good, with 60-day mortality less than 1% for those with a negative sputum culture, there may still be benefit in ascertaining other causes of respiratory symptoms. These benefits might include reduction in morbidity, avoidance of inappropriate anti-TB medication, and allowance for earlier initiation of antiretroviral therapy by identification of causes other than TB. One study of adult primary health care attendees in Zimbabwe determined the aetiology of cough of three weeks or longer duration using standardised investigations including sputum-based diagnostics and X-ray.[387] In this study, common causes of cough other than TB were pneumonia and non-pneumonic lower respiratory tract infection, asthma, post-TB fibrotic lung disease and cardiac disease. In the context of ambulatory primary health care attendees, HIV-associated conditions such as *Pneumocystis jiroveci* pneumonia, cryptococcal lung disease, and Kaposi's sarcoma were relatively rare.[387] Additional rapid diagnostics suitable for point-of-care detection of common bacterial and viral respiratory pathogens,[388] and development of point-of-care X-ray technologies,[151] might allow more accurate diagnosis and appropriate treatment of

a broader set of conditions. Sequelae of pulmonary TB disease are well recognised, in the form of fibrotic lung disease, bronchiectasis, chronic obstructive pulmonary disease (COPD), persistent cavitation, and pulmonary aspergilloma.[389] The burden of chronic lung disease might be expected to increase in coming years in southern Africa as a result of large numbers of HIV-associated TB cases since the 1990s and increasing rates of long-term survival with antiretroviral therapy, interlinked with occupational and environmental exposures.[390,391] There is a need for surveillance to monitor the true burden and impact of chronic respiratory diseases in southern Africa.[392] Concurrently, research is needed to establish diagnostic and therapeutic strategies for chronic respiratory diseases.

7.2 Limitations

7.2.1 Statistical power for primary outcome

The trial was underpowered to detect a significant difference in the primary outcome. This was due most importantly to the lower than anticipated proportion of culture-positive TB cases amongst those investigated for TB. For the initial sample size calculation, the assumption was that 25% of people being investigated for pulmonary TB would have a positive sputum culture but the actual figure was only 13%. The assumption of 25% was based upon preliminary data for yield of sputum culture examinations in Hlabisa sub-district and in other South African studies. In the Xpert demonstration study, the yield of sputum culture at the Cape Town site was 24%.[46] In other South Africa studies using culture to investigate adults with symptoms suggestive of pulmonary TB, the yield of sputum culture was approximately 30%.[152,393] In the TB-NEAT study, the overall yield of sputum

culture was 24%, although this ranged from 18-38% across the different study sites.[240]

There are likely to be a number of reasons for the lower than anticipated culture positivity observed during the study. There was substantial attrition in the processes leading to a culture result, with just over three-quarters (77%) of sputum specimens yielding a valid result. The first problem was leakage of sputum specimens during transit. Overall 11% of specimens for culture leaked during transit from the clinic to the provincial culture laboratory. Measures were taken during the study to limit specimen leakage – this included reinforcing instructions to participants and health care workers about the proper closure of specimen containers, use of different types of specimen container, and the routine use of plastic paraffin film to seal specimen containers prior to transport. However, the proportion of specimens that leaked remained similar throughout the study. The storage and transportation of specimens was done through the routine systems operated by the National Health Laboratory Service (NHLS) but this was complex, with initial transit from the clinic to the district hospital, and then onwards to the provincial reference laboratory.

There is actually very limited information in the literature about leakage of sputum specimens during transit, even though this is recognised as an important logistic step in the framework of TB diagnosis.[195] In one study also from Hlabisa sub-district, 2.4% (8/335) of sputum specimens leaked during transit from hospital laboratory to Durban.[394] In one other South African study, 3.7% of specimens collected at primary health care clinics for smear microscopy leaked in transit to a centralised laboratory.[106] In two studies exploring transport of sputum specimens to

centralised laboratories in India, 4.2% (51/1210) and 1.7% (3/175) leaked during transit.[395,396] It was also noted recently as a significant problem during the South African national TB drug resistance survey, although the extent of the problem was not quantified.[397] There are no data provided by the NHLS quantifying the extent of the problem in the routine laboratory system. It is an important reminder that in laboratory studies focused on diagnostic test accuracy, loss of specimens due to leakage is unlikely to be documented and that evaluation of the true effectiveness of diagnostics needs to examine the entire process from initial presentation with symptoms to diagnosis and treatment.

Contamination of sputum cultures occurs due to the overgrowth of bacteria in the liquid culture medium. The bacteria can be present in the sputum sample or can be introduced from the environment during laboratory procedures. The likelihood of contamination can be influenced by the quality of sputum specimens, quality and duration of storage, and laboratory decontamination procedures. The overall rate of contamination in this study was 8.3% (or 9.7% if excluding culture specimens with no valid result). This is comparable to the pooled proportion of 8.6% with the MGIT system in a meta-analysis including ten studies from high-income settings.[398] In South African studies, contamination with the MGIT system has varied from 3.0% to 16.7% [218,220,246,393]; and in one study in the Zambian national reference laboratory the contamination rate was as high as 29.6%.[399] As results from sputum culture defined the study population and outcome, further measures could have been taken to optimise the yield of sputum culture. It may be that the use of both solid and liquid culture media would have increased the yield and reduced contamination rates. It is also likely that the collection of two sputum specimens for

culture would have improved the yield, although this would have brought additional logistical challenges and the provision of three spot sputum specimens may not have been possible for some participants.

The lower than expected culture positivity could also be partly explained by the characteristics of the study population. The median duration of cough was two weeks for all those investigated and three weeks for culture-positive cases. Historically, individuals were defined as TB suspects and investigated in the presence of a cough for longer than two-three weeks, on the basis that most acute viral and bacterial respiratory tract infections would be expected to resolve within this timeframe. Now, in the context of HIV infection, investigations for TB are recommended for cough of any duration.[400] Given that this study was at primary health care level and included a mixture of passive and active case finding, it is possible that this was close to a true representative sample of clinic attendees with current cough and it is a reminder that the majority of people with cough do not have TB, even in a community with an extremely high burden of disease.

7.2.2 Evaluation of feasibility and broader impact of the point-of-care strategy

The initial design of the trial incorporated an assessment of the operational feasibility of both strategies. Unfortunately, data on all feasibility indicators were not collected. In particular, information was not collected on hands-on user time, user performance or user appraisal. In general, the diagnostic system operated well under both strategies with few interruptions in power supply and no requirement for system maintenance throughout the study (other than routine annual calibration of modules). At times the temperature exceeded the recommended maximum temperature both for operation of the GeneXpert system and for storage of cartridges at the primary health

care clinic yet there was no evidence that this adversely affected the performance of the system.

One of the most reliable and easily measurable indicators of feasibility was the proportion of Xpert tests with an indeterminate result (error, invalid or no result). Encouragingly, there was no significant difference between the two trial arms in this indicator, and if anything the proportion was marginally lower under the point-of-care nurse-operated strategy (3.1% of initial Xpert tests) than under the laboratory strategy (4.7%). Overall, this was similar to the proportions reported in other clinical studies, and lower than reported for routine programme implementation (see section 3.5).

The incomplete data on operational feasibility and the lack of economic evaluation limits the conclusions that can be drawn as to whether point-of-care Xpert could be implemented more widely. This also highlights the need to compile a comprehensive evidence base about the impact of a new diagnostic tool or strategy that goes beyond analysis of effectiveness. The Impact Assessment Framework (IAF) comprises five interconnected elements: effectiveness analysis, equity analysis, health systems analysis, scale-up analysis, policy analysis. [401]The main focus of the trial was the clinical impact of a point-of-care strategy but use of this framework to guide the research might have enabled collection of a broader set of data to inform policy decisions.

7.3 Trial design

The cluster randomised trial incorporated an unusual design with clusters defined as participants enrolled during a two-week time block. This design allowed for randomisation to one or other diagnostic strategy and was considered logistically easier to implement than individual randomisation. Whilst there are examples of similar cluster randomised trials with time blocks in the literature, these are relatively rare.[144,338-342] In general, the main reasons for selecting a cluster randomised design are: where an intervention is to be applied to groups of individuals; where the population-level effect of an intervention is to be measured; or where there is a need to avoid contamination from individuals in the same community being randomised to different trial arms.[402] The intervention in this trial was delivered at the individual level and a design incorporating individual randomisation would theoretically have been possible and indeed would have been statistically the most efficient design. Both the TB-NEAT study and the study in Zimbabwe comparing Xpert to smear microscopy used individually randomised designs.[240,298] Implementing a trial with individual randomisation at the single primary health care clinic would have posed some logistical difficulties due to the need for the study team to switch between diagnostic strategies on the same day. For this reason it was considered appropriate to adopt a cluster randomised design.[402]

Alternative designs to address the principal research hypothesis were also considered. If more resources had been available, a cluster randomised design with individual health care facilities as units of randomisation would have been possible. Alternatively, if inclusion of more health care facilities was possible, a stepped wedge design could have been used whereby all clinics would have commenced the

trial using the laboratory strategy and then would have adopted the point-of-care strategy at different time points. A stepped wedge design was used for the XTEND study comparing Xpert to smear microscopy in 20 laboratories across several districts in South Africa.[297] However, such a design would have created additional challenges for analysis and ultimately was not possible with the time and resources available. Finally, quasi-experimental designs, such as a non-randomised pre- and post-intervention study were considered.[403] These are generally the most common type of study used in diagnostic research and there are several examples in TB diagnostic research, both with the line probe assay,[128,129,191,192] and with Xpert MTB/RIF.[130,228,232] These may be logistically the easiest to design and implement. However, the lack of randomisation is the key weakness of such designs and inappropriate conclusions can be drawn on the basis of associations without evidence of a causal association. This type of design would have faced real challenges in this environment, particularly with the outcome of mortality, as significant reductions in mortality were documented in the area during the trial period.[372,373]

The randomised design did provide well-balanced groups in the study. The only imbalance in baseline characteristics was in the CD4+ T-cell count of HIV-infected participants, which was marginally lower in the point-of-care arm than in the laboratory arm (247 cells/ μ l vs. 280 cells/ μ l). However, the imbalance between the arms in culture positivity was unexpected and could not be clearly explained from the data available. This does raise the possibility that there were some other systematic differences in participants that would also have had the potential to affect the outcomes.

An important concept for the design of cluster randomised trials is that of the between-cluster coefficient of variation (κ).^[336] This is a measure of the variation between clusters in the main outcome of interest. In general, κ is usually ≤ 0.25 and many large community randomised trials in TB have used values of 0.20-0.25.^[24,404,405] For this trial, there were no prior data to inform an estimate of the coefficient of variation. Given that the clusters were defined by time blocks, limited variation between clusters was expected for the primary outcome and therefore a value of $k = 0.05$ was selected for the sample size calculation. This value of κ corresponded to a range of proportions appropriately treated in the laboratory arm of 77-94% for individual clusters. The estimated value of κ based on the trial data was 0.11. This should be a useful guide to future studies employing similar designs with clusters defined by blocks of time.

The trial was designed to measure the time to appropriate treatment for all culture-positive cases and specifically for rifampicin-resistant cases. This time to event data required survival analysis techniques, specifically Cox proportional hazards regression. Cox regression assumes that the ratio of hazards comparing the trial arms is constant over time. In practice, this might have been an unreasonable assumption as according to the trial design there was expected to be significant early difference in treatment initiation due to the anticipated earlier diagnosis under the point-of-care strategy. Indeed the same-day treatment initiation in the point-of-care arm meant that some individuals reached the endpoint on day zero, which creates problems for survival analysis. For the Cox regression models of time to appropriate anti-TB treatment and time to appropriate drug-resistant TB treatment the proportional

hazards assumption was not met. Therefore any hazard ratio from these analyses would have been unreliable and they were not reported. Kaplan-Meier survival curves were plotted and compared using the log rank test. Although there are other analysis methods that might be appropriate in the setting of non-proportional hazards,[406] discussion of these was beyond the scope of this thesis.

7.4 Generalisability

Many of the laboratory systems functioned relatively well during the study. Whilst aspects of the laboratory strategy incorporated routine systems, the use of Xpert within the laboratory was confined to testing samples from the trial and involved specific trial personnel and so might not have been truly representative of a real world laboratory setting. Participant numbers were such that the system was only testing around 20-30 specimens per week. Data from routine implementation of Xpert in KwaZulu-Natal show that the laboratory turnaround time of 48 hours was exceeded with almost one-third of samples.[361] Separate data also from KwaZulu-Natal has detailed the delays in laboratory processes when Xpert was introduced into a single central hospital laboratory in Durban.[301] In the Durban study, the overall time from sputum collection to return of results to the health facility was 6.4 days, somewhat longer than the time in the laboratory arm in this study.[301]

The majority of participants were HIV-infected. Around 40% of HIV-infected participants were on ART at the time of enrolment and many others were undergoing preparation prior to commencing ART. This was therefore a population already engaged in care and with reasons to return to the clinic other than for receipt of TB test results. Whether or not individuals with a documented risk of drug-resistant TB

are more likely to return for TB test results is not known, but it is plausible that concern about drug resistance or familiarity with clinic procedures from previous TB episodes might make people more likely to return. It is therefore possible that the study population, although selected because of prioritisation for Xpert testing,[326] might have different risk for diagnostic default from HIV-uninfected individuals without risk of drug-resistant TB. This could therefore have affected the ability to detect an advantage to the POC strategy and could also limit the generalisability of the findings to other settings with lower HIV prevalence and lower rates of drug-resistant TB.

7.5 Lessons for future diagnostic research

There is relatively limited experience with clinical trials of diagnostic tests or strategies and the knowledge gained from this study should inform future diagnostic research. It is critical in diagnostic studies to decide whether the outcomes are to be measured in all those investigated or only in those found to have the disease, in this case TB. One of the main challenges of diagnostic trials is that a large number of suspects need to be enrolled in order to identify a suitable number of cases. In this study, given that the rate of culture positivity was almost half of that anticipated, enrolment of double the number of suspects would have been required to achieve the sample size for the primary outcome and this was not possible for logistical and financial reasons.

Clinical trials for TB diagnostics are of growing importance as new technologies emerge to meet the recognised demand.[401,407,408] Experience with diagnostic trials is more limited than with vaccine and drug trials, and the design, conduct and

analysis of such trials can be complex.[47,409] The design of clinical trials and selection of endpoints has been relatively consistent for TB vaccines,[410,411] and for anti-TB drugs.[412] In the few clinical trials involving Xpert, all have employed different primary and secondary endpoints, summarised in Table 7-1.[240,297,298] The different endpoints reflect some differences in study populations, interventions, and underlying hypotheses, and there are also differences in whether the primary outcome is measured amongst all participants investigated for TB or only amongst participants with TB disease. As clinical trials will increasingly be necessary to evaluate and compare the effectiveness of new TB diagnostics, it would be useful to seek consensus on appropriate designs and endpoints for clinical trials.

The poor performance of culture and drug susceptibility testing created problems not only for the diagnostic accuracy analysis but also for the clinical outcomes as this was the gold standard diagnostic used to define the population for analysis and the primary endpoint itself. The problem of an imperfect gold standard is well recognised in diagnostic accuracy research and various solutions have been proposed to deal with this.[413-416] In future research beyond diagnostic accuracy studies, careful thought should be given as to the role of a reference standard and how imperfect reference tests might affect the evaluation of impact.

Table 7-1 Comparison of endpoints in randomised trials investigating impact of Xpert MTB/RIF

| | TB-NEAT [240] | XTEND [297] | Mupfumi <i>et al.</i> [298] | This study |
|----------------------------|---|---|---|--|
| Intervention | Point-of-care Xpert | Xpert | Xpert | Point-of-care Xpert |
| Comparator | Point-of-care smear microscopy | Smear microscopy | Fluorescent microscopy | Laboratory Xpert |
| Randomisation | Individual | Cluster (health facilities) | Individual | Cluster (time blocks) |
| Study population | Adults with TB symptoms | Adults with TB symptoms | HIV-infected adults due to start ART (with or without TB symptoms) | HIV-infected adults with TB symptoms and adults with suspected DR-TB |
| Primary endpoint | TB morbidity in culture-positive cases who had commenced anti-TB treatment | Mortality at 6 months in all participants | Proportion diagnosed with TB or died within 3 months (composite endpoint) | Proportion of culture-positive cases initiated on appropriate anti-TB treatment within 30 days |
| Secondary endpoints | Time to TB diagnosis Time to anti-TB treatment initiation Proportion of culture-positive cases not started on anti-TB treatment Proportion of culture-positive cases lost to follow-up Feasibility of point-of-care Xpert | Proportion with positive index test Proportion started on anti-TB treatment at 6 months Loss to follow-up | - | Time to appropriate anti-TB treatment for culture-positive cases Time to appropriate anti-TB treatment for culture-positive rifampicin-resistant cases Mortality at 60 days in all participants Time to ART initiation for HIV-infected participants eligible for ART |

ART, antiretroviral therapy; DR-TB, drug-resistant TB

An exploratory analysis was performed using only the Xpert result to define cases requiring treatment, regardless of culture result. The results were similar, with no evidence that the proportion of Xpert-positive cases initiated on appropriate anti-TB treatment within 30 days was different between the two strategies. If this analysis had been chosen as the primary analysis, it would not have combined the assessment of rapidity of diagnosis with accuracy of Xpert under the different positioning strategies. This was the rationale for using the results of culture/DST as the gold standard to define cases, as it was not known whether different diagnostic performance of Xpert under the two strategies might affect the outcomes selected for the study. In particular, at the time of study design, there was genuine concern about the problem of false positive rifampicin resistance results and whether this was due to a technical issue with the assay or whether this might be operator dependent. In reality, the performance of Xpert was similar under both strategies and there was only one false positive rifampicin resistance result so this therefore had no influence on the main outcomes. One concern about using Xpert positivity to define cases in need of treatment was the possibility of false positive results for the detection of *M. tuberculosis*, particularly in the context of non-viable bacilli in previously treated individuals.[417] There were a few cases where this was a possibility but the majority of those not on treatment at enrolment with positive Xpert but negative culture had not previously received anti-TB treatment and so there must have been other explanations for the discordance. In the absence of data conclusively explaining the discordance and of prospective data on clinical outcomes without treatment in these

individuals, no conclusions can be drawn about whether such cases truly require treatment or not.

