

1       **Performance of the new automated Abbott RealTime MTB assay for rapid detection of**  
2       ***Mycobacterium tuberculosis* complex in respiratory specimens**  
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22 **ABSTRACT**

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24 Purpose

25 The automated high-throughput Abbott RealTime MTB real-time PCR assay has been recently launched  
26 for *Mycobacterium tuberculosis* complex (MTBC) clinical diagnosis. This study would like to evaluate  
27 its performance.

28 Methods

29 We first compared its diagnostic performance with the Roche Cobas TaqMan MTB assay on 214 clinical  
30 respiratory specimens. Prospective analysis of a total 520 specimens was then performed to further  
31 evaluate the Abbott assay.

32 Results

33 The Abbott assay showed a lower limit of detection at 22.5 AFB/ml, which was more sensitive than the  
34 Cobas assay (167.5 AFB/ml). The two assays demonstrated significant difference in diagnostic  
35 performance (McNemar's test;  $P=0.0034$ ), which the Abbott assay presented significantly higher area  
36 under curve (AUC) than the Cobas assay (1.000 vs 0.880;  $P=0.0002$ ). The Abbott assay demonstrated  
37 extremely low PCR inhibition on clinical respiratory specimens. The automated Abbott assay required  
38 only very short manually handling time (0.5 hour), which could help to improve the laboratory  
39 management. In the prospective analysis, the overall estimates for sensitivity and specificity of the  
40 Abbott assay were both 100% among smear-positive specimens, whereas the smear-negative specimens  
41 were 96.7% and 96.1% respectively. No cross-reactivity with non-tuberculosis mycobacterial species was  
42 observed. The superiority in sensitivity of the Abbott assay for detecting MTBC in smear-negative  
43 specimens could further minimize the risk in MTBC false negative detection.

44 Conclusion

45 The new Abbott RealTime MTB assay has good diagnostic performance which can be a useful diagnostic  
46 tool for rapid MTBC detection in clinical laboratories.

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48 (Word count: 238 words)

49 Keywords: *Mycobacterium tuberculosis*, real-time PCR, clinical diagnosis

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52 **INTRODUCTION**

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54 Tuberculosis can cause high mortality and it has been a global public health problem for decades  
55 [1]. In highly endemic region like Hong Kong, acid-fast bacilli (AFB) smear microscopy and  
56 mycobacterial culture on selective solid media (Lowenstein-Jensen and Stonebrink medium) was used  
57 as conventional methods for *Mycobacterium tuberculosis* complex (MTBC) detection [2-5]. However,  
58 due to the low sensitivity of AFB smear and the long turn-around-time of mycobacterial culture, PCR-  
59 based detection assays have been introduced for rapid detection of MTBC DNA from clinical specimens  
60 [6]. Commercial assays such as the Cobas Amplicor MTB or the Cobas TaqMan MTB assay (Roche  
61 Diagnostics, Switzerland) has been widely used in clinical mycobacteriology laboratories for direct  
62 specimen detection of MTBC DNA in the recent 20 years [7, 8]. These assays could significantly reduce  
63 the diagnostic time from weeks to hours, which can greatly improve patient care and infection control.

64 Recently, Abbott Molecular (Des Plaines, IL) has introduced an automated RealTime MTB  
65 PCR assay for *in vitro* diagnostic purpose. This *in-vitro* diagnostic (CE-IVD) marked assay includes an  
66 automated extraction step, which can manage a maximum of 96 specimens in a single batch, and a real-  
67 time PCR-based amplification that specifically targets two MTBC genes, the insertion sequence 6110  
68 (IS6110) and protein antigen b (PAB) in a single reaction [9, 10]. The presence of multiple copies of  
69 IS6110 gene in some MTBC can certainly increase the diagnostic sensitivity of the assay.

70 In this study, we compared the diagnostic performance of the new Abbott assay with the Roche  
71 Cobas TaqMan MTB assay (Roche Diagnostics, Switzerland). A prospective analysis of the Abbott  
72 system was also performed on 535 clinical respiratory specimens requesting for mycobacterium culture.  
73 The performance of the Abbott system was further compared with the conventional AFB smear staining  
74 and culture results.

