DIAGNOSTIC TEST OF SPUTUM GENEXPERT MTB/RIF FOR SMEAR NEGATIVE PULMONARY TUBERCULOSIS

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Abstract. The objective of this study was to evaluate the performance of the GeneXpert MTB/RIF sputum test for diagnosing pulmonary tuberculosis (TB) among patients sputum acid-fast bacillus (AFB) smear negative results in Thailand, a country with a high prevalence of pulmonary tuberculosis. We studied 151 patients who presented to Srinagarind Hospital, Khon Kaen, Thailand with a 2 week or more history of fever and/or cough and an abnormal chest radiograph between 2010 and 2014; these patients had at least 2 negative sputum AFB smear results. Of these, 76 were diagnosed as having either confirmed or probable pulmonary TB: the 32 confirmed cases were those with a positive sputum culture for *Mycobacterium tuberculosis* (MTB) and the 44 probable case were those with clinical and radiographic findings consistent with TB and who had a response to anti-TB therapy. Seventy-five cases were diagnosed as not having pulmonary TB. Of the 32 patients with a positive sputum culture for MTB, 26 had a positive GeneXpert MTB/RIF sputum test. Compared to sputum culture for MTB the GeneXpert MTB/RIF test gave a sensitivity of 83.9% (95% CI: 66.3-94.5) and a specificity of 92.1% (95% CI: 83.6-97), a positive predictive value (PPV) of 81.3% (95% CI: 63.6-92.8) and a negative predictive value (NPV) of 93.3% (95% CI: 85.1-97.8). The GeneXpert MTB/RIF test had a fair sensitivity and specificity for diagnosing smear negative pulmonary TB. It may be useful for diagnosing pulmonary TB in patients with a negative sputum AFB smear. The assay is faster than culture and can detect rifampicin resistant strains of MTB.

Keywords: pulmonary tuberculosis, negative AFB smear, diagnostic test, GeneXpert MTB/RIF sputum test

INTRODUCTION

Tuberculosis (TB) is a common infection requiring a long duration of treatment. It can be transmitted via respiratory droplets from active pulmonary tuberculosis patients. In 2014, the World Health Organization (WHO) estimated 9.0 million people developed TB and 1.5 million died (WHO, 2014a). The proportion of new cases with multidrug-resistant TB (MDR-TB) is estimated to be 3.5% worldwide; much higher levels of resistance and poorer outcomes occur in some parts of the world (WHO, 2014a). Conventional sputum acid-fast bacillus (AFB) staining

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is the first step in TB diagnosis. The average turnaround time (TAT) to obtain the results of AFB staining is about 24 hours (ATS, 2000). Early treatment of active pulmonary TB patients is important to reduce the risk of spread to others. Hobby *et al* (1973) found there must be at least 5,000 to 10,000 bacilli per milliliter of specimen to be detected on AFB staining. Only 10-100 bacilli per milliliter are needed for a positive culture (Yeager *et al*, 1967). If two sputum samples for AFB staining are negative, the next step is culture and susceptibility testing for mycobacteria, which requires 3 to 4 weeks for liquid media and 6 to 8 weeks for solid media (ATS, 2000). Culture results can differentiate *Mycobacterium tuberculosis* from non-tuberculous mycobacterium (NTM) and detect mono-, poly-, and multi-drug resistant tuberculosis (MDR-TB) strains. The main limitation of culture is the time it takes to get a result (ATS, 2000).

The GeneXpert MTB/RIF assay uses a reverse transcriptase polymerase chain reaction (RT-PCR) to detect the TB specific *rpoB* gene; it is simple and the results can be obtained within 100 minutes (Boehme *et al*, 2010). Mutation of the *rpoB* is associated with resistance to rifampicin (RIF) (Boehme *et al*, 2010). This rapid test was endorsed by the WHO in December 2010 for diagnosing pulmonary TB; 108 countries had access to the GeneXpert MTB/RIF test by June 2014 (WHO, 2014b).

The WHO reported the estimated incidence of TB in Thailand during 2014 was 80,000 cases, of whom 8,100 died (WHO, 2014a). Thailand is one of the top 22 countries with a high prevalence of TB (WHO, 2014a). Only 32,887 cases in Thailand (41.1%) were confirmed by sputum AFB staining to be TB (WHO, 2014a). A difficulty in diagnosis is smear negative pulmonary TB and extrapulmonary TB.

