



Research article

Diagnostic performance of the Anyplex MTB/NTM real-time PCR in detection of *Mycobacterium tuberculosis* complex and nontuberculous mycobacteria from pulmonary and extrapulmonary specimensAjcharaporn Sawatpanich^a, Suthidee Petsong^a, Somying Tumwasorn^a, Suwatchareeporn Rotcheewaphan^{a,b,*}^a Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand^b Center of Excellence in Systems Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

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ABSTRACT

Accurate and rapid diagnosis of mycobacterial infections is significant for appropriate treatment. In this study, we retrospectively evaluated the performance of the Anyplex MTB/NTM real-time detection assay (Anyplex MTB/NTM) compared to mycobacterial culture in detecting *Mycobacterium tuberculosis* complex (MTBC) and nontuberculous mycobacteria (NTM) in 9,575 clinical specimens. For MTBC detection, the sensitivity, specificity, PPV, NPV, and percent agreement of the Anyplex MTB/NTM were 79.7%, 94.5%, 64.4%, 97.4%, and 92.9%, respectively. In pediatric patient (age ≤ 15) specimens, the Anyplex MTB/NTM demonstrated 84.8% sensitivity and 95.8% specificity. For NTM detection, the sensitivity, specificity, PPV, NPV, and percent agreement were 44.9%, 97.7%, 36.7%, 98.4%, and 96.2%, respectively. The sensitivity of the Anyplex MTB/NTM was enhanced in acid-fast bacilli (AFB) smear-positive specimens which was 97.7% and 80% for MTBC and NTM detection, respectively. The Anyplex MTB/NTM is a rapid tool for detection and differentiation of MTBC and NTM in clinical specimens.

1. Introduction

Mycobacterium tuberculosis complex (MTBC) is the causative agent of tuberculosis (TB) which is a challenging global public health issue. In 2020, Thailand was listed as one of the 30 high TB burden countries with high incidence of multidrug-resistant tuberculosis (MDR-TB) [1]. Therefore, rapid and accurate identification of active TB cases is the most significant strategy to provide adequate patient care, management, and infection control. At present, there are various tests available for TB diagnosis. The smear microscopy by acid-fast staining is a rapid and simple method to presumptively identify acid-fast organisms including MTBC and nontuberculous mycobacteria (NTM) but its sensitivity varies depending on the quality and quantity of clinical specimens [2, 3, 4, 5]. Culture method is still considered as the diagnostic gold standard for active TB. However, the isolation of MTBC from clinical specimens by culture could take at least 1–2 months, resulting in a delay of TB treatment and increased risk of disease transmission, morbidity, and mortality [3, 6, 7]. Therefore, reliable and rapid molecular nucleic acid amplification tests (NAATs) are essential for early detection of TB infection. The

Centers for Disease Control and Prevention (CDC) has recommended the application of the molecular method to diagnose all suspected pulmonary TB patients [8]. Direct MTBC detection by NAATs are used widely aiming for rapid and accurate results. These include the Conformite Europeenne (CE)-marked commercial assays such as Anyplex MTB/NTM real-time detection assay (Anyplex MTB/NTM) (Seegene Inc., Republic of Korea) [9] and FDA-approved tests such as Xpert MTB/RIF (Cepheid, USA) [10, 11]. Some NAATs can not only detect MTBC organisms but also detect gene mutations associated with TB drug resistance such as Xpert MTB/RIF assay detecting rifampicin resistance (*rpoB* gene) and Anyplex II MTB/MDR detection [12, 13] and GenoType MTBDRplus [14, 15] detecting both rifampicin resistance and isoniazid resistance (*inhA* and *katG* genes).

The Anyplex MTB/NTM is a multiplex real-time PCR for the direct detection of MTBC by targeting insertion sequence 6110 (*IS6110*) [16] and *mpb64* gene [17] and NTM by targeting the partial region of 16S rRNA gene from clinical specimens which are sputum, culture cells, fresh tissue, or bronchial washing within 2 h [18]. This retrospective study aimed to determine the diagnostic performance of the Anyplex

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MTB/NTM for MTBC and NTM detection in pulmonary and extrapulmonary clinical specimens from adult and pediatric patients in Thailand, a high TB burden country.

