

# Xpert MTB/RIF Results in Patients With Previous Tuberculosis: Can We Distinguish True From False Positive Results?

Grant Theron,<sup>1,2a</sup> Rouxjeane Venter,<sup>2</sup> Greg Calligaro,<sup>1</sup> Liezel Smith,<sup>1</sup> Jason Limberis,<sup>1</sup> Richard Meldau,<sup>1</sup> Duncan Chanda,<sup>1,3</sup> Aliasgar Esmail,<sup>1</sup> Jonny Peter,<sup>1</sup> and Keertan Dheda<sup>1,4</sup>

<sup>1</sup>Lung Infection and Immunity Unit, Division of Pulmonology and University of Cape Town Lung Institute, Department of Medicine, University of Cape Town, and <sup>2</sup>DST/NRF of Excellence for Biomedical Tuberculosis Research, and MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa; <sup>3</sup>Institute for Medical Research and Training, Lusaka, Zambia; and <sup>4</sup>Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, South Africa

**Background.** Patients with previous tuberculosis may have residual DNA in sputum that confounds nucleic acid amplification tests such as Xpert MTB/RIF. Little is known about the frequency of Xpert-positive, culture-negative (“false positive”) results in retreatment patients, whether these are distinguishable from true positives, and whether Xpert’s automated filter-based wash step reduces false positivity by removing residual DNA associated with nonintact cells.

**Methods.** Pretreatment patients (n = 2889) with symptoms of tuberculosis from Cape Town, South Africa, underwent a sputum-based liquid culture and Xpert. We also compared Xpert results from dilutions of intact or heat-lysed and mechanically lysed bacilli.

**Results.** Retreatment cases were more likely to be Xpert false-positive (45/321 Xpert-positive retreatment cases were false-positive) than new cases (40/461) (14% [95% confidence interval {CI}, 10%–18%] vs 8% [95% CI, 6%–12%]; *P* = .018). Fewer years since treatment completion (adjusted odds ratio [aOR], 0.85 [95% CI, .73–.99]), less mycobacterial DNA (aOR, 1.14 [95% CI, 1.03–1.27] per cycle threshold [*C<sub>T</sub>*]), and a chest radiograph not suggestive of active tuberculosis (aOR, 0.22 [95% CI, .06–.82]) were associated with false positivity. *C<sub>T</sub>* had suboptimal accuracy for false positivity: 46% of Xpert-positives with *C<sub>T</sub>* > 30 would be false positive, although 70% of false positives would be missed. *C<sub>T</sub>*’s predictive ability (area under the curve, 0.83 [95% CI, .76–.90]) was not improved by additional variables. Xpert detected nonviable, nonintact bacilli without a change in *C<sub>T</sub>* vs controls.

**Conclusions.** One in 7 Xpert-positive retreatment patients were culture negative and potentially false positive. False positivity was associated with recent previous tuberculosis, high *C<sub>T</sub>*, and a chest radiograph not suggestive of active tuberculosis. Clinicians may consider awaiting confirmatory testing in retreatment patients with *C<sub>T</sub>* > 30; however, most false positives fall below this cut-point. Xpert can detect DNA from nonviable, nonintact bacilli.

**Keywords.** tuberculosis; diagnosis; Xpert; false positivity.

Xpert MTB/RIF (Xpert; Cepheid) is an automated nucleic acid amplification test (NAAT) for *Mycobacterium tuberculosis* and rifampicin resistance [1–3], endorsed by the World Health Organization and the US Food and Drug Administration [4, 5]. Xpert is increasingly deployed in many countries as the initial diagnostic test for tuberculosis [6].

Xpert is used routinely in patients who have previously had tuberculosis [6, 7]. This is despite evidence that approximately 30% of patients who are microbiologically cured after 6 months of treatment are Xpert positive [8], a proven correlation between retreatment status and diminished specificity [9–11], and

several case reports detailing false-positive (FP) Xpert results in retreatment cases [12–14]. Detectable mycobacterial DNA, which can be extracellular or associated with nonintact cells (and hence is not culturable), is a possible cause of this false positivity, which may trigger unwarranted treatment and unnecessarily expose patients to toxic drugs, delay establishing the correct underlying diagnosis and its appropriate treatment, and escalate healthcare costs. Although the manufacturer recommends that Xpert always be used in conjunction with culture [15], culture capacity is not mandatory for Xpert’s use in the field [7] and, even in high-burden countries such as South Africa that do have culture capacity, most Xpert-positive patients do not receive culture, as per the national algorithm [16].