Srinagarind Hospital is a tertiary care hospital in northeastern Thailand where 400 new TB cases are registered each year at the TB clinic (Reechaipichitkul, 2012, unpublished data). Of these, 15-20% are new smear positive pulmonary TB patients, 30-40% are new smear negative pulmonary TB patients and the remainder are extrapulmonary TB patients (Reechaipichitkul, 2012, unpublished data). The GeneXpert MTB/RIF assay has been used at Srinagarind Hospital to diagnose TB since 2010, especially in sputum smear negative cases suspected to have TB or suspected to have rifampicin resistance.

The objective of the present study was to evaluate the performance of sputum GeneXpert MTB/RIF assay testing to diagnose TB in AFB sputum smear negative patients presenting to Srinagarind Hospital, Thailand.

MATERIALS AND METHODS

The laboratory results and patient charts of patients presenting to the TB clinic during 2010-2014 were retrospectively reviewed. Inclusion criteria for our study were: 1) patient signs and symptoms consistent with TB, including prolonged fever and/or cough of more than 2 weeks, 2) an abnormal chest radiograph, 3) sputum AFB smear negative results from two specimens, 4) availability of sputum GeneXpert MTB/RIF and mycobacterium culture results and 5) receiving treatment at Srinagarind Hospital. Exclusion criteria were: 1) extrapulmonary TB and 2) culture results reporting normal flora contamination because of being an inadequate specimen.

Data obtained from the patient charts included: age, sex, occupation, clinical signs and symptoms, history of underlying diseases, chest radiograph results,

sputum AFB smear results, sputum GeneXpert MTB/RIF assay results, sputum mycobacteria culture results, mycobacteria drug susceptibility testing results, treatment regimen and outcome.

A confirmed case of pulmonary TB was defined as a sputum culture positive for *M. tuberculosis*. A probable case of pulmonary TB was defined as a patient with a sputum culture negative for MTB who responded clinically and radiographically to anti-TB drug treatment (WHO, 2010). The GeneXpert MTB/RIF assay was conducted and the results recorded for each patient studied.

We used descriptive statistics to describe the demographic data, means and standard deviations (SD) for continuous variables and numbers and percentages for categorical variables. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and 95% confidence intervals (CI) for the GeneXpert MTB/RIF assay. We used SPSS, version 17.0 (IMB, Armonk, NY) and STATA, version 11.0 (Stata, College Station, TX) to conduct the statistical calculations.

This study was approved by the Research Ethics Committee (HE581037), Khon Kaen University, Khon Kaen, Thailand.

RESULTS

During the 5 year study period, the GeneXpert MTB/RIF assay was performed 499 times, 80% (400/499) were from respiratory specimens. One hundred fifty-one patients had signs and symptoms consistent with pulmonary tuberculosis but had negative sputum AFB smears performed at least twice on each patient. The diagnoses of these 151 cases are shown in Table 1. Seventy-six subjects were diagnosed as having either confirmed or probable TB with a negative sputum smear for AFB results: the 32 confirmed cases had a sputum culture positive for *M. tuberculosis* and the other 44 probable cases improved clinically with anti-TB treatment. The other 75 cases were diagnosed as not having pulmonary TB. The final diagnoses in those without active pulmonary TB were old pulmonary TB (n=18), bacterial pneumonia (n=11), bronchogenic carcinoma (n=6), bronchiectasis (n=5), pulmonary nocardiosis (n=3), metastatic lung cancer (n=3), lung abscess (n=2), pulmonary cryptococcosis (n=2), *P. jirovecii* (n=2), volume overload (n=2), pulmonary hypertension (n=2), *M. abscessus* (n=1), empyema thoracis (n=1), germ cell tumor (n=1), lymphoma (n=1), pulmonary alveolar hemorrhage (n=1) and no definite pulmonary disease (n=14).

The characteristics of the subjects with and without suspected pulmonary TB are shown in Table 2. Age, sex, duration of symptoms, clinical presentation, cough, fever, weight loss, hemoptysis, history of underlying autoimmune disease, history of diabetes mellitus, radiographic findings of minimal fibropatchy infiltration or patchy alveolar infiltration did not significantly differ between the tuberculosis and nontuberculosis patients (Table 2).

The mean age of all 151 cases was 59.6 (SD 16.2) years; 100 patients (66.2%) were male (Table 2). Their occupations were agriculture (31.8%), government service (17.9%), employee (9.3%), business (7.9%), monk (4.0%), student (3.3%), prisoner (1.3%), and no occupation (24.5%). Fifty-one cases (34%) were from Khon Kaen Province. The median duration of symptoms was 14 days. Common symptoms were chronic cough (57.6%), fever (47.7%) and weight loss (27.2%). Forty-three point seven percent of patients (66/151) had an

Table 1
Diagnoses of 151 cases with signs and symptoms consistent with tuberculosis.

