Optimising Xpert-Ultra and culture testing to reliably measure tuberculosis prevalence in the community: findings from surveys in Zambia and South Africa

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ABSTRACT

Objectives Prevalence surveys remain the best way to assess the national tuberculosis (TB) burden in many countries. Challenges with using culture (the reference standard) for TB diagnosis in prevalence surveys have led to increasing use of molecular tests (Xpert assays), but discordance between these two tests has created problems for deciding which individuals have TB. We aimed to design an accurate diagnostic algorithm for TB prevalence surveys (TBPS) that limits the use of culture.

Design TBPS in four communities, conducted during 2019.

Setting Three Zambian communities and one South-African community included in the TBPS of the Tuberculosis Reduction through Expanded Anti-retroviral Treatment and Screening study.

Participants Randomly sampled individuals aged ≥15 years. Among those who screened positive on chest X-ray or symptoms, two sputum samples were collected for field Xpert-Ultra testing and a third for laboratory liquid-culture testing. Clinicians reviewed screening and test results; in Zambia, participants with Mycobacterium tuberculosis-positive results were followed up 6–13 months later. Among 946 culture-negative individuals, 917 were Xpert-negative, 12 Xpert-trace-positive and 17 Xpert-positive (grade very low, low, medium or high), with Xpert categorised as the highest grade of the two sample results. Among 63 culture-positive individuals, 8 were Xpert-negative, 9 Xpert-trace-positive and 46 Xpert-positive. Counting trace-positive results as positive, the sensitivity of Xpert-Ultra compared with culture was 87% (95% CI 76% to 94%) using two samples compared with 76% (95% CI 64% to 86%) using one. Specificity was 97% when trace-positive results were counted as positive and 98% when trace-positive results were counted as negative. Most Xpert-Ultra-positive/culture-negative discordance was among individuals whose Xpert-positive results were trace-positive or very low grade or they reported previous TB treatment. Among individuals with both Xpert-Ultra results grade low or above, the positive-predictive-value was 90% (27/30); 3/30 were plausibly false-negative culture results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The Tuberculosis Reduction through Expanded Anti-retroviral Treatment and Screening prevalence survey is the first to have both collected two sputum samples for Xpert-Ultra testing (rather than one) on all sputum-eligible individuals, as well as collect a third sputum sample for liquid culture testing using mycobacteria growth indicator tubes.

⇒ Substantial efforts were made to ensure that culture testing was of high quality, with various quality control and quality assurance procedures in place.

⇒ In Zambian communities, all individuals whose sputum samples tested positive on one or both of Xpert-Ultra and culture were followed up 6–13 months later, and this provided additional information that was valuable for assessing whether discordant test results were ‘false-positive’ Xpert results or ‘false-negative’ culture results.

⇒ The prevalence survey was conducted in two countries, rather than only one, and in settings with relatively high HIV prevalence and relatively high prevalence of individuals with previous tuberculosis treatment, strengthening the generalisability of study findings.

⇒ Our study was large enough to identify important patterns, but it was not large enough to estimate the sensitivity of Xpert-Ultra (using two sputum samples) compared with culture with high precision and additional evidence from other surveys would be valuable.
INTRODUCTION

Tuberculosis (TB) is a major cause of ill health, one of the top causes of death worldwide and, until the COVID-19 pandemic, the leading global cause of death from a single infectious agent. In countries where routine national surveillance systems cannot yet be relied on to provide accurate information about the number of people who develop TB each year, national TB prevalence surveys (TBPS) are currently the best way to directly measure the burden of TB disease in the community and assess trends. Findings from these surveys also provide insights to inform policy, planning and programmatic action.

National TBPS among individuals aged ≥15 years were completed in >50 countries with a high burden of TB in Asia and Africa during 2007–2020. All survey participants were screened using a chest X-ray and an interview about TB symptoms, with sputum samples collected for diagnostic testing for TB among those who screened positive, following an established and standardised methodology. Until 2015, diagnostic testing was done using smear microscopy and the reference standard of solid or liquid culture, following WHO guidance. In most countries it was challenging to achieve high-quality culture testing; challenges included the need for specialised staff and laboratory facilities, multiple processing steps, the large number of samples to be processed in already-busy laboratories, and the difficulty of maintaining a cold chain over long distances and transport times.

During 2015–2020, 11 national TBPS used a rapid molecular test—Xpert M. tuberculosis (MTB)/Rifampicin (RIF) or Xpert-Ultra—as a primary diagnostic alongside reference-standard culture testing, with the molecular test being cheaper than culture and much simpler and quicker to implement. Discordance between culture and Xpert results was observed in all surveys, consistent with previously reported findings from clinical settings of lower sensitivity (around 88% for Xpert-Ultra, around 85% for Xpert MTB/RIF and specificity (around 96% for Xpert-Ultra, around 98% for Xpert MTB/RIF) of Xpert compared with culture, and of lower specificity among individuals who report previous TB treatment compared with those who report no previous TB treatment. However, the amount of discordance in TBPS was larger than anticipated and created challenges in interpretation of results and in deciding which individuals to count as TB cases.

Among individuals who tested positive on Xpert but negative on culture, it is possible that the Xpert result was a ‘false-positive’ for being a TB case on the day of the survey. Such ‘false-positives’ are possible because a molecular test can detect DNA from dead as well as live bacilli (and this is well recognised), whereas a culture-negative result indicates no growth of MTB bacilli. In TBPS the true prevalence of TB among those who screen positive is expected to be around 2%–5% (lower than in clinical settings where individuals have presented as unwell) and (with specificity of Xpert-Ultra around 96%) the proportion of Xpert-positive results that are ‘false-positives’ may be similar to the proportion that are ‘true-positives’. An alternative to the ‘false-positive-Xpert’ explanation is that the culture result was a ‘false-negative’. ‘False-negative’ culture results may be a particular challenge in TBPS as most individuals who are diagnosed with TB in this context (ie, in the community rather than after presenting at a clinic with symptoms) have paucibacillary disease, their sputum samples contain relatively fewer bacilli, and if a proportion of the initially-live bacilli die during the transportation and/or laboratory processing steps then the number that remain may be below the limit of detection of culture testing.