More than 700 000 patients with a history of tuberculosis were diagnosed in 2013 [17]; however, there are limited data about the frequency of Xpert false positivity in retreatment patients [18] and what factors, if any, may guide clinical practice [10]. We therefore examined the relationship between Xpert results (including *M. tuberculosis* complex-specific quantitative information), routinely collected clinical information, and

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<sup>a</sup>Present affiliation: DST/NRF of Excellence for Biomedical Tuberculosis Research, and MRC Centre for Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa.

Correspondence: K. Dheda, University of Cape Town, H47 Old Main Bldg, Groote Schuur Hospital, Observatory 7925, South Africa (keertan.dheda@uct.ac.za).

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culture results in a large cohort of patients evaluated for tuberculosis in the high-burden, high-human immunodeficiency virus (HIV) setting of Cape Town, South Africa. To interrogate claims that Xpert does not detect free DNA because of a preamplification wash step [19–22], which would potentially reduce the risk of an FP result in retreatment cases, we performed a laboratory-based substudy to ascertain whether Xpert can detect DNA from lysed nonviable cells.

## METHODS

### Patient Recruitment

We analyzed data from 3166 patients who had symptoms suggestive of tuberculosis. Patients were recruited from primary care clinics or hospitals in Cape Town, South Africa, as part of studies that evaluated the utility of Xpert. Patients included in the final analysis were Xpert positive and had cycle threshold ( $C_T$ ) data, were either culture positive or negative, had a known previous tuberculosis status, had not been on treatment for >48 hours, and had not taken antituberculosis treatment 60 days prior to testing. This study was approved by the University of Cape Town Faculty of Health Sciences Ethics Committee.

### Diagnostic Tests

Two paired sputum specimens were collected at recruitment; 1 was randomly selected for an Xpert test, and the other was used for a BACTEC MGIT 960 liquid culture (Becton, Dickinson, and Co). If patients were unable to expectorate sputum, sputum induction with hypertonic saline was performed. Tuberculosis morbidity score [23] data and chest radiograph (CXR) data were collected in a subset of patients, as determined by the parent protocol.

### Xpert MTB/RIF Cell Lysis Experiment

To assess whether Xpert detected nonviable cells, 1 mL of *M. tuberculosis* H37Rv in phosphate-buffered saline and 0.25% Tween 80 (10 000, 1000, 500, and 0 colony-forming units [CFU] mL<sup>-1</sup>) was added to Xpert sample buffer (2:1 ratio) and, after 15 minutes of incubation with intermittent shaking, 2 mL was added to the Xpert cartridge (direct Xpert). In parallel, a 1.5-mL aliquot of each concentration underwent heat treatment (80°C, 1 hour), followed by mechanical disruption using Lysing Matrix B tubes (0.1 mm zirconium beads; MP Biochemicals) and a Fast Prep-24 machine (MP Biochemicals) (6.5 meters per second for three 30-second intervals with 1 minute resting on ice between intervals). After bead-beating, the lysate was allowed to settle for 2 minutes and 1 mL of supernatant was used for Xpert (lysed Xpert). Ten 10- $\mu$ L aliquots of each dilution (direct and lysed) were plated on Middlebrook 7H10 agar supplement with oleic acid albumin dextrose complex and incubated for 6 weeks at 37°C to check for viability. This experiment was performed in triplicate.

### Statistical Analysis

Xpert-positive, culture-positive patients were defined as true positive (TP) and Xpert-positive, culture-negative patients

were defined as false positive (FP). The  $\chi^2$  test was used for comparisons between proportions. The Mann–Whitney test was used to compare differences in nonparametric continuous data. Multivariable logistic regression was performed to adjust for potential confounding. A backward elimination strategy using the likelihood ratio test was used to finalize each model. Analyses were performed using GraphPad Prism version 6.0 (GraphPad Software) and Stata version 13 (StataCorp) software. All statistical tests are 2-sided at  $\alpha = .05$ .