2. Materials and methods

2.1. Specimen collection and processing

A total of 9,575 clinical specimens (Table 1) were obtained from 6,520 patients who were suspected of mycobacterial infections during September 2015 and December 2019 at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Patient information (sex, age) was collected. All specimens were submitted for routine direct examination [acid-fast bacilli (AFB) staining, Kinyoun method], mycobacterial culture, and molecular detection without additional specimens requested for this study purpose. This study was approved by the Institutional Review Board (IRB) of the institute with the exemption of patient consent (IRB No. 251/60).

2.2. Mycobacterial culture and identification

Clinical specimens were processed using the sodium hydroxide-N-acetyl-L-cysteine-sodium citrate (NaOH/NALC-Na citrate) method [19]. Briefly, clinical specimens were decontaminated and digested with an equal volume of NaOH/NALC-Na citrate digestion solution (2% NaOH, 0.5% NALC, and 1.45% Na-citrate) for 15 min at room temperature. Phosphate buffer (pH 6.8) was added to the processed samples to a final volume of 40 ml, mixed by inversion, and centrifuged at 3,000 xg for 15 min at 12 °C. The supernatant was then discarded, and the sediment was resuspended in a new 2.5 ml phosphate buffer. However, sterile specimens with limited quantity, such as small amounts of cerebrospinal fluid (CSF) and tissue biopsies were processed for culture without digestion-decontamination. A 500 µl of well-mixed sediment was added to the mycobacterial growth indicator tubes (MGIT) (Becton Dickinson, USA) and incubated in the BD BACTEC MGIT 960 System for 6 weeks or until the signal turned positive. In addition, 200 µl of sediment was inoculated on Ogawa solid medium (in-house preparation) for 8 weeks at 37 °C. Mycobacterial identification to species/complex level was achieved by using a line probe assay which was either GenoType MTBC VER 1.X, GenoType Mycobacterium CM VER 2.0, GenoType Mycobacterium AS VER 1.0, or GenoType NTM-DR VER 1.0 (Hain Lifescience, Germany) [20, 21, 22, 23].

2.3. Real-time PCR by Anyplex MTB/NTM real-time detection assay

The Anyplex MTB/NTM real-time detection assay V2.0 (Anyplex MTB/NTM) (Seegene Inc., Republic of Korea) was applied for the direct detection of mycobacteria from clinical specimens. For nucleic acid extraction, briefly, an equal volume of 4% NaOH solution was added to 0.5 ml of respiratory specimens. Samples were incubated for 15 min at room temperature and centrifuged at 14,000 rpm for 5 min at room temperature. The pellets were washed with one ml of phosphate-buffered saline (PBS), pH 7.4 and centrifuged at 14,000 rpm for 5 min at room temperature. The pellets were then resuspended in 200 µl of PBS. Extrapulmonary specimens (0.5–1 ml), stool (0.1 ml diluted in 1.5 ml PBS), and tissue [pre-treated with tissue lysis buffer (Bioneer corporation, Republic of Korea) and 20 µl of 10 mg/ml proteinase K] were centrifuged at 14,000 rpm for 5 min at room temperature. The pellets from pulmonary, extrapulmonary specimens, and stools were resuspended in 200 µl of PBS and the supernatant from tissue processing were subjected for DNA extraction using a magLEAD®12gC Automated Nucleic Acid Extraction System (Precision System Science Co., Japan) following the manufacturer's instructions [20]. The final volume of eluted DNA was 50 µl.

A 5 µl of extracted DNA used as PCR template was mixed with 15 µl of PCR amplification mixture for real-time PCR detection following the manufacturer's instructions [18]. The PCR reactions were run on a

Table 1. Clinical specimens in this study.