The TREATS (Tuberculosis Reduction through Expanded Anti-retroviral Treatment and Screening) project followed the HPTN 071 (PopART) trial, with one study endpoint being TB prevalence measured in a TBPS across 21 communities in Zambia and South Africa. We conducted an ‘intensive diagnostic phase’ (IDP) of the TBPS in 4 communities in 2019, with IDP aiming to include a better understanding of the Xpert-culture test discordance in TBPS and to design an accurate and culture-minimising diagnostic algorithm that could subsequently be implemented in the remaining study communities. We also aimed to contribute evidence towards WHO recommendations on alternative diagnostic algorithms that could be used in future national TBPS, and towards updated WHO guidelines on active case-finding for TB.

METHODS

Study design

The TREATS study was conducted during 2017–2021, across 21 urban and peri-urban communities in Zambia and the Western Cape province of South Africa. These same communities were included in the HPTN 071 (PopART) trial that was conducted during 2013–2018, in which each community was randomised to one of 3 trial arms; Arms A and B received the ‘full’ or ‘intermediate’ PopART intervention respectively, and arm C received standard-of-care. The PopART intervention consisted of population-level screening for TB, combined with universal testing and treatment for HIV.

TB prevalence, measured in a random sample of individuals aged ≥15 years, is one of the outcomes of the TREATS study. The target sample size was 3000–4000 participants in each Arm C community, and 1500–2000 participants in each Arm A and Arm B community, to have good study power to compare TB prevalence between the 14 arm A and B communities (combined) and the 7 arm C communities.
IDP of TBPS

The IDP was implemented in 2019 in the first four communities in which the TBPS was conducted, three Zambian communities (all in Lusaka, one in each of arms A, B, and C) and 1 South African community (in Khayelitsha, in arm A). All findings reported in this paper are from these 4 IDP communities.

Field procedures, all study communities

Within each community, random sampling was structured according to geographically defined blocks of around 200 households. For every randomly selected block, all households were visited by a research assistant; where an adult household member was found at home, permission was sought to enumerate (list) all household members. In enumerated households, an individual was eligible to participate if they were a community resident aged ≥15 years. Eligible individuals were given barcoded invitation cards, and invited to attend a mobile field site (MFS) that included a mobile laboratory housed in a truck.

All individuals who attended the MFS and consented to participate in the survey followed a defined order of procedures. First, questionnaire information including TB symptoms, previous history of TB treatment and previous HIV testing history, was collected. A digital chest X-ray was taken, with images read using a computer-aided-detection (CAD) system called CAD4TB (V.5.0, Delft Imaging, the Netherlands) that provided a score between 0% and 100% representing the probability that an individual has TB. HIV testing was offered to individuals who did not self-report they were HIV-positive.

‘Sputum-eligible’ individuals were those who had a cough for ≥2 weeks or who had ≥2 among 5 ‘TB suggestive’ symptoms (cough of any duration, unexpected weight loss for ≥2 weeks, night sweats for ≥2 weeks, chest pains for ≥2 weeks, fever for ≥2 weeks), or an X-ray score above a predefined threshold (≥40% during the IDP), or who did not have an X-ray done. All sputum-eligible individuals were requested to provide two ‘on-the-spot’ sputum samples (S1 and S2, taken ≥30 minutes apart), for Xpert-Ultra testing within the next 24 hours, and to return the following day (day 2) to receive the results. On this ‘day 2’ they met a medical officer, who reviewed all available screening and test results alongside information including self-reported previous TB treatment and HIV status, re-enquired about TB symptoms, recorded their interpretation of the chest X-ray, and made decisions on referral to TB or other care.

IDP, sample collection for culture

In the IDP, all sputum-eligible individuals who returned on day 2 were asked to provide a third ‘on-the-spot’ sputum sample (S3) for laboratory culture testing. S3 samples were batched and kept in a refrigerator until they were transported later the same day to the laboratory in a cooler box, with each batch including a ‘dummy’ sputum sample of known bacterial load (very low grade on Xpert testing) to monitor whether bacterial viability was reduced during transportation. In Zambia, samples were taken to the Zambart central laboratory in Lusaka, and in South Africa to the National Health Laboratory Service (NHLS) laboratory in Greenpoint, Cape Town.

Sample processing and interpretation of test results as negative or positive

For samples S1 and S2, Xpert-Ultra testing was conducted in the truck laboratory at the MFS, according to the manufacturer’s standard operating procedures. For each sample, the test result was classified using the semi-quantitative categories of the test read-out, as MTB not detected, MTB-trace detected, MTB detected very low, low, medium or high; or, as invalid, error or no result. In some of our analyses, we simplified these categories to Xpert-negative (MTB not detected), Xpert-trace-positive and Xpert-positive (grade very low or above).

At the culture laboratory, S3 samples were decontaminated using the N-acetyl-L-cysteine-sodium-hydroxide (NALC-NaOH) method. After decontamination, the dissolved sediment was inoculated onto two mycobacteria growth indicator tubes and incubated for 42 days or until growth was observed. Decontamination of the S3 samples was done in batches, with each batch including one low-MTB-bacillary-load positive control and one negative control sample. Xpert-Ultra testing was conducted on the leftover sediment, according to the manufacturer’s instructions.

Growth-positive cultures were tested using culture ZN staining and MPT64 antigen testing to distinguish MTB from non-tuberculous mycobacteria (NTM) and from contamination, and those that showed acid-fast bacilli or were MPT64 positive were tested using the line probe assay (LPA) for common NTM (LPA-CM, HAIN). The culture outcome for each tube was defined based on the combination of the three test results as follows: (1) negative (no growth observed) (2) MTB (3) NTM (4) non-interpretable (when test results were conflicting) or (5) contaminated (online supplemental figure S1).