7.6 Recommendations for implementation

The evidence generated in this study suggests that Xpert MTB/RIF can be delivered at the point of care in a rural primary health care clinic and diagnostic accuracy comparable to laboratory implementation can be achieved. Whilst there were advantages to point-of-care placement, including earlier initiation of appropriate anti-TB treatment and reduced number of clinic visits prior to treatment, an effect on the proportion of culture-positive pulmonary TB cases initiated on appropriate anti-TB treatment within 30 days could not be demonstrated. Cost-effectiveness analyses have suggested that point-of-care placement of Xpert MTB/RIF at current prices would need to produce substantial clinical benefits to offset the increased costs associated with clinic deployment in South Africa.[418] Increased costs were at least partly due to loss of efficiency because of lower testing volumes at each implementation site. Further cost-effectiveness analyses are needed, but it is likely that further significant price reductions would be required to justify routine decentralisation of Xpert MTB/RIF beyond district hospitals in the immediate future. However, an argument exists that where the primary health care infrastructure allows and where sufficient volumes of testing can be maintained then point-of-care placement could bring important benefits.

7.7 Recommendations for future research

It remains important that new TB diagnostic technologies and strategies are subjected to rigorous evaluation with patient-relevant outcomes in real world settings.[47,48,419] It is likely that developments in molecular technologies may bring competing systems to the market and there needs to be a solid framework to guide evaluation and implementation of such tools. It is important that studies are designed and appropriately powered to address hypotheses and to detect differences of clinical and public health significance.

One of the critical gaps in knowledge relates to the impact of diagnostic strategies on TB transmission. Whilst mathematical modelling can address certain questions, our fundamental understanding of TB transmission remains poor. With advances in molecular epidemiology enabling better understanding of transmission, this presents an opportunity for integrated research to gain insight into the impact of different diagnostic strategies on transmission. Of particular interest in this regard would be the impact of different diagnostic strategies on nosocomial transmission within primary health care facilities and hospitals. Whilst this research focused on the primary health care setting and ambulatory TB suspects, there is a need to explore the impact of point-of-care strategies in an inpatient setting with hospitalised adults. In addition to determining the effect on clinical

outcomes, of particular interest would be whether near-patient testing can facilitate triage and isolation of patients and whether this could have an impact on nosocomial transmission.

This study evaluated a single diagnostic test and used only sputum specimens to detect *M. tuberculosis*. It is possible that diagnostic algorithms involving a combination of diagnostic tests may offer the potential for improved detection of TB and improved outcomes. There is some evidence that urine-based testing, either with Xpert MTB/RIF or with tests to detect urinary lipoarabinomannan (LAM), may be complementary to sputum Xpert testing.[263,420] In particular, urine-based tests may be better at detecting individuals with disseminated or miliary disease that may be missed by sputum testing or that may be unable to expectorate sputum.[260,263] Whilst such combination testing may be more suited to the inpatient setting and hospitalised adults, there is also the potential to explore combined algorithms at primary health care level.

Detection of tuberculosis remains the priority when investigating respiratory symptoms in high burden settings. However, it remains the case that the majority of people with respiratory symptoms do not have active TB disease. Again there is the need to explore whether other diagnostic tests (for example multiplex PCR for detection of bacterial and viral respiratory tract pathogens) could be used in combination with TB diagnostics at the point of care in order to better inform treatment decisions. There is also likely to be an increasing need to improve the detection and management of

chronic respiratory diseases within the framework of primary health care in Africa.

7.8 Concluding remarks

New TB diagnostic strategies are required to improve TB control in high burden settings, particularly in southern Africa with high levels of anti-TB drug resistance and HIV co-infection. In the study presented in this thesis, the impact of a point-of-care diagnostic strategy was explored for people at the highest risk of TB mortality in a rural area at the epicentre of the TB and HIV epidemics in South Africa. Whilst it was not possible to demonstrate benefit from the point-of-care strategy in terms of the primary outcome, important evidence was generated that should inform future diagnostic strategies and diagnostic research. Point-of-care placement allowed for earlier initiation of appropriate anti-TB treatment, in some cases same-day initiation, which could plausibly impact on TB transmission and reduce both patient and health system costs. Point-of-care placement also reduced attrition within the diagnostic process, with fewer specimens untested due to leakage in transit. This observation, coupled with high rates of leakage of culture specimens, highlighted the potential impact that could be achieved by relatively simple interventions, such as procurement of high quality specimen containers and ensuring correct closure of containers at clinic level.

In this study the main reasons that culture-positive cases were not initiated on treatment within 30 days were missed diagnosis by Xpert (due to

suboptimal sensitivity) or the presence of drug-resistant TB and the resultant delay in accessing treatment at the specialist drug-resistant TB unit. Despite the suboptimal sensitivity of Xpert compared to the defined gold standard of culture, the diagnostic systems based around sputum culture in a centralised laboratory performed poorly, due to high rates of leakage during transit and relatively high contamination rate. As a result, the actual yield of Xpert under both strategies was greater than culture.

Certainly with the evidence available now, future clinical trials of Xpert MTB/RIF should not need to use culture as a gold standard to define cases requiring treatment and clinical outcomes could be measured in cases as defined by Xpert result. However, as molecular technologies are developed with improved sensitivity, [421]but consequently with potential for reduced specificity, there may still be a need for culture as a gold standard in clinical trials exploring clinical impact. However, the selection of appropriate outcomes in future diagnostic research will depend on the specific question being addressed and the intervention being evaluated.

Overall, the study highlights that improvements in the diagnostic cascade to get all TB cases on treatment in a timely fashion will require a combination of technological advances (tests with improved sensitivity), optimisation of simple systems such as sputum specimen collection and transport, and broader strengthening of health systems to limit delays between diagnosis and treatment.

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Appendices

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| Appendix Q | Published manuscript in Trials: 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 |

Appendix A Details of the GeneXpert system and Xpert MTB/RIF assay

The GeneXpert® diagnostic system is a self-contained device which integrates automated sample processing and real-time amplification and detection of infectious pathogens. The system was originally developed in the United States for the detection of agents of bioterrorism, particularly the causative agent of anthrax (*Bacillus anthracis*).[1] A wide range of important infectious agents can now be detected using pathogen-specific cartridges within the same GeneXpert system: methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, *Clostridium difficile*, influenza, group B streptococci, *Chlamydia trachomatis* and *Neisseria gonorrhoea*. An assay for the measurement of human immunodeficiency virus (HIV) viral load is currently in development.[2]

The Xpert® MTB/RIF assay was developed collaboratively by three partner organisations: Cepheid, a commercial molecular diagnostics company; the Foundation for Innovative New Diagnostics (FIND), a non-profit organisation; and the University of Medicine and Dentistry of New Jersey (UMDNJ). The assay was based on the use of molecular beacon technology (Figure A-1),[3,4] which had been exploited for the rapid detection of drug-resistant *M. tuberculosis*. [5-7]

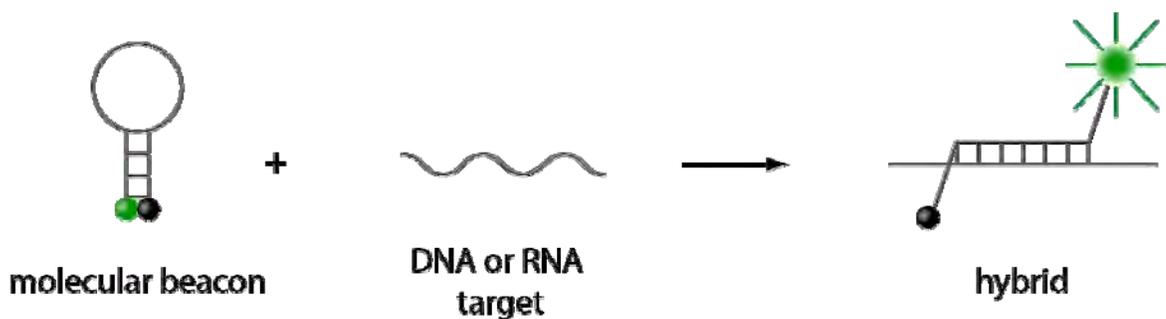


Figure A-1 Molecular beacons as hybridization probes

Molecular beacons are designed to detect specific target DNA sequences and are highly specific, easily discriminating sequences that differ from one another by a single nucleotide substitution. The target sequences for the molecular beacon probes are contained within the 81bp core region of the *rpoB* gene, which encodes the RNA polymerase enzyme (Figure A-2). Mutations within this region are highly predictive of rifampicin resistance and ~96% of all rifampicin-resistant strains contain mutations in this region.[8] The use of this target sequence allows for the simultaneous detection of the pathogen (*M. tuberculosis*) and the most important form of drug resistance.



Figure A-2 Core region of *rpoB* gene with target DNA sequence of the molecular beacon probes (A-E)

Sample pre-treatment is minimal: treatment reagent (sodium hydroxide and isopropanol) is added to unprocessed sputum, which liquefies the sputum and inactivates *M. tuberculosis* (rapid killing by 6-7 log₁₀). After 15 minutes incubation at room temperature, 2mls of digested sputum is transferred to the sample chamber of the cartridge (Figure A-3). These are the only manual steps required and all further steps are fully automated in the closed system.



Figure A-3 Manual steps for sample preparation

The single-use Xpert MTB/RIF cartridges contain i) chambers for holding sample and reagents, ii) a valve body composed of a plunger and syringe barrel, iii) a rotary valve system for controlling the movements of fluids between chambers, iv) an area for capturing, concentrating, washing and lysing cells, v) lyophilized real-time PCR reagents and wash buffers and vi) an integrated PCR reaction tube that is automatically filled by the instrument (Figure A-3). The cartridge contains lyophilised *Bacillus globigii* spores, which function as an internal sample processing and PCR control.

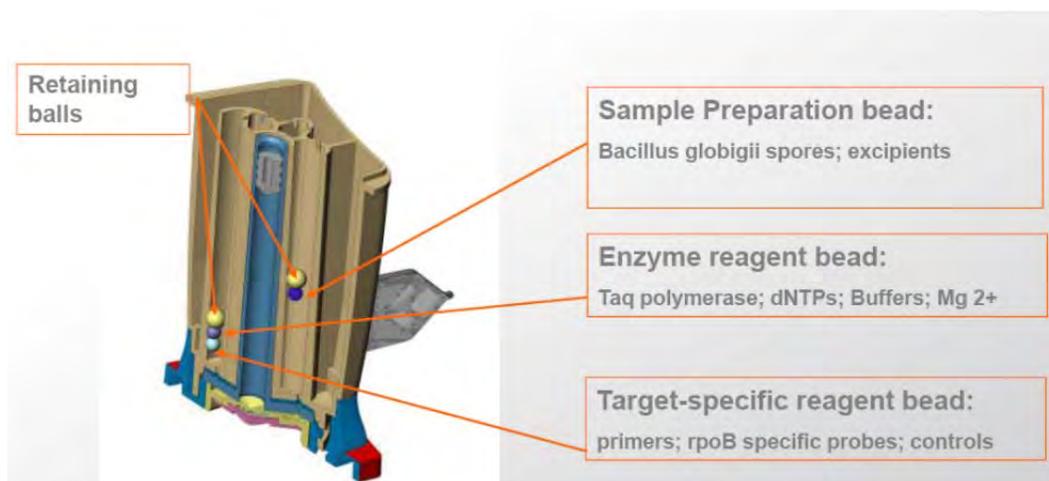


Figure A-4 Interior of an Xpert MTB/RIF cartridge

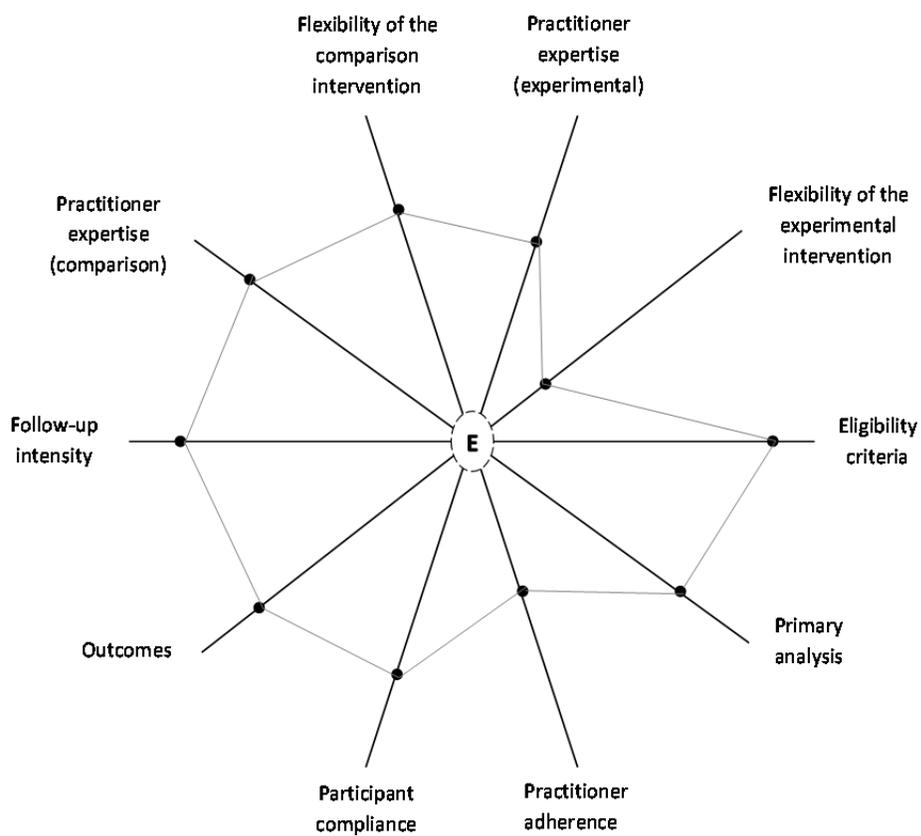
Each GeneXpert module incorporates a syringe drive, rotary drive and a sonic horn. The sonic horn delivers ultrasonic energy necessary to lyse the raw specimen and release nucleic acids contained within, while the combination of the syringe drive and rotary drive moves liquid between cartridge chambers in order to wash, purify and concentrate these nucleic acids. After the automated extraction is complete, the nucleic acid concentrate is moved into the cartridge reaction chamber where amplification and detection takes place.

Mycobacterium tuberculosis is detected if at least two of the five probes (A-E) produce a positive signal with a cycle threshold (C_t) of ≤ 38 cycles and within two cycles of each other. *Mycobacterium tuberculosis* is not detected if there is a positive signal from the *B. globigii* control without a positive *M. tuberculosis* signal. If the *B. globigii* internal control fails then the test is reported as invalid.

The definition of resistance in the original Xpert MTB/RIF assay (version G3) is based on the ΔC_t (difference between highest and lowest C_t for the *M. tuberculosis* probes). Under the original assay algorithm, rifampicin resistance was defined with a $\Delta C_t > 3.5$. Following early reports of suboptimal specificity for the detection of rifampicin resistance,[9-12] the algorithm was later changed (to improve specificity of the assay) such that resistance was then defined with $\Delta C_t > 5$. [13] Subsequently, the cartridge and software were modified (version G4), particularly with regard to the probe B beacon sequence, to reduce false rifampin resistance results.[14,15]

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Appendix B PRECIS (pragmatic-explanatory continuum indicator summary) wheel

Appendix C Eligibility form

GeneXpert TB study
Eligibility_v1.0_June2011



Subject ID

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Subject initials

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Inclusion Criteria

- | | | |
|--|---------------------------|--------------------------|
| 1. Age 18 years or older | <input type="radio"/> Yes | <input type="radio"/> No |
| 2. Current cough | <input type="radio"/> Yes | <input type="radio"/> No |
| 3. Confirmed HIV infection <u>OR</u> high risk of MDR-TB | <input type="radio"/> Yes | <input type="radio"/> No |

Note: All Inclusion Criteria must be answered YES, to be included in study.