	Specimens	No. (%)
Pulmonary	Bronchoalveolar lavage	2023 (21.1%)
	Endotracheal aspirate	178 (1.9%)
	Sputum	3105 (32.4%)
	Tracheal secretion	16 (0.2%)
	Total	5322 (55.6%)
Extrapulmonary	Biopsy, tissue, autopsy	1297 (13.5%)
	Blood	2 (0.02%)
	Body fluid	2041 (21.3%)
	Bone marrow	19 (0.2%)
	Eye specimen (Vitreous fluid, aqueous fluid, cornea scraping)	10 (0.1%)
	Gastric wash	85 (0.9%)
	Stool	66 (0.7%)
	Urine	153 (1.6%)
	Sinus wash, nasopharyngeal suction	2 (0.02%)
	Abscess, discharge	578 (6.0%)
	Total	4253 (44.4%)
	Grand total	9575 (100%)

CFX96™ Real-Time PCR system (Bio-Rad Laboratories Ltd., USA) with thermocycles of an initial hold at 50 °C for 5 min, 95 °C for 15 min, and 45 cycles of 95 °C for 30 s, and 60 °C for 1 min with fluorescence detection on the FAM (MTBC), Cal Red 610 (genus *Mycobacterium*), and Quasar 670 (internal control) channel. Result interpretation was performed automatically by Seegene Viewer 2.0 software according to the manufacturer's threshold and cutoff values (FAM C_T of <40 for MTBC detection, Cal Red 610 C_T of <38 with negative MTBC for NTM detection) [18].

2.4. Statistical analysis

The evaluation of frequency and percentage, analytical sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and percent agreement of the Anyplex MTB/NTM was performed using a 95% confidence interval (95% CI) (p < 0.05). The correlation of AFB smear microscopy, Anyplex MTB/NTM, and mycobacterial culture were analyzed using Chi-square statistics (p < 0.05). Descriptive analysis was performed for patient sex and age information. All statistical analyses were performed using the SPSS program (IBM SPSS Statistics, version 26.0).

3. Results

A total of 9,575 clinical specimens were submitted for AFB smear microscopy, mycobacterial culture, and Anyplex MTB/NTM during September 2015 and December 2019. The specimens were collected from 6,520 patients [3,013 (46.2%) female and 3,507 (53.8%) males] with age range <1–102 years old (mean ± SD, 55.06 ± 20.71). Of these specimens, 5,322 (55.6%) were pulmonary specimens and 4,253 (44.4%) were extrapulmonary specimens (Table 1).

A total of 226/9,575 (2.4 %) of clinical specimens which were 168/226 (74.3%) pulmonary specimens and 58/226 (25.7%) extrapulmonary specimens were positive by AFB smear microscopy. For mycobacterial culture, out of a total of 1,336/9,575 (14%) clinical specimens had mycobacteria isolated. MTBC and NTM grew in 1,060/9,575 (11.1%) and 276/9,575 (2.9%) specimens, respectively. From these culture-positive specimens, mycobacteria isolates were recovered from 966/1,336 (72.3%) pulmonary and 370/1,336 (27.7%) extrapulmonary specimens, respectively. Of 226 AFB smear-positive specimens, 30/226 (13.3%) and 172/226 (76.1%) specimens had NTM and MTBC recovered from culture, respectively.

The diagnostic performance of the Anyplex MTB/NTM for detection of pulmonary and extrapulmonary TB was evaluated and compared to mycobacterial culture. The Anyplex MTB/NTM could detect MTBC in 845/1,060 (79.7%) of the MTBC-culture positive specimens, which were 580/845 (68.6%) pulmonary specimens and 265/845 (31.4%) extrapulmonary specimens. In addition, MTBC nucleic acids were detected by the Anyplex MTB/NTM in 468 (5.5%) of 8,515 MTBC-culture negative specimens (Table 2A). The overall performance of the Anyplex MTB/NTM for MTBC detection from clinical specimens were 79.7% sensitivity, 94.5% specificity, 64.4% PPV, 97.4% NPV, and 92.9% percent agreement (Table 2A). The performance for MTBC detection in pulmonary specimens was 78.8% sensitivity, 94.6% specificity, 70% PPV, 96.5% NPV, and 92.4% percent agreement. For extrapulmonary specimens, the sensitivity, specificity, PPV, NPV, and percent agreement were 81.8%, 94.4%, 54.6%, 98.4%, and 93.4%, respectively (Table 2A).