The ‘final’ S3 culture result was defined based on the combination of the culture outcomes from the two tubes. S3 was classified as culture-positive if ≥1 tube result was positive for MTB. Among S3 that were not culture-positive for MTB, they were classified as culture-negative if ≥1 tube was negative or ≥1 tube was positive for NTM. The result was classified as contaminated if both tubes were contaminated, and as non-interpretable if either both tubes were non-interpretable or one was non-interpretable and one was contaminated. In our analyses, a ‘valid’ culture result was one that was culture-positive or culture-negative and from a batch where the positive control grew and the negative control did not.

Clinical review

The Xpert-Ultra and culture test results were jointly reviewed by 3–5 experienced infectious-disease clinicians, alongside individual characteristics including TB symptoms, the chest X-ray image and its CAD score, HIV status.
and self-reported history of previous TB treatment. For each individual, a judgement was made on whether or not they had TB, using categories no TB, TB with microbiological evidence, clinical TB, possible TB, unlikely to be TB, or unable to evaluate.

**Follow-up of individuals in IDP communities, in Zambia**

Zambian IDP participants who had positive culture or Xpert-Ultra test results or an X-ray CAD-score ≥70%, were followed up 6–13 months later at their home in order to understand clinical and TB treatment trajectories after survey participation. In analyses presented here, we considered only those individuals whose Xpert-Ultra and culture results were discordant.

At follow-up, a questionnaire was administered that inquired about whether the individual reported for TB or HIV care, if TB treatment was started and completed, and current TB symptoms. All were offered to have a digital chest X-ray taken at a nearby site using a ‘backpack’ mobile X-ray (Delft Light, Delft Imaging, the Netherlands). Participants were asked to provide one ‘on-the-spot’ sputum sample for Xpert-Ultra testing if they had tested culture or Xpert-positive in the prevalence survey but had not yet started TB treatment, or if they reported current ‘TB-suggestive’ symptoms or had an X-ray CAD score ≥70% at follow-up.

**Data collection, outcomes and explanatory variables, and analysis**

MFS data were captured digitally into an electronic data management system (DMS) specifically designed for the TREATS TBPS, while culture data were captured using each laboratory’s DMS.

In our analyses, we considered culture as the reference standard to which we compared Xpert-Ultra testing. First, we cross-tabulated Xpert-Ultra (using S1 and S2) and culture (from S3) test results, to summarise the level of discordance. Second, we quantified the additional value of taking two sputum samples (S1 and S2) for Xpert-Ultra testing, compared with only one (S1), for increasing the sensitivity of Xpert-Ultra testing against the reference standard of culture. Third, we estimated the specificity and positive-predictive-value (PPV) of Xpert-Ultra testing (using two sputum samples) compared with culture, with and without considering ‘Xpert-trace-positive’ results as Xpert-positive. Fourth, we considered pairs of S1 and S2 Xpert-Ultra results, and the grade of the result from each sample, to estimate the PPV of different combinations of results compared with culture. We present these analyses overall, and stratified according to self-reported current and previous TB treatment, HIV status (positive if tested HIV-positive in the survey or self-reported as HIV-positive, negative if tested HIV-negative in the survey or self-reported their last HIV test result was negative and it was within the previous 12 months), TB symptoms and X-ray CAD score.

After these analyses were completed, we gave detailed consideration to individuals with discordant Xpert-Ultra and culture test results, to attempt an assessment of the balance of ‘false-positive’ Xpert-Ultra test results versus ‘false-negative’ culture results, informed by the medical officer review on ‘day 2’ of field procedures, the clinical review and the later follow-up findings.

Analyses were restricted to individuals with a negative, trace-positive or positive Xpert-Ultra result on each of S1 and S2, and a valid culture result.

**Patient and public involvement**

Our key findings have been shared as part of community dissemination meetings, in meetings with health officials and programme implementers, and with WHO representatives who are members of the study advisory group.

**RESULTS**

**Participation, sputum eligibility, provision of sputum samples and valid test results**

In Zambian communities, 51% (8922/17 574) of eligible individuals participated, 18% of participants were sputum-eligible, 92% of sputum-eligible individuals had Xpert-Ultra results from two sputum samples, 69% of individuals with Xpert-Ultra results submitted S3, and 68% of those who submitted S3 had a valid culture result (figure 1). The corresponding figures for the South African community were 63% (2048/3234), 24%, 81%, 94% and 88%. In Zambia, most (86%, 270/315) of the 315 invalid culture results were due to the S3 sample having a test result that was ‘sample contaminated’, whereas in the South African community few (11%, 5/45) of the 45 invalid culture results were due to the S3 sample being contaminated (figure 1).

In Zambia, there was a strong association between the grade of the Xpert result (from testing of S1 and S2 samples) and whether the culture result (from testing of the S3 sample) was valid. The culture result was contaminated for around 30% of Xpert-negative individuals and those whose highest result was trace-positive, compared with 0% for those with medium or high-grade Xpert results (online supplemental table S1).

**Xpert-Ultra and culture results and their discordance**

In Zambian communities, 6.0% (41/681) were culture-positive, 6.2% (42/681) were Xpert-positive (≥very low grade) on S1 or S2, and for 1.2% (8/681) the highest Xpert result was trace-positive (table 1). In the South African community 6.7% (22/328) were culture-positive, 6.4% (21/328) were Xpert-positive (≥very low grade) and for 4.0% (13/328) the highest Xpert result was trace-positive.

In Zambian communities, among 42 individuals who were Xpert-positive on S1 or S2, 34 were culture-positive and 8 were culture-negative, with a PPV of 81%; among 8 individuals whose highest Xpert results were trace-positive, 2 were culture-positive, with a PPV of 25% (table 1). In the South African community, the corresponding values were a PPV of 57% (12/21) and 54% (7/13).
Figure 1  Flow diagram of participation and inclusion in the analysis. *discordant results between Zn and MPT64 MTB identification. LFU = Lost to follow-up; QA = Quality assured; CXR = Chest x-ray; S1/S2/S3 spot sample 1, 2, 3. Values in bold are the ones to which attention is drawn in the article text.
Sensitivity of Xpert-Ultra, among culture-positive individuals

The sensitivity of Xpert-Ultra increased when trace-positive results were considered as positive rather than negative, especially in South Africa, and from testing two samples rather than relying on only one (table 2).