Exclusion Criteria

- | | | |
|--|---------------------------|--------------------------|
| 1. Severely unwell requiring admission to hospital | <input type="radio"/> Yes | <input type="radio"/> No |
| 2. Previous MDR/XDR-TB diagnosis or treatment | <input type="radio"/> Yes | <input type="radio"/> No |
| 3. Diagnosis or suspicion of extrapulmonary TB only (no cough) | <input type="radio"/> Yes | <input type="radio"/> No |
| 4. Unable to give informed consent | <input type="radio"/> Yes | <input type="radio"/> No |

Note: All Exclusion Criteria must be answered NO, to be included in study.

Did this subject meet the eligibility requirements for this study? Yes No

Has this subject given consent to participate in the study? Yes No

Completed by:

Date completed:

Please return to Dr Lessells, Africa Centre for Health and Population Studies

Appendix D Information sheet (English & isiZulu)

TB diagnostic study

Information sheet

Introduction

You are invited to consider participating in a research study about the diagnosis of pulmonary tuberculosis (TB) in adults. This document gives you information about the study that will be discussed with you. Once you understand the study, and if you agree to take part, you will be asked to sign a consent form, or make a mark on the form in front of a witness.

Explanation of what we are trying to do

The aim of this study is to find out whether a new diagnostic test for TB leads to improved outcomes for patients. We particularly want to explore whether there is a difference if the test is carried out at Hlabisa Hospital or at the primary health care clinic.

At the moment, if you have symptoms suggestive of TB, you are asked to cough up a sputum sample and this is sent to the hospital for a test called *sputum smear microscopy*. The main problem with this system is that the test is not very good at detecting TB, particularly in people who have HIV infection. It can also take time for the laboratory to process and the result is not always back at the clinic by the time you return for results. Many people then require further tests (e.g. chest X-ray) and to see a doctor before a TB diagnosis can be made. As a result there is often a significant delay before TB treatment is started, meaning that you could become more unwell and also that you could pass on TB to other people.

The new diagnostic test that will be used in this study has the potential to identify more cases of TB and to identify them more quickly than current diagnostic tests meaning that people will get the correct treatment earlier than at present. It also has the advantage of being able to identify cases of multidrug-resistant TB (MDR-TB) - people with MDR-TB will not get better with standard TB treatment and so they require different treatment. It is not yet known whether these features of the test lead to better outcomes for patients. This is what we plan to examine in this study and information from this study will help to decide how this new test should be used in this area and other similar areas in South Africa.

The study is comparing two different strategies for using the new diagnostic test. It will already have been decided which system will be in place at the clinic based on the week that you attend clinic. For half the participants in the study, sputum samples which you cough up will be sent to Hlabisa Hospital where the test will be done and results returned to the nurse at your clinic. This is similar to what happens at the moment. For the other half, the test will be done at the clinic on the same day (takes about two hours) and the result will allow the nurse to decide on that same day whether you need treatment and which specific treatment you need.

Invitation to participate

The main benefits of this test are expected to be seen in people who are suspected of having pulmonary TB and are either at high risk for having MDR-TB or are HIV-infected. You are being invited to participate in this study because the information you have given suggests that you are in one or other of these categories. The study is expected to enrol a total of 2000 participants over a period of 18 months. Participants will be recruited from KwaMatsane clinic.

What does it mean to be involved in this study?

If you agree to participate in this study, you will be interviewed by a nurse at the time of your clinic visit. The nurse will ask you some questions about your health that will take no longer than 10 minutes and will then collect two sputum samples. If you are unable to cough up sputum then you may be invited to undergo a routine procedure to produce a sample. This procedure uses sterile water or salt water to irritate the airway, increase secretions, promote coughing, and produce a specimen.

If the test is to be done at the hospital then you will be able to go home and will be asked to return to the clinic for the result 2-3 days later. If the test is to be done at the clinic then you will be asked to wait two hours at the clinic and then the result will be used by the nurse to decide that day on your treatment plan. In either group you may be referred for further tests (e.g. chest X-ray) and may be referred to a doctor for further assessment. One of your sputum samples will also be sent to Durban for additional testing (TB culture) as happens currently.

We will also invite you to come for review at the clinic after two months (this will be at the same time as your routine visit if you are on treatment) – this is so that we can assess your health, go over all your laboratory results, confirm that you are on appropriate TB treatment if you need it, and decide whether you need any additional treatment. If you are unable to come to the clinic then with your consent we will contact you by telephone or by home visit.

Will the study use any other information about me?

With your consent, the information from this study will be linked to information held on databases at the Africa Centre. This includes information from the HIV & TB programmes in addition to information from the household and individual health survey (for those people who have taken part in the survey).

Are there any risks to being in the study?

There are no specific risks to your health involved from participating in this study. The only foreseen discomfort is extra time spent at the clinic. If any clinic visits are extra to those expected for your medical care then you will receive travel reimbursement (value R50). All possible steps will be taken to ensure the confidentiality of your medical records.

Are there any benefits to being in the study?

The main benefit to being in the study is that you have access to this new diagnostic test for TB and as a result you will get appropriate treatment faster than at present. At present this test is only available in a small number of locations in South Africa.

What if I do not want to take part?

Taking part in this study is entirely voluntary. Refusal to participate will not in any way affect your care and treatment at the clinic. If you decide to withdraw from the study at any stage, you will not be penalised for doing so. The processes for diagnosis and treatment of TB will then take place as normal and will be co-ordinated by the TB nurse at your clinic.

Who will have access to this information?

All information that is collected will be kept confidential. Only the researchers will have access to any information that identifies you. Results from the study will be given to the Department of Health, the National Health Laboratory Service, and other interested researchers in the form of presentations and

publications in medical journals. In all these cases, the identity of individual participants will not be revealed.

Who can you contact for more information on this study?

If you need more information or there is something you do not understand concerning this study, you can contact the following people:

Dr Richard Lessells, Project Principal Investigator

Africa Centre for Health and Population Studies

035 550 7500 (office) 072 580 3864 (cell phone)

Mduduzi Mahlinza, Head of Community Liaison Office

Africa Centre for Health and Population Studies

035 550 7500 (office)

Who can you contact for complaints/problems?

Biomedical Research Ethics Administration

Address: University of KwaZulu-Natal, Research Office, Westville Campus, Govan Mbeki Building, Private Bag X54001, Durban 4000, KwaZulu-Natal

Telephone: 031 260 4769

Fax: 031 260 4609

email: BREC@ukzn.ac.za

Isingeniso

Uyamenywa ukuba ubambe iqhaza ocwaningweni olumayelana nokuhlonzwa kwesifo sokuvuvukalelwa ngamaphaphu kubantu abadala. Le ncwajana ikumika ulwazi mayelana nocwaningo okuzoxorwa ngalo nawe. Uma sewuluqonda ucwaningo, kanti futhi uma uvuma ukubamba iqhaza, uzocelwa ukuba usayine ifomu lokuvuma, noma ufake uphawu efonini phambi kukufakazi.

Incazelo ngesizama ukukwenza

Inhloso yalolu cwaningo ukuba kutholakale ukuthi ngakube ukuhlonzwa kokuhlolwa okusha kwe-TB kuhlola ekwenzeni ngcono imiphumela ezigulini. Sifuna ukucwaninga ukuthi ngakube ukhona yini umehluko uma ukuhlola kwenziwa esibhedlela sakwaHlabisa noma emitholampilo wendawo.

Okwamanje, uma ngabe unezimpawu eziyizinkomba ze-TB, uyacelwa ukuba ukhiphe izikhwehlela kanti zizothumyelwa esibhedlela ziyohlolwa okubizwa ngokuthi yi – *sputum smear microscopy*. Inkinga enkulu ngalolu hlelo ukuthi ukuhlolwa akukuhle ekuhlonzeni i-TB, ikakhulukazi kubantu abanegciwane lengculazi. Kungathatha isikhathi eside ukuthi ilaboretari ihlole kanti nemiphumela akuvamile ukuthi ibuyiselwe emitholampilo ngesikhathi sokubuya kwakho uzobhaka imiphumela. Abantu abaningi bayaye bese bedinga ukuhi baphinde bahlolewe (isb. Ukuhlolwa kwesifuba nge-X-ray) kanye nokubonana nodokotela ngaphambi kokuhlonzwa kweTB. Ngakho-ke kujwayeleke ukuthi kube nokubambezeleka ngaphambi kokuqalwa kokudla amaphilisi, okuchaza ukuthi ungaqhubeka nokugula kanye nokuthi ungadlulisela i-TB kwabanye.

Uhlelo olusha lokuhlola oluzosetshenziswa kulolu cwaningo kumokwenzeka ukuthi luhlonze ezinye iziqubu ze-TB kanti ukuzihlonza ngokushesha kumalokhu okwenzeka manje kusho ukuthi abantu bazothola ukwelashwa okuyikho ngokushesha kumamanje. Futhi linobuhle bokuhlona ezinye iziqubu zesifo esingazweli emakhambini (multidrug-resistant TB (MDR-TB) – abantu abane-MDR-TB ngeke babe ngcono ngokusebenzisa amaphilisi ajwayelekile e-TB ngakho-ke badinga ukwelashwa okwehlukile. Akukaziwa okwamanje ukuthi lezi zimpawu zokuhlolwa zizohlela emiphumeleni engcono yini ezigulini. Yilokhu esihlela ukukubhaka kulolu cwaningo kanti ulwazi olutholakele kulolu cwaningo luzosiza ekunqumeni ukuthi lokhu kuhlola okusha kungasetshenziswa yini kule ndawo nasezindaweni ezifana nayo eNingizimu Afrika.

Ucwaningo luqhathanisa izindlela ezimbili ezahlukene ekulandeleni ukuhlola okusha. Kuzobe sekunqunywe ukuthi yiluphi uhlelo oluzosetshenziswa emitholampilo oya kuwo. Ingxenywe yababambe iqhaza ocwaningeni, amasampula ezikhwehlela owakhiphile azothumyelwa esibhedlela sakwaHlabisa laphe kuzokwenziwa khona ukuhlola bese imiphumela ibuyiselwa kumhlengikazi emitholampilo wakho. Lokhu kuyefana nalokhu okwenzeka manje. Okunye ukuhlola kuzokwenziwa kwenye ingxenywe emitholampilo ngosuku olufanayo (kuthatha cishe amahora amabili) kanti imiphumela iyovumela umhlengikazi ukuba athathe isinqumo ngalo usuku ukuthi ngakube uyakudinga yini ukwelashwa nokuthi yiluphi uhlobo lokwelashwa oludingayo.

Isimemo sokubamba iqhaza

Inzuzo enkulu yalokhu kuhlola kulindeleke ukuthi kubonakale ebanrwini okusolakala ukuthi banesifo sokuvuvukalelwa ngamaphaphu futhi besebungozini bokuba ne-MDR-TB noma mhlawumbe batheleleke ngegciwane lesandulela ngculazi. Uyamenywa ukuba ubambe iqhaza kulolu cwaningo ngoba ulwazi olumikezile lukhomba ukuthi umgomo wabo abakulolu hlelo. Ucwaningo kulindeleke ukuba lubhalise abantu abayizi – 2000 esikhathini esiyizinyanga eziyi – 18. ababambe iqhaza bazouxenxa emitholampilo waKwaMsane.

Kusho ukuthini ukubamba iqhaza ocwaningweni?

Uma uvuma ukubamba iqhaza kulolu cwaningo, uzokwenziwa inhlolelwazi ngumhlengikazi ngesikhathi sokuhambela kwakho emholampilo. Umhlengikazi uzokubuzisa imibuzo ngenpilo yakho okuyothatha imizuzu engangaphezu kwe-10 futhi oyothatha amasampula amabili esikhwehlela. Uma ungakwazi ukukhipha izikhwehlela kunokwenzeka ukuthi ucelwe ukuba ulandele inqubo ethile yokuba kukhishwe isikhwehlela kuwena. Le nqubo isebenzisa amanzi ahloliwe noma anosawoti ukuze athinte umgudu womoya, ukhuphule okusakutshazi ukuze ukhwehlele bese ukhipha isikhwehlela.

Uma ukuhlola kwenzelwa esibhedlela uyokwazi ukubuyela ekhaya futhi uyocelwa ukuba ubuyele emholampilo ukuba uyolanda imiphumela ezinsukwini ezimbi-2 noma ezi-3. Uma ngabe ukuhlola kuzokwenzelwa emholampilo uyocelwa ukuba ulinde amahora amabili emholampilo kanti umhlengikazi uyobe esekunika imiphumela oyobe esenquma ngalolo suku ngohlelo lokwelashwa kwakho. Uyothumyelwa kunoma liphi iqembu ukuze kwenziwe okanye ukuhlola (isb. Kwesifuba nge-X-ray) kanti futhi ungadluliselwa kudokotela ukuthi enze okanye ukuhlola. Esinye sezikhwehlela zakho siyothumyelwa eThekwini ukuze kuqutshelwe nokuhlola (isimo se-TB) njengoba kwenzeka namanje.

Siyophinde sikumemele futhi emholampilo emva kwezinyanga ezimbili ukuze sibuyekeze (lokhu kuyoqondana nesikhathi oza ngaso emholampilo uma ungolashwayo) – inhloso yalokhu wukuba kuhlolwe isimo sempilo yakho, kubhekiswe yonke imiphumela yokuhlolwa kwakho, kuqinisekise ukuthi welashelwa ngokuyikho i-TB uma ukudinga, bese unquma ukuthi uyakudinga yini okanye ukwelashwa. Uma ungaphumeleli ukufika emholampilo, ngenjenge yakho siyokuthinta ngocingo noma sikuvakashele ekhaya.

Ngabe ucwaningo luzosebenzisa olunye ulwazi ngami?

Ngenjenge yakho, ulwazi olutholakale kulolu cwaningo luyoxhunyaniswa nocwaningo olugcinwe kusigcina-lwazi e-Africa Centre. Lokhu kubandakanya ulwazi olutholakale ezinhlelweni ze-HIV & TB ekwengezeni olwazini olutholakale emakhaya kanye nenhlolelo yezempilo yangayedwana (kulabo Bantu ababambe iqhaza kwinhlolelo).

Ngabe bukhona ubungozi ngokubamba kwami iqhaza ocwaningweni?

Abukho ubungozi empilweni yakho obuthintekayo ekubambeni kwakho iqhaza kulolu cwaningo. Kuphela okubonakalayo kokungaphatheki kahle kungaba yisikhathi esengezelelwe ozosichitha emholampilo. Noma yikuphi ukuhambela kwakho emholampilo okwengezelelwe kulokho okulindelekile kokwelashwa kwakho ngakho-ke uyoxephezela ngezindleko zokuhamba (inani lama – R50). Zonke izinyathelo ezinqala ziyothathwa ukuba kuqinisekise ubumfihlo bemininingwane yokwelashwa kwakho.

Ngabe ikhona inzuzo ngokuba socwaningweni?

Inzuzo enkulu yokuba socwaningweni ukuba nelungelo lokuthola ukuhlolwa i-TB ngendlela entsha okuyokwenza ukuba uthole ukwelashwa okuyikho ngokushesha kunamanje. Okwamanje lokhu kuhlola kutholakala ezindaweni ezimbalwa eNingizimu Afrika.

Kwezakalani uma ngingathandi ukubamba iqhaza?

Ukubamba iqhaza kulolu cwaningo kungokuzikhethela. Ukwenqaba ukubamba iqhaza ngeke kukhinyabeze ukusakekelwa kanye nokwelashwa kwakho emtholampilo. Uma unquma ukuyeka ocwaningweni noma nini, ngeke ujeziswe ngalokho. Izinqubo zokuhlonda kanye nokwelashwa kwe-TB kuyoba sekuqhutshwa ngokujwayelekile kanti kuyokwenganyelwa wumhlengikazi obhekelele i-TB emtholampilo wangakini.

Ubani oyofinyelela kulolu lwazi?

Lonke ulwazi oluqoqiwe luyogcinwa luyimfihlo. Kuyoba ngabacwaningi kuphela abayofinyelela kunoma yiluphi ulwazi oluneminimigwane yakho. Imiphumela yocwaningo iyonikerwa uMnyango WezeMpilo, i-National Health Laboratory Service, kanye nabacwaningi abanothando uma befuna ukwenza izethulo kanye nemibhalo eshicilelwe ezincwadini zezempilo. Kukona konke lokhu, ulwazi ngobamba iqhaza ngeke luvurwe.

Ngubani ongaxhumana naye ngolunye ulwazi kulolu cwaningo?

Uma udinga olunye ulwazi noma kukhona ongakuqondi ngocwaningo ungaxhumana nala bantu abalandelayo:

Dr. Richard Lessells, Project Principal Investigator

Africa Centre for Health and Population Studies

035 550 7500 (ehhovisi) 072 580 3864 (umakhalekhalakwini)

Mkhuzi Mahlirza, Head of Community Liaison Office

Africa Centre for Health and Population Studies

035 550 7500 (ehhovisi)

Who can you contact for complaints/problems?

