MAJOR ARTICLE

Xpert MTB/RIF Testing in a Low Tuberculosis Incidence, High-Resource Setting: Limitations in Accuracy and Clinical Impact

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(See the Editorial Commentary by Salfinger on pages 977–9.)

Background. Xpert MTB/RIF, the first automated molecular test for tuberculosis, is transforming the diagnostic landscape in low-income countries. However, little information is available on its performance in low-incidence, high-resource countries.

Methods. We evaluated the accuracy of Xpert in a university hospital tuberculosis clinic in Montreal, Canada, for the detection of pulmonary tuberculosis on induced sputum samples, using mycobacterial cultures as the reference standard. We also assessed the potential reduction in time to diagnosis and treatment initiation.

Results. We enrolled 502 consecutive patients who presented for evaluation of possible active tuberculosis (most with abnormal chest radiographs, only 18% symptomatic). Twenty-five subjects were identified to have active tuberculosis by culture. Xpert had a sensitivity of 46% (95% confidence interval [CI], 26%–67%) and specificity of 100% (95% CI, 99%–100%) for detection of *Mycobacterium tuberculosis*. Sensitivity was 86% (95% CI, 42%–100%) in the 7 subjects with smear-positive results, and 28% (95% CI, 10%–56%) in the remaining subjects with smear-negative, culture-positive results; in this latter group, positive Xpert results were obtained a median 12 days before culture results. Subjects with positive cultures but negative Xpert results had minimal disease: 11 of 13 had no symptoms on presentation, and mean time to positive liquid culture results was 28 days (95% CI, 25–47 days) compared with 14 days (95% CI, 8–21 days) in Xpert/culture-positive cases.

Conclusions. Our findings suggest limited potential impact of Xpert testing in high-resource, low-incidence ambulatory settings due to lower sensitivity in the context of less extensive disease, and limited potential to expedite diagnosis beyond what is achieved with the existing, well-performing diagnostic algorithm.

Keywords. tuberculosis; diagnostics; molecular testing; point-of-care.

The Xpert MTB/RIF assay ("Xpert"; Cepheid, Sunnyvale, California) is an automated nucleic acid amplification test for sputum specimens that can detect both *Mycobacterium tuberculosis* and rifampin resistance within 2 hours, and requires minimal hands-on time. When tested in high-incidence settings, usually with spontaneously expectorated sputum, Xpert is highly accurate (sensitivity of 88%, specificity of 98%) [1]. Due to its excellent performance characteristics, Xpert is transforming the diagnostic landscape in the developing world and is now used in >80 countries [2].

Xpert has also been recently approved by the US Food and Drug Administration (FDA) and Health Canada [3]. Nevertheless, it is conceivable that important factors in evaluating the performance characteristics of the test, such as patient population, stage of disease, methods

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for obtaining sputum sample (spontaneous vs induced), and accuracy of routine smear and culture tests, may differ between high- and low-resource settings. Yet, the performance of the test has not been studied in routine use in high-resource, tertiary-care settings with low incidence of tuberculosis [4, 5]. It is therefore critical to generate evidence on whether existing data and policies are transferrable to these settings [2].

In Canada, the current tuberculosis incidence is 4.6 per 100 000 population, with two-thirds of cases among immigrants [6]. Most pulmonary tuberculosis disease in Canada (as in other low-incidence settings) is smear negative (66%), and therefore diagnosed only by liquid culture–based techniques that typically take 2–3 weeks to provide a result [7]. Delays in diagnosis and treatment can increase patient morbidity and mortality [8, 9]. Although smear-negative cases are less infectious than smear-positive cases, they may account for up to one-fifth of all secondary transmission [10, 11]. Furthermore, the suspicion of tuberculosis has economic and resource implications for the healthcare system, as patients may be hospitalized for respiratory isolation while undergoing the relevant investigations.

The Xpert assay may enhance accurate and rapid detection, as it can detect up to 67% of smear-negative cases [1]. In addition, it might be suitable for use at the point of care as the test's sample reagent has potent tuberculocidal properties, thus largely eliminating biosafety concerns [12]. With the use of Xpert at the point of care and the availability of results within hours, patients can potentially be diagnosed with tuberculosis at their first visit, which would conceivably shorten the time to treatment and reduce transmission. However, there are limited data on the use of Xpert at the point of care, outside of laboratories [13, 14].