In Zambia, 87.8% (36/41) of culture-positive individuals had a positive (grade very low or above) or trace-positive Xpert result on S1 or S2, a gain in sensitivity of 10% compared with the 78.0% (32/41) with a positive result using S1 alone (table 2). In South Africa, 86.4% (19/22) of culture-positive individuals had a positive or trace-positive Xpert result on S1 or S2, a gain in sensitivity of 36% compared with the 50.0% (11/22) with a positive result on S1 alone. Combining the two countries, the sensitivity of Xpert-Ultra compared with culture was 87% (95% CI 76% to 94%) with two sputum samples and trace-positive results counted as positive.

With trace-positive results counted as negative, the gain in sensitivity from Xpert testing of two samples rather than one was modest in both countries, at ~5%, for example a gain from 78.0% to 82.9% in Zambia (table 2). With trace-positive results counted as positive, the gain in sensitivity from testing two samples rather than one was larger, at 7% in Zambia and 18% in South Africa, and was larger in HIV-negative compared with HIV-positive individuals and in those with ≤1 symptom compared with those with more symptoms (table 2).

Specificity and PPV of Xpert-Ultra, when categorised as the highest value from S1 and S2

There was a loss in specificity of ~1% in Zambia and ~2% in South Africa when trace-positive results were counted as positive rather than negative (table 3). In Zambia, specificity was 97.8% when trace-positive results were counted as positive and 98.7% when trace-positive results were counted as negative, while in South Africa the corresponding figures were 95.1% and 97.1%, respectively. The loss in specificity when trace-positive results were counted as positive was low (<1%) among individuals who self-reported no previous TB treatment, but higher (~5%) among those reporting previous TB treatment.

In Zambia, the PPV of Xpert was much higher among individuals with positive results compared with those whose highest test result was trace-positive, at 81% compared with 25% (tables 1 and 3). In South Africa, the corresponding figures were lower and more similar, at 57% and 54%, respectively. However, the country-specific estimates of the PPV among individuals whose highest test result was trace-positive were based on relatively low numbers, and there was no statistical evidence they differed by country (p=0.37, Fisher’s exact test).

In both countries, the PPV of a positive or trace-positive Xpert result was higher among individuals who self-reported no previous TB treatment than among those reporting previous TB treatment. For example, among individuals in Zambia who self-reported no previous TB treatment the PPV among Xpert-positive individuals was 91% and among those whose highest Xpert result was trace-positive it was 40%, with corresponding figures of 67% and 0% among those reporting previous TB treatment.

Among the few individuals who reported they were currently on TB treatment, in both Zambia (0/3) and South Africa (0/2) the PPV of a positive Xpert result was 0%, and in South Africa the PPV of a trace-positive result was also 0% (0/2) (table 3).

PPV of various combinations of two Xpert-Ultra results, compared with culture

The PPV of Xpert-Ultra was highest for individuals with a positive result of at least low grade on both S1 and S2, and a grade of medium or high on at least one (table 4); in Zambia the PPV was 100% (15/15) and in South Africa it was 83% (5/6). In South Africa, the PPV was <60% for other combinations of test results. In Zambia, the PPV was high for both samples low grade (86%, 6/7), one sample low and the other very low grade (75%, 6/8) and both very low grade (100%, 2/2),

| Table 1 | Concordance and discordance of Xpert-Ultra result (highest value among S1 and S2) and culture result on S3, and positive-predictive-value (PPV) of Xpert-Ultra compared with culture |
|----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Zambian communities                          | Culture                                      | Xpert-Ultra                                   |
|                                             | Positive | Negative | Total | PPV of Xpert |
| Positive (≥very low grade)                   | 34       | 8        | 42    | 81%          |
| Trace-positive                               | 2        | 6        | 8     | 25%          |
| Negative                                    | 5        | 626      | 631   |              |
| Total                                       | 41       | 640      | 681   |              |
| South African community                      | Culture                                      | Xpert-Ultra                                   |
|                                             | Positive | Negative | Total | PPV of Xpert |
| Positive (≥very low grade)                   | 12       | 9        | 21    | 57%          |
| Trace-positive                               | 7        | 6        | 13    | 54%          |
| Negative                                    | 3        | 291      | 294   |              |
| Total                                       | 22       | 306      | 328   |              |
| Zambian and South African communities        | Culture                                      | Xpert-Ultra                                   |
|                                             | Positive | Negative | Total | PPV of Xpert |
| Positive (≥very low grade)                   | 46       | 17       | 63    | 73%          |
| Trace-positive                               | 9        | 12       | 21    | 43%          |
| Negative                                    | 8        | 917      | 925   |              |
| Total                                       | 63       | 946      | 1009  |              |
## Table 2  Sensitivity of 1 or 2 samples (S1 and S2) tested with Xpert-Ultra, compared with a third sample (S3) that tested culture-positive