## RESULTS

Of the 3166 patients, we excluded 263 (8%) patients (73 had did not have a positive- or negative-culture result, 86 were on treatment >48 hours, 104 Xpert-positive patients were missing  $C_T$  data, and 14 were missing data on their previous tuberculosis history). Of the remaining 2889 patients with a known culture status, 837 (29%) were culture positive and 782 (27%) were Xpert positive. A total of 1220 (42%) patients were retreatment cases. A summary of the demographic and clinical characteristics of the cohort is shown according to previous tuberculosis status in Table 1. Retreatment patients were more likely to be older and HIV-infected. Differences in Xpert  $C_T$  and years since completion of previous antituberculosis treatment in new and retreatment patients are shown in Figure 1.

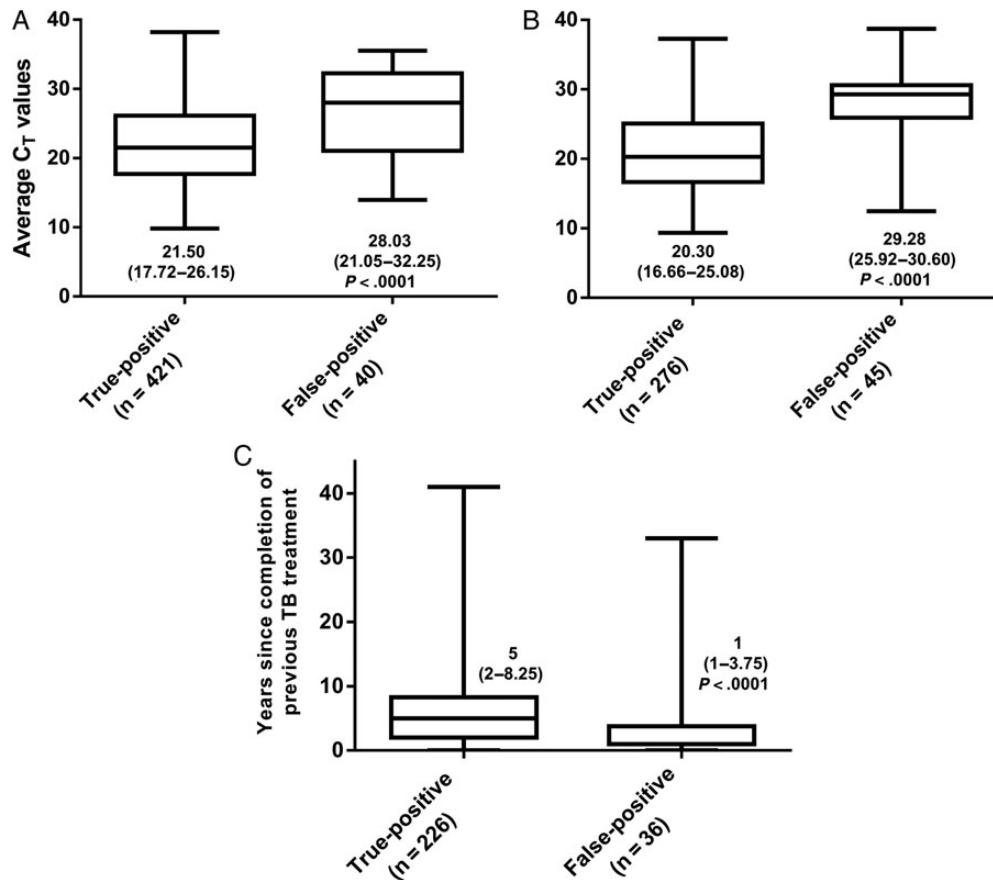
### Xpert MTB/RIF False Positivity in Patients With Newly Diagnosed Tuberculosis

Xpert had a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 83% (95% confidence interval [CI], 80%–86%), 97% (95% CI, 95%–98%), 91% (95% CI, 88%–94%), and 93% (95% CI, 91%–94%), respectively, in new patients. Forty of 461 (9%) Xpert-positive results were FP.