Final diagnosis	No. (%)
Smear negative pulmonary TB (n = 76)	
M. tuberculosis culture positive (confirmed case)	32 (21.2)
M. tuberculosis culture negative (probable case)	44 (29.1)
Not pulmonary TB (n = 75)	
Old pulmonary TB	18 (11.9)
Bacterial pneumonia	11 (7.3)
Bronchogenic carcinoma	6 (4.0)
Bronchiectasis	5 (3.3)
Pulmonary nocardiosis	3 (2.0)
Metastatic lung cancer	3 (2.0)
Lung abscess	2 (1.3)
Pulmonary cryptococcosis	2 (1.3)
Pneumocystis jirovecii	2 (1.3)
Volume overload	2 (1.3)
Pulmonary hypertension	2 (1.3)
M. abscessus	1 (0.7)
Empyema thoracis	1 (0.7)
Germ cell tumor	1 (0.7)
Lymphoma	1 (0.7)
Pulmonary alveolar hemorrhage	1 (0.7)
No definite pulmonary disease	14 (9.3)

underlying disease. The most common underlying disease was autoimmune diseases (14.6%). Seventy-seven patients were tested for anti-HIV antibodies, 8 (10.4%) were positive. Other medical co-morbidities included diabetes mellitus (11.2%), malignancy (6.0%), post-kidney transplantation (5.3%), nephrotic syndrome (2.0%), cirrhosis (1.3%), psoriasis (0.7%), pemphigus vulgaris (0.7%) and corneal transplant (0.7%). Eighty-five cases had no medical co-morbidities.

The chest X-ray findings are summarized in Table 3. The most common radiographic abnormality seen was a minimal fibropatchy infiltration (34.4%), follow by patchy alveolar infiltration (31.1%), miliary infiltrations (7.9%), cavitary lesions (7.3%), reticulonodular lesions (3.9%), pleural effusion (3.3%), lung mass (3.3%), interstitial infiltration (2.6%), honeycombing (2.6%), pulmonary congestion (1.3%), mediastinal mass (1.3%) and atelectasis (0.7%).

Compared to sputum culture for MTB as the gold standard to diagnose TB, the sensitivity of the GeneXpert MTB/RIF assay was 83.9% (95% CI: 66.3-94.5), the specificity was 92.1% (95% CI: 83.6-97), the positive predictive value (PPV) was 81.3% (95% CI: 63.6-92.8), the negative predictive value (NPV) was 93.3% (95% CI: 85.1-97.8), the positive likelihood ratio (LR+) was 10.6 (95% CI: 4.8-23.3) and the negative likelihood ratio (LR-) was 0.18 (95% CI: 0.07-0.39) (Table 4).

Using probable and confirmed cases of pulmonary TB, the sensitivity of the GeneXpert MTB/RIF assay was 64.5%

Table 2
Demographic characteristics of subjects with and without suspected pulmonary TB.

Characteristic	Smear negative pulmonary TB patients, *N*=76	Not pulmonary TB patients, *N*=75	Total *N*=151
Mean age in years (SD)	59.6 (16.2)	54.2 (17.0)	59.6 (16.2)
Male, *n* (%)	53 (69.4)	47 (62.7)	100 (66.2)
Occupations, *n* (%)			
Agriculture	21 (27.6)	27 (36.0)	48 (31.8)
Government service	15 (19.7)	12 (16.0)	27 (17.9)
Employee	8 (10.5)	6 (8.0)	14 (9.3)
Business	6 (7.9)	6 (8.0)	12 (7.9)
Monk	4 (5.3)	2 (6.7)	6 (4.0)
Student	2 (2.6)	3 (4.0)	5 (3.3)
Prisoner	0 (0)	2 (2.7)	2 (1.3)
No occupation	20 (26.3)	17 (22.7)	37 (24.5)
Median duration of symptoms in days (first, third quartile)	14 (3, 30)	14 (1, 30)	14 (3, 30)
Symptoms, *n* (%)			
Cough	40 (53.3)	47 (61.8)	87 (57.6)
Fever	37 (48.7)	35 (46.7)	72 (47.7)
Weight loss	26 (34.2)	15 (20.0)	41 (27.2)
Hemoptysis	12 (15.8)	16 (21.3)	28 (18.5)
Anorexia	13 (17.1)	6 (8.0)	19 (12.6)
Underlying disease, *n* (%)			
Autoimmune diseases	11 (14.5)	11 (14.7)	22 (14.6)
DM	11 (14.5)	6 (8.0)	17 (11.2)
Malignancy	3 (3.9)	6 (8.0)	9 (6.0)
HIV	4 (5.3)	4 (5.3)	8 (5.3)
Post-kidney transplantation	5 (6.6)	3 (4.0)	8 (5.3)
Nephrotic syndrome	0 (0)	3 (4.0)	3 (2.0)
Cirrhosis	2 (2.6)	0 (0)	2 (1.3)
Psoriasis	0 (0)	1 (1.3)	1 (0.7)
Pemphigus vulgaris	0 (0)	1 (1.3)	1 (0.7)
Corneal transplantation	0 (0)	1 (1.3)	1 (0.7)