For pediatric patients (age ≤ 15), a total of 346 clinical specimens from pulmonary [93/346 (26.9%)] and extrapulmonary [253/346 (73.1%)] sites were submitted for MTBC detection. Overall, the sensitivity, specificity, PPV, NPV, and percent agreement for TB diagnosis were 84.8%, 95.8%, 68.3%, 98.4% and 94.8%, respectively, in all specimens (Table 3). The sensitivity of the Anyplex MTB/NTM was 100% (95% CI, 54.1–100) in AFB-positive specimens ($N = 7$). The performance for TB diagnosis in pulmonary specimens was 100% sensitivity, 98.8% specificity, 90.9% PPV, 100% NPV, and 98.9% percent agreement (Table 3). For extrapulmonary specimens, the Anyplex MTB/NTM had sensitivity, specificity, PPV, NPV, and percent agreement of 78.3%, 94.8%, 60%, 97.8%, and 93.3%, respectively (Table 3).

For NTM detection, the Anyplex MTB/NTM detected NTM nucleic acids in 124/276 (44.9%) of NTM culture-positive specimens and 214/9,299 (2.3%) NTM culture-negative specimens (Table 2B). Ninety-eight/124 (79%) and 26/124 (21%) of those NTM-PCR positive specimens were pulmonary and extrapulmonary specimens, respectively. The NTM detected by the Anyplex MTB/NTM in those 124 NTM-PCR positive specimens were recovered from mycobacterial cultures and identified by line probe assays as *M. abscessus* complex, *M. avium* complex, *M. chelonae*, *M. fortuitum* group, *M. goodii*, *M. haemophilum*, *M. kansasii*, *M. scrofulaceum*, *M. simiae*, and *Mycobacterium* sp. (Table 4). The two most prevalent NTM species isolated from all clinical specimens by culture were *M. abscessus* complex [97/276 (35.1%)] and *M. avium* complex [68/276 (24.6%)] (Table 4). The diagnostic performance of the Anyplex MTB/NTM to detect NTM in all clinical specimens were 44.9% sensitivity, 97.7% specificity, 36.7% PPV, 98.4% NPV, and 96.2% percent agreement (Table 2B). For pulmonary specimens, the Anyplex MTB/NTM

had 42.6% sensitivity, 96.6% specificity, 36.3% PPV, 97.4% NPV, and 94.3% percent agreement. For extrapulmonary specimens, the sensitivity, specificity, PPV, NPV, and percent agreement were 56.5%, 99%, 38.2%, 99.5%, and 98.5%, respectively (Table 2B).

In addition, the performance of the Anyplex MTB/NTM was evaluated based on direct AFB smear results which were AFB-positive (226/9,575) and AFB-negative (9,349/9,575) specimens. For AFB smear-positive specimens, the Anyplex MTB/NTM could detect MTBC in 189/226 (83.6%) specimens and identify NTM in 25/226 (11%) specimens. The sensitivity and specificity of the Anyplex MTB/NTM were 97.7% and 61.1% for MTBC detection and 80% and 99.5% for NTM detection, respectively (Table 5). For AFB-negative specimens, mycobacterial detection rate by the Anyplex MTB/NTM was 1,124/9,349 (12%) for MTBC detection and 313/9,349 (3.3%) for NTM detection. The performance for MTBC detection was 76.2% sensitivity, 94.7% specificity, 60.2% PPV, 97.4% NPV, and 93% percent agreement. The Anyplex MTB/NTM had 40.7% sensitivity, 97.7% specificity, 31.9% PPV, 98.4% NPV, and 96.2% percent agreement for NTM detection (Table 5).