## METHODS AND MATERIALS

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### Specimen collection and sample processing

A total of 535 sputum specimens were available from adult patients with chest symptoms and/or chest radiographic infiltrates of undetermined origin between 4 December 2013 and 24 March 2014 in Queen Mary Hospital and Chest Clinics. Clinical and treatment history of these patients were collected for culture-PCR discrepancy analysis. All clinical specimens were first examined by fluorescence Auramine O and Ziehl-Neelsen staining for AFB. The sputum specimens were then decontaminated and concentrated as described previously [2]. The digested sediments were divided equally for mycobacterial culture and subsequent PCR assays. Mycobacterial cultures were grown on Lowenstein-Jensen and Stonebrink medium with incubation for 6 weeks. Positive AFB cultures were identified as described previously [7, 11]. The species identification of non-tuberculosis mycobacteria (NTM) was determined by 16S rRNA gene sequencing [12].

For the first 214 specimens collected during the study, parallel run on the Roche Cobas TaqMan MTB assay and the Abbott RealTime MTB assay would be performed. For the other 221 specimens collected afterwards, only the Abbott assay would be performed. For specimens demonstrated to be PCR positive, they were further confirmed by using the Cobas TaqMan MTB assay. On the other hand, the limit of detection (LOD) for both assays was further evaluated by 8 serial dilutions of the *M. tuberculosis* H37Rv control strain (0-100,000 AFB per ml) spiking into AFB negative sputum. The number of AFB in each dilution was confirmed by actual count on bacterial culture colonies grown on Middlebrook 7H10 agar after 6 weeks incubation. This study protocol has been approved by the institutional review board (IRB Reference Number: UW 12-309).

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### Cobas TaqMan MTB assay

The Cobas TaqMan MTB assay which included 2 major steps, the DNA extraction and PCR amplification was conducted according to the manufacturer's instructions [13]. Briefly, 0.5 ml of each digested sediment was mixed with same volume of wash solution and centrifuged. The supernatant was discarded and the pellet was lysed. Finally, the mixture was incubated and neutralized. MTB DNA was then extracted by using the Cobas Amplicor Respiratory Specimen Preparation Kit (Roche Diagnostics, Switzerland). Fifty microliters of DNA extract were used for each PCR reaction by using the Cobas

TaqMan 48 Analyzer (Roche Diagnostics, Switzerland) with positive and negative controls. A maximum of 48 samples could be performed per run. One negative control and 1 positive control were included in each run, therefore allowing a maximum of 46 specimens to be processed per run.

#### **Abbott RealTime MTB automated assay**

The RealTime MTB automated assay included a simple manual sample inactivation step and the automated sample preparation and amplification steps. A maximum of 96 samples could be performed per run. One negative control and 1 positive control were included in each run, therefore allowing a maximum of 94 specimens to be processed per run. For sample inactivation, 0.5ml of each digested sediment was manually mixed with 1.5ml inactivation reagent (0.4M NaOH, 60% Isopropanol and 0.18% Tween-20). The mixtures were incubated for at least 1 hour but no more than 24 hours before loading into the Abbott *m2000sp* instrument for nucleic acid extraction. Sample preparation step was then performed automatically in the Abbott *m2000sp* instrument by using the *mSample Preparation* System DNA kit. Twenty-five microliters of extraction eluate were then mixed with 25 µl amplification reagent mix from the RealTime MTB Amplification Reagent Kit. The sample amplification step was then performed in the Abbott *m2000rt* real-time PCR thermocycler with the “RealTime MTB assay application” protocol.

#### **Statistical analysis**

The diagnostic performance of PCR was evaluated against that of bacterial culture plus medical history including chest X-ray and clinical coughing symptoms as gold standard. The sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated based on the results of concurrently performed mycobacterial cultures. The area under curve (AUC) calculated from the receiver operating characteristic (ROC) curve that generated by the MedCalc software version 14.12.0 (MedCalc Software, USA) were compared between the two methods for performance evaluation. The correlation of the two assays was also compared by the McNemar’s test.