SD, standard deviation; TB, tuberculosis; DM, diabetes mellitus; HIV, human immunodeficiency virus.

(95% CI: 52.7-72.1), the specificity was 93.3% (95% CI: 85.5-97.8), the positive predictive value (PPV) was 90.7% (95% CI: 79.7-96.9), the negative predictive value (NPV) was 72.2% (95% CI: 62.1-80.8), the positive likelihood ratio (LR+) was 9.6 (95% CI: 4.1-22.9) and the negative likelihood ratio (LR-) was 0.38 (95% CI: 0.28-0.52) (Table 4).

Twenty-eight cases had positive result for MTB with the GeneXpert MTB/RIF assay but a negative culture for MTB. Six cases had a negative result for MTB with the GeneXpert MTB/RIF assay but a positive culture for MTB. Ninety-one cases had a negative result on both the GeneXpert MTB/RIF assay and on culture for MTB. Twenty-six cases were positive

Table 3
Chest radiograph findings among study subjects.

Chest X-ray results	Smear negative pulmonary TB patients (*N*=76) *n* (%)	Not pulmonary TB patients (*N*=75) *n* (%)	Total (*N*=151) *n* (%)
Minimal fibropatchy infiltration	31 (40.8)	21 (28)	52 (34.4)
Patchy alveolar infiltration	25 (32.9)	22 (29.3)	47 (31.1)
Miliary infiltrations	8 (10.5)	4 (5.3)	12 (7.9)
Cavitary lesions	7 (9.2)	4 (5.3)	11 (7.3)
Reticulonodular lesions	1 (1.3)	5 (6.8)	6 (3.9)
Pleural effusion	3 (3.9)	2 (2.7)	5 (3.3)
Lung mass	0 (0)	5 (6.7)	5 (3.3)
Interstitial infiltration	1 (1.3)	3 (4.0)	4 (2.6)
Honeycombing	0 (0)	4 (5.3)	4 (2.6)
Mediastinal mass	0 (0)	2 (2.7)	2 (1.3)
Pulmonary congestion	0 (0)	2 (2.7)	2 (1.3)
Atelectasis	0 (0)	1 (1.3)	1 (0.7)

TB, tuberculosis.

on both the GeneXpert MTB/RIF assay and on culture for MTB. The concordance between these two tests was 77.5% (κ=0.46). One case had negative result on both the GeneXpert MTB/RIF assay and culture for MTB, but grew out *M. abscessus* instead. The GeneXpert MTB/RIF assay detected 5 RIF-resistant MTB sputum samples, but only 1 was confirmed by drug susceptibility testing (DST).

DISCUSSION

TB diagnosis primarily relies on sputum smear for AFB microscopy, which has a lower sensitivity and specificity than culture (ATS, 2000). AFB staining of the sputum to diagnose TB has a sensitivity of only 50-60% and a specificity of 89% (Lipsky *et al*, 1984; Siddiqi *et al*, 2003). The microbiological identification of *M. tuberculosis* on culture is the gold standard to diagnosis TB (ATS, 2000). The conventional culture technique for MTB, however, does not provide a rapid diagnosis, needs more mycobacteria to grow and requires sophisticated laboratory facilities with adequate biological safety, which may not be possible in resource limited settings (ATS, 2000).