4. Discussion

This retrospective study evaluated the diagnostic performance of the multiplex real-time PCR test for direct detection of MTBC and NTM from clinical specimens in a tertiary hospital in Thailand. The clinical specimens in this study were submitted to direct examination by AFB smear microscopy, mycobacterial culture, and Anyplex MTB/NTM. The AFB smear microscopy is a rapid and inexpensive method to detect AFB in the clinical specimens. However, the sensitivity of AFB smear may vary (12.5–75%) [4, 5, 24] depending on quality and quantity of clinical specimens, smearing, staining, and smear reading. In our study, the overall sensitivity of the AFB smear microscopy to detect AFB was 15.1% compared to the mycobacterial culture (data not shown). The mycobacterial culture of clinical specimens on both solid and liquid media is still the gold standard method for mycobacterial diagnosis. This method provides mycobacterial growth for further investigations such as mycobacterial identification and drug susceptibility test but is time-consuming. Therefore, rapid methods like NAATs are required for the diagnosis of mycobacterial infection and the detection of gene-associated drug resistance. Currently, commercial NAATs are widely used for rapid diagnosis of MTBC and/or NTM infections with variable diagnostic performance [5, 9, 10, 13, 25, 26].

The Anyplex MTB/NTM has been used for differential diagnosis of MTBC and NTM with internal control. This test was validated for testing

Table 2. The diagnostic performance of the Anyplex MTB/NTM for MTBC (A) and NTM (B) detection (A) MTBC detection.

(A) MTBC detection									
Specimens	Culture-positive		Culture-negative		Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	Percent agreement (%) (95%CI)
	PCR +	PCR -	PCR +	PCR -					
Pulmonary	580	156	248	4338	78.8 (75.7–81.7)	94.6 (93.9–95.2)	70 (66.8–73.2)	96.5 (96–97)	92.4 (91.7–93.1)
Extrapulmonary	265	59	220	3709	81.8 (77.1–85.8)	94.4 (93.6–95.1)	54.6 (50.1–59.1)	98.4 (98–98.8)	93.4 (92.7–94.2)
Overall	845	215	468	8047	79.7 (77.2–82.1)	94.5 (94–95)	64.4 (61.7–67)	97.4 (97–97.7)	92.9 (92.4–93.4)
(B) NTM detection									
Specimens	Culture-positive		Culture-negative		Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	Percent agreement (%) (95%CI)
	PCR +	PCR -	PCR +	PCR -					
Pulmonary	98	132	172	4920	42.6 (36.1–49.3)	96.6 (96.1–97.1)	36.3 (30.6–42.3)	97.4 (96.9–97.8)	94.3 (93.7–94.9)
Extrapulmonary	26	20	42	4165	56.5 (41.1–71.1)	99 (98.7–99.3)	38.2 (26.7–50.8)	99.5 (99.3–99.7)	98.5 (98.2–98.9)
Overall	124	152	214	9085	44.9 (39–51)	97.7 (97.4–98)	36.7 (31.5–42.1)	98.4 (98.1–98.6)	96.2 (95.8–96.6)

Table 3. The diagnostic performance of the Anyplex MTB/NTM for MTBC detection in clinical specimens from pediatric patients (age ≤15).

Specimens	Culture-positive		Culture-negative		Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	Percent agreement (%) (95%CI)
	PCR +	PCR -	PCR +	PCR -					
Pulmonary	10	0	1	82	100 (69.2–100)	98.8 (93.5–100)	90.9 (58.7–99.8)	100 (95.6–100)	98.9 (96.8–100)
Extrapulmonary	18	5	12	218	78.3 (56.3–92.5)	94.8 (91.1–97.3)	60 (40.6–77.3)	97.8 (94.8–99.3)	93.3 (90.2–96.4)
Overall	28	5	13	300	84.8 (68.1–94.9)	95.8 (93–97.8)	68.3 (51.9–81.9)	98.4 (96.2–99.5)	94.8 (92.5–97.1)

different types of samples which are sputum, culture cells, fresh tissue, and bronchial washing [18]. However, our laboratory has validated and applied this test for other types of specimens as shown in Table 1. In this study, the Anyplex MTB/NTM has demonstrated acceptable sensitivity and specificity for the detection of MTBC in clinical specimens (Table 2A). From a previous study, the sensitivity, specificity, PPV, and NPV of the Anyplex MTB/NTM for diagnosis of MTBC were 100%, 96%, 93%, and 100%, respectively [9]. However, it was conducted in a low-prevalence TB country with a small number of samples.