## RESULTS

### Limit of detection for the Abbott RealTime MTB assay

Serial dilutions of the MTB standard strain H37Rv were used to compare the LOD of the Abbott and Cobas assays. The average LOD for each assay was determined by the average of 2 serial dilution sets of H37Rv (Table 1). The Cobas TaqMan MTB assay was found to have an average LOD at 167.5 AFB/ml ( $C_p$  value = 44.7) while the Abbott assay demonstrated to have an average LOD at 22.5 AFB/ml ( $C_t$  value = 39.0).

### Comparative analysis of the Abbott RealTime MTB and Roche Cobas TaqMan MTB assays

The first 214 clinical sputum specimens received from the chest clinic were subjected in parallel to the Cobas TaqMan MTB and Abbott assays. No PCR inhibition was observed in the Abbott RealTime MTB assay, while there were 23 specimens demonstrated PCR inhibition in the Cobas TaqMan MTB assay and they were removed from the comparative analysis. The other 191 non-inhibited specimens were eligible for the analysis of diagnostic performance of the two assays (Table 2).

The overall diagnostic sensitivity was 100% for the Abbott assay and 76.1% for the Cobas assay, while the overall diagnostic specificity for the Abbott and Cobas assay was both 99.3% (Table 2). The McNemar's test results ( $P=0.0034$ ) showed that the two assays had significant difference in diagnostic performance for clinical respiratory specimens. The ROC curve for the two assays showed that the AUC was 1.000 for the Abbott assay with optimal  $C_t$  cut-off value at 40.7 and 0.880 for the Cobas assay at optimal  $C_p$  cut-off value at 46.7 (Figure 1). Significant difference could be observed between the AUC of the two assays ( $P=0.0002$ ).

About the processing time, the Abbott RealTime MTB assay required 6 hours for the detection of 46 specimens plus 2 controls in each batch. The sample inactivation, nucleic acid extraction and PCR amplification mixture preparation steps took 4 hours while the amplification needed 2 hours. The automated extraction and detection design of the Abbott RealTime MTB assay could minimize the actual manual handling time to 0.5 hour. For handling the same number of specimens by using the Cobas TaqMan MTB assay, 1.5 hour was required for nucleic acid extraction and another 0.5 hour was required for PCR amplification mixture preparation. The PCR amplification step required another 4 hours for completion, which made the total processing time to 6 hours. Since the extraction and PCR amplification

mix preparation for the Cobas assay required manual processing, the actual labor handling time for this assay could be up to 2 hours for handling every 46 specimens.

#### **Prospective analysis of diagnostic performance for Abbott RealTime MTB assay**

In order to further evaluate the clinical performance of the Abbott assay, a total of 532 sputum samples were collected within the study period. All specimens were tested by culture, smear staining and the Abbott assay (Table 3). After excluding 12 culture contaminated specimens, results of 520 specimens were included for diagnostic performance analysis. Among the 520 specimens, 42 of them were confirmed to be NTM including *M. avium* complex, *M. abscessus/chelonae*, *M. celatum*, *M. kansasii*, *M. gordonae*, *M. neoaurum* and *M. xenopi* by 16S rRNA gene sequencing. All these 42 NTM were determined as PCR negative in the Abbott RealTime MTB assay.

#### Smear positive specimens

A total of 63 out of 520 specimens (12.1%) were AFB smear positive. All specimens showed concordant results (48 positives and 15 negatives) between the Abbott RealTime MTB assay and MTB culture after reviewing the medical history of the discrepant cases. The Abbott assay was found to have 100% sensitivity and 100% specificity among the smear positive specimens after discrepancies had been resolved.