A case of smear negative pulmonary TB was defined by the WHO (2010) as a case with at least two negative AFB smears but a positive TB culture, or two negative AFB smears and radiographical abnormalities consistent with active pulmonary TB and treatment for TB with a clinical responses (WHO, 2010). A problem in this type of patient is delayed diagnosis and treatment, resulting in possible transmission of TB; even though smear negative pulmonary TB is less infectious than smear positive TB patients. In the Netherlands, patients with smear-negative, culture-positive pulmonary TB are responsible for 13% of TB transmissions (Tostmann *et al*, 2008). High burden TB countries with adequate resources should have TB control efforts to prevent transmission from smear-negative, culture-positive pulmonary TB patients to others.

Patients with a cough and/or fever for more than 2 weeks should have a

Table 4
Diagnostic performance of the GeneXpert MTB/RIF assay.

Performance[a]	GeneXpert MTB/RIF assay % (95% CI)
Sensitivity	
Culture confirmed TB patients	83.9 (66.3-94.5)
Probable and culture confirmed cases	64.5 (52.7-72.1)
Specificity	
Culture confirmed TB patients	92.1 (83.6-97.0)
Probable and culture confirmed cases	93.3 (85.5-97.8)
Positive predictive value	
Culture confirmed TB patients	81.3 (63.6-92.8)
Probable and culture confirmed cases	90.7 (79.7-96.9)
Negative predictive value	
Culture confirmed TB patients	93.3 (85.1-97.8)
Probable and culture confirmed cases	72.2 (62.1-80.8)
Positive likelihood ratio (LR+)	
Culture confirmed TB patients	10.6 (4.8-23.3)
Probable and culture confirmed cases	9.6 (4.1-22.9)
Negative likelihood ratio (LR-)	
Culture confirmed TB patients	0.18 (0.07-0.39)
Probable and culture confirmed cases	0.38 (0.28-0.52)

[a]Culture confirmed TB patients (*n*=32); Probable and culture confirmed cases (*n*=76); Not having pulmonary TB cases (*n*=75).

chest radiograph and sputum AFB staining. The differential diagnosis of diseases that mimic smear negative pulmonary tuberculosis includes infectious and noninfectious diseases (Colebunders and Bastian, 2000). Bacterial pneumonia is first in the differential diagnosis of disease that mimic TB among both HIV-positive and HIV-negative individuals, while *P. jirovecii*, cryptococcosis and nocardiosis are also important among HIV-positive patients and patients who have received immunosuppressive drugs (Colebunders and Bastian, 2000; Nyamande *et al*, 2007). For patients with noninfectious disease, bronchogenic carcinoma and metastatic lung cancer are sometimes difficult to differentiate from pulmonary tuberculosis (Colebunders and Bastian, 2000; Bhatt *et al*, 2012). In our study, the most common infectious disease among patients without TB was bacterial pneumonia and the most common noninfectious disease was bronchogenic carcinoma, similar to previous reports (Colebunders and Bastian, 2000; Nyamande *et al*, 2007; Bhatt *et al*, 2012). Old pulmonary TB was also a common diagnosis in this study, although the physician suspected relapse or re-infection of TB; however, the sputum smear for AFB was negative and the culture for MTB was negative. Some of the other diseases found in our study that differed from previous reports (Colebunders and Bastian, 2000; Nyamande *et al*, 2007; Bhatt *et al*, 2012) included infected bronchiectasis, pulmonary nocardiosis, metastatic lung cancer, lung abscess, pulmonary cryptococcosis, *P. jirovecii*, volume over load, pulmonary hypertension, *M. abscessus*, empyema thoracis, germ cell tumor, lymphoma, and pulmonary alveolar hemorrhage.