In this study, the Anyplex MTB/NTM showed an overall sensitivity and specificity of 79.7% and 94.5% for MTBC detection in all clinical specimen types. The Anyplex MTB/NTM demonstrated a comparable sensitivity (78.8% vs 81.8%) and specificity (94.6% vs 94.4%) for MTBC detection in pulmonary and extrapulmonary specimens (Table 2A). Compared to AFB smear microscopy, the Anyplex MTB/NTM markedly increased sensitivity for screening MTBC in clinical specimens (79.7% vs 15.1%). In addition, the Anyplex MTB/NTM can detect MTBC nucleic acids in 468 (5.5%) of 8,515 MTBC-culture negative specimens.

However, the nucleic acids detected by the Anyplex MTB/NTM may be derived from dead bacteria as remaining DNA in treated TB patients. Moreover, it could be explained by the limit of detection of the Anyplex MTB/NTM which can detect MTBC of up to 10 copies of IS6110 per reaction [18]. Additional information, such as clinical manifestations and imaging evaluation, would be beneficial for confirmation of TB diagnosis in patients with MTBC-PCR positive, culture-negative specimens.

From these results, the Anyplex MTB/NTM is suitable for the detection and differentiation of MTBC and NTM in a variety of specimen types. Moreover, the sensitivity of the Anyplex MTB/NTM for MTBC detection was enhanced in AFB smear-positive specimens (97.7%) compared to AFB smear-negative specimens (76.2%) (Table 5). However, the specificity of Anyplex MTB/NTM for detection of MTBC was reduced in smear-positive specimens (61.1%) which was also observed in the evaluation of the Anyplex Plus MTB/NTM detection kit from a previous study [25]. The explanation for this reduced specificity could be from non-specific detection of dead AFB in clinical specimens by the Anyplex MTB/NTM. Moreover, this study demonstrated that the Anyplex MTB/NTM was a

Table 4. Nontuberculous mycobacteria isolated from mycobacterial culture and detected by the Anyplex MTB/NTM.

Specimens	<i>Mycobacterium</i> spp.	No. of specimens with NTM isolated by mycobacterial culture	No. of specimens with NTM detected by Anyplex MTB/NTM (%)	
Pulmonary	<i>M. abscessus</i> complex	78	41 (52.6%)	
	<i>M. abscessus</i> complex and <i>M. avium</i> complex	1	1 (100%)	
	<i>M. avium</i> complex	56	18 (32.1%)	
	<i>M. chelonae</i>	4	4 (100%)	
	<i>M. fortuitum</i> group	18	8 (44.4%)	
	<i>M. genavense</i>	1	0	
	<i>M. gordonae</i>	11	1 (9.1%)	
	<i>M. gordonae</i> and <i>M. avium</i> complex	1	0	
	<i>M. haemophilum</i>	2	2 (100%)	
	<i>M. kansasii</i>	30	13 (43.3%)	
	<i>M. lentiflavum</i>	2	0	
	<i>M. scrofulaceum</i>	9	5 (55.6%)	
	<i>M. shimoidei</i>	1	0	
	<i>M. simiae</i>	7	3 (42.9%)	
	<i>Mycobacterium</i> sp.	9	2 (22.2%)	
	Total		230	98 (42.6%)
Extrapulmonary	<i>M. abscessus</i> complex	18	12 (66.7%)	
	<i>M. avium</i> complex	10	6 (60%)	
	<i>M. chelonae</i>	3	0	
	<i>M. fortuitum</i> group	2	0	
	<i>M. gordonae</i>	1	0	
	<i>M. haemophilum</i>	3	3 (100%)	
	<i>M. kansasii</i>	3	1 (33.3%)	
	<i>M. marinum</i>	1	1 (100%)	
	<i>M. scrofulaceum</i>	3	2 (66.7%)	
	<i>M. szulgai</i>	1	0	
	<i>Mycobacterium</i> sp.	1	1 (100%)	
	Total		46	26 (56.5%)
	Grand total		276	124 (44.9%)

Table 5. The diagnostic performance of the Anyplex MTB/NTM for MTBC and NTM detection according to AFB smear results.