#### Smear negative specimens

For smear negative specimens, a total of 457 out of 520 specimens (87.9%) were collected. There were 425 specimens showing concordant results (78 positives and 377 negatives) between the Abbott RealTime assay and culture after reviewing the clinical symptoms and medical history of the discrepant cases. There were still 2 PCR positive but culture negative cases remaining unresolved. The Abbott assay showed a 100% sensitivity and 99.5% specificity among the smear negative specimens after resolving the discrepancies.

#### Overall performance of Abbott RealTime MTB assay

Overall, the RealTime MTB assay yielded 520 (99.6%) concordant results (126 positives and 394 negatives) with the MTB culture. The overall sensitivity, specificity, PPV and NPV of the Abbott

192 assay were 100%, 99.5%, 98.4%, and 100% respectively (Table 3). According to the statistical findings,  
193 the ROC curve for the RealTime MTB assay showed that the area under curve was 0.999 (95% CI: 0.991-  
194 1.000).



## DISCUSSION

Early diagnosis and initiating treatment are vital steps for tuberculosis infection control and patient care. Due to the low sensitivity of smear staining and the long incubation time of MTB bacterial culture, PCR-based tests with high sensitivity and short turn-a-round time had been widely used in clinical mycobacteriology laboratories. In the present study, we evaluated the diagnostic performance for the new Abbott RealTime MTB automated high-throughput real-time PCR assay (maximum 94 clinical specimens per batch) on clinical respiratory specimens. The performance of this new assay was also compared with the Roche Cobas TaqMan MTB assay.

The new Abbott assay was demonstrated to be nearly 10-fold more sensitive than the Cobas assay. This could be due to duo-targets (multiple copies IS6110 gene and the MTBC specific PAB gene) used in Abbott assay, whereas the Cobas TaqMan assay targets solely the single copy 16S rRNA gene in MTBC. The high sensitivity of the Abbott assay could benefit the detection of low MTBC DNA load in clinical respiratory specimens. The McNemar's test results of the two assays demonstrated the significant difference in diagnostic sensitivity of the two assays. The significantly higher AUC in Abbott assay showed that the new Abbott assay has better diagnostic performance in comparing to the widely used Roche Cobas TaqMan MTB assay.

Through this evaluation, around 10% of PCR inhibition could be found by using the Cobas assay, whereas the Abbott assay did not show any inhibition among the respiratory specimens. The low PCR inhibition rate of the Abbott system could be explained by the use of multiple silica beads washing steps during the DNA extraction. These washes could significantly minimize the amount of PCR inhibitors present in the nucleic acid eluates. On the other hand, the Cobas assay used crude DNA alkaline lysis method to extract nucleic acid. The cell debris and other crude proteins in the DNA extract could become inhibitors during PCR amplification. Among these 23 Cobas PCR inhibited specimens found in this evaluation, 6 of them were actually MTB culture positive and MTB DNA could be detected in all 6 specimens by the Abbott assay. The Abbott assay seems to have advantage in handling clinical specimens with PCR inhibitors.

Other than the clinical performance, the ease of handling was also compared. Although both the Abbott and Cobas assays required 6 hours for processing, we found that the actual manual handling time for the Abbott system (0.5 hours) is much less than that of the Cobas TaqMan MTB system (2 hours).

The difference is mainly due to the fully automated extraction steps in the Abbott assay versus the manual alkaline lysis extraction steps in the Cobas assay. The shortening of labor manual handling time can benefit the human resources management in clinical laboratories.

From the prospective analysis in this study, the Abbott assay demonstrated high sensitivity (100%) and specificity (99.5%) on respiratory specimens. These performance values of the Abbott system were better than those reported in various studies for the Cobas TaqMan MTB assay [8, 14-17]. The high sensitivity and specificity indicated that the chance of production of false positives and negatives in the Abbott system was low. The high overall PPV (98.4%) and NPV (98.4%) of the Abbott assay indicated that the new system has high precision and specificity to MTBC and it is reliable for excluding NTM in clinical specimens.

For the smear positive specimens, the Abbott assay showed perfect sensitivity and specificity on both culture positive and negative specimens. This indicates that the Abbott system has superior performance in detection of MTBC in AFB smear positive specimens. Among the smear negative specimens, high sensitivity (100%) PPV (97.5%) was also observed for the Abbott assay.