Ngubani ongaxhumana naye ngezikhalo/ngozinkinga?

i-Biomedical Research Ethics Administration

Ikheli: University of KwaZulu-Natal, Research Office, Westville Campus, Govan Mbeki Building, Private Bag X54001, Durban 4000, KwaZulu-Natal

Ucingo: 031 260 4769

ifeksi: 031 260 4609

i-e-mail: BREC@ukzn.ac.za

Appendix E Consent form (English & isiZulu)

TB diagnostic study

Consent form

I..... agree to be part of the **TB diagnostic study**.

I have been informed about the study and I fully understand the purpose and the procedures of the study. I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.

I understand the implications of joining the study and that I may be asked additional information regarding my health and my treatment during study visits. I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting my medical care.

I understand that the study staff may need to look at my clinic records and that information from this study may be linked to information held about me from the Hlabisa HIV & TB programmes and from the surveillance activities at the Africa Centre.

If I have any further questions or concerns related to this study I understand that I may contact the researcher (Dr Lessells) at the Africa Centre [035 550 7500]

If I have any questions or concerns about my rights as a study participant or if I am concerned about an aspect of the study or the study team then I may contact Biomedical Research Ethics Administration [031 260 4769]

Signature of study participant

Date

Signature of witness

Date

Subject ID

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Mina.....ngiyavuma ukuba yingxenyeyocwanningo lokuhlonzwa kwesifo sofuba. Ngazisiwe ngocwanningo futhi ngiyiqonda ngokugcwele inhloso kanye nenqubo yalo. Nginikiwe ithuba lokubuzaimibuzo ngocwanningo futhi nganelisekile ngzimpendolo.

Ngiyayiqonda imithelela yokungenela ucwanningo nokuthi kungenzeka ngibuzwe imininingwane eyengeziwe ngempilo kanye nokwelashwa kwami ngesikhathi ngifikele ucwanningo. Ngiyaqinisa ukuthi ukubamba kwami iqhaza kungokuzikhethela nokuthi ngingaphuma noma yinini ngaphandle kokuphazamiseka ekwelashweni kwami.

Ngiyaqonda ukuthi umsebenzi wocwanningo kungenzeka adinge ukubheka irekhodi lami lasemtholampilo nokuthi imininingwane yalolu cwanningo kungenzeka ixhunyaniswe naleyo yami esezinhlalweni ze-Hlabisa HIV & TB kanye nalezo ezibhekwe e- Africa Centre.

Uma nginemibuzo noma ukukhathazeka okuqondene nocwanningo ngingaxhumana nomcwanningi (uDkt. Lessells) e-Africa Centre ku-[035 550 7500]

Uma nginemibuzo noma ukukhathazeka okuqondene namalungelo ami njengobamba iqhaza ocwanningweni noma nginokukhathazeka ngokwenzeka ocwanningweni noma ngeqembu locwanningo ngingaxhumana nabe- Biomedical Research Ethics Administration ku-[031 260 4769]

.....
 Isishicilelo sobamba iqhaza

.....
 Usuku

.....
 Isishicilelo sikafakazi

.....
 Usuku

Appendix G Baseline case report form (CRF)

GeneXpert TB study
Baseline visit_v1.0_June2011



Subject ID

K - [] [] [] []

Subject initials

[] []

1. WEIGHT & HEIGHT

a. Weight [] [] [] [] . [] kg

b. Height [] [] [] cm

2. CURRENT SYMPTOMS

a. Which of the following TB symptoms does the patient have currently?

| Symptoms | Yes | No | Duration (weeks) |
|--------------|-----------------------|-----------------------|------------------|
| Cough | <input type="radio"/> | <input type="radio"/> | [] [] |
| Weight loss | <input type="radio"/> | <input type="radio"/> | [] [] |
| Fever | <input type="radio"/> | <input type="radio"/> | [] [] |
| Night sweats | <input type="radio"/> | <input type="radio"/> | [] [] |

b. Has he/she already attended clinic or hospital with these symptoms? Yes No

c. Does the patient have any other symptoms at present? Yes No

d. If yes, which symptoms (tick all that apply)?

- Chest pain Haemoptysis Dyspnoea Lymph node swelling
 Anorexia Diarrhoea Abdominal pain Vomiting
 Headache Back pain Joint pain Other (specify below)

Other symptoms.....

3. CURRENT TB

a. Is the patient currently on treatment for active TB disease? Yes No → SECTION 4

b. Date started [Y] [Y] [Y] [Y] [M] [M] [D] [D]

c. TB type: Smear positive PTB Smear negative PTB EPTB NK

d. TB regimen: 1 (HRZE) 2 (HRZES) NK

Completed by:

Date completed:

GeneXpert TB study
Baseline visit_v1.0_June2011



Subject ID

K -

Subject initials

4. PREVIOUS TB

a. Has the patient previously had TB treatment of any duration? Yes No → SECTION 5

b. List all previous TB episodes (if more than 3 then use continuation sheet):

| Year started | | | | Type | Regimen | Outcome |
|----------------------|----------------------|----------------------|----------------------|---|--|---|
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="radio"/> PTB <input type="radio"/> EPTB <input type="radio"/> NK | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> NK | <input type="radio"/> Cured <input type="radio"/> Completed <input type="radio"/> Failure <input type="radio"/> Defaulted <input type="radio"/> NK |
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="radio"/> PTB <input type="radio"/> EPTB <input type="radio"/> NK | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> NK | <input type="radio"/> Cured <input type="radio"/> Completed <input type="radio"/> Failure <input type="radio"/> Defaulted <input type="radio"/> NK |
| <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="text"/> | <input type="radio"/> PTB <input type="radio"/> EPTB <input type="radio"/> NK | <input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> NK | <input type="radio"/> Cured <input type="radio"/> Completed <input type="radio"/> Failure <input type="radio"/> Defaulted <input type="radio"/> NK |

5. ISONIAZID PREVENTIVE THERAPY

a. Is the patient currently on IPT? Yes No → SECTION 6

b. Date started

6. RISK OF MDR-TB

a. Which of the following criteria are met for this patient?

| | Yes | No |
|---|-----------------------|-----------------------|
| Failure of regimen 1 – smear positive at month 3 or later | <input type="radio"/> | <input type="radio"/> |
| Failure of regimen 2 – smear positive at end of regimen 2 | <input type="radio"/> | <input type="radio"/> |
| Smear non-converter during regimen 1 | <input type="radio"/> | <input type="radio"/> |
| Smear non-converter during regimen 2 | <input type="radio"/> | <input type="radio"/> |
| Household contact of drug-resistant TB case | <input type="radio"/> | <input type="radio"/> |
| Suspect with previous history of TB treatment | <input type="radio"/> | <input type="radio"/> |
| Health care worker | <input type="radio"/> | <input type="radio"/> |
| Prison inmate within last 12 months | <input type="radio"/> | <input type="radio"/> |

Completed by:

Date completed:

Please return to Dr Lessells, Africa Centre for Health and Population Studies

Page 2 of 3

GeneXpert TB study
Baseline visit_v1.0_June2011



Subject ID

K - [] [] [] []

Subject initials

[] []

7. HIV INFECTION

- a. HIV status Positive Negative Never tested Not disclosed
- b. If positive, date of first positive test [Y | Y | Y | Y | M | M | D | D]
- c. If negative, date of last negative test [Y | Y | Y | Y | M | M | D | D]
- d. Is patient currently on antiretroviral therapy? Yes No
- e. Date of ART initiation [Y | Y | Y | Y | M | M | D | D]
- f. Has patient ever taken antiretroviral therapy? Yes – ART Yes – PMTCT only No
- g. Is patient on co-trimoxazole? Yes No
- h. Latest CD4 count [] [] [] [] cells/ μ l ND NK
- i. Latest viral load [] [] [] [] [] [] copies/ml Below LDL ND NK
- j. ART group [] [] []

Completed by:

Date completed:

Please return to Dr Lesells, Africa Centre for Health and Population Studies

Page 3 of 3

Appendix H Follow-up case report form (CRF)

GeneXpert TB study
Follow-up visit_v1.1_Jan 2012

Subject ID

| | | | | |
|---|--|--|--|--|
| K | | | | |
|---|--|--|--|--|

Subject initials

| | |
|--|--|
| | |
|--|--|

Date

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Y | Y | Y | Y | M | M | D | D |
|---|---|---|---|---|---|---|---|

Weight

| | | | | | |
|--|--|--|--|--|----|
| | | | | | kg |
|--|--|--|--|--|----|

1. METHOD OF DATA COLLECTION

a. What is the primary method used to collect this information?

- Patient clinic visit Phone with patient Phone with family/other
 Home visit Other (specify)

2. VITAL STATUS

a. What is the patient's current status?

- Alive → SECTION 3
 Dead → END Date of death

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Y | Y | Y | Y | M | M | D | D |
|---|---|---|---|---|---|---|---|

 NK → END

3. CURRENT TB TREATMENT

a. Is the patient currently on TB treatment?

- Yes No → SECTION 4

b. Has the TB regimen changed since the enrolment visit

- Yes No

c. Date current regimen started

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Y | Y | Y | Y | M | M | D | D |
|---|---|---|---|---|---|---|---|

d. Basis of diagnosis:

- Xpert Smear Culture Chest X-ray
 Other (specify) NK

e. TB type

- Pulmonary Extrapulmonary (specify)..... NK

f. TB regimen:

- 1 (HRZE) 2 (HRZES) MDR XDR

g. How many clinic attendances before TB treatment commenced?
(Not including clinic visit at enrolment)

[] NK

h. How many OPD/cough clinic attendances before TB treatment commenced?

[] NK

i. Has the patient been admitted to hospital since enrolment?

- Yes → If yes, duration of hospital stay (days) [] NK
 No

i. Did the patient have a chest X-ray?

- Yes No

Completed by:

Date completed:

Please return to Dr Lessells, Africa Centre for Health and Population Studies

Page 1 of 2

Subject ID - Subject initials **4. NO CURRENT TB TREATMENT**

a. Have any of the following been done since enrolment visit?

- Chest X-ray Physician assessment OPD/cough clinic attendance
 Hospital admission Other investigations (*specify*)

b. Does the patient still have any of the following symptoms?

- Cough Yes No
 Weight loss Yes No
 Fever Yes No
 Night sweats Yes No

c. Has patient received culture result? Yes Nod. If yes, result Positive Negative Contaminated Not processed/leaking**5. HIV INFECTION**

a. Has HIV test been done since enrolment visit?

- Yes → Result: Positive Negative Not disclosed
 No
 Known positive

b. Has CD4 cell count been done since enrolment visit?

- Yes → Result: cells/μl NK
 No

c. Has patient started antiretroviral therapy since enrolment visit?

- Yes → Date of commencement
 No
 NK

d. Has patient started co-trimoxazole since enrolment visit?

- Yes → Date of commencement
 No
 NK

e. Has patient started isoniazid preventive therapy since enrolment visit?

- Yes → Date of commencement
 No
 NK

Completed by:

Date completed:

Appendix I Ethics approval London School of Hygiene and Tropical Medicine

LONDON SCHOOL OF HYGIENE
& TROPICAL MEDICINE

ETHICS COMMITTEE



APPROVAL FORM

Application number: 5926

Name of Principal Investigator Dr Richard John Lessells

Faculty Infectious and Tropical Diseases

Head of Faculty Professor Simon Croft

Title: 'Impact of a novel molecular TB diagnostic system in patients at high risk of TB mortality in rural South Africa: a pragmatic cluster randomised trial'.

This application is approved by the Committee.

Chair of the Ethics Committee

Date01 April 2011.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.

Appendix J Ethics approval University of KwaZulu-Natal



UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Ncedoile Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604799 • Fax: 27 31 2604809
Email: BRAC@ukzn.ac.za
Website: <http://research.ukzn.ac.za/ResearchEthics/ResearchEthics11415.aspx>

23 June 2011

Dr J Lessells
Africa Centre for Health & Population Studies
Mtubatuba
KwaZulu-Natal
3935

Dear Dr Lessells

PROTOCOL: Impact of a novel molecular TB diagnostic system in patients at high risk of TB mortality in rural South Africa: a pragmatic cluster randomised trial. REF: BF033/11

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was provisionally approved by a quorate meeting of BREC on 08 March 2011 pending appropriate responses to queries raised. Your responses dated 18 April 2011 to queries raised on 23 March 2011 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 23 June 2011.

This approval is valid for one year from 23 June 2011. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at <http://research.ukzn.ac.za/ResearchEthics11415.aspx>.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The following Committee members were present at the meeting that took place on 08 March 2011:

| | |
|------------------------|------------------------------------|
| Professor D Wassenaar | Chair |
| Professor V Rambiritch | Pharmacology |
| Dr Z Khumalo | KZN Health (External) |
| Prof L Puckree | Physiotherapy |
| Professor S Collings | Psychology |
| Dr R Govender | Family Medicine |
| Dr S Paruk | Psychiatry |
| Dr T Hardcastle | Surgery - Trauma |
| Professor D J Pudifin | Medicine |
| Prof R Bhimma | Paediatrics and Child Health |
| Dr U Govind | Private Pract. - Gen. Practitioner |
| Dr MA Sathar | Medicine |
| Mr C Schembri | Legal Advisor |

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely



PROFESSOR D R WASSENAAR
Chair: Biomedical Research Ethics Committee

Appendix K Ethics approval KwaZulu-Natal Department of Health



health

Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
Private Bag x9051
Pietermaritzburg
3200
Tel.: 033 – 3953189
Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM084/11
Enquiries : Mrs G Khumalo
Telephone : 033 – 3953189

15 June 2011

Dear Dr R J Lesselis

Subject: Approval of a Research Proposal

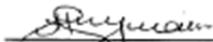
1. The research proposal titled '**Impact of a novel molecular TB diagnostic system in patients at high risk of TB mortality in rural South Africa: a pragmatic cluster randomised trial**' was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby **approved** for research to be undertaken at KwaMsane clinic.

2. You are requested to take note of the following:
 - a. Make the necessary arrangement with the identified facility before commencing with your research project.
 - b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.
3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

For any additional information please contact Mrs G Khumalo on 033-3953189.

Yours Sincerely


Mrs E Snyman

Interim Chairperson, Health Research Committee

KwaZulu-Natal Department of Health

Date: 24/06/2011

Appendix L Study approval Hlabisa Hospital



health

Department:
Health
PROVINCE OF KWAZULU-NATAL

HLABISA HOSPITAL

Physical address: Saunder Street, Hlabisa 3937
Postal address: Private Bag x 5001, Hlabisa 3937
Tel.: 035 838 1003, Fax.: 035 838 1103,
Email.: dawn.zungu@kznhealth.gov.za/
www.kznhealth.gov.za

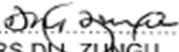
25TH May 2011

RE: IMPACT OF A NOVEL MOLECULAR TB DIAGNOSTIC SYSTEM IN PATIENTS AT RISK OF TB MORTALITY IN RURAL SOUTH AFRICA: A PRAGMATIC CLUSTER RANDOMISED TRIAL (PRINCIPAL INVESTIGATOR: DR RICHARD LESSELS)

Dear Sir/Madam

I hereby grant permission for the above mentioned research study to be conducted in the health facilities in Hlabisa Sub District, specifically at KwaMsane Primary Health Care Clinic and Hlabisa Hospital

Yours sincerely


.....
MRS D.L. ZUNGU
CHIEF EXECUTIVE OFFICER

25 May 2011

**Appendix M Study approval Africa Centre for Health & Population Studies
Community Advisory Board**



26 August 2010

Biomedical Research Ethics Committee
Medical Research Administration
Nelson R Mandela School of Medicine
Private Bag x7
Congella
4013

Dear Sir/Madam

Impact Of A Novel Molecular TB Diagnostic System On Clinical Outcomes Of Multidrug-Resistant TB In Rural South Africa (Principal Investigator: Dr Richard Lessells)

The above study has been granted approval by the Community Advisory Board (CAB) to the Africa Centre for Health and Population Studies.

The background to the study and the study outline was presented by Dr Lessells to the CAB during two separate meetings on 29th July and 26th August. Questions and clarifications about the study were addressed by Dr Lessells. The CAB members considered the benefits and risks of the study to individual participants and to the community as a whole. The CAB then granted the study its unconditional approval.

Yours sincerely


CAB Chairperson

26-08-2010
Date


CAB Secretary

26/08/10
Date

Management Committee Chairperson: T.K. Mkhwanazi
Members: B. Biyase, S. Buthezi, T. Mgenge, T. Ntombela, P. Mkhwanazi, N. Simelane
Postal: PO Box 189, Mbatuba 3935, South Africa Physical: Africa Centre, R518 en route to Hlabisa, Somkhale
Tel: +27 (0)35 550 7500 Fax +27 (0)35 550 7565 E-mail: info@afriacentre.ac.za
Website: www.afriacentre.ac.za

Appendix N Trial registration Current Controlled Trials

ISRCTN18642314 - Impact of a novel molecular tuberculosis...