With this study, we aim to improve the understanding of the accuracy and the potential impact of Xpert in a low-incidence, high-resource setting.

METHODS

Study Participants

Consecutive patients aged ≥18 years, referred to the Montreal Chest Institute Tuberculosis Clinic for evaluation of suspected active pulmonary tuberculosis, were recruited. The institutional review board of the McGill University Health Centre, Montreal, Canada, approved the study.

Specimen Collection/Processing

Sputum samples, induced using 3% hypertonic saline solution and an ultrasonic nebulizer, were collected from all patients with possible/suspected pulmonary tuberculosis. Two samples were obtained from all consenting patients on the day of enrollment. The first sample was processed in standard fashion in the clinical microbiology laboratory, including smear (Auramine O method) and liquid culture (Mycobacterium Growth Indicator Tube, Becton Dickinson). The second sample was used for Xpert testing. Two additional samples were collected for smear and culture.

The Xpert test was performed at the tuberculosis clinic according to the standard protocol for unprocessed samples, per the manufacturer [15]. Further information is available in Supplementary Appendix A and Supplementary Figure E1.

When we began the study, Xpert had been endorsed by the World Health Organization [16]. Approval of the test by Health Canada followed in 2012. Because Xpert was done outside the hospital-approved clinical lab, test results obtained as part of the study were not made available for clinical decision making. However, following approval of the test by Health Canada (after enrollment of 394 subjects), the microbiology laboratory was alerted of any positive Xpert result and a conventional nucleic acid amplification test (NAAT) (Cobas TaqMan MTB, Roche Diagnostics, Switzerland) was performed. Of note, clinical laboratory protocol is to perform the NAAT on all smear-positive specimens and mycobacterial isolates growing on culture; it is performed only by request on smear-negative specimens.

The reference standard was liquid culture on 3 processed samples, followed by phenotypic culture-based drug susceptibility testing (DST) at the provincial reference laboratory [17]. For all discrepant results (ie, rifampin resistant on Xpert but susceptible on DST), sequencing of the *rpoB* gene was performed (Supplementary Appendix B). From January 2012 onward, all *M. tuberculosis* isolates were also routinely typed using mycobacterial interspersed repetitive units typing. From these data, we determined that 1 positive culture result (Xpert negative) could have been due to cross-contamination in the laboratory and therefore was excluded from all analyses.

To assess the limit of detection of Xpert and the potential impact of hypertonic saline on the performance of Xpert, we added bacille Calmette-Guerin (BCG) at concentrations of 250 colony-forming units (CFU)/mL (n = 6), 125 CFU/mL (n = 8), 62 CFU/mL (n = 8), and 31 CFU/mL (n = 10) to normal and 3% hypertonic saline, then submitted these samples for Xpert sample preparation and testing as above.

Statistical Analysis

All data were collected and entered into a database by one of the study authors (A. D. A.). Another study author (C. M. D.) cross-checked a subset of data. The analysis was done using Stata/SE 12.0 software (StataCorp, College Station, Texas).

We calculated sensitivity, specificity, and exact binomial confidence intervals of Xpert compared to the culture reference standard. We assessed the accuracy of rifampin resistance testing on Xpert compared with culture-based DST.

We assessed clinical impact of all diagnostic methods by examining the interval from procuring the first sample to obtaining the relevant diagnostic result. Furthermore, we obtained the

time from first sputum collection to treatment initiation and the days of empiric treatment given prior to culture confirmation. We compared this with the time when the Xpert result would have been available to the physician, if results had been shared (Supplementary Figure E2).

We used Standards for the Reporting of Diagnostic Accuracy [18, 19] for reporting the study results.

RESULTS

Between October 2011 and May 2013, we enrolled 502 consecutive patients who presented to the tuberculosis clinic for evaluation of possible active tuberculosis (Supplementary Appendix C). The median age of subjects was 44 years (interquartile range [IQR], 31–61 years) and 44% were female (Table 1). Persons referred for immigration-related screening constituted the largest number of subjects (294 [59%]), and only 93 subjects were born in Canada (18.5%). Many (44%) were born in countries with very high tuberculosis prevalence (>100/100 000). Only 12 subjects were infected with human immunodeficiency virus, and 15 subjects had other immunocompromising comorbidities (5%). A history of prior active tuberculosis was reported by 111 subjects (22%).