Organisms	AFB smear	Culture-positive		Culture-negative		Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	PPV (%) (95%CI)	NPV (%) (95%CI)	Percent agreement (%) (95%CI)
		PCR +	PCR -	PCR +	PCR -					
MTBC	Positive	168	4	21	33	97.7 (94.2–99.4)	61.1 (46.9–74.1)	88.9 (83.5–93)	89.2 (74.6–97)	88.9 (84.8–93)
	Negative	677	211	447	8014	76.2 (73.3–79)	94.7 (94.2–95.2)	60.2 (57.3–63.1)	97.4 (97.1–97.8)	93 (92.4–93.5)
NTM	Positive	24	6	1	195	80 (61.4–92.3)	99.5 (97.2–100)	96 (79.6–99.9)	97 (93.6–98.9)	96.9 (94.6–99.2)
	Negative	100	146	213	8890	40.7 (34.5–47.1)	97.7 (97.3–98)	31.9 (26.8–37.4)	98.4 (98.1–98.6)	96.2 (95.8–96.5)

reliable and accurate assay for diagnosis of TB in pediatric patients (Table 3). The Anyplex MTB/NTM had 100% sensitivity and 98.8% specificity for MTBC detection in pulmonary specimens which was superior to its performance in extrapulmonary specimens (Table 3).

At present, the number of NTM-infected cases has increased from various infections in immunocompromised hosts with HIV infection, immunodeficiency diseases, and patients with structural lung diseases. Microbiologic diagnosis of NTM infection is challenging because NTM is ubiquitous in an environment and can contaminate clinical specimens during specimen collection and processing [27]. Consequently, the diagnosis of NTM infection cannot rely on only microbiologic laboratory results. The clinical, radiographic, and microbiologic criteria are necessary for diagnosing NTM pulmonary diseases [27]. This study evaluated the performance of the Anyplex MTB/NTM for detection of NTM in clinical specimens. The Anyplex MTB/NTM showed poor sensitivity (44.9%) but high specificity (97.7%) for NTM detection. The low sensitivity (40.8%) for NTM detection was observed in a previous study that validated an alternative version of Seegene Anyplex (Anyplex MTB/NTMe assay) [28]. However, the performance of the Anyplex MTB/NTM was enhanced in AFB smear-positive specimens with 80% sensitivity and 99.5% specificity compared to its performance (40.7% sensitivity and 97.7% specificity) in AFB smear-negative specimens (Table 5). In addition, the Anyplex MTB/NTM could detect nucleic acids from various species of NTM as shown in Table 4. However, they were identified and reported as *Mycobacterium* sp. by this assay.

A limitation of this study was the diversity of quantity and quality of the clinical specimens received. Some specimens were improperly collected or insufficient in volume for testing, which could affect the yield of results. In addition, this study involved patients who were new and treated TB and NTM cases which could affect the quantity and viability of the mycobacteria detected by the Anyplex MTB/NTM and culture results. Therefore, the false positive results from remaining nucleic acids of mycobacteria in treated patients detected by the Anyplex MTB/NTM could impact the statistical analysis. The limitation of using the Anyplex MTB/NTM in a routine laboratory in a high TB burden area is its absence of the concomitant detection of antibiotic resistance. The detection of drug-resistant tuberculosis requires additional tests such as the Seegene Anyplex II MTB/MDR detection or Anyplex MTB/MDR/XDR assay in the laboratory workflow. In conclusion, the Anyplex MTB/NTM is a rapid tool for detection and differentiation of MTBC and NTM in clinical specimens. It is a suitable and useful tool for early detection of mycobacteria in clinical specimens, especially AFB smear-positive specimens.

Declarations

Author contribution statement

Ajcharaporn Sawatpanich: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Suthidee Petsong: Performed the experiments.

Somying Tumwasorn: Contributed reagents, materials, analysis tools or data.

Suwatcareeporn Rotcheewaphan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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