**Table 1. Demographic and Clinical Characteristics of Patients Included in the Analysis Who Had Symptoms Suggestive of Tuberculosis**

Characteristic	No Previous TB (n = 1669)	Previous TB (n = 1220)	P Value
Demographic characteristics			
Female sex, No. (%)	773/1617 (48)	548/1181 (46)	.463
Age, y, median (IQR)	36 (28–46)	39 (32–49)	<.001
Clinical characteristics			
HIV-infected, No. (%)	695/1617 (42)	623/1198 (53)	<.001
TB morbidity score, median (IQR)	5 (3–6)	5 (3–6)	.654
Test characteristics			
Culture-positive, No. (%)	506/1669 (30)	331/1220 (27)	.062
Time-to-positivity, d, median (IQR)	12 (8–17)	13 (8–17)	.352
Xpert MTB/RIF-positive, No. (%)	461/1669 (28)	321/1220 (26)	.434
$C_T$ , median (IQR)	21.20 (17.92–26.64)	22.00 (17.23–27.06)	.309
CXR compatible with active TB, No. (%)	402/928 (43)	329/708 (46)	.204

Abbreviations:  $C_T$ , cycle threshold; CXR, chest radiograph; HIV, human immunodeficiency virus; IQR, interquartile range; TB, tuberculosis.



**Figure 1.** Box-and-whisker plot comparison of Xpert MTB/RIF quantitative information (cycle threshold [ $C_T$ ] values) in true-positive (Xpert-positive, culture-positive) and false-positive (Xpert-positive, culture-negative) specimens for new (A) or retreatment (B) patients, and a comparison of the years since the completion of previous tuberculosis (TB) treatment in Xpert-positive retreatment patients according to culture status (C). Median values with interquartile ranges in parentheses are shown.

### Correlates of Xpert MTB/RIF False Positivity

New tuberculosis patients with an FP Xpert were, compared to those who were TP, more likely to have higher median Xpert  $C_T$  (28.03 [interquartile range {IQR}, 21.20–32.23] vs 21.50 [IQR, 17.72–26.10];  $P < .001$ ) and more likely to be female (25/40 [63%] of FP cases were women vs 187/414 [45%] of TP cases; odds ratio [OR], 2.02 [95% CI, 1.04–3.95]). In a multivariate logistic regression analysis (Table 2), each unit increase in  $C_T$  was associated with a 14% increase in the relative risk (adjusted OR [aOR], 1.14 [95% CI, 1.08–1.21]) of Xpert false positivity, presuming the other variables held constant. Morbidity in patients with tuberculosis symptom score data ( $n = 168$ ) was similar in those with an FP or TP Xpert result (median, 4 [IQR, 2–6] vs 4 [IQR, 4–5];  $P = .389$ ).

Where CXR data ( $n = 193$ ) were available, FP patients were less likely to have a CXR compatible with active tuberculosis than TP patients (9/19 [47%] vs 144/174 [83%];  $P = .001$ ) and, when included in a multivariable logistic regression model with previous tuberculosis and  $C_T$ , a CXR compatible with active tuberculosis was associated with a 79% reduction

in the relative odds of Xpert false positivity (aOR, 0.21 [95% CI, .08–.57]; [Supplementary Table 2](#)).

### Receiver Operating Characteristic Curve Analyses

The area under the curve (AUC) for  $C_T$  (0.70 [95% CI, .61–.80]) did not increase when CXR (0.78 [95% CI, .71–.86]) was included (Figure 2) (receiver operating characteristic [ROC] curves for  $C_T$  only, and not those who also had CXR data, are shown in [Supplementary Figure 1](#)). At a rule-out cut-point ( $C_T > 14.22$ ) for Xpert false positivity (selected based on 95% sensitivity; ie, 95% of the 40 FP Xperts fell above this cut-point),  $C_T$  alone had a specificity, NPV and negative likelihood ratio (LR) of 6%, 93%, and 0.78, respectively (Table 3). At a rule-in cut-point (selected based on 95% specificity) of  $>32.19$ ,  $C_T$  had a sensitivity, PPV, and positive LR of 3%, 33%, and 5.5, respectively. At a cut-point ( $C_T > 27.08$ ) corresponding to Youden index,  $C_T$  had a sensitivity, specificity, NPV, PPV, positive LR, and negative LR of 55%, 80%, 21%, 95%, 2.79, and 0.56, respectively.