<http://www.controlled-trials.com/isrctn/pf/18642314>[\[Print \]](#)[\[Close \]](#)**Impact of a novel molecular tuberculosis (TB) diagnostic system in patients at high risk of TB mortality in rural South Africa**

| | |
|-----------------------------------|--|
| ISRCTN | ISRCTN18642314 |
| ClinicalTrials.gov Identifier | |
| Public title | Impact of a novel molecular tuberculosis (TB) diagnostic system in patients at high risk of TB mortality in rural South Africa |
| Scientific title | Impact of a novel molecular tuberculosis (TB) diagnostic system in patients at high risk of TB mortality in rural South Africa: a pragmatic cluster randomised controlled trial |
| Acronym | N/A |
| Serial number at source | Wellcome Trust grant number 090999/Z/09/Z |
| Study hypothesis | Timely initiation of appropriate tuberculosis (TB) treatment will be improved when the diagnostic system is positioned at the primary health care clinic (point-of-care) compared to when it is positioned centrally at the district hospital laboratory |
| Lay summary | Lay summary under review |
| Ethics approval | 1. University of KwaZulu-Natal Biomedical Research Ethics Committee (BF033/11) 2. London School of Hygiene and Tropical Medicine Ethics Committee (5926) Approval pending as of 15/06/2011 |
| Study design | Single-centre cluster randomised controlled trial |
| Countries of recruitment | South Africa |
| Disease/condition /study domain | Pulmonary tuberculosis |
| Participants - inclusion criteria | 1. TB suspect (defined as cough of any duration) 2. Age 18yrs or older 3. Confirmed human immunodeficiency virus (HIV) infection and/or high risk for multi-drug-resistant tuberculosis (MDR-TB) |
| Participants - exclusion criteria | 1. Severely unwell requiring admission to hospital 2. Previous MDR/extensively drug-resistant tuberculosis (XDR-TB) diagnosis or treatment 3. Diagnosis or suspicion of extra-pulmonary TB only 4. Unable to give informed consent |
| Anticipated start date | 04/07/2011 |
| Anticipated end date | 31/12/2012 |
| Status of trial | Ongoing |
| Patient information material | Not available in web format, please use the contact details below to request a patient information sheet |
| Target number of participants | 2000 |
| Interventions | Comparison between two delivery strategies for the GeneXpert system and Xpert MTB/RIF test: Positioning at district hospital vs positioning at primary health care clinic |
| Primary outcome measure(s) | Proportion of TB cases initiated on appropriate TB treatment within 30 days of enrolment |

| | |
|-------------------------------------|--|
| Secondary outcome measure(s) | <ol style="list-style-type: none"> 1. Time to initiation of appropriate TB treatment 2. Time to initiation of appropriate MDR-TB treatment 3. All-cause mortality at 60 days 4. Hospital admissions in first 60 days 5. Time to initiation of antiretroviral therapy (for eligible HIV-infected participants) |
| Sources of funding | Wellcome Trust (UK) (090999/Z/09/Z) |
| Trial website | |
| Publications | |
| Contact name | Dr Richard Lessells |
| Address | Africa Centre for Health and Population Studies PO Box 198 |
| City/town | Mtubatuba |
| Zip/Postcode | 3935 |
| Country | South Africa |
| Tel | +27 (0)35 550 7500 |
| Fax | +27 (0)35 550 7565 |
| Email | riessells@africacentre.ac.za |
| Sponsor | London School of Hygiene and Tropical Medicine (UK) |
| Address | Research Grants & Contracts Office Keppel Street |
| City/town | London |
| Zip/Postcode | WC1E 7HT |
| Country | United Kingdom |
| Tel | +44 (0)20 7927 2626 |
| Fax | +44 (0)20 7580 5636 |
| Email | patricia.henley@lshtm.ac.uk |
| Sponsor website | http://www.lshtm.ac.uk |
| Date applied | 15/06/2011 |
| Last edited | 17/06/2011 |
| Date ISRCTN assigned | 17/06/2011 |

Appendix O Trial registration South African National Clinical Trials Registry

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

| | | | | | |
|-----------------|------|------------|------------------|-------|-----|
| Application ID: | 2568 | DOH Number | DOH-27-0711-3568 | Page: | 1/2 |
|-----------------|------|------------|------------------|-------|-----|

Applicant Details

| | |
|--|---|
| Organisation : | Africa Centre for Health and Population Studies |
| Applicant Type : | Academic Investigator |
| Contact Name : | Dr Richard Lessells |
| Address : | Africa Centre for Health & Population Studies PO Box 198 Mtubatuba 3935 KwaZulu-Natal South Africa |
| Telephone : | 035 550 7500 |
| Fax : | 035 550 7565 |
| E-mail : | rlessells@africacentre.ac.za |
| Responsible Contact person (for public) | Mr Mduduzi Mahlinza |
| Telephone : | 035 550 7686 |
| Research contact person | Dr Richard Lessells |
| Telephone : | 035 550 7657 |

Trial Application Details

| | |
|--------------------------------------|---|
| Issue Date : | 2011/03/14 |
| Sponsors : | London School of Hygiene and Tropical Medicine |
| Primary Sponsor : | London School of Hygiene and Tropical Medicine |
| FundingType : | Grant Funded |
| Research Site Names : | Africa Centre for Health and Population Studies |
| Primary Research Site Name : | Africa Centre for Health and Population Studies |
| Total National Budget for Trial : | R 3,400,000 |
| Protocol / Grant Reference Number | ITCRBC86 |

Study Descriptive Information

| | | |
|-------------------------------|---|--------|
| Brief Title of Study : | Impact of a TB diagnostic system on clinical outcomes | |
| Full Title of Study : | Impact of a novel molecular TB diagnostic system in patients at high risk of TB mortality in rural South Africa: a pragmatic cluster randomised trial | |
| Anticipated Start Date : | 2011/06/01 | |
| Anticipated End Date : | 2013/01/30 | |
| Target Sample Size : | 2000 | |
| Study Phase : | Other | |
| Study Scope : | Single Site | |
| Study Type : | Interventional | |
| Disease Type Heading : | Bacterial and Fungal Diseases | |
| Disease Type Condition : | Tuberculosis | |
| Intervention Name (Generic) : | Automated real-time PCR for M. tuberculosis and rifampicin resistance | |
| Intervention Duration : | No. | Type |
| | 18 | Months |

NHREC

South African Human Research Electronic Application System

TRIAL APPLICATION

| | | | | | |
|-----------------|------|------------|------------------|-------|-----|
| Application ID: | 2568 | DOH Number | DOH-27-0711-3568 | Page: | 2/2 |
|-----------------|------|------------|------------------|-------|-----|

Interventional

| | |
|---------------------|------------|
| Intervention Type : | Device |
| Purpose : | Diagnostic |
| Allocation : | Randomised |
| Masking : | Open |
| Control : | Active |
| Assignment : | Parallel |
| Endpoints : | Efficacy |

Study Descriptive Information

| | |
|--|--|
| Recruitment Status as at Date: | 2011/03/14 |
| Recruitment Status : | Not Yet Recruiting |
| Gender : | Both |
| Ethnicity : | All |
| Age : | From 18 Years To Years |
| Qualifying Disease Condition for Inclusion : | TB suspect (cough, fever, night sweats, or weight loss) |
| Major Exclusion Criteria : | Previous diagnosis of MDR/XDR-TB Severely unwell requiring admission to hospital Suspicion of extrapulmonary TB only Unable to give informed consent |
| Key Primary Outcome : | Proportion of TB cases initiated on appropriate TB treatment within one month of initial clinic visit (TB case defined as anyone with confirmed TB diagnosis, whether from Xpert MTB/RIF test or from sputum culture) |
| Key Secondary Outcomes : | 1. Time to initiation of appropriate TB treatment 2. All-cause mortality at two months 3. Episodes of hospital attendance +/- admission 4. Time to ART initiation (for HIV-infected patients not yet on ART, and eligible for ART) 5. Diagnostic performance of Xpert MTB/RIF (sensitivity and specificity for M. tuberculosis detection and for MDR-TB detection; compared to gold-standard MGIT culture & DST/LPA) |

Committees

| Ethics Committee : | Approval Status | Ethics Number | Ethics Date |
|--------------------------|-----------------|---------------|-------------|
| KwaZulu Natal University | Approved | BF033/11 | 2011/06/23 |

Appendix P Published manuscript in *Journal of Infectious Diseases*: Evaluation of tuberculosis diagnostics: establishing an evidence base around the public health impact

Evaluation of Tuberculosis Diagnostics: Establishing an Evidence Base Around the Public Health Impact

Richard J. Lessells,^{1,2} Graham S. Cooke,^{2,3} Marie-Louise Newell,^{2,4} and Peter Godfrey-Faussett¹

¹Department of Clinical Research, London School of Hygiene and Tropical Medicine, London, United Kingdom; ²Africa Centre for Health and Population Studies, University of KwaZulu-Natal, Mtubatuba, KwaZulu-Natal, South Africa; ³Department of Infectious Diseases, Imperial College, London, and ⁴UCL Institute of Child Health, London, United Kingdom

The limitations of existing tuberculosis diagnostic tools are significantly hampering tuberculosis control efforts, most noticeably in areas with high prevalence of human immunodeficiency virus (HIV) infection and antituberculosis drug resistance. However, renewed global interest in tuberculosis research has begun to bear fruit, with several new diagnostic technologies progressing through the development pipeline. There are significant challenges in building a sound evidence base to inform public health policies because most diagnostic research focuses on the accuracy of individual tests, with often significant limitations in the design, conduct, and reporting of diagnostic accuracy studies. Diagnostic accuracy studies may not be appropriate to guide public health policies, and clinical trials may increasingly be required to determine the incremental value and cost-effectiveness of new tools. The urgent need for new diagnostics should not distract from pursuing rigorous scientific evaluation focused on public health impact.

Global control of the tuberculosis epidemic is a public health priority [1, 2]. The targets for reduction in tuberculosis prevalence and mortality linked to the Millennium Development Goals and enshrined in the STOP TB Global Plan 2006–2015 will not be achieved with current interventions [3, 4]. There is an acute need for improved tuberculosis diagnostics as one critical component of the public health response to the tuberculosis epidemic.

The rapid growth of the human immunodeficiency virus (HIV) epidemic and the emergence of antituberculosis drug resistance have highlighted the major deficiencies in current diagnostic technologies both

for pathogen detection and for diagnosis of drug resistance [5]. In most high-burden countries, sputum smear microscopy remains the principal tool for diagnosing active disease; however, operationally, its sensitivity for pulmonary tuberculosis can be as low as 20% [6, 7]. Sputum culture and drug susceptibility testing are available in certain settings, but their impact is limited by the long duration and complexity of the laboratory processes [8]. Additional challenges are faced in developing diagnostics for extrapulmonary tuberculosis, pediatric tuberculosis, and latent tuberculosis infection [9–11].

The STOP TB Global Plan 2006–2015 included the target that, “by 2010, simple, robust, affordable technologies for use at peripheral levels of the health system will enable rapid, sensitive detection of active tuberculosis at the first point of care” [4, p. 24]. Although this has not been achieved, there have been developments in the tuberculosis diagnostic field, and promising technologies have entered the clinical sphere [6, 12–15]. Most promising has been the Xpert MTB/RIF system, an automated molecular test that simultaneously detects *Mycobacterium tuberculosis* and mutations associated with rifampicin resistance [16, 17]. It is hoped that the

Correspondence: Richard J. Lessells, MBChB, Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT, United Kingdom (richard.lessells@lshtm.ac.uk).

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renewed global focus on tuberculosis will in the next few years lead to the further proliferation of diagnostic technologies in parallel with advances in therapeutics and vaccines.

It is the responsibility of the global scientific community to correctly evaluate these new technologies so that proven effective and cost-effective diagnostics can be adopted, thus generating the greatest public health impact. The importance of diagnostic research in the overall tuberculosis research agenda has been highlighted by many different groups [2, 15, 18–22]. However, huge gaps in funding for tuberculosis research and tuberculosis control remain [1, 2, 23]; this should force us to rethink how diagnostic research can be most effectively targeted and rationalized to inform public health policies.

This article focuses on the framework for evaluation of new diagnostics: at the outset, we look at the potential benefits of new diagnostics, and then we discuss different methodologies to evaluate diagnostic performance with a view to their ultimate implementation. Our focus throughout is on diagnostic tests for detection of active tuberculosis disease and/or drug resistance in high-burden countries.

POTENTIAL IMPACT OF NEW TUBERCULOSIS DIAGNOSTICS

It has been hypothesized that a test more sensitive than sputum microscopy for tuberculosis would be the diagnostic intervention that would alleviate the greatest burden of infectious disease in developing countries [24]. More specifically, one mathematical model of the global tuberculosis epidemic suggested that a new rapid diagnostic test with 100% sensitivity, 100% specificity, and 100% access could prevent 625 000 deaths annually (equivalent to 36% of all tuberculosis-related deaths) [25]. Other models have derived fairly consistent estimates of mortality reductions of 17%–23% from a more sensitive rapid tuberculosis diagnostic, despite exploring different epidemics [26–28]. In one model, the estimated benefit in terms of mortality from a new diagnostic test was equivalent in magnitude to that expected from a novel vaccine or an optimized 2-month treatment regimen for active disease [26]. This highlights 2 important points: (1) no single intervention will have the impact required to meet tuberculosis control targets; thus, scaled-up investment in research and implementation of diagnostics, drugs, and vaccines will be required; and (2) because new diagnostics could have an equivalent impact to new drugs or vaccines, evaluation of diagnostics should be as rigorous as evaluation of drugs and vaccines.

EXISTING FRAMEWORK FOR TUBERCULOSIS DIAGNOSTIC RESEARCH AND DEVELOPMENT

The fact that sputum smear microscopy remains the cornerstone of tuberculosis diagnosis in most high-burden countries is

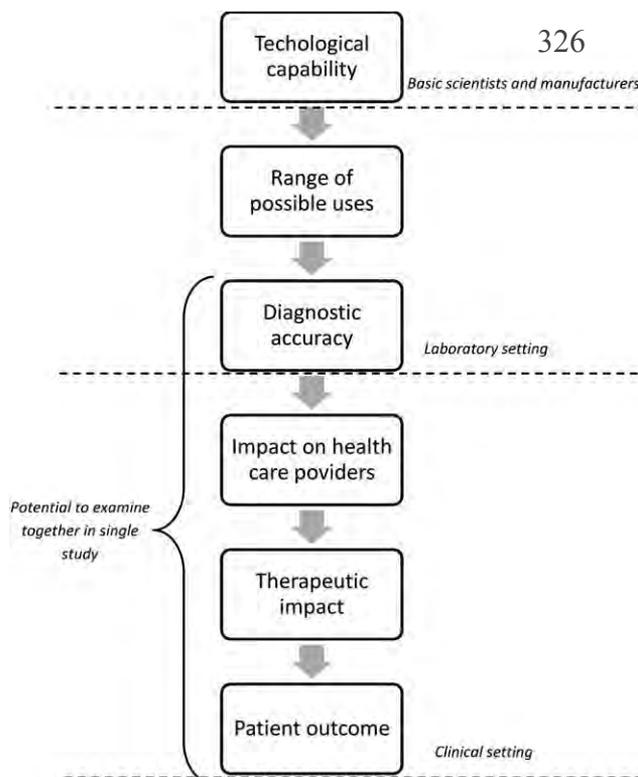


Figure 1. Stepwise approach to evaluation of diagnostic technologies.

testament to the relative paucity of research and development in the diagnostic arena and the failure to translate research findings into policy. In medicine broadly, diagnostic research tends to be performed in stepwise fashion, with basic science leading to laboratory-based performance evaluation and then to clinical studies (Figure 1) [29]. This structure inherently tends to exclude the perspectives of end users in the conception and development of diagnostics, although more recently in the tuberculosis field, organizations have assisted this process by defining the ideal specifications for a point-of-care test [30].

In the tuberculosis field, the process of diagnostic development has rarely gone beyond diagnostic accuracy studies to assess the impact in clinical practice on clinical decision making, patient outcomes, and health system costs [13, 31, 32]. This is in part explained by the fact that the regulatory framework for in vitro diagnostic devices usually does not require evidence beyond performance data. Diagnostic accuracy studies are an important part of the evaluation process. However, there is much potential for bias in such studies, and diagnostic accuracy might vary widely between different clinical settings and populations [33–36].

In the field of diagnostic accuracy research, there have been certain key initiatives aimed at improving and standardizing research methodologies and reporting: the guidelines for diagnostic evaluation produced by the TDR Diagnostics Evaluation Expert Panel (DEEP) [37], the Quality Assessment of Diagnostic

| | |
|----|---|
| 1 | Was the spectrum of patients representative of the patients who will receive the test in practice? |
| 2 | Were selection criteria clearly described? |
| 3 | Is the reference standard likely to correctly classify the target condition? |
| 4 | Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests? |
| 5 | Did the whole sample, or a random selection of the sample, receive verification using a reference standard of diagnosis? |
| 6 | Did patients receive the same reference standard regardless of the index test result? |
| 7 | Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)? |
| 8 | Was the execution of the index test described in sufficient detail to permit replication of the test? |
| 9 | Was the execution of the reference standard described in sufficient detail to permit its replication? |
| 10 | Were the index test results interpreted without knowledge of the results of the reference standard? |
| 11 | Were the reference standard results interpreted without knowledge of the results of the index test? |
| 12 | Were the same clinical data available when test results were interpreted as would be available when the test is used in practice? |
| 13 | Were uninterpretable/intermediate test results reported? |
| 14 | Were withdrawals from the study explained? |

Figure 2. The Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.