A sizeable fraction of subjects were referred for evaluation in the context of an immigration screen that yielded a chest radiograph with a possible tuberculosis-related abnormality (22%; Table 2). Others were contacts of active tuberculosis cases, who had positive tuberculin skin tests (5%). Only 18% had symptoms suggestive of active tuberculosis (ie, fever, cough, night sweats, weight loss) and overall, 74% had an abnormal chest radiograph (19% of these with findings highly suggestive of active tuberculosis, ie, cavitation and/or apical fibronodular disease; Table 2).

Twenty-five subjects were identified to have active culture-confirmed tuberculosis. Eleven subjects had smear positive results, but only 7 of these were identified to have *M. tuberculosis* disease (3 had nontuberculous mycobacteria and 1 was had false-positive results [culture/NAAT negative]).

Noninterpretable Results on Xpert

Noninterpretable results were obtained in 44 (8.8%) samples overall. For most of these tests (37/44 [84%]) the internal control failed. If Xpert yielded an invalid result, repeat testing was

Table 1. Demographic and Clinical Characteristics

Variables	No.	%
Subjects total	502	100
Age group, y		
18–29	76	15.2
30–49	223	44.4
>50	203	40.4
Sex		
Female	223	44.4
Male	279	55.6
Born in Canada	93	18.5
Tuberculosis prevalence in country of birth (all forms, per 100 000)		
Low (≤25)	52	10.4
Medium (26–50)	14	2.8
High (51–100)	120	23.9
Very high (>100)	223	44.4
Status in Canada		
Canadian-born citizen	93	18.5
Foreign-born citizen	62	12.4
Immigrant	294	58.6
Foreign-born student	12	2.4
Work permit	12	2.4
Other	29	5.8
Comorbidities		
Diabetes	6	1.2
Malnutrition	0	0
End-stage renal disease	1	0.2
History of malignancy	2	0.4
Treatment with immunosuppressive medications	6	1.2
HIV testing result		
Negative	37	7.4
Positive	12	2.4
History of tuberculosis		
Active	111	22.1
Latent	9	1.8
Close contact with tuberculosis patient	31	6.2

Abbreviation: HIV, human immunodeficiency virus.

Table 2. Symptoms and Radiographic Findings

Variables	No.	%
Total subjects[a]	502	100
Symptoms		
Fever	17	3.4
Cough	85	16.0
Hemoptysis	14	2.8
Chest pain	10	2.0
Shortness of breath	6	1.2
Night sweats	12	2.4
Weight loss	23	4.6
Any symptom	91	18.1
Radiographic findings		
Apical fibronodular disease	64	12.8
Cavitation	10	2.0
Granuloma	36	7.2
Costophrenic angle blunting	14	2.8
Other abnormality	285	56.8

[a] No clinical information was available for 10 subjects.

Table 3. Xpert MTB/RIF Assay Results

Result	No. of Samples	No. of Tuberculosis Cases[a]	True-Positive Xpert	False-Positive Xpert	Sensitivity, % (95% CI)	Specificity, % (95% CI)	No. Invalid[b] (%)
Xpert results (1st test)[c]	501	25[d]	11	1	46 (26–67)	99.8 (99–100)	44 (8.8)
By smear result							
Positive	11	7	6	0	86 (42–100)	100 (29–100)	1 (9.1)
Negative	425	18[d]	5	1	29 (10–56)	99.8 (99–100)	40 (8.6)
By cartridge version							
G3	143	10	6	0	67 (30–93)	100 (97–100)	17 (11.9)
G4	358	15	5	1	33 (12–62)	99.7 (98–100)	27 (7.5)

Abbreviations: CI, confidence interval; MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

[a] Culture-confirmed tuberculosis cases.

[b] Invalid or erroneous result.

[c] One subject with contaminated culture result excluded.