### Xpert MTB/RIF False Positivity in Retreatment Tuberculosis Patients

Xpert had a sensitivity, specificity, PPV, and NPV of 84% (95% CI, 79%–87%), 94% (95% CI, 93%–96%), 85% (95% CI, 82%–

**Table 2. Factors Associated With Xpert False Positivity in New and Retreatment Cases**

	Univariate Analysis				Multivariate Logistic Regression	
	True-Positive Xpert (n = 421)	False-Positive Xpert (n = 40)	OR (95% CI)	P Value	Adjusted OR (95% CI)	P Value
<b>New TB Patients (n = 461)</b>						
Demographic variables						
Age, y, median (IQR)	33 (27–43)	32 (25–44)	1.00 (.97–1.02)	.780	...	...
Female, No. (%)	187/414 (45)	25/40 (63)	2.02 (1.04–3.95)	.039	...	...
Smoker, No. (%)	125/347 (36)	13/30 (43)	1.36 (.64–2.89)	.427	...	...
Clinical variables						
HIV-infected, No. (%)	162/404 (58)	21/39 (50)	1.74 (.90–3.37)	.099	...	...
Xpert information						
TB-specific C <sub>T</sub> values, median (IQR)	21.50 (17.72–26.10)	28.03 (21.20–32.23)	1.14 (1.07–1.21)	<.001	1.14 (1.08–1.21)	<.001
<b>Retreatment Patients (n = 321)</b>						
Demographic variables						
Age, y, median (IQR)	37 (30–45)	41 (21–48)	1.03 (1–1.07)	.030	...	...
Female, No. (%)	108/274 (39)	15/42 (36)	1.17 (.60–2.30)	.647	...	...
Smoker, No. (%)	100/229 (44)	18/32 (56)	1.66 (.79–3.50)	.184	...	...
Clinical variables						
HIV-infected, No. (%)	122/273 (45)	20/43 (47)	1.08 (.57–2.05)	.823	...	...
Previous TB treatment not completed, No. (%)	55/239 (23)	26/39 (26)	1.15 (.53–2.51)	.719	...	...
Years since previous TB treatment stopped or completed, median (IQR)	2 (0–5)	1 (0–1)	0.92 (.85–.99)	.033	0.91 (.84–.99)	.048
Xpert information						
TB-specific C <sub>T</sub> values, median (IQR)	20.30 (16.71–25.05)	29.28 (26.18–30.60)	1.27 (1.18–1.37)	<.001	1.25 (1.15–1.35)	<.001

Versions of this table for all patients (Supplementary Table 1) or restricted to the subset of patients with chest radiographic data (Supplementary Table 2) are provided in the Supplementary Data. Abbreviations: CI, confidence interval; C<sub>T</sub>, cycle threshold; HIV, human immunodeficiency virus; IQR, interquartile range; OR, odds ratio; TB, tuberculosis.

90%), and 94% (95% CI, 92%–95%) in retreatment patients. Forty-five of 321 (14%) Xpert-positive results were false positive ( $P = .018$  compared to new cases).

#### Correlates of Xpert MTB/RIF False Positivity

Although retreatment patients with an FP Xpert were older than those with a TP Xpert in a univariate analysis (Median [IQR], 41 [21–48] vs 37 [30–45] years;  $P = .030$ ), after multivariable adjustments were performed, only C<sub>T</sub> (aOR, 1.25 [95% CI, 1.15–1.35];  $P < .001$ ) and the number of years since stopping treatment for the previous episode of tuberculosis (aOR, 0.91 [95% CI, .84–.99];  $P = .048$ ) were independent predictors of Xpert FP (Table 2). There was no correlation between C<sub>T</sub> and years since stopping treatment for previous tuberculosis ( $P = .427$ ; Supplementary Figure 2). When radiographic data were available, a CXR compatible with active tuberculosis was also an independent predictor of Xpert false positivity (aOR, 0.22 [95% CI, .06–.82]; Supplementary Table 2).