Accuracy Studies (QUADAS) tool [38], and the Standards for the Reporting of Diagnostic Accuracy Studies (STARD) initiative [39, 40]. The DEEP guidelines outline best practice in the design and conduct of diagnostic evaluations, with focus on performance characteristics and operational feasibility. QUADAS is a quality assessment tool to be used specifically for the assessment of diagnostic accuracy studies included in systematic reviews. The tool consists of 14 items (Figure 2); the majority involve sources of bias, with a few relating to variability and quality of reporting. The objective of the STARD initiative is to improve the quality of reporting of diagnostic accuracy studies. The 25-item checklist (Figure 3) allows the reader to judge the potential for bias (internal validity) and the generalizability and applicability (external validity) of the study.

A systematic review that used both QUADAS and STARD criteria to assess tuberculosis diagnostic accuracy studies published during 2004–2006 showed significant deficiencies in methodology and reporting of studies [41]. Unfortunately, more widespread use of the STARD system has not been apparent in recent years. As a further example, of the 10 published studies evaluating the diagnostic accuracy of the Genotype MTBDR*plus* assay (published during 2007–2010) [42–51], only one manuscript explicitly mentions STARD [51]. Additional efforts are required by researchers, research funders, journal editors, and policy makers to encourage the use of these tools, with the aim of improving the quality and validity of this element of the evidence base.

THE NEED FOR HIGH-QUALITY EVIDENCE TO INFORM PUBLIC HEALTH POLICIES

Public health policies and guidelines are now usually informed by a systematic approach to judging the relevant evidence. In the tuberculosis field, the World Health Organization (WHO) convenes expert groups to assess the available evidence for a specific

intervention (eg, diagnostic test), and this group then presents their findings to the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) for consideration and endorsement. The system to assess the evidence now adopted by many organizations, including WHO, is the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system, which incorporates judgments on the quality of evidence (high, moderate, low, or very low) and on the strength of any recommendation (initially categorized as strong or weak; now incorporates “conditional,” whereby national programs should consider implementation based on their own situation) [52, 53].

The GRADE system is based around the concept of patient-important outcomes, and as such, evidence from diagnostic interventions creates additional challenges. Studies using indirect outcomes (eg, diagnostic accuracy studies) will usually provide lower-quality evidence because of the uncertainty about outcomes important to patients and the potential for bias [54]. It is important to be clear that the rating of low quality in this context does not necessarily imply that studies were conducted poorly, but that data from the study are not optimal for deriving public health recommendations.

GOING BEYOND DIAGNOSTIC ACCURACY STUDIES—THE NEED FOR IMPACT DATA

In the STOP TB New Diagnostics Working Group blueprint for the evaluation of diagnostics, the next step after diagnostic accuracy studies are demonstration studies, which include patient outcomes (Figure 4) [55]. These demonstration studies are designed to assess the scaled-up test performance and to determine patient-level outcomes. This is the stage of the evaluation process that should start to inform policy. It is stated in this document that patient-important outcomes should be assessed (eg, time to initiation of treatment, time to smear and/or culture conversion, and treatment outcome) and

| Section | Item | |
|--------------------------------|------|---|
| TITLE/ABSTRACT/KEYWORDS | 1 | Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity') |
| INTRODUCTION | 2 | State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups |
| METHODS | | |
| <i>Participants</i> | 3 | The study population: inclusion and exclusion criteria, setting and locations where the data were collected |
| | 4 | Participant recruitment: was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard? |
| | 5 | Participant sampling: was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected |
| | 6 | Data collection: was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)? |
| <i>Test methods</i> | 7 | The reference standard and its rationale |
| | 8 | Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard |
| | 9 | Definition of and rationale for the units, cut-offs, and/or categories of the results of the index tests and the reference standard |
| | 10 | The number, training, and expertise of the persons executing and reading the index tests and the reference standard |
| | 11 | Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers |
| <i>Statistical methods</i> | 12 | Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals) |
| | 13 | Methods for calculating test reproducibility, if done |
| RESULTS | | |
| <i>Participants</i> | 14 | When study was done, including beginning and ending dates of recruitment |
| | 15 | Clinical and demographic characteristics of the study population (e.g. age, sex, spectrum of presenting symptoms, comorbidity, current treatment, recruitment centers) |
| | 16 | The number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended) |
| <i>Test results</i> | 17 | Time interval from the index tests to the reference standard, and any treatment administered between |
| | 18 | Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition |
| | 19 | A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the results by the results of the reference standard |
| | 20 | Any adverse events from performing the index tests or the reference standard |
| <i>Estimates</i> | 21 | Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals) |
| | 22 | How indeterminate results, missing responses, and outliers of the index tests were handled |
| | 23 | Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done |
| | 24 | Estimates of test reproducibility, if done |
| DISCUSSION | 25 | Discuss the clinical applicability of the study findings |

Figure 3. Standards for the Reporting of Diagnostic Accuracy Studies (STARD) checklist.

that “these impact-related data should be compared to historical data recorded prior to implementation of the new test in routine clinical practice” [55, p. 62]. This use of historical data

is problematic as a method of assessing any health care intervention and would not generally be accepted by regulatory bodies in the field of drugs or vaccines [56]. It is difficult to be

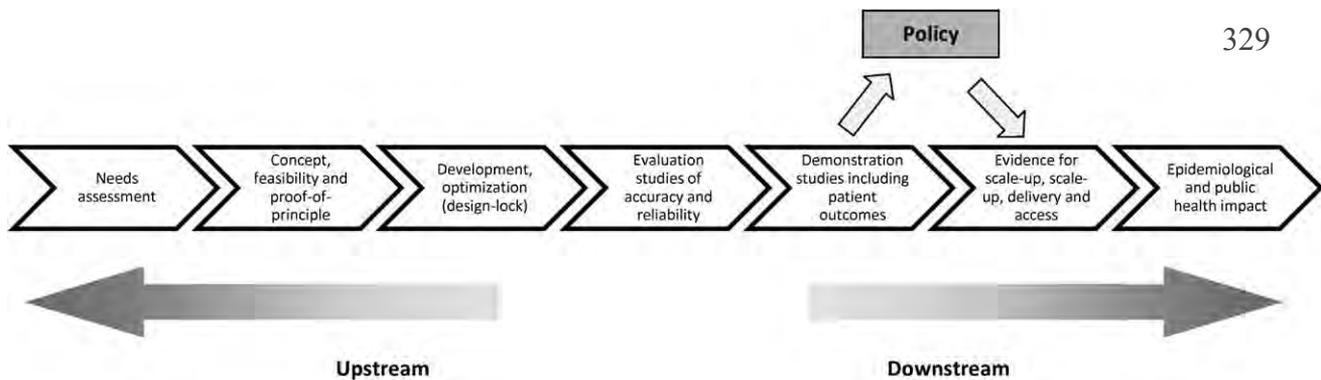


Figure 4. The pathway for evaluation of new diagnostics (from the STOP TB New Diagnostic Working Group).

sure that any comparison is fair; there are potential sources of bias, and consequently, the risk is that the value of the intervention can be exaggerated.

Two organizations that have been instrumental in driving forward development and evaluation of diagnostic technologies for tuberculosis are the Foundation for Innovative New Diagnostics and the WHO TDR program (Special Programme for Research and Training in Tropical Diseases). Demonstration studies are key elements of their tuberculosis projects, which aim to determine the feasibility, impact, and cost-effectiveness of the diagnostic test under evaluation. The evidence from these studies is a key element assessed by the expert groups and reported to STAG-TB. If we take the example of the Genotype MTBDR*plus* assay, preliminary data regarding patient-important outcomes from the South African demonstration projects seemed relatively disappointing because the median turnaround times did not meet their predefined objective of 7 days; of the patients with multidrug-resistant tuberculosis who were identified, only 28% were started on appropriate therapy on the basis of the test result (42% had therapy delayed until results of conventional drug susceptibility testing were available) [57]. Although these results were based only on preliminary data analysis and are understandable during implementation of a new technology, there has, to our knowledge, been no further published evidence from high-burden settings on patient-important outcomes. However, the test has been introduced into routine practice in some countries, and its use is now being scaled up [58].

It is generally considered that the optimal methodology for assessing the clinical impact of any intervention, including diagnostics, is the randomized controlled trial (RCT) [59–61]. This is the methodology least prone to bias in estimating the benefits and risks of any intervention. Data from RCTs can additionally be used to perform economic evaluation, a step of major importance for policy makers. The relative shortage of RCTs in diagnostic research, in contrast to therapeutic and vaccine research, is likely to be explained by a combination of factors: lack of emphasis on this level of evidence by manufacturers and regulatory authorities, limited funding and poor

coordination of diagnostic research, and logistical and ethical challenges. There are features specific to diagnostic trials that complicate trial design and implementation. In a tuberculosis diagnostic study, the population of interest might be persons with suspected pulmonary tuberculosis (eg, individuals with cough). Inevitably, the majority of participants will not have tuberculosis; thus, the potential effect size on the total cohort resulting from improved diagnosis is relatively small. However, we have to include the entire cohort in a trial if we want to capture comprehensive outcome data (to balance benefits and harms).

To reveal the value of well-designed RCTs in diagnostic research, it is worthwhile to stop studying tuberculosis and consider malaria, another global health priority. Malaria rapid diagnostic tests (RDTs) have been shown to have good diagnostic accuracy [62], and mathematical models have suggested that implementation of RDTs could lead to significant public health benefits in settings where malaria is endemic [63]. Trials were designed to assess the performance of the tests in a field setting and to measure the impact on health care providers, therapeutic decisions, and patient outcomes [64–67]. Three of these trials showed that, despite good diagnostic accuracy, there was no reduction in incorrect antimalarial treatment with the use of RDTs [64–66]; of more concern, one trial even showed a significant reduction in correct antimalarial treatment [66]. These trials have provided vital information for the further development and implementation of RDTs. The results of these trials highlight the fact that a diagnostic test is only ever a vehicle to guide therapies; it is never of therapeutic benefit, and it is the treatment decision that will impact on patient outcomes.

CONCEPTUALIZING CLINICAL TRIALS OF TUBERCULOSIS DIAGNOSTICS

The first step in any trial is to determine the hypothesis that is to be tested because this will inform the trial design. It is important to consider the likely position of the new test in the diagnostic

process. In the case of a test for active pulmonary tuberculosis, we need to decide how the test will be introduced in the existing diagnostic structure, which includes sputum microscopy, sputum culture, drug-susceptibility testing, and chest radiography. It could be proposed as a replacement for ≥ 1 of these tests, as an addition to these tests, or as a means of triage, for example, to target sputum culture and/or drug-susceptibility testing. This decision is in turn likely to depend on the proposed benefits of the new test (eg, whether it is more rapid, more sensitive, more specific, less technical, safer, or less expensive). Furthermore, we need to consider the outcomes of interest, whether related to benefit or harm; these may be appropriate or inappropriate commencement of tuberculosis treatment, outcomes during treatment (smear or culture conversion), final treatment outcomes (cure or completion), and mortality.

One possible reason to explain the lack of RCTs in diagnostic research is the perception that diagnostic tests carry minimal or no risk. Although the test is unlikely to harm the patient, the consequences of the test (eg, the therapeutic decision) may confer harm, as shown in the example of RDTs of malaria. What risks might we expect in a trial of a tuberculosis diagnostic? Consider a hypothetical trial comparing clinical outcomes between a rapid molecular tuberculosis test and the standard-of-care diagnostic pathway (Figure 5). At a basic level, this trial will tell us whether the benefits from earlier correct diagnosis or exclusion of tuberculosis outweigh the risks from incorrect classification of disease (false-negative or false-positive results). The benefits would seem to be self-evident but need to be quantified. The risks are more complicated and will be context specific. False-negative diagnoses will result in appropriate treatment being withheld, with potential for poorer outcomes. False-positive diagnoses also carry risk, however, because alternative diagnoses may not be considered and, therefore, not treated, and patients may be exposed to potentially toxic therapy. For diagnosis of drug resistance, the risks from incorrect classification are even more complicated. False-negative results of genotypic testing may lead to inappropriate treatment with first-line regimens, with consequent adverse outcomes, including amplification of drug resistance. False-positive results may lead to inappropriate treatment with multidrug-resistant tuberculosis regimens, with lower efficacy against sensitive strains and with risks of severe toxicity.

These examples highlight another challenge with tuberculosis diagnostic research (and common to much diagnostic research), which is the lack of a perfect gold standard with which to compare new tests. If our new test is potentially more sensitive than the existing test (as might be the case with molecular tests, compared with sputum culture), this will affect any analysis. The lack of a gold standard often requires a construct gold standard that comprises information from the reference test with additional clinical information and follow-up information [68]. Of further concern, discrepancies between

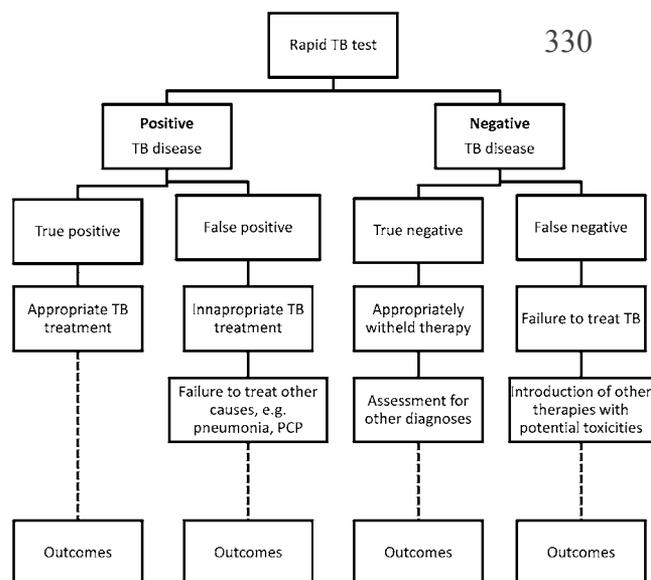


Figure 5. Potential impact of false-positive and false-negative tuberculosis diagnoses in a hypothetical trial comparing a rapid molecular test to tuberculosis culture.

phenotypic and genotypic drug-susceptibility results can be extremely difficult to interpret, and it is not always clear which is the more reliable measure of drug resistance [69]. In many ways, these issues reinforce the need for well-designed clinical trials because thorough interpretation of the tests may only be possible with meticulously collected baseline and follow-up clinical data.

PRACTICAL TRIAL DESIGNS

If the outcomes of interest are individual-level outcomes (eg, treatment initiation and mortality), a clinical trial with individual randomization would be the logical and statistically most efficient design. However, because there will be information regarding the diagnostic performance from the laboratory-based evaluation, the question arises, if the test is shown to have comparable accuracy to an existing test but has other advantages (ie, more rapid and/or less invasive), is it ethical to conduct an RCT with individual randomization? Critical to this decision is whether there is equipoise regarding the clinical outcome. Equipoise with regard to clinical outcomes of a diagnostic strategy arises, for example, when the consequences of misdiagnosis are severe (eg, HIV-infected patients who receive a misdiagnosis of tuberculosis who are dying of another HIV-related illness) or when failure to diagnose does not lead to mistreatment or poorer outcomes (eg, patients prescribed tuberculosis treatment regardless of the test result).

Individual randomization may, however, present considerable logistical challenges in certain health care settings, and for this reason, cluster randomized designs may be considered with

health care units (eg, hospitals, clinics, and mobile teams) as clusters. Cluster randomized designs are increasingly used in public health research. The principal reasons for considering such a design are as follows: if the intervention is to be delivered to groups rather than individuals, if the outcome is to be measured at a population level, or to avoid contamination by individuals in the same community who are randomized to different trial arms [70]. However, there is also an acceptance that cluster randomization may also be appropriate in settings where it offers greater logistical convenience, compared with an individually randomized trial, although cluster RCTs generally require larger sample sizes and have added challenges in design, analysis, and ethics [70–72].

A further modification of the cluster randomized design is the phased implementation or stepped-wedge design [70, 73]. The key features of this design are that all clusters receive the intervention by the end of the trial, and the order in which the clusters receive the intervention is decided at random. This is particularly appropriate when there is preexisting evidence that the intervention may have a beneficial effect and when assigning clusters to the control arm for the duration of the trial might be ethically unacceptable. This might be particularly suited to evaluation of certain diagnostic technologies, for which there is evidence from initial diagnostic accuracy studies that suggests beneficial effect.