[d] One subject with positive culture, negative smear, and invalid Xpert result.

performed either on the same sample (if sufficient volume) or a repeat sample. All repeat testing resulted in an interpretable result (5 subjects did not return for repeat testing). Whereas noninterpretable results decreased somewhat with the change from the G3 to the G4 cartridge (11.9% for G3 [95% confidence interval {CI}, 7.1%–18.4%] vs 7.5% for G4 [95% CI, 5.0%–10.8%]), the number of invalid results still exceeded that reported in the literature (Table 3) [3]. Therefore, we requested an evaluation by the manufacturer. The manufacturer discovered that 1 lot accounted for 91% of all invalid tests but only 70% of all tests (odds ratio, 5.5 [95% CI, 1.3–23.9] for this lot vs all other lots). Further evaluation of reasons for invalid results is ongoing with the manufacturer, but similarly high invalid rates have not been described from other sites.

Xpert Accuracy

Xpert detected 11 of 25 subjects with culture-confirmed tuberculosis, for a sensitivity of 46% (95% CI, 27%–67%) and a specificity of 99.8% (95% CI, 98.7%–100%) for detection of culture-positive tuberculosis (Table 3). The sensitivity was improved in subjects with smear-positive results (86% [95% CI, 42%–100%]) compared with only 29% sensitivity in subjects with smear-negative results (95% CI, 10%–56%). Although sensitivity appeared to be lower with the G4 cartridge (33% [95% CI, 12%–62%]) compared with the G3 cartridge (67% [95% CI, 30%–93%]), the CIs were wide and overlapping (Table 3).

One subject had false-positive results on Xpert (culture negative; no documented pretreatment). In that case, the Xpert result was confirmed by a positive NAAT in the clinical microbiology lab. Eight other subjects who were treated for tuberculosis based on clinical grounds (not culture confirmed) were Xpert negative (no NAAT done).

Rifampin Resistance Results on Xpert

Only 2 isolates were labeled rifampin resistant by Xpert testing. Culture-based DST confirmed only 1 of the 2 to be rifampin resistant. Sequencing of the *rpoB* gene on the isolate that provided a discrepant result between Xpert and culture-based DST identified a mutation in the 511 locus (Leu > Pro) that is captured by probe A of the Xpert assay (Supplementary Appendix B).

Evaluation of Low Sensitivity

Evaluation of the limit of detection of Xpert yielded 100% detection of BCG at a concentration as low as 62 CFUs/mL and 80% at a concentration of 31 CFU/mL in normal saline, thus suggesting an even lower limit of detection than that described in the original validation studies on sputum samples [2]. Furthermore, the sensitivity of Xpert was the same in samples with hypertonic saline as with normal saline.

Most participants with culture-positive tuberculosis had minimal disease (Table 4). This is suggested by the fact that only 7 of 25 (28%) subjects with culture-positive tuberculosis had smear-positive results, only 12 (44%) had symptoms at presentation, and 2 subjects had no radiographic abnormalities at all. Two of 7 subjects (18%) who had only 1 positive culture (out of 3) were Xpert positive, whereas 9 of 12 subjects (75%) with 3 positive cultures were Xpert positive. In addition, a longer period to culture positivity was noted for subjects with Xpert-negative, culture-positive tuberculosis (28 days [95% CI, 25–47 days]) compared with Xpert-positive/culture-positive cases (14 days [95% CI, 8–21 days]), suggesting a lower bacillary load. The mean cycle threshold value for all Xpert- and culture-positive subjects also was high at 28.2 (SD, 2.9), suggesting a low bacillary burden even in those subjects who were Xpert positive [20]. The presence of symptoms upon enrollment

Table 4. Xpert Result by Subject Characteristic

Characteristic	Total Tuberculosis Cases No.[a]	Xpert Positive	
		% (No.)	95% CI
Age			
<35 y	13	46 (6)	17–77
>35 y	11	46 (5)	19–75
Sex			
Female	6	33 (2)	4–78
Male	18	50 (9)	26–74
Country of origin			
Canada	0	0	0
Other	24	46 (11)	26–67
Tuberculosis prevalence in country of origin			
Low/medium	7	43 (3)	10–82
High/very high	17	47 (8)	23–72
History of tuberculosis			
No	22	46 (10)	24–68
Yes	2	50 (1)	1–98
Immunocompromising illness			
No	24	46 (11)	26–67
Yes	0	0	0
Symptoms[b]			
No	13	15 (2)	2–45
Yes	11	82 (9)	48–98
Radiographic abnormalities			
No	2	0	0–84
Yes	22	50 (11)	28–72
No. of cultures positive			
1	7	29 (2)	4–71
2–3	17	53 (9)	28–77
Time to liquid culture positivity			
>3 wk	13	23 (3)	5–54
<3 wk	11	73 (8)	39–94

Abbreviation: CI, confidence interval.

