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Suboptimal specificity of Xpert MTB/RIF among treatment-experienced patients

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To the editor

The Xpert® MTB/RIF assay (Cepheid, Sunnyvale, USA) is strongly recommended by the World Health Organization (WHO) as an initial diagnostic test for treatment-experienced patients of any retreatment category. [1–3] Yet, retreatment TB suspects have been infrequently included in studies of Xpert, [4] likely because current-generation PCR-based tests are unable to determine *M. tuberculosis* viability. [5] Indeed, Xpert is known to frequently remain positive at end of standard short course therapy, [6] with case reports emerging of Xpert false-positivity up to five years post-treatment completion. [7, 8] Further, 56% (n=3485/6285) of specificity data informing the most recent Cochrane meta-analysis [4] was derived from validation and demonstration studies, [9, 10] which may be optimistic due to selection bias related to post-enrolment exclusions. [7] Not surprisingly, there have been increasing calls to clarify guidelines for use of Xpert among treatment-experienced patients. [11]

To address these concerns, we prospectively enrolled individuals with history of prior treatment in a high HIV prevalence, limited resource setting (Harare, Zimbabwe) over a two-year (2011–2013) period. We hypothesized that among individuals with history of prior

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treatment, specificity would be lower than that reported in pooled summaries, and would correlate with mean cycle threshold (i.e., mycobacterial load) and time since prior treatment completion. All participants provided written informed consent, and ethical approval was obtained from the Medical Research Council of Zimbabwe and the UCSF Human Research Protection Program. Notified cases were categorized according to the outcome of their most recent course of treatment [1] as either (1) “recurrent TB” (TB following cure or completion of treatment of a previous TB episode), or (2) “prevalent retreatment TB” (treatment failure (acid-fast bacilli (AFB) sputum smear-positivity at month 5 or later)). The reference standard for *M. tuberculosis* detection was a positive result on solid (Löwenstein-Jensen (LJ) media), liquid (BBL™ MGIT™ Mycobacterial Growth Indicator Tubes (Becton Dickinson, Sparks, MD)), or microscopic-observation drug-susceptibility (MODS) culture (TB MODS Kit™, Hardy Diagnostics, Santa Maria, CA USA). [12, 13] Xpert false detection of active TB (“Xpert false-positive”) was defined as Xpert-positivity in absence of any of the three culture modalities being positive. The Biomedical Research and Training Institute (BRTI) Tuberculosis Laboratory within the National Microbiology Reference Laboratory (NMRL) is a centre for Trials of Excellence in Southern Africa (TESA). The most recent Centre for American Pathologists (CAP) assessment in 2014 demonstrated 100% agreement for isoniazid, rifampicin, ethambutol, and streptomycin resistance testing.

During the study, we enrolled 380 ambulatory retreatment TB cases, representing approximately 65% of all retreatment TB cases notified to the Harare City Health Department during this time period. One hundred forty-nine (43.4%) patients had recurrent TB, and 194 (56.6%) were prevalent retreatment cases. The diagnostic accuracy of Xpert for *M. tuberculosis* detection was evaluated among 149 patients with recurrent TB. Most (n=111/149, 74.5%) had HIV comorbidity with a median CD4 count of 177 (IQR, 83–350). The median time from completion of previous TB treatment to clinical re-presentation and Xpert testing was 19.6 months (IQR, 7.9–62.9 months). One hundred twenty-seven (85.2%) Xpert tests were generation 4. Of the 149 patients included in the analysis, 24 (16.1%) had culture-positive rifampicin (RMP)-resistant tuberculosis; 65 (43.6%) had culture-positive RMP-susceptible tuberculosis; and 60 (40.2%) had clinically defined tuberculosis. The overall sensitivity of Xpert was 92.1% (95% CI 84.5–96.8; n=82/89) (Table). The sensitivity was 97.5% (95% CI 91.4–99.7; n=79/81) for smear- and culture-positive cases and 37.5% (95% CI 8.5–75.5; n=3/8) for smear-negative, culture-positive cases. The sensitivity of Xpert was not significantly affected by HIV status (p=0.22). The false-positive rate among patients with recurrent TB was 13.3% (95% CI 5.9–24.6). Mean (SD) cycle threshold (C_T) was lower for true- versus false-positive results, (21.3 (5.0) vs. 28.0 (5.3) cycles, respectively; p<0.01). HIV co-infection was more common among individuals with false-(100%) rather than true-positive (69.5%; p=0.07) results. In multivariate analysis, mean C_T independently predicted false detection of active TB (p<0.01), though time since prior TB treatment completion (p=0.58) did not. Model accuracy of mean C_T alone was high (area under the curve (AUC), 0.82, 95% CI 0.62–1.0), and each unit increase in C_T (holding time since completion of prior TB treatment fixed) was associated with a 23.0% (95% CI 6.0–42.0) increased risk of Xpert false-positivity.

Approximately 700,000 cases of recurrent TB were notified in 2013, [1] with many-fold higher numbers presenting as recurrent TB suspects. Rapid treatment initiation in clinical

practice and enrolment in clinical trials critically relies on the "rule-in" value of Xpert. Although meta-analyses have documented a high pooled specificity for *M. tuberculosis* detection (99%, 95% CI 90–100), [4] these pooled estimates included a small proportion of treatment-experienced patients. We found that among patients with recurrent TB (history of prior TB treatment), up to one in seven can be expected to be Xpert false-positive for active TB. These results are similar to other studies where stratification of patients by history of prior TB treatment was possible. [14] In our study, higher relative mean cycle threshold predicted false-positivity, with values over 30 (Xpert quantitation result "very low") having a likelihood ratio (LR+) of 5.4 and a specificity of 91% for false-positivity. However, these findings are preliminary and should be examined in larger independently cross-validated cohorts. [15]

Our study has limitations. First, as noted, our sample size precluded intensive cross-validation of the predictive accuracy of covariates for false-positive Xpert determinations. The AUC describes how well models can rank order cases and non-cases, but is not a function of the actual predicted probabilities. Second, our criteria for false detection of active TB (multiple negative reference cultures in the setting of a positive Xpert) would be strengthened by systematic withholding of antituberculosis medications. However, this would be ethically infeasible in a programmatic setting given the imperfect negative predictive value of Xpert and patient immunosuppression. Still, some proportion of "false-positive" Xpert results may have actually been true-positive, as has been noted in other studies from high HIV-burden settings. [16, 17]

In conclusion, patients with history of prior TB are exceptional both for an elevated likelihood of harbouring nonviable organisms and an increased pre-test probability of TB. Within this group, we found that Xpert results with low mean cycle thresholds (Xpert quantitation "high" or greater) are unlikely to be false-positive. Clinicians should consider quantitative cycle threshold in addition to RMP-resistance determination in interpreting Xpert results among retreatment TB suspects.

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Table

Accuracy of Xpert MTB/RIF for *M. tuberculosis* detection among patients with recurrent TB

	Sensitivity			Specificity	PPV	NPV
	All Culture-Positive	Smear-Positive, Culture-Positive	Smear-Negative, Culture-Positive	Culture-Negative	% (95% CI)	% (95% CI)
Correct — no./total no. (%)	82/89 (92.1)	79/81 (97.5)	3/8 (37.5)	52/60 (86.7)	91.1	88.1
95% CI	84.5–96.8	91.4–99.7	8.5–75.5	75.4–94.1	84.5–96.8	77.1–95.1