|                      | Trace-positive classified as negative | Trace-positive classified as positive | Culture-positive |
|----------------------|--------------------------------------|--------------------------------------|------------------|
|                      | S1 n* %                              | S1 and S2 n† %                       | S1 n‡ %          |
|                      |                                      |                                      | S1 and S2 n§ %   |
|                      |                                      |                                      | N¶               |
| **Zambian communities** |                                      |                                      |                  |
| Overall              | 32 78.0                               | 34 82.9                              | 33 80.5          |
|                      |                                      |                                      | 36 87.8          |
|                      |                                      |                                      | 41               |
| HIV status**         |                                      |                                      |                  |
| HIV-negative         | 19 76.0                               | 21 84.0                              | 19 76.0          |
|                      |                                      |                                      | 22 88.0          |
|                      |                                      |                                      | 25               |
| HIV-positive         | 10 76.9                               | 10 76.9                              | 11 84.6          |
|                      |                                      |                                      | 11 84.6          |
|                      |                                      |                                      | 13               |
| Self-reported previous TB treatment |                                      |                                      |                  |
| No                   | 28 75.7                               | 30 81.1                              | 29 78.4          |
|                      |                                      |                                      | 32 86.5          |
|                      |                                      |                                      | 37               |
| Yes, previous        | 4 100.0                               | 4 100.0                              | 4 100.0          |
|                      |                                      |                                      | 4               |
| Self-reported cough ≥2 weeks and/or ≥2 TB symptoms |                                      |                                      |                  |
| No                   | 18 69.2                               | 20 76.9                              | 19 73.1          |
|                      |                                      |                                      | 22 84.6          |
|                      |                                      |                                      | 26               |
| Yes                  | 14 93.3                               | 14 93.3                              | 14 93.3          |
|                      |                                      |                                      | 14 93.3          |
|                      |                                      |                                      | 15               |
| X-ray CAD score††    |                                      |                                      |                  |
| <40                  | 1 100.0                               | 1 100.0                              | 1 100.0          |
| 40–49                | 1 25.0                                | 1 25.0                               | 2 50.0           |
| ≥50                  | 29 82.9                               | 31 88.6                              | 30 85.7          |
|                      |                                      |                                      | 32 91.4          |
| **South African community** |                                      |                                      |                  |
| Overall              | 11 50.0                               | 12 54.5                              | 15 68.2          |
|                      |                                      |                                      | 19 86.4          |
|                      |                                      |                                      | 22               |
| HIV status**         |                                      |                                      |                  |
| HIV-negative         | 8 61.5                                | 9 69.2                               | 8 61.5           |
|                      |                                      |                                      | 11 84.6          |
| HIV-positive         | 1 16.7                                | 1 16.7                               | 5 83.3           |
|                      |                                      |                                      | 5 83.3           |
|                      |                                      |                                      | 6               |
| Self-reported previous TB treatment |                                      |                                      |                  |
| No                   | 9 52.9                                | 10 58.8                              | 11 64.7          |
|                      |                                      |                                      | 14 82.4          |
|                      |                                      |                                      | 17               |
| Yes, previous        | 2 40.0                                | 2 40.0                               | 4 80.0           |
|                      |                                      |                                      | 5 100.0          |
|                      |                                      |                                      | 5               |
| Self-reported cough ≥2 weeks and/or ≥2 TB symptoms |                                      |                                      |                  |
| No                   | 9 50.0                                | 10 55.6                              | 12 66.7          |
|                      |                                      |                                      | 16 88.9          |
|                      |                                      |                                      | 18               |
| Yes                  | 2 50.0                                | 2 50.0                               | 3 75.0           |
|                      |                                      |                                      | 3 75.0           |
|                      |                                      |                                      | 4               |
| X-ray CAD score††    |                                      |                                      |                  |
| <40                  | 1 100.0                               | 1 100.0                              | 1 100.0          |
| 40–49                | 2 66.7                                | 2 66.7                               | 3 100.0          |
| ≥50                  | 8 47.1                                | 9 52.9                               | 11 64.7          |
|                      |                                      |                                      | 14 82.4          |
|                      |                                      |                                      | 17               |
| **Zambian+South African communities** |                                      |                                      |                  |
| Overall              | 43 68.3                               | 46 73.0                              | 48 76.2          |
|                      |                                      |                                      | 55 87.3          |
|                      |                                      |                                      | 63               |

* n=number tested Xpert-Ultra-positive on S1, with grade very low, low, medium, or high counted as positive, that is, trace-positive results classified as negative. % =n/N, with N=number tested culture-positive on S3.
† n=number tested Xpert-Ultra-positive on S1 and/or S2, with grade very low, low, medium, or high counted as positive, that is, trace-positive results classified as negative. % =n/N, with N=number tested culture-positive on S3.
‡ n=number tested Xpert-Ultra-positive on S1, with grade trace-positive, very low, low, medium, or high counted as positive, that is, trace-positive results classified as positive. % =n/N, with N=number tested culture-positive on S3.
§ n=number tested Xpert-Ultra-positive on S1 and/or S2, with grade trace-positive, very low, low, medium, or high counted as positive, that is, trace-positive results classified as positive. % =n/N, with N=number tested culture-positive on S3.
¶ N=denominator for analysis, number tested culture-positive.
** Among individuals who tested culture-positive in Zambian communities, 3 were of unknown HIV status (they did not self-report HIV-positive, did not self-report an HIV-negative test in the previous 12 months, and did not accept the offer of HIV testing as part of the TBPS). In the South Africa community there were also three culture-positive individuals whose HIV status was unknown. Excluding these individuals, the denominator of culture-positive individuals was 38 in Zambia and 19 in South Africa.
†† Among individuals who tested culture-positive in Zambian communities, 1 had missing data on their chest X-ray CAD score. In the South African community there were also one culture-positive individual with missing data on their chest X-ray. Excluding these individuals, the denominator of culture-positive individuals was 40 in Zambia and 21 in South Africa. The 3 X-ray CAD score categories were chosen to distinguish individuals who were sputum-eligible based on TB symptoms but not on X-ray (<40), ‘borderline’ sputum-eligibility based on their X-ray score (40–49) and those whose X-ray CAD score was 50% or higher (≥50 CAD score).
TB, tuberculosis; TBPS, TB Prevalence Surveys.
|                      | Zambian communities | South African community |
|----------------------|---------------------|-------------------------|
|                      | Overall             | Overall                 |
|                      | HIV-negative        | HIV-negative            |
|                      | HIV-positive        | HIV-positive            |
|                      | Self-reported TB    | Self-reported TB        |
|                      | treatment           | treatment               |
|                      | No                  | No                      |
|                      | Yes, previous       | Yes, previous           |
|                      | Yes, current        | Yes, current            |
|                      | X-ray CAD score     | X-ray CAD score         |
|                      | <40                 | 40–49                   |
|                      | ≥50                 |                          |
|                      | Culture-negative    |                          |