[a] One subject with positive culture and invalid Xpert result.

[b] The only variable for which confidence intervals do not overlap.

into our study was the one variable that was predictive of Xpert positivity (Table 4).

Potential Clinical Impact Evaluation

The Xpert result for all subjects was available within 2 hours, on average. However, given that subjects were not always enrolled on initial presentation to the tuberculosis clinic, the time between the first sample and the positive Xpert result for culture-confirmed cases was a median of 25 hours (IQR, 3–93 hours; n = 11). A positive smear result was reported within 26 hours (IQR, 25–51 hours), and a positive culture result was reported after 516 hours (ie, median 22 days; IQR, 336–720) (Figure 1).

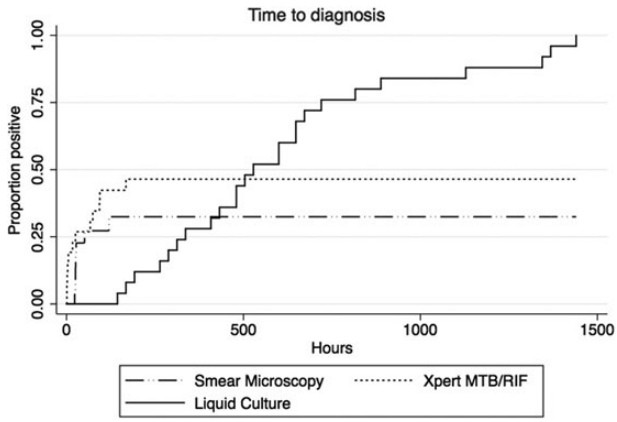

Figure 1. Sensitivity and time to positivity of different diagnostic methods. Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

The median time to treatment initiation (from initial sample provided) was 1 day for smear-positive cases (IQR, 0–2 days) and 26 days for smear-negative cases (IQR, 4–30 days). For 13 of the 18 smear-negative cases, Xpert was negative and therefore would have not influenced treatment decisions. For the remaining 5 subjects who had smear-negative but Xpert-positive results, treatment would potentially have been started a median of 12 days (IQR, 4–23 days) sooner, if results had been shared with the physicians. Treatment initiation would have been only 1 day earlier, at best, for smear-positive cases.

Subjects with smear-positive results who were ultimately identified not to have tuberculosis in this study were not started on tuberculosis therapy while awaiting culture results, likely because species confirmation by existing NAAT in the clinical lab was usually done within a day of the positive smear, and suspicion of clinicians was low. Thus, Xpert would not have had any impact in preventing unnecessary tuberculosis treatment and possibly contact investigations in these subjects.

DISCUSSION

The Xpert assay has been shown to effectively and rapidly diagnose tuberculosis in low-resource settings where diagnosis has hitherto depended primarily on smear microscopy. In such settings, introduction of Xpert can potentially decrease morbidity associated with diagnostic delay, dropout, and mistreatment even if some persons with smear-negative active tuberculosis are still missed because of imperfect sensitivity [3, 11]. However, the impact of the technology in low-incidence, high-resource settings with full mycobacterial culture and DST capability has not been adequately studied. With the recent FDA approval of this technology, it is important to generate evidence in low-tuberculosis-incidence settings.

Our study highlights that in a high-resource, ambulatory, tertiary-care setting, subjects are likely to present early in their disease course with minimal disease, in part detected as a result of active immigration screening. This is suggested by the substantial number of asymptomatic subjects in our study. Furthermore, the time to positivity of mycobacterial cultures in our participants was longer than the expected average for liquid cultures, suggesting a low bacillary load—notably in those with negative Xpert results [21].

The preponderance of paucibacillary disease likely accounts for the limited sensitivity of Xpert observed. Although the results for smear-positive samples are within the range of previous observations in low-resource settings, the sensitivity of Xpert for smear-negative samples is substantially lower than that reported in a recent systematic review (68% sensitivity) [3]. However, that review involved subjects who were symptomatic on presentation, whereas in our study only 18% of subjects were symptomatic.