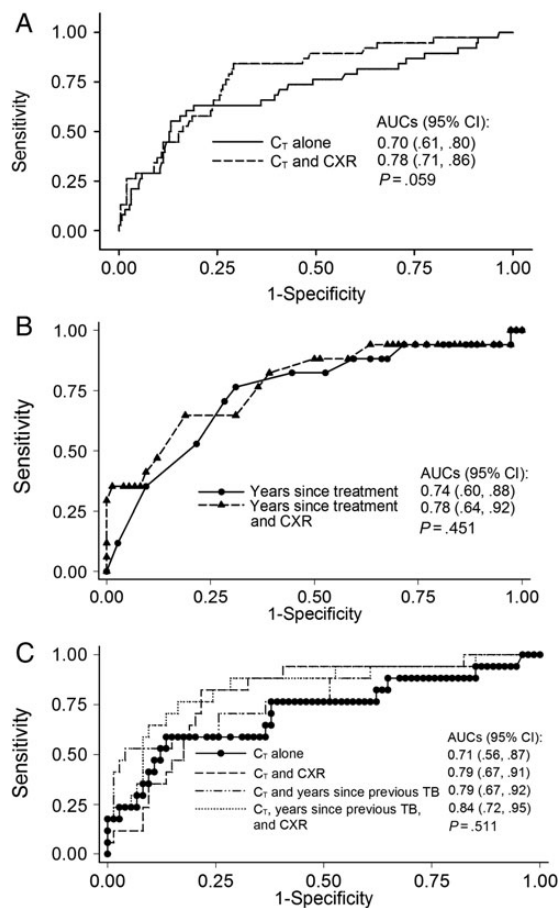
#### Receiver Operating Characteristic Curve Analyses

ROC curve AUCs of 0.83 (95% CI, .76–.90), 0.83 (95% CI, .75–.91), 0.78 (95% CI, .79–.86), and 0.84 (95% CI, .72–.95) were obtained for C<sub>T</sub> alone, a model incorporating C<sub>T</sub> and the number of years since stopping treatment, a model

incorporating C<sub>T</sub> and CXR, and a model incorporating all 3 variables, respectively (Figure 2). C<sub>T</sub> had, at a cut-point for ruling out Xpert false positivity (selected based on 95% sensitivity; ie, 95% of the 45 FP Xperts in retreatment patients fell above this cut-point), a specificity, NPV, and negative LR of 36%, 98%, and 0.12, respectively (cut-point >18.28), whereas at a rule-in cut-point (selected based on 95% specificity), it had a sensitivity, PPV, and positive LR of 27%, 46%, and 5.26, respectively (cut-point >30.56) (Table 3). At a cut-point (>28.36) corresponding to Youden index, C<sub>T</sub> had a sensitivity, specificity, NPV, PPV, positive LR, and negative LR of 64%, 90%, 52%, 94%, 6.59, and 0.39, respectively.

#### Detection of DNA From Nonviable Bacilli

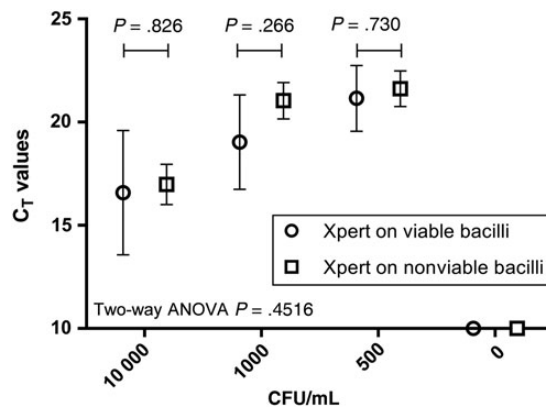
Each dilution of bacilli (10 000, 1000, and 500 CFU mL<sup>-1</sup>) was detected as positive when Xpert was done directly or on lysate. Similar C<sub>T</sub>s (SEM) were obtained (direct vs lysed): 16.58 (0.70) vs 16.98 (0.98;  $P = .826$ ), 19.03 (0.53) vs 21.04 (0.88;  $P = .266$ ), and 21.15 (0.37) vs 21.62 (0.86;  $P = .730$ ) for the 10 000, 1000, and 500 CFU mL<sup>-1</sup> dilutions, respectively (Figure 3). The 0 CFU mL<sup>-1</sup> dilutions were undetected. After 6 weeks of incubation, each aliquot used for direct Xpert grew the expected number of CFUs, whereas no growth was observed from the aliquots of heat inactivated, bead-beaten bacilli.



**Figure 2.** Receiver operating characteristic curves of Xpert MTB/RIF cycle threshold values and clinical information for the prediction of Xpert MTB/RIF false positivity in new (A) or retreatment (B and C) patients. Abbreviations: AUC, area under the curve; CI, confidence interval;  $C_T$ , cycle threshold; CXR, chest radiograph; TB, tuberculosis.