|                      | PPV                 | Specificity             |
|                      | n=culture-positive  | n=culture-negative      |
|                      | N=Xpert-positive    | N=Xpert-negative or trace-positive | N=Xpert-negative | Culture-negative |
|                      | N=Xpert-trace-positive | n             | %         | n            | %         | N         |
|                      | n/N*                | n/N†                  | %         | n            | %         | N         |
| Overall              | 34/42               | 81                    | 2/8       | 25           | 632       | 98.7      | 626       | 97.8      | 640       |
| HIV status           |                     |                       |           |              |           |           |           |           |           |
| HIV-negative         |                     |                       |           |              |           |           |           |           |           |
| HIV-positive         |                     |                       |           |              |           |           |           |           |           |
| Self-reported previous TB treatment |                     |                       |           |              |           |           |           |           |           |
| No                   | 30/33               | 91                    | 2/5       | 40           | 509       | 99.4      | 506       | 98.8      | 512       |
| Yes, previous        | 4/6                 | 67                    | 0/3       | 0            | 120       | 98.4      | 117       | 95.9      | 122       |
| Yes, current         | 0/3                 | 0                     | /         | /            | 3         | 50.0      | 3         | 50.0      | 6         |
| X-ray CAD score      |                     |                       |           |              |           |           |           |           |           |
| <40                  | 1/2                 | 50                    | /         | /            | 142       | 99.3      | 142       | 99.3      | 143       |
| 40–49                | 1/2                 | 50                    | 1/2       | 50           | 189       | 99.5      | 188       | 98.9      | 190       |
| ≥50                  | 31/37               | 84                    | 1/6       | 17           | 284       | 97.9      | 279       | 96.2      | 290       |

Continued
Table 3  Continued

|                          | PPV            |                      | Specificity       |
|--------------------------|----------------|----------------------|------------------|
|                          | n=culture-positive | N=Xpert-positive | n=Xpert-negative or trace-positive | n=Xpert-negative | Culture-negative |
|                          | N=Xpert-positive | N=Xpert-trace-positive |                         |                  |                  |
| Self-reported cough ≥2 weeks and/or ≥2 TB symptoms | 10/17 59 6/12 50 | 222 96.9 216 94.3 229 | 75 97.4 75 97.4 77 |
| No                       | 2/4** 50 1/1 100 | 45 100 45 100 45 | 83 98.8 83 98.8 84 |
| Yes                      | 9/16 56 5/11 45 | 160 95.8 154 92.2 167 |

X-ray CAD score

|                   | PPV            |                      | Specificity       |
|-------------------|----------------|----------------------|------------------|
|                   | n=culture-positive | N=Xpert-positive | n=Xpert-negative or trace-positive | n=Xpert-negative | Culture-negative |
|                   | N=Xpert-positive | N=Xpert-trace-positive |                         |                  |                  |
| <40               | 1/1 100 / / | 45 100 45 100 45 | 83 98.8 83 98.8 84 |
| 40–49             | 2/3 67 1/1 100 | 83 98.8 83 98.8 84 |
| ≥50               | 9/16 56 5/11 45 | 160 95.8 154 92.2 167 |

* n/N, n=number tested culture-positive, N=number tested Xpert-positive with grade very low, low, medium, or high on one or both of S1 and S2.<br>† n/N, n=number tested culture-positive, N=number with highest Xpert-Ultra result across S1 and S2 a trace-positive result.<br>‡% calculated as n/N, with n=number who tested Xpert-negative or Xpert-trace-positive on S1 and S2, and N=number who tested culture-negative for Mycobacterium tuberculosis.<br>§% calculated as n/N, with n=number who tested Xpert-negative on S1 and S2, and N=number who tested culture-negative for M. tuberculosis.<br>¶Among the 5/19 who tested culture-negative, 3 reported they were currently on TB treatment and two reported no previous TB treatment.<br>**Among the two in four who tested culture-negative, one reported they were currently on TB treatment and one reported previous TB treatment.<br>PPV, positive predictive value; TB, tuberculosis.
### Table 4  Comparison of combinations of Xpert-Ultra test results from two sputum samples (S1 and S2) compared with culture on a third sputum sample (S3)