Most studies thus far published on Xpert also used expectorated sputum, whereas all sputum samples in this study were obtained by induction. It is conceivable that the dilution of the sample in the process of sputum induction results in even smaller numbers of CFUs in the cartridge. This may contribute to the lower sensitivity of Xpert in our setting, particularly as no concentration step was done prior to Xpert (with the intent to minimize processing steps and equipment as well as biosafety concerns in the clinic), whereas smear microscopy and culture were done on concentrated samples. An effect of hypertonic saline (used for the sputum induction) on the performance of Xpert appears unlikely as the pH of the sample obtained with induction is likely to be only minimally different from expectorated sputum. Furthermore, an evaluation of 32 samples using BCG to compare normal and hypertonic saline did not show any difference.

A decreased sensitivity of Xpert in induced sputa has also been described in preliminary results from a study of South African adults and in the package insert data for Xpert, based on a small number of samples [15]. However, studies in children have shown adequate sensitivity of Xpert in induced sputum [17]. It is conceivable that adults with paucibacillary disease are more likely not to produce sputum, whereas children may have many more reasons why they cannot provide a spontaneous sputum sample (eg, inability to follow instructions), which could explain the discrepant finding.

Concerns have been raised about the limited specificity of Xpert for rifampin resistance detection and thus its positive predictive value in a setting with low prevalence of multidrug resistance [22, 23]. In this study, only 2 subjects were labeled as having rifampin-resistant tuberculosis by Xpert, of which only 1 subject had confirmed resistance on culture-based DST. Sequencing of the *rpoB* gene of the isolate with the discrepant result suggested a mutation that was associated with increased failure and relapse rates in recent studies [24, 25]. This finding raises some concern about the predictive validity of phenotypic testing for rifampin susceptibility and its use as the gold standard for confirmation of the Xpert rifampin resistance test. Sequencing for confirmation of rifampin resistance detected on Xpert is therefore recommended [26].

In addition, our study highlights the limited potential impact of Xpert on time to diagnosis and likely also on treatment decisions in a setting where (1) the standard diagnostic algorithm with smear and culture, supplemented by confirmatory NAAT in the laboratory, performs well; (2) there are excellent logistics for transport and analysis of samples and communication of results; and (3) physicians are experienced in the diagnosis and care of tuberculosis subjects (in our tuberculosis clinic, all subjects are seen by pulmonologists).

However, in settings where these conditions are not met (eg, smear microscopy not done on site, NAATs not available, and physicians less experienced in diagnosing tuberculosis), Xpert may still have an important role. The value of the test may be even further increased in more remote areas or in confined populations within a high-resource country, especially if there is a substantial community burden of tuberculosis. A study is currently under way to examine this hypothesis and evaluate the role of Xpert in Aboriginal communities in the Canadian Arctic. Preliminary findings support a potential role for the new technology in this remote setting, where there is limited on-site laboratory capacity (personal communication, G. Alvarez) [6].

Furthermore, Xpert may be useful in an inpatient setting, where patients typically present later in their disease. In this setting, Xpert may also reduce the time in respiratory isolation for patients suspected of having tuberculosis, and thus result in cost savings [26, 27].

In summary, we found that the impact of Xpert testing in a low-incidence, high-resource ambulatory setting is limited. These findings underscore a recommendation in the Canadian Tuberculosis Standards that allows the use of the Xpert MTB/RIF assay in laboratories, but cautions that the use of Xpert should not replace conventional smears and cultures, and recommends that all Xpert results should be confirmed by routine smears and cultures [7].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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References