## DISCUSSION

Our key findings are as follows: (1) patients with an FP Xpert are more likely to have previous tuberculosis (and to have had this more recently), low mycobacterial DNA load (measured by  $C_T$ ), and a CXR not compatible with active tuberculosis; (2)



**Figure 3.** Comparison of Xpert MTB/RIF cycle threshold values (mean  $\pm$  SEM) from a dilution series of bacilli, showing similar  $C_T$  when Xpert MTB/RIF was performed on intact bacilli or nonviable, heat- and mechanically-lysed bacilli. Three experimental replicates were performed. Abbreviations: ANOVA, analysis of variance; CFU, colony-forming units;  $C_T$ , cycle threshold; SEM, standard error of the mean.

about 1 in 7 Xpert-positive results in retreatment patients will be FP; (3)  $C_T$  predicts false positivity, but has suboptimal discriminatory power (a specificity of 10% at a rule-out cut-point [95% sensitivity], and a sensitivity of 20% at a rule-in cut-point [95% specificity]) that is not enhanced by the incorporation of additional variables; (4) using a cut-point of  $C_T > 30$  in retreatment patients, 7 of 10 FP cases will be missed; however, about half of the patients falling above this cut-point will be FP; and (5) Xpert detects DNA from nonviable cells that are not intact, thereby suggesting that free DNA—and not just DNA from intact cells—is detected by Xpert.

Early evaluations of Xpert [25] contributed the majority of data to meta-analyses of test accuracy [26, 27]; however these studies excluded patients who were culture-negative and treated based on symptoms (including many Xpert-positive patients), despite the known poor specificity of empirical treatment [28, 29]. This led to calls that Xpert's specificity might be overestimated, especially in retreatment cases [12, 17]. A reanalysis of the pooled data found that, when these early evaluations were excluded, no significant change in specificity occurred; however,

**Table 3. Accuracy of Cycle Threshold Values for Predicting Xpert MTB/RIF False Positivity in New and Retreatment Patients**

Test Use	Suggested $C_T$ Cut-point	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
<b>New patients (n = 461)</b>							
Rule-in	>32.19	3 (2–4)	95 (92–97)	34 (19–54)	93 (90–95)	5.51 (2.87–10.60)	0.76 (.63–.92)
Rule-out	>14.22	95 (82–99)	6 (4–9)	9 (6–12)	93 (76–99)	1.02 (.94–1.09)	0.78 (.19–3.22)
Youden index <sup>a</sup>	>27.08	55 (39–70)	80 (76–84)	21 (14–30)	95 (92–97)	2.79 (1.99–3.92)	0.56 (.40–.79)
<b>Retreatment patients (n = 321)</b>							
Rule-in	>30.56	27 (15–42)	95 (91–97)	46 (27–66)	89 (85–92)	5.26 (2.60–10.63)	0.77 (.65–.92)
Rule-out	>26.80	95 (84–99)	36 (30–42)	20 (15–26)	98 (92–100)	1.39 (1.33–1.66)	0.12 (.03–.49)
Youden index <sup>a</sup>	>28.36	64 (49–78)	90 (86–93)	52 (38–65)	94 (90–96)	6.59 (4.33–10.01)	0.39 (.27–.58)

Abbreviations: CI, confidence interval;  $C_T$ , cycle threshold; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

<sup>a</sup> Defined as the best compromise between sensitivity and specificity assuming equal weighting [24].

when patients' history of previous tuberculosis was included as a covariate, a trend between an increased prevalence of retreatment cases and diminished specificity existed [9].

The specificity of Xpert in retreatment cases in our study was 95% (95% CI, 93%–96%), indicating that 1 in 20 culture-negative patients will be FP by Xpert. This is less than the specificity reported in meta-analyses that included (99% [95% CI, 98%–99%]) [9] or excluded (98% [95% CI, 97%–99%]) data from the initial Xpert validation studies [25]. Our specificity is also less than that reported previously in retreatment cases in South Africa (99% [95% CI, 98%–100%]) [11], but higher than that seen among retreatment cases in Harare (87% [95% CI, 75%–94%]) [10]. As suggested by others [10, 11], our study indicates that about 1 in 7 Xpert-positive retreatment patients will be FP. In settings such as Cape Town, South Africa, where approximately 1 in 4 tuberculosis notifications are retreatment cases (approximately 7500 per annum) [30], this represents a potentially large public health problem.