| Zambian communities                      | South African community                                      |
|------------------------------------------|-------------------------------------------------------------|
| **Overall**                              |                                                             |
| 1. Both ≥low, ≥1 medium/high             | 1. Both ≥low, ≥1 medium/high                                |
|   Culture-positive                        | 5                                                           |
|   Culture-negative                        | 1*                                                          |
|   Total                                   | 6                                                           |
|   PPV                                     | 83%                                                         |
| 2. Both low                               | 2. Both low                                                 |
|   Culture-positive                        | 1                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 2                                                           |
|   PPV                                     | 50%                                                         |
| 3. 1 low, 1 very low                      | 3. 1 low, 1 very low                                        |
|   Culture-positive                        | 2                                                           |
|   Culture-negative                        | 2                                                           |
|   Total                                   | 4                                                           |
|   PPV                                     | 50%                                                         |
| 4. Both very low                          | 4. Both very low                                            |
|   Culture-positive                        | 1                                                           |
|   Culture-negative                        | 2                                                           |
|   Total                                   | 3                                                           |
|   PPV                                     | 33%                                                         |
| 5. 1 ≥very low, 1 negative               | 5. 1 ≥very low, 1 negative                                  |
|   Culture-positive                        | 2                                                           |
|   Culture-negative                        | 3                                                           |
|   Total                                   | 5                                                           |
|   PPV                                     | 40%                                                         |
| 6. ≥1 trace                               | 6. ≥1 trace                                                 |
|   Culture-positive                        | 8                                                           |
|   Culture-negative                        | 6                                                           |
|   Total                                   | 14                                                          |
|   PPV                                     | 57%                                                         |
| 7. Both negative                          | 7. Both negative                                            |
|   Culture-positive                        | 3                                                           |
|   Culture-negative                        | 291                                                         |
|   Total                                   | 294                                                         |
| **Self-reported no previous TB treatment**|                                                             |
| 1. Both ≥low, ≥1 medium/high             | 1. Both ≥low, ≥1 medium/high                                |
|   Culture-positive                        | 3                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 4                                                           |
|   PPV                                     | 75%                                                         |
| 2. Both low                               | 2. Both low                                                 |
|   Culture-positive                        | 1                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 2                                                           |
|   PPV                                     | 50%                                                         |
| 3. 1 low, 1 very low                      | 3. 1 low, 1 very low                                        |
|   Culture-positive                        | 2                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 3                                                           |
|   PPV                                     | 67%                                                         |
| 4. Both very low                          | 4. Both very low                                            |
|   Culture-positive                        | 0                                                           |
|   Culture-negative                        | 0                                                           |
|   Total                                   | 0                                                           |
|   PPV                                     | 100%                                                        |
| 5. 1 ≥very low, 1 negative               | 5. 1 ≥very low, 1 negative                                  |
|   Culture-positive                        | 2                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 3                                                           |
|   PPV                                     | 67%                                                         |
| 6. ≥1 trace                               | 6. ≥1 trace                                                 |
|   Culture-positive                        | 8                                                           |
|   Culture-negative                        | 6                                                           |
|   Total                                   | 14                                                          |
|   PPV                                     | 57%                                                         |
| 7. Both negative                          | 7. Both negative                                            |
|   Culture-positive                        | 3                                                           |
|   Culture-negative                        | 291                                                         |
|   Total                                   | 294                                                         |
| **Self-reported previous TB treatment, not currently on TB treatment** |                                                             |
| 1. Both ≥low, ≥1 medium/high             | 1. Both ≥low, ≥1 medium/high                                |
|   Culture-positive                        | 2                                                           |
|   Culture-negative                        | 0                                                           |
|   Total                                   | 2                                                           |
|   PPV                                     | 100%                                                        |
| 2. Both low                               | 2. Both low                                                 |
|   Culture-positive                        | 0                                                           |
|   Culture-negative                        | 0                                                           |
|   Total                                   | 0                                                           |
|   PPV                                     | /                                                           |
| 3. 1 low, 1 very low                      | 3. 1 low, 1 very low                                        |
|   Culture-positive                        | 0                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 1                                                           |
|   PPV                                     | 0%                                                          |
| 4. Both very low                          | 4. Both very low                                            |
|   Culture-positive                        | 0                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 1                                                           |
|   PPV                                     | 0%                                                          |
| 5. 1 ≥very low, 1 negative               | 5. 1 ≥very low, 1 negative                                  |
|   Culture-positive                        | 0                                                           |
|   Culture-negative                        | 1                                                           |
|   Total                                   | 1                                                           |
|   PPV                                     | 0%                                                          |
| 6. ≥1 trace                               | 6. ≥1 trace                                                 |
|   Culture-positive                        | 3                                                           |
|   Culture-negative                        | 4                                                           |
|   Total                                   | 7                                                           |
|   PPV                                     | 43%                                                         |
| 7. Both negative                          | 7. Both negative                                            |
|   Culture-positive                        | 0                                                           |
|   Culture-negative                        | 117                                                         |
|   Total                                   | 117                                                         |
|   PPV                                     | 100%                                                        |
but low when one sample was positive with grade very low or above while the other was negative, or ≥1 result was trace-positive.

Most individuals with low/low or low/very low combinations of Xpert results tested culture-positive, so we considered whether the six with culture-negative results might be ‘false-negatives’ (online supplemental table S2). In Zambia, one individual reported they were currently on TB treatment, compatible with Xpert identifying MTB DNA but the live bacillary load being zero or very low, and the Xpert result might be a ‘false-positive’. Two individuals reported TB symptoms, had an abnormal X-ray, and the culture result was positive for an NTM, compatible with them having NTM disease or with them having tuberculosis mixed infection with the faster-growing NTM out-competing MTB on culture; the culture result might be a ‘false-negative’.

In South Africa, one individual reported previous TB treatment and had an abnormal X-ray but reported no TB symptoms, the medical officer considered their X-ray consistent with past but not current TB, and the Xpert result might be a ‘false-positive’. Two individuals reported TB symptoms and no previous TB treatment, the pellet of the sputum sample used for culture tested Xpert-negative, the medical officer considered the X-ray suggestive of TB, and the clinical review judged they had TB on the day of the survey; the culture result might be a ‘false-negative’.

Follow-up findings, among individuals with discordant Xpert-Ultra and culture results

There were 19 individuals in Zambian communities with discordant Xpert-Ultra and culture results in the prevalence survey (tables 1, 3 and 4), 16 were traced at follow-up, and information collected for 15.

Among the four individuals who tested culture-positive and Xpert-negative in the TBPS, the culture-positive result was likely correct for all of them (online supplemental table S3).

Among the 11 individuals who tested Xpert-positive (including trace-positive results as positive) but culture-negative, for 7 the Xpert result was plausibly a false-positive (online supplemental table S3). Three of these seven individuals had been on TB treatment at the time of the prevalence survey. Among the other four individuals, all had no or only one TB symptom at the time of the prevalence survey, at follow-up they all reported they had not started TB treatment and that they had no TB symptoms, and for three the X-ray score at follow-up was similar to or lower than in the prevalence survey.

For 4 of the 11 individuals who tested Xpert-positive but culture-negative, the culture-negative result was plausibly a false-negative culture result (ie, it was plausible that the individual had TB but this was not identified on culture testing). Among these four individuals, for three the culture result was culture-positive for an NTM, and for one individual there was no mycobacterial growth on culture (online supplemental table S3).
DISCUSSION

Key findings

Our study confirmed the discordance between Xpert-Ultra and culture results reported by previous studies, and that the PPV of Xpert-positive results is lower among individuals who report previous TB treatment than in those who report no previous treatment.\(^4\)\(^7\)\(^8\) We found that Xpert-Ultra has high sensitivity (around 87% compared with culture) to detect TB in the general adult population in a prevalence survey, if two sputum samples are tested and trace-positive results are considered positive. However, the PPV of Xpert trace-positive results was low (compared with culture), and among individuals who reported they were currently on TB treatment the PPV of Xpert-positive results was 0% (though based on only five individuals).