1. Steingart K, Sohn H, Schiller I, et al. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev **2013**; 1:CD009593.
2. World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system. Geneva, Switzerland: WHO, **2011**.
3. Food and Drug Administration. FDA permits marketing of first U.S. test labeled for simultaneous detection of tuberculosis bacteria and resistance to the antibiotic rifampin. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm362602.htm. Accessed 25 July 2013.
4. Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaitre N. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. J Clin Microbiol **2011**; 49:1772–6.
5. Moure R, Martin R, Alcaide F. Effectiveness of an integrated real-time PCR method for detection of the *Mycobacterium tuberculosis* complex in smear-negative extrapulmonary samples in an area of low tuberculosis prevalence. J Clin Microbiol **2012**; 50:513–5.
6. Public Health Agency of Canada. Tuberculosis in Canada 2010—prerelease. Ottawa: Public Health Agency of Canada, **2010**.
7. Pai M, Minion J, Jamieson F, Wolfe J, Behr M. Canadian Tuberculosis Standards. 7th ed. Canadian Resp J **2013**; 20(suppl A):16A–22A.
8. Sreeramareddy CT, Kishore PV, Menten J, Van den Ende J. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. BMC Infect Dis **2009**; 9:91.
9. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. BMC Public Health **2008**; 8:15.
10. Behr MA, Warren SA, Salamon H, et al. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. Lancet **1999**; 353:444–9.
11. Tostmann A, Kik SV, Kalisvaart NA, et al. Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. Clin Infect Dis **2008**; 47:1135–42.
12. Banada PP, Sivasubramani SK, Blakemore R, et al. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. J Clin Microbiol **2010**; 48:3551–7.
13. Clouse K, Page-Shipp L, Dansey H, et al. Implementation of Xpert MTB/RIF for routine point-of-care diagnosis of tuberculosis at the primary care level. S Afr Med J **2012**; 102:805–7.
14. Van Rie A, Page-Shipp L, Hanrahan CF, et al. Point-of-care Xpert(R) MTB/RIF for smear-negative tuberculosis suspects at a primary care clinic in South Africa. Int J Tuberc Lung Dis **2013**; 17:368–72.
15. Cepheid. Xpert MTB/RIF assay (package insert). Sunnyvale, CA: Cepheid, **2013**.
16. World Health Organization. Policy framework for implementing new tuberculosis diagnostics. Available at: http://www.who.int/tb/laboratory/whopolicyframework_rev_june2011.pdf. Accessed 1 March 2013.
17. Kent PA, Kubica GP. Public health mycobacteriology—a guide for the level III laboratory. Atlanta, GA: US Department of Health and Human Services, Public Health Service, Centers for Disease Control, **1985**.
18. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD Initiative. Ann Intern Med **2003**; 138:40–4.
19. Bossuyt PM, Reitsma JB, Bruns DE, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med **2003**; 138:W1–12.
20. Friedrich SO, Rachow A, Saathoff E, Singh K. Assessment of the sensitivity and specificity of Xpert MTB/RIF assay as an early sputum biomarker of response to tuberculosis treatment. Lancet Respir Med **2013**; 1:462–70.
21. Bark CM, Gitta P, Ogwang S, et al. Comparison of time to positive and colony counting in an early bactericidal activity study of anti-tuberculosis treatment. Int J Tuberc Lung Dis **2013**; 17:1448–51.
22. Van Rie A, Mellet K, John MA, et al. False-positive rifampicin resistance on Xpert(R) MTB/RIF: case report and clinical implications. Int J Tuberc Lung Dis **2012**; 16:206–8.
23. Williamson DA, Roberts SA, Bower JE, et al. Clinical failures associated with rpoB mutations in phenotypically occult multidrug-resistant *Mycobacterium tuberculosis*. Int J Tuberc Lung Dis **2012**; 16:216–20.
24. Somoskovi A, Deggim V, Ciardo D, Bloemberg GV. Diagnostic implications of inconsistent results obtained with the Xpert MTB/Rif assay in detection of *Mycobacterium tuberculosis* isolates with an rpoB mutation associated with low-level rifampin resistance. J Clin Microbiol **2013**; 51:3127–29.
25. Van Deun A, Maug AK, Bola V, et al. Rifampicin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol **2013**; 51:2633–40.
26. Iademarco MF. Availability of an assay for detecting *Mycobacterium tuberculosis*, including rifampin-resistant strains, and considerations for its use—United States, 2013. MMWR Morb Mortal Wkly Rep **2013**; 62:821–4.
27. Choi HW, Miele K, Dowdy D, Shah M. Cost-effectiveness of Xpert(R) MTB/RIF for diagnosing pulmonary tuberculosis in the United States. Int J Tuberc Lung Dis **2013**; 17:1328–35.