New molecular diagnostic techniques have an important role in the early identification of *M. tuberculosis* by reducing the length of time to diagnosis and rapid detection of resistant strains (Soini and Musser, 2001). Popular rapid tests include the GeneXpert MTB/RIF assay and the line probe assay (LPA) (Soini and Musser, 2001). The LPA is recommended for use in smear positive TB patients in whom resistance is suspected, since the LPA can detect isoniazid and rifampicin resistance. The standard turnaround time (TAT) for the LPA is 2 - 3 days (Brossier *et al*, 2006; Lin and Desmond, 2014). The GeneXpert MTB/RIF assay is easier to do and can be used for both AFB smear positive and smear negative respiratory specimens to identify *M. tuberculosis* and detect rifampicin resistance using a probe for the *rpoB* gene, which accounts for more than 95% of mutations associated with rifampicin resistance (Boehme *et al*, 2010; Lin and Desmond, 2014). Because the TAT of the GeneXpert MTB/RIF assay in clinical practice is only 2 hours, it is faster than AFB staining in some places and needs only 131 colony forming units (CFU/ml) (95% CI: 106-176) for detection (Lawn *et al*, 2013). The GeneXpert MTB/RIF assay is more sensitive than sputum smear microscopy in detecting TB and has an accuracy similar to a culture (Boehme *et al*, 2010; Lawn *et al*, 2013). For this reason, it may be substituted for AFB staining or used as an additional test in suspected TB cases with a negative AFB smear (Lawn *et al*, 2013). The reagent in the assay is designed to reduce the viability of *M. tuberculosis* in the sputum and thus reduce the biohazard risk (Boehme *et al*, 2010; WHO, 2007). A limitation of molecular diagnosis is that a positive result may be obtained from dead bacilli (WHO, 2007). In patients who have recently been treated with anti-TB drugs, a molecular test cannot differentiate between old TB and active TB (WHO, 2014b). This should be confirmed by mycobacterial culture to detect viable bacilli. This means the follow-up sputum test after treatment cannot use the molecular technique. This is different from the culture which can be used to detect growth of MTB (WHO, 2014b). Only on culture can drug susceptibility testing (DST) be performed on all first and second line anti-tuberculosis drugs (Soto *et al*, 2013).

In this study, the clinical manifestations of patients with smear negative pulmonary tuberculosis did not differ from other diseases. Therefore, noninvasive investigations, such as molecular techniques and mycobacterial culture, and invasive investigations, such as bronchoscopy with tissue biopsy are needed for accurate diagnosis (WHO, 2014b).

Molecular techniques can compliment culture to increase the yield for TB diagnosis. The specificity of the GeneXpert MTB/RIF assay was high (>90%) in this study. Five cases were positive with the GeneXpert MTB/RIF assay and negative on culture for *M. tuberculosis*. They all improved spontaneously without anti-TB drug treatment and were considered to all be old pulmonary TB patients. This reduced the specificity of the GeneXpert MTB/RIF assay. This finding is similar to other studies regarding the performance of the GeneXpert MTB/RIF assay (Chang *et al*, 2012; Steingart *et al*, 2014). According to a meta-analysis of the GeneXpert MTB/RIF assay using 15 studies (Chang *et al*, 2012), the sensitivity and specificity were 75.0% and 98.2% for AFB smear-negative specimens and 98.7% and 98.2% for smear-positive specimens, respectively (Chang *et al*, 2012). Since the GeneXpert MTB/RIF assay is more expensive than

AFB staining and the incidence of rifampicin resistant strains in new cases is currently low, the WHO does not recommend the GeneXpert MTB/RIF assay for new smear positive cases of pulmonary TB (WHO, 2014b). It has been recommended for new AFB smear negative clinically suspected TB cases and in previously treated cases of suspected MDR-TB (WHO, 2014b). For non-respiratory specimens, the GeneXpert MTB/RIF assay has a sensitivity in detecting MTB of only 33.3% but a specificity of 99.7% (Bunsow *et al*, 2014). A Cochrane Database Systematic Review (CDSR) evaluating the role of the Xpert MTB/RIF assay among adults with pulmonary tuberculosis conducted during 2014 reported that as an add-on test following a negative smear microbiology result, the Xpert MTB/RIF assay had a pooled sensitivity of 67% [95% Credible Interval (CrI) 60% to 74%] and a pooled specificity of 99% (95% CrI 98% to 99%) among 21 studies with 6,950 participants (Steingart *et al*, 2014). In comparison with smear microscopy, the Xpert MTB/RIF assay increased TB detection of culture-confirmed cases by 23% (95% CrI 15% to 32%) in 21 studies among 8,880 participants (Steingart *et al*, 2014). The data of the present study support the performance of the GeneXpert MTB/RIF assay for use in smear negative pulmonary tuberculosis in Thailand, similar to other developing, high burden TB countries (Zmak *et at*, 2013; Iram *et al*, 2015).

In conclusion, the GeneXpert MTB/RIF assay is a fairly sensitive and specific test for the rapid diagnosis of AFB smear negative pulmonary tuberculosis. In addition to conventional AFB staining and mycobacterial culture, the GeneXpert MTB/RIF assay is recommended for patients who are clinically suspected to have pulmonary tuberculosis but have two smear-negative AFB results. This molecular technique uses genotype detection of *M. tuberculosis* and may detect dead bacilli. Each case should be confirmed by phenotypic drug susceptibility testing (DST) from culture. Rapid diagnosis and prompt treatment of pulmonary TB, even in smear negative patients, can decrease transmission of the infection to others and may decrease severity of the disease.

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