Considering pairs of Xpert-Ultra results (from S1 and S2), and the grade of each test result, was more informative than simply classifying individuals as Xpert-positive, Xpert-trace-positive or Xpert-negative. Specifically, among individuals who tested Xpert-positive on both of these two sputum samples, with each of them at least low grade and at least one of them medium or high grade, the PPV of Xpert-Ultra was very high (and the ‘false-positive’ rate correspondingly very low). The PPV was also high among individuals with a low grade test result on both sputum samples. On the other hand, the PPV of pairs of Xpert-Ultra results that included very low or trace-positive results, or were discordant (one sample positive, one sample negative), was considerably lower.

We also found evidence of ‘false culture-negative for MTB’ results among individuals with combinations of trace-positive, very low and low grade Xpert-Ultra results. In Zambia, but not in South Africa, most of these ‘culture-negative’ results were culture-positive for an NTM rather than with no mycobacterial growth on culture; for those that were NTM-positive it is possible that the NTM out-competed MTB on culture. For the ‘false culture-negative’ results for which there was no mycobacterial growth on culture (and perhaps also for those that were culture-positive for an NTM), explanations include that the sample contained live MTB bacilli when it was collected but the bacilli died during transportation or culture processing, or that the individual had paucibacillary disease and the (single) sample that was collected for laboratory culture did not (by chance) include any live MTB bacilli.

Consistency with previously reported findings

If anything, the level of discordance we found was less than in previous national TBPS, facilitated by taking two sputum samples for Xpert-Ultra testing and various procedures to try to ensure that culture testing was of high quality. The evidence that we found of some ‘false-negative’ culture results, as well as some ‘false-positive’ Xpert-Ultra results, is consistent with previously suggested explanations for the discordance between Xpert-Ultra and culture results that has been observed repeatedly in national TBPS.

Generalisability of findings and study strengths and limitations

Our key findings were consistent between Zambian and South African communities, and among HIV-negative and HIV-positive individuals, suggesting our findings could have wide generalisability. Our study was large enough to identify important patterns, but it was not large enough to estimate the sensitivity of Xpert-Ultra (using two sputum samples) compared with culture with high precision and additional evidence from other surveys would be valuable.

The proportion of individuals with a likely ‘false-negative’ culture result, among those with pairs of Xpert-Ultra results that were low/low or low/very low, was higher in the South African community compared with the Zambian communities, but this comparison was based on very small numbers. One plausible explanation is that the sputum decontamination procedures used in the South African NHLS laboratory were harsher than those used in the Zambart laboratory, as suggested by the much lower culture contamination rate among the samples from the South African community compared with those from the Zambian communities.

In Zambia, the considerable proportion (27%) of samples whose culture result was ‘culture contaminated’ (which we classified as an ‘invalid’ result and did not include in analyses) was a limitation. The strong association that was observed between the grade of the Xpert test result, and whether the culture result was contaminated, is probably explained by overgrowth by non-mycobacterial organisms being more likely when the amount of mycobacteria in the sample is zero or very low compared with when it is relatively high (one hypothesis is that when the mycobacterial load is (very) low then non-mycobacterial organisms have a more favourable environment in which to out-compete the mycobacteria\(^1\)\(^5\). Culture decontamination methods need to balance being too harsh (and then killing MTB mycobacteria) against being insufficient to achieve a low contamination rate.

Implications of findings

The high sensitivity of Xpert-Ultra, if two sputum samples are collected and trace-positive results are counted as positive, may be high enough to justify using Xpert-Ultra as the primary diagnostic test in prevalence surveys, with culture used only as a confirmatory test. Xpert-Ultra has the advantage, compared with culture, that it can be conducted in the community, with test results on the same day as sample processing, and implemented in a way that is robust and reproducible across settings. Using culture for confirmation of positive Xpert-Ultra results, but not on all sputum-eligible individuals, would massively reduce (by >90%) the number of individuals for whom culture testing is needed. Among the samples that are still collected, there could then be an increased focus on their quality, and on transporting and processing them well.
Our findings indicate that one possible diagnostic algorithm could be to classify an individual as having prevalent TB if they test Xpert-Ultra positive with a grade of low or above on both of 2 sputum samples; and to consider that individuals with other combinations of positive (including trace-positive) Xpert-Ultra results should be confirmed with culture. A second option would be to use culture confirmation as in option 1, but in addition to use culture confirmation for all individuals who have Xpert-Ultra positive (including trace-positive) results and also self-report current or previous TB treatment (online supplemental figure S2). A third option would be to use culture confirmation on all individuals with Xpert-positive (including trace-positive) results.

In our study, only one sputum sample was collected for culture testing. If two samples were collected then there would be two opportunities to identify MTB on culture, the number of individuals with an overall (across two samples) ‘false-negative’ culture result would be reduced, and the performance of culture as a confirmatory test improved.

In the context of active case-finding for TB in the community, our findings show that if Xpert-Ultra alone is used as a diagnostic test then there will be ‘false-positives’, that is, individuals with an Xpert-Ultra positive result who do not have current TB disease. To limit overtreatment for TB, it will be important to take the grade of the Xpert result and clinical information (including on TB treatment history, TB symptoms and X-ray reading) into account when making decisions about referral for TB treatment, and if possible to use culture as a confirmatory test.

CONCLUSION

The sensitivity of Xpert-Ultra can be high in TBPS, if two sputum samples are collected and trace-positive results counted as positive. Following Xpert-Ultra testing of two sputum samples, culture in TBPS could be reserved for participants with discordant, trace-positive, or very low grade Xpert-Ultra results, or with a history of current or previous TB treatment, as a confirmatory test; this would massively reduce the use of culture in such surveys.

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