If randomization is not deemed to be appropriate or feasible, alternative prospective trial designs, often termed quasi-experimental designs, may still be able to generate evidence on the effectiveness of diagnostics [74]. An example would be the pre- and postimplementation study in which outcomes are measured during a pre-intervention phase and subsequently during a postintervention phase. Although the lack of randomization threatens the internal validity (no firm conclusion can be made with regard to the effect of the intervention unless the effect size is large), there may conversely be a gain in external validity (improved generalizability of findings if fewer patients are excluded than in conventional RCTs).

Retrospective studies may be the only methodology to obtain outcome data in circumstances in which a diagnostic is widely implemented on the basis of performance characteristics. Such pre- and postimplementation analyses have been used in high-resource settings to estimate the impact of molecular resistance testing on detection and treatment of multidrug-resistant tuberculosis [75, 76].

Whether a clinical trial is justified in the evaluation of diagnostics will ultimately depend on the balance between the benefit to be gained by accurately establishing the impact of a new tool and the costs of running a large clinical trial and potentially delaying full-scale implementation of an effective intervention. These decisions are not straightforward, and collaboration between scientists and policy makers is vital to determine when diagnostic trials are necessary.

CONCLUSIONS

Recent developments in tuberculosis diagnostics have led to much optimism, but we still lack the tools that meet the needs of patients in high-burden countries. The next 10–20 years will hopefully see further developments in diagnostic technology. We need to ensure that the framework for evaluating diagnostic tools is best suited to ensuring that the tools with the greatest public health impact and cost-effectiveness are implemented and that those with minimal impact are developed further or are discarded. Diagnostic accuracy studies are an important early step in the evaluation process but do not produce sufficient evidence to inform public health policies. Well-designed prospective studies (including RCTs) should be integrated in the research pathway to provide reliable information on therapeutic impact, patient outcomes, and cost-effectiveness. This new era of tuberculosis diagnostics should be accompanied by a new era for diagnostic research focused clearly on the evaluation of public health impact.

Notes

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Appendix Q Published manuscript in *Trials*: 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

STUDY PROTOCOL

Open Access

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

Richard J Lessells^{1,2*}, Graham S Cooke^{2,3}, Nuala McGrath^{2,4}, Mark P Nicol⁵, Marie-Louise Newell^{2,6} and Peter Godfrey-Faussett¹

Abstract

Background: Tuberculosis control in sub-Saharan Africa has long been hampered by poor diagnostics and weak health systems. New molecular diagnostics, such as the Xpert® MTB/RIF assay, have the potential to improve patient outcomes. We present a cluster randomised trial designed to evaluate whether the positioning of this diagnostic system within the health system has an impact on important patient-level outcomes.

Methods/Design: This pragmatic cluster randomised clinical trial compared two positioning strategies for the Xpert MTB/RIF system: centralised laboratory versus primary health care clinic. The cluster (unit of randomisation) is a 2-week time block at the trial clinic. Adult pulmonary tuberculosis suspects with confirmed human immunodeficiency virus infection and/or at high risk of multidrug-resistant tuberculosis are enrolled from the primary health care clinic. The primary outcome measure is the proportion of culture-confirmed pulmonary tuberculosis cases initiated on appropriate treatment within 30 days of initial clinic visit. Univariate logistic regression will be performed as the primary analysis using generalised estimating equations with a binomial distribution function and a logit link.

Conclusion: Diagnostic research tends to focus only on performance of diagnostic tests rather than on patient-important outcomes. This trial has been designed to improve the quality of evidence around diagnostic strategies and to inform the scale-up of new tuberculosis diagnostics within public health systems in high-burden settings.

Trial registration: Current Controlled Trials ISRCTN18642314; South African National Clinical Trials Registry DOH-27-0711-3568.

Keywords: Tuberculosis, Multidrug-resistant tuberculosis, HIV, Molecular diagnostics, Point-of-care systems, Clinical trial

Background

Control of the tuberculosis (TB) epidemic in sub-Saharan Africa is a major public health challenge [1,2]. The epidemic has been exacerbated by the co-existent explosive human immunodeficiency virus (HIV) epidemic and the emergence of drug-resistant *Mycobacterium tuberculosis* strains leading to high mortality rates

[2,3]. Enshrined in Millennium Development Goal 6 and the Stop TB Partnership Global Plan 2006–2015 are the targets to reduce TB prevalence and TB mortality rates by 50% (compared to 1990) by 2015 and to eliminate TB as a public health problem by 2050 [4,5]. At current rates of progress these targets will not be achieved in sub-Saharan Africa. New interventions and improved strategies for delivery of interventions are urgently required.

TB control at present relies primarily on the diagnosis and treatment of individuals with active TB disease. Early case detection and initiation of appropriate antituberculous

* Correspondence: richard.lessells@shttm.ac.uk

¹Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

²Africa Centre for Health and Population Studies, University of KwaZulu-Natal, Mtubatuba, South Africa

Full list of author information is available at the end of the article

therapy is necessary not only to reduce mortality but also to interrupt transmission. TB microscopy (still the most common diagnostic method in use worldwide) is poorly equipped to control the current TB epidemic in sub-Saharan Africa given its poor sensitivity, particularly in HIV co-infection, and inability to detect drug resistance [6]. Additionally, the placement of diagnostics in centralised facilities distant from where patients seek care contributes to significant delays [7,8] and default [9-13] before initiation of treatment. The impact of this is illustrated most starkly in multidrug-resistant TB (MDR-TB), where delays in culture and drug susceptibility testing (DST) techniques mean that 50% of patients have died by the time their culture/DST result is available [14,15].

The development of novel molecular tools, in particular the Xpert[®] MTB/RIF assay, offers new opportunities to tackle these problems. This is a fully automated, closed cartridge diagnostic system that utilises hemi-nested polymerase chain reaction (PCR) and molecular beacon technology to detect the presence of *Mycobacterium tuberculosis* and rifampicin-resistant mutations directly from clinical samples in less than 2 h [16-18]. The World Health Organization (WHO) recommended the system be implemented in high-burden settings on the basis of initial data from validation and demonstration studies [19-21]. Many countries are now moving ahead with implementation and there is a need for research to address key questions in the early phase of implementation so as to inform future scale-up [21]. One critical question relates to the optimal positioning of the diagnostic system within different health systems, and this is the focus of the research study.

The primary objective is to test the hypothesis that timely initiation of appropriate TB treatment when the diagnostic system is positioned at the primary health care clinic (point of care) is different from when the diagnostic system is positioned centrally at the district hospital laboratory. Secondary objectives are:

- To evaluate the impact of Xpert MTB/RIF positioning on additional clinical outcomes (mortality, hospital admission, time to initiation of antiretroviral therapy)
- To explore the cost-effectiveness of Xpert MTB/RIF implementation at primary health care clinic level
- To compare the operational feasibility of Xpert MTB/RIF placement at the primary health care clinic level and district hospital laboratory level.

Methods/Design

Setting

The trial is being conducted in Hlabisa health sub-district, uMkhanyakude district, northern KwaZulu-

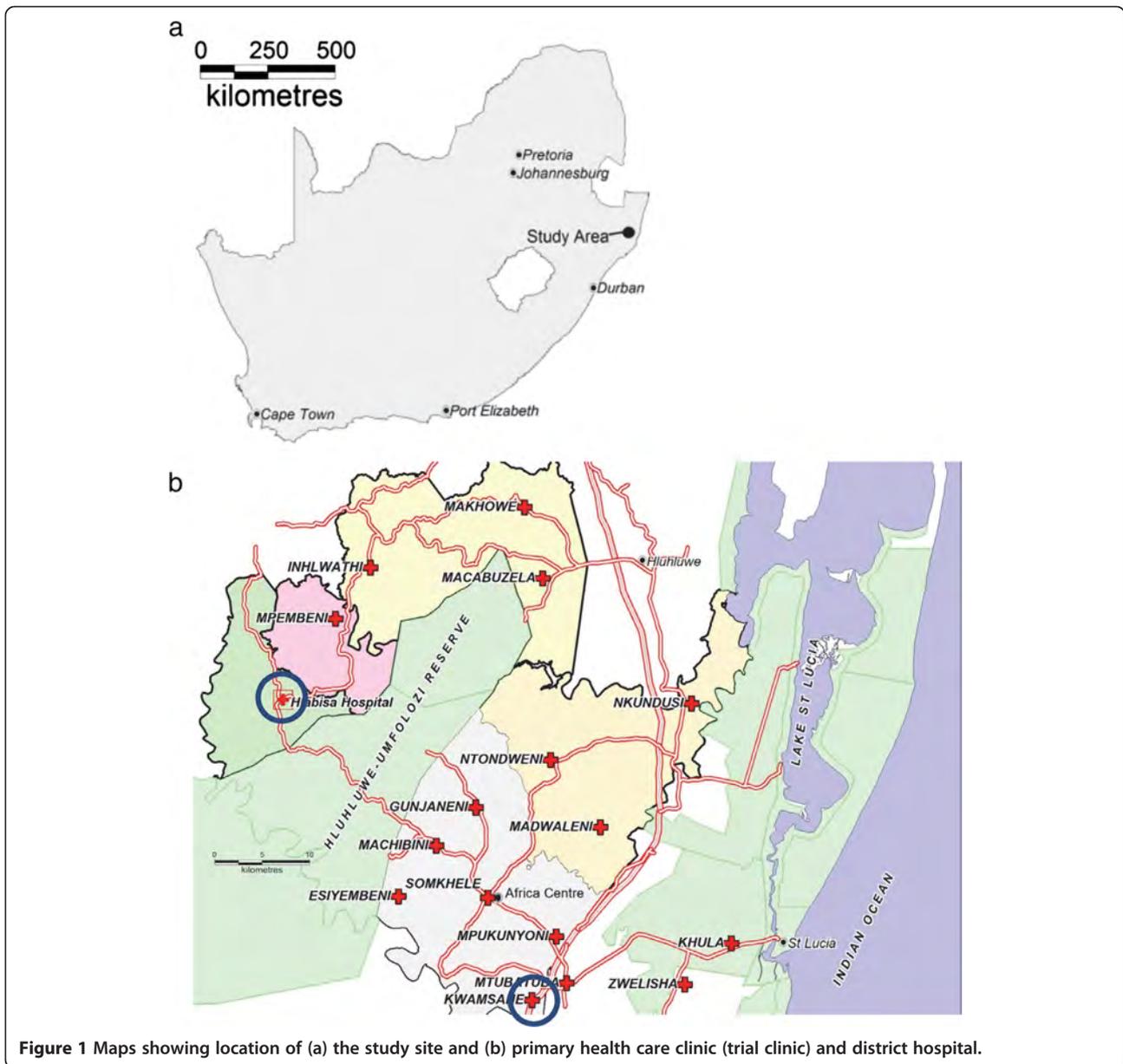
Natal, South Africa (Figure 1). This area has an extremely high dual burden of TB and HIV: the TB notification rate for the sub-district in 2010 was 1,130/100,000; HIV seroprevalence in the adult population (≥ 15 years) within the Africa Centre surveillance area was 24.1% in 2010; in 2008, 76% of TB cases were associated with HIV infection [22]. In the years 2000–2006 HIV and TB accounted for 71.5% of deaths in young adults (25–49 years) in the Africa Centre surveillance area [23]. HIV and TB treatment and care are delivered at 17 primary health care (PHC) clinics through decentralised collaborative programmes. Participants are recruited from the largest PHC clinic that is situated within a small urban township in the south of the sub-district, approximately 60 km by road from the district hospital.

Study design

The study is a pragmatic cluster randomised clinical trial comparing two positioning strategies for the Xpert MTB/RIF system: positioning at centralised laboratory level (district hospital laboratory) versus positioning at primary health care clinic level (point of care). The cluster (unit of randomisation) is a 2-week time block at the primary health care clinic (clinic blocks), and clusters are randomly assigned to the district hospital laboratory strategy or point-of-care strategy. The trial schema is shown in Figure 2.

Participants

Adult (≥ 18 years) pulmonary TB suspects with confirmed HIV infection and/or at high risk of MDR-TB are included after giving informed consent. These criteria were defined because of the high risk for mortality in these groups and prioritisation for Xpert MTB/RIF testing, in line with the WHO recommendations [20]. A TB suspect is defined for the purposes of the trial as an individual with a current cough (of any duration) with or without other symptoms. High risk of MDR-TB is defined according to national and international guidelines and incorporates the following categories: failure of the standard treatment regimen (2HRZE/4HR), failure of the re-treatment regimen (2HRZES/1HRZE/5HRE), acid-fast bacilli (AFB) smear non-conversion at month 2 or 3 of the standard or re-treatment regimen, relapse or return after default, any other previous TB (4 or more weeks of TB treatment), household contact with a known MDR-TB case, prison inmate within the last 12 months and health care worker [24,25]. Participants are excluded if they report a previous diagnosis of MDR-TB or extensively drug-resistant TB (XDR-TB), are severely unwell requiring admission to hospital, or are unable to give informed consent. Participants are recruited between the times of 0800 and 1630, Monday to Friday.



Interventions

All participants provide two spontaneously expectorated sputum specimens on the day of enrolment (spot specimens). The first sputum specimen is submitted for Xpert MTB/RIF testing. The second specimen is submitted for Mycobacterial Growth Indicator Tube (MGIT) culture, line probe assay (LPA) ± phenotypic drug susceptibility testing (DST). In both strategies, the specimen for culture/LPA/DST is transported daily (in the afternoon) to the National Health Laboratory Service (NHLS) laboratory at the district hospital and then onwards to the provincial NHLS referral laboratory. The results of this are used to define TB cases and to define the primary outcome measure.

District hospital laboratory strategy

Sputum specimens are transported on a daily basis to the National Health Laboratory Service (NHLS) laboratory at the district hospital using the routine sample transport system. Xpert MTB/RIF testing is performed by a trained laboratory technician at the earliest convenience (within 24 h of the specimen being received in the laboratory) and printed results are returned to the clinic using the same routine transport system. Under this strategy, participants are requested to return for results after 72 h.

Point-of-care (POC) strategy

The diagnostic system is located at the primary health care in a dedicated room close to the TB clinic (Figure 3).

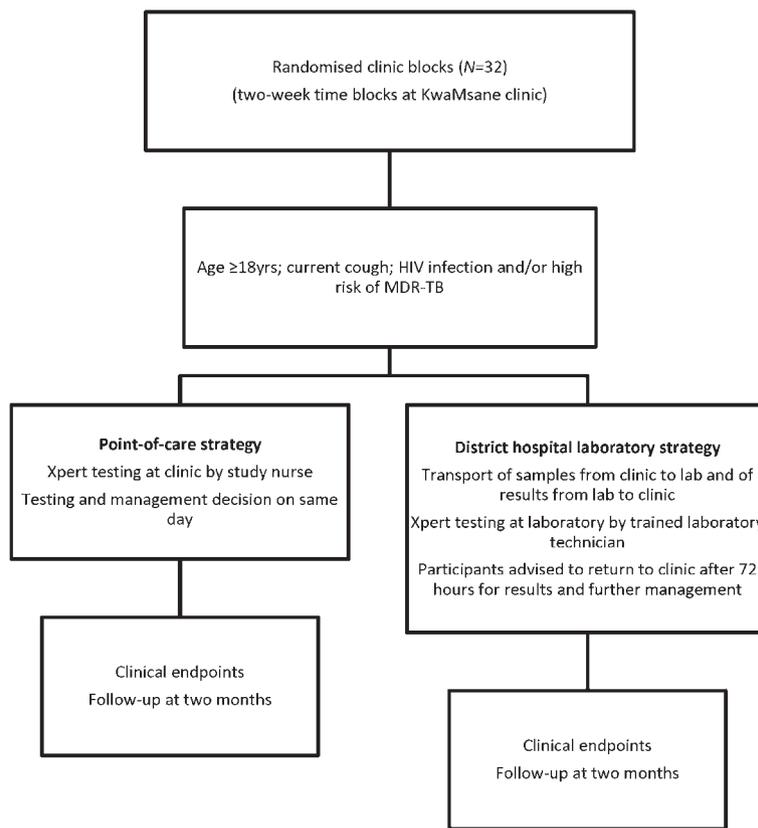


Figure 2 Trial schema.



Figure 3 Professional nurse operating the Xpert MTB/RIF system at the primary health care clinic.

Xpert MTB/RIF testing is performed immediately by the study nurse, on the same day where possible. Participants are invited to wait for the result (approximately 2 h) or, if they are unable to wait or it is towards the end of the working day, they are advised to return the following day.

Outcome measures

The observational unit for all analyses is the individual participant. The primary outcome for the study is the proportion of culture-confirmed pulmonary TB cases initiated on appropriate TB treatment within 30 days of initial clinic visit (appropriate treatment defined according to results of LPA \pm phenotypic DST on the culture isolate).