We found  $C_T$  to differentiate poorly between TP or FP Xpert results. For example, at an optimized rule-out cut-point ( $C_T > 26.80$ ; 95% sensitivity) in retreatment patients, only a third of true-positive patients would be correctly classified, and only 1 in 5 FP Xpert results would be correctly classified. Conversely, 70% of FP cases would be missed at an optimized rule-out cut-point ( $C_T > 30.56$ ; 95% specificity), and less than half of the Xpert-positive results with  $C_T$  above this cut-point would be correctly classified as FP. Although suboptimal for use in routine clinical practice, this result suggests that clinicians should be cautious in interpreting Xpert-positive results in retreatment patients with  $C_T > 30$ , and that they may wish to await the results of confirmatory culture-based testing before starting treatment. This study, as well as others that have demonstrated  $C_T$  to be a useful proxy of bacterial load [31–33] and infectiousness [34–36], suggests that laboratories should consider routinely reporting these values.

Our study is the first to describe an inverse association between Xpert FP results and the time since previous treatment was stopped, and the utility of CXR in discriminating Xpert TP from FP patients. Although these tools reduced the odds of an FP result, they did not, unfortunately, improve upon the relatively poor discriminatory ability of  $C_T$  alone. This is because several TP patients had recently been treated for active tuberculosis (which is reflective of our high transmission setting), or had a CXR not suggestive of active tuberculosis.

As Xpert does not detect DNA from nontuberculous mycobacteria [37], almost all positive results likely reflect the true detection of *M. tuberculosis* complex DNA [32]; however, this does not always correspond to the presence of active disease caused by viable, intact bacilli. Our research shows that the on-board sample processing system of Xpert is unable to remove genomic DNA from nonintact, nonviable cells, which may be present in retreatment cases. This is likely the mechanism by which Xpert FP occurs, and suggests that Xpert's

automated mechanism to isolate intact bacilli prior to DNA extraction requires optimization if false positivity due to the detection of extracellular DNA or DNA in nonintact, nonviable cells is to be minimized. Notably, a study observed Xpert to effectively remove large numbers of amplicons in spiked sputum, preventing detection [37]; however, unlike our study, this earlier work used free DNA of low molecular weight.

Our study has limitations. Other causes of Xpert false positivity include variations in specimen quality and bacterial load in the different samples used for Xpert and culture, and the overlapping stochastic limits of detection of these 2 tests, which can cause false-negative reference standard results (and hence false-positive Xpert results, which may be minimized by repeated cultures). Culture itself is an imperfect reference standard with incomplete sensitivity, although it is used widely in both clinical practice and research. Several factors may underpin this incomplete sensitivity including sampling error, differential immune reactivity in retreatment cases, and technical reasons, among others. Furthermore, we lacked long-term systematic clinical outcome data to incorporate into a reference standard; however, this lacks specificity as empiric overtreatment is frequent in high-burden settings [28, 38, 39], patients without tuberculosis can still improve when on antituberculosis treatment, and patients with tuberculosis and a concomitant infection (eg, *Pneumocystis*) can still fail to improve. Laboratory error and sample cross-contamination are, as always, potential sources of error; however, Xpert is a closed system that generates few aerosols [40], and we performed Xpert in a quality-assured laboratory separate to that used for culture. Finally, it should also be noted that these findings, which are from a high-burden setting with a high intensity of transmission and where retreatment tuberculosis is relatively common, should undergo further validation, especially in different settings.

In summary, patients with a history of tuberculosis, more recent previous tuberculosis, and a CXR incompatible with active tuberculosis are at a higher risk of Xpert false positivity; however, these do not add discriminatory power over and above  $C_T$  alone. Although most FP cases would be missed, clinicians should treat  $C_T > 30$  in retreatment cases with caution. Further investigation is needed to discriminate NAAT FP patients from TP patients, including research into technologies that exclude DNA from nonintact cells (such as propidium monoazide or ethidium monoazide staining [41, 42]) or detect messenger RNA in live bacilli [43]. This is important as next-generation NAATs, such as Xpert Ultra, will purportedly have a sensitivity approaching that of culture [44], and hence be more likely to detect low quantities of residual tuberculosis DNA and have poor specificity in patients who have previously had tuberculosis.

### Supplementary Data

Supplementary materials are available at <http://cid.oxfordjournals.org>. Consisting of data provided by the author to benefit the reader, the posted

materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

## Notes

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