Secondary outcomes at an individual level are the following, with all time-to-event analyses using the initial clinic visit as time zero:

- All-cause mortality in TB suspects and MDR-TB suspects at 60 days
- Time to initiation of appropriate TB treatment (days) for culture-confirmed pulmonary TB cases
- Time to initiation of MDR-TB treatment (for MDR-TB cases confirmed by culture/LPA/DST)
- Proportion of TB suspects and MDR-TB suspects with at least one hospital attendance within 60 days
- Time to initiation of antiretroviral therapy (ART) for HIV-infected TB suspects and MDR-TB suspects not yet receiving but eligible for ART
- Sensitivity and specificity of Xpert MTB/RIF
 - for *M. tuberculosis* detection (compared to reference standard of single MGIT culture)
 - for detection of rifampicin resistance (compared to reference standard of phenotypic DST \pm LPA)

Sample size

The study was designed to detect an increase from 85% to 95% in the proportion of culture-confirmed pulmonary TB cases initiated on appropriate treatment within 30 days. Sample size was calculated with the equation of Hayes and Bennett, using the coefficient of variation (κ) [26]. With $\kappa = 0.05$ and a cluster size of 12 culture-positive cases, we would need 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 at 60 days, so we would need 208 culture-positive TB cases in each arm. Based on the assumption that 25% of TB suspects would have a positive MGIT culture, we would require enrolment of 1,664 TB suspects. The total sample size will therefore be 32 clusters and 1,664 individual participants.

The coefficient of variation (κ) is small, but as the clusters are clinic time blocks rather than geographic areas or

health care facilities, minimal variation is expected between clusters. This value of κ corresponds to a range of proportions appropriately treated within 30 days in the district hospital laboratory arm of 77-94%.

For the secondary endpoint of all-cause mortality within 60 days, the analysis will incorporate all participants (all suspects), regardless of presence or absence of TB disease. The sample size of 32 clusters and 60 participants per cluster gives approximately 80% power to detect a 33% reduction in mortality from 12% in the district hospital laboratory arm to 8% in the point-of-care arm, with 95% confidence.

Randomisation

The allocation schedule for random assignment of 2-week time blocks was computer generated, using random permuted blocks of eight. Allocation for each clinic block was placed into sealed envelopes by the statistician; the principal investigator opens the envelope on the Friday before the start of a new 2-week block and communicates the allocation for the next 2 weeks to study staff.

Implementation

Health care workers at the primary health care clinic identify potential participants. All individuals reporting cough are referred to the study nurse. Eligibility criteria are checked by the nurse, and subjects meeting the inclusion criteria are provided spoken and written information about the study in isiZulu and/or English; those willing to participate are taken through the informed consent process and are asked to provide a signature or thumbprint on the consent form.

A baseline assessment is performed by the study nurse. Demographic information, current symptoms, previous TB history, risk factors for drug resistance, HIV status, and history of ART use are documented on a case report form.

With both strategies, clinical decisions are made by the study nurse on the basis of the Xpert MTB/RIF result and according to pre-defined algorithms. TB patients without resistance to rifampicin are commenced on standard anti-TB therapy (4HRZE/2HR) by the study nurse. All patients with rifampicin-resistant TB are reported to the trial physician on the same day and are subsequently referred to the specialist drug-resistant TB treatment centre in Durban. Management of suspects with a negative Xpert MTB/RIF follows existing protocols for smear-negative TB suspects: oral antibiotics are prescribed and patients are advised to return if symptoms do not improve after 14 days. Patients who remain symptomatic following this course of antibiotics can be referred to the district hospital for chest X-ray and physician review. Results from MGIT culture and DST are

returned through the routine laboratory system and are used to guide clinical management where appropriate.

Outcome evaluation

To ascertain the primary and secondary outcomes, at enrolment all participants are allocated a review date 2 months from the enrolment visit. Participants are invited to attend clinic for review but are also invited to consent to telephonic follow-up and/or home visit in case clinic visit is not possible. Additional contact details are provided for at least one other family member (or other person designated by participant) at enrolment, wherever possible. Participants are told that, when attending the clinic for the follow-up visit, they will be reimbursed with a ZAR 50 grocery voucher (approximately equivalent to USD 6). Outcome data pertaining to TB treatment initiation, additional investigations, hospital attendances and admissions, and ART initiation (where appropriate) are collected on a case report form by the study nurse. In the event that no contact is made with patient or with named contact persons, follow-up information is collected from the clinic TB registers and the operational HIV programme database – permission to use these data is also included in the informed consent process.

Statistical analysis

Analysis of baseline characteristics will be performed to characterise the study population and to identify baseline imbalances between the study arms in order to decide whether any covariates need to be adjusted for in the final analyses. The baseline data will include: age, sex, body mass index (BMI), history of previous TB, HIV infection status, CD4+ cell count, and use of antiretroviral therapy and isoniazid preventive therapy.

All final analyses will be intention-to-treat analyses performed at the individual level taking account of within-cluster correlation. The definition of TB cases for the primary outcome will be based on MGIT culture positivity. The proportion of TB cases initiated on appropriate TB treatment within 30 days will be based on whether the appropriate treatment regimen was commenced within 30 days of the initial clinic visit—appropriate regimens are defined according to drug susceptibility pattern and with reference to national guidelines (Table 1) [27]. The primary analysis will include only TB cases not on TB treatment at the time of enrolment, i.e. excluding smear non-converters or failures still on treatment. The primary outcome is a binary variable (initiation of appropriate treatment or not) so univariate logistic regression will be performed as the primary analysis using generalised estimating equations (GEE) with a binomial distribution function and a logit link [28]. The odds ratio will be reported with 95% confidence intervals

and a *p*-value from the Wald test. This method will allow for the correlation between observations (within clusters) without needing to specify a distributional assumption for the correlations. In addition, important individual-level characteristics that are unbalanced between arms will be included in the model as covariates. For the secondary outcomes with binary variables, GEE models will also be fitted with a binomial distribution function and a logit link. For the secondary outcomes with time-to-event measures, Cox proportional hazard models will be fitted with the shared frailty option to account for the cluster randomisation [29]. Hazard ratios will be presented with 95% confidence intervals.

The diagnostic performance of Xpert MTB/RIF will be compared between the two arms. Estimation of sensitivity and specificity of Xpert MTB/RIF for the detection of *M. tuberculosis* against the reference standard of single MGIT culture will be based on complete case analysis (participants with paired valid Xpert MTB/RIF and MGIT culture results) and will only include individuals not on TB treatment at the time of enrolment. Estimation of sensitivity and specificity of Xpert MTB/RIF for the detection of rifampicin resistance against the reference standard of genotypic ± phenotypic DST on the culture isolate will be based on participants with *M. tuberculosis* detected by Xpert MTB/RIF and with a positive MGIT culture and valid drug susceptibility test (LPA ± phenotypic DST) results. This will include individuals on TB treatment at the time of enrolment (e.g. participants with AFB smear non-conversion or treatment failure).

Economic evaluation

In addition to evaluating the effectiveness of point-of-care positioning of Xpert MTB/RIF, data from the trial will be combined with those from a costing analysis to explore the cost-effectiveness of point-of-care placement. Health system costs will be obtained through monitoring of study expenditure and interviews with health service management. Collection of data relating to patient and household costs will be nested within the trial—this will involve additional data collected from a subset of patients at baseline and at the 2-month follow-up to determine direct and indirect costs incurred during the diagnostic process. The framework for costing analysis is presented in Table 2. The health system costs and patient costs will be combined with the outcome data to generate an average incremental cost per TB case appropriately treated.

Operational feasibility

The study will also compare the operational feasibility of Xpert MTB/RIF implementation at the hospital

Table 1 Definitions of appropriate initial anti-TB drug regimen for primary outcome measurement

| Case definition* | Appropriate initial anti-TB drug regimen |
|--|--|
| <i>M. tuberculosis</i> susceptible to rifampicin and isoniazid | Isoniazid + rifampicin + pyrazinamide + ethambutol |
| <i>M. tuberculosis</i> with mono-resistance to isoniazid | Isoniazid + rifampicin + pyrazinamide + ethambutol |
| <i>M. tuberculosis</i> with mono-resistance to rifampicin | Standardised second-line regimen§ with isoniazid |
| Multidrug-resistant <i>M. tuberculosis</i> (MDR-TB)† | Standardised second-line regimen§ |
| Extensively drug-resistant <i>M. tuberculosis</i> (XDR-TB)‡ | Standardised XDR-TB regimen |

* Case definition based on results of MGIT culture + line probe assay + phenotypic DST.

† MDR-TB defined as resistance to rifampicin and isoniazid.

‡ XDR-TB defined as MDR plus resistance to ofloxacin and kanamycin.

§Standardised regimen according to national guidelines (kanamycin/amikacin + fluoroquinolone + ethionamide + cycloserine/terizidone ± pyrazinamide ± ethambutol) [27].

||Standardised regimen according to national guidelines (capreomycin + fluoroquinolone + ethionamide + cycloserine/terizidone + PAS + clofazimine) [27].

laboratory and at the primary health care clinic. This encompasses an assessment of the performance and robustness of the system, as well as evaluation of the practicality of operating the system at the laboratory and at the clinic. The key indicators to be assessed are displayed in Table 3. Data on these indicators will be collected throughout the trial.

Ethical considerations

The study has been approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BF033/11), the Ethics Committee of the London School of Hygiene and Tropical Medicine (5926), and the Health Research Committee of the KwaZulu-Natal Department of Health (HRKM084/11). Permission for the study was granted by Hlabisa Hospital and by the Community Advisory Board of the Africa Centre for Health and Population Studies.

Given that the units of randomisation are time blocks, it is not possible for individuals to consent to randomisation. Individual consent for participation remains important given that the intervention is delivered to individuals and that individual-level data are collected at enrolment and at follow-up.

There are TB suspects who are not eligible for this study and therefore will not have access to Xpert MTB/RIF testing within the study (suspects who are neither HIV-infected nor at high risk for MDR-TB). The justification for this is that these groups were a lower priority for this intervention given the much lower mortality rates and these suspects continue to receive diagnostic evaluation including sputum microscopy ± culture/LPA/DST according to national guidelines. At the time of study design it was predicted that, were Xpert MTB/RIF to be implemented in South Africa, the WHO recommendations would be followed (use in HIV-infected and those at high risk of MDR-TB). Although the national roll-out plan went beyond this in incorporating its use for all TB suspects, Xpert MTB/RIF has not yet been

installed in Hlabisa sub-district and is therefore not yet available in the sub-district outside the trial.

Discussion

Evaluation of diagnostic tools provides different challenges than those of therapeutic interventions. Diagnostic accuracy studies are usually the starting point for evaluation of new technologies, yet to inform public health policies and implementation it is crucial to evaluate patient-important outcomes [30]. The ultimate

Table 2 Components of cost analysis

| Health service costs | Patient and household costs |
|---|--|
| Direct costs associated with diagnostic services | Direct costs |
| <i>Fixed</i> | <i>Transport</i> |
| Building space | Transport to/from clinic (patient ± carer) |
| Utilities | Transport to/from hospital (patient ± carer) |
| GeneXpert machine | Non-transport |
| Staff training | Medication |
| Internal/external QC | OPD attendance |
| GeneXpert calibration | X-rays |
| <i>Variable</i> | GP consultation |
| Xpert MTB/RIF tests | Traditional healer consultation |
| Consumables (gloves, N95 masks) | |
| Specimen transport | |
| Staff work time (based on time analysis) | |
| GeneXpert maintenance | |
| Direct costs associated with medical services | Indirect costs |
| First-line TB therapy | Lost time (salary) at work (patient ± carer) |
| MDR-TB therapy | |
| Hospital admission | |
| OPD attendance | |
| Clinic attendance | |

Table 3 Indicators for operational feasibility evaluation

| Indicator | Method of measurement |
|---|--|
| Power supply | Time log for power cuts/ generator use |
| Operating temperature for GeneXpert machine | Temperature log |
| Storage temperature for Xpert MTB/RIF kits | Temperature log |
| Hands-on user time | Activity log |
| Indeterminate results | GeneXpert software |
| Data errors (incomplete identifiers etc.) | GeneXpert software |
| Maintenance needs | Requirement for supplier support |
| Training requirements | Recording of initial and follow-up training sessions |
| Supervision requirements | Log of assistance from other laboratory staff/PI |
| Waste management | Recording of problems with disposal of used cartridges |
| User appraisal | Regular appraisal by laboratory staff and study staff |
| User performance | Regular independent observation of staff performance |

impact of a new diagnostic test should be measured by its capacity to generate beneficial outcomes. A randomised trial is the most rigorous design to evaluate clinical outcomes from a diagnostic intervention. Individual randomisation was considered logistically challenging and potentially disruptive in the context of a busy clinic and laboratory system, although this would have been the most efficient statistical design [28]. Cluster randomisation by health care facility was not possible given the limited resources. Therefore cluster randomisation by time block was the preferred study design to maximise internal validity and to minimise disruption to clinic services. Other trial designs were considered (non-randomised controlled trial with allocation by day/week/month or controlled before and after study) but were felt to be inferior in addressing the research hypothesis, mainly because of the potential for bias and therefore loss of internal validity. There have been few published trials where the unit of randomisation is a time block rather than a geographical or organisational cluster. The trials that have been published have not used consistent methods for sample size calculation—some have adjusted appropriately for cluster variation [31-33] whereas others have arbitrarily inflated the sample size from that for an individual RCT [34] and others have based the sample size on the numbers available to participate [35].

Blinding of patients or of health care workers is not feasible in this pragmatic diagnostic trial because allocation to trial arms involves different actions by the patient and the clinical staff. As a result of this, the outcomes

are as objective as possible to limit potential bias from differential ascertainment of outcomes in the two arms. The possibility of differential recruitment into the trial arms exists but will be minimised by standardised referral criteria for the clinic health care workers; recruitment will be monitored by reviewing the clinic records to ascertain what proportion of patients with cough were referred to the study during each 2-week block. This will be reported if there is a major imbalance in recruitment to the two trial arms. There is a further risk of selection bias if there is differential non-participation. The proportions of eligible subjects consenting by trial arm will be monitored and will be reported accurately at the conclusion of the trial. There is some risk of contamination between the arms if, over time with point-of-care testing, the health workers see the importance and the effect of receiving the test result in a timely fashion and this then improves their ability to encourage all suspects to return and receive their result. This would tend to bias the findings towards the null hypothesis. We will explore this by assessment of the variability in the proportion returning for their test result by cluster and by time period.

The evaluation of diagnostic accuracy is not the primary focus of the trial and the reference standard of a single culture could be considered an imperfect gold standard. In the initial Xpert MTB/RIF clinical validity studies, the reference standard used results of liquid and solid culture on two specimens (four culture results in total) [17]. Conversely, in the later demonstration studies, the reference standard varied between study sites and in some sites included results of only a single culture [18]. Observational data from the district in 2007 suggested that 5% of all culture-positive cases were multidrug-resistant [36]. Given that we will preferentially include suspects with a high risk of MDR-TB, we expect the overall proportion with MDR-TB to be at least 10%. It is possible that the impact of Xpert MTB/RIF positioning may be different for the drug-susceptible and drug-resistant cases. If this is the case then a higher or lower than expected proportion with MDR-TB could modify the effect of point-of-care placement, and this will be explored in secondary analyses.

There are a number of trials evaluating the impact of Xpert MTB/RIF in different settings and with different research hypotheses. Information about research projects is collated by the TREAT TB Xpert Research Mapping Project [37]. Several studies are examining point-of-care implementation but, to our knowledge, this is the only study directly comparing point-of-care use to centralised laboratory use. There is already some evidence from South Africa of the feasibility of implementation at the primary health care level, although several operational challenges were experienced when

implemented within a very large urban clinic [38,39]. This study should provide direct evidence of any benefits of point-of-care positioning as well as further information about the costs and logistical challenges of such strategies. This can also be considered as a proof-of-principle study that will help to understand the benefits of bringing diagnostics closer to patients, and this will have broader relevance as we continue to develop and evaluate diagnostic technologies suitable for point-of-care use [40,41].

Trial status

The study received final ethical approval in June 2011. Enrolment commenced on 22 August 2011. Enrolment is scheduled to complete in March 2013 and follow-up will be complete in May 2013.

Abbreviations

ART: Antiretroviral therapy; DST: Drug susceptibility testing; GEE: Generalised estimating equation; HIV: Human immunodeficiency virus; MDR-TB: Multidrug-resistant TB; MGIT: Mycobacterial growth indicator tube; NHLS: National Health Laboratory Service; PCR: Polymerase chain reaction; PHC: Primary health care; TB: Tuberculosis; WHO: World Health Organization; XDR-TB: Extensively drug-resistant TB.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RJL and PGF conceived and designed the trial, with additional input from GSC, NM, MPN and MLN. NM helped with the statistical design and analysis plan. RJL wrote the first draft of the manuscript. All authors contributed to revision of the manuscript and approved the final version.

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Author details

¹Department of Clinical Research, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. ²Africa Centre for Health and Population Studies, University of KwaZulu-Natal, Mtubatuba, South Africa. ³Department of Infectious Disease, Imperial College, London, UK. ⁴Academic Unit of Primary Care and Population Sciences and Academic Unit of Social Sciences, University of Southampton, Southampton, UK. ⁵Division of Medical Microbiology, University of Cape Town, Cape Town, South Africa. ⁶UCL Institute of Child Health, London, UK.

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