In conclusion, the diagnostic performance of the CE-IVD Abbott RealTime MTB assay was comparable to the widely using Roche Cobas TaqMan MTB assay. The new Abbott assay has both high sensitivity and specificity to either AFB smear positive and negative respiratory specimens. The automated extraction steps of the Abbot system can significantly shorten the labor manual handling time and this can benefit the laboratory management. Therefore, our study results suggest that this new Abbott RealTime MTB assay can be a good candidate for routine *M. tuberculosis* complex detection in clinical laboratories.

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**FIGURE AND TABLE LEGENDS**

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**TABLE 1**

Limit of detection of the Abbott RealTime MTB and Roche Cobas TaqMan MTB assays

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**TABLE 2**

Diagnostic performance comparison of the Abbott RealTime MTB and Roche Cobas TaqMan MTB

assays, with *M. tuberculosis* culture results as the gold standard

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**TABLE 3**

Diagnostic performance of the Abbott RealTime MTB assay on 520 clinical specimens

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**FIGURE 1**

ROC curve of the Cobas TaqMan MTB and Abbott RealTime MTB assays. The area under curve (AUC)

was 1.000 with  $C_t$  cut-off at 40.7 for the Abbott assay and 0.880 with  $C_p$  cut-off at 46.7 for the Cobas

assay.

**TABLE 1**      Limit of detection of the Cobas TaqMan MTB and RealTime MTB assays

Serial dilutions	Average actual concentration (AFB/ml)	Abbott RealTime MTB		Roche Cobas Taqman MTB	
		Interpretation	Target C <sub>t</sub> value (±SD)	Interpretation	Target C <sub>p</sub> value (±SD)
A	100000	Positive	25.35±0.72	Positive	33.4±1.06
B	10000	Positive	28.95±0.70	Positive	36.8±0.85
C	755	Positive	35.70±2.03	Positive	42.5±0.64
D	167.5	Positive	38.38±0.40	Positive	44.7±0
E	79	Positive	38.41±0.28	Negative	ND
F	22.5	Positive	38.63±0.52	Negative	ND
G	1	Negative	ND	Negative	ND
H	0	Negative	ND	Negative	ND

ND, Not detected; SD, Standard deviation

**TABLE 2** Diagnostic performance comparison of the Abbott RealTime MTB and Roche Cobas TaqMan MTB assays, with *M. tuberculosis* culture results as the gold standard

Assay	Smear result	Performance (% [95% CI])*				AUC
		Sensitivity	Specificity	PPV	NPV	
Abbott	Positive (22)	100 (100-100)	100 (100-100)	100 (100-100)	100 (100-100)	
RealTime	Negative (192)	100 (100-100)	99.3 (97.9-100)	97.0 (91.1-100)	100 (100-100)	
MTB	All (214)	100 (100-100)	99.3 (98.0-100)	97.9 (93.7-102.0)	100 (100-100)	1.000 (0.980-1.000)
Cobas	Positive (22)	92.9 (79.4-100)	100 (100-100)	100 (100-100)	75.0 (32.6-100)	
TaqMan	Negative (192)	68.8 (52.7-84.8)	99.3 (97.9-100)	95.7 (87.3-100)	93.4 (89.4-97.3)	
MTB	All (214)	76.1 (63.8-88.4)	99.3 (98.0-100)	97.2 (91.9-100)	92.9 (88.9-96.9)	0.880 (0.825-0.922)

CI, confidence Intervals; PPV, positive predictive value; NPV, negative predictive value;

AUC, area under receiver operating characteristic (ROC) curve

\* The results were adjusted after discrepancy analysis.

**TABLE 3** Diagnostic performance of the Abbott RealTime MTB assay on 520 clinical specimens

Specimen group (no. of specimens)	Result for Abbott RealTime MTB assay		Result for MTB culture <sup>#</sup>		% (95% CI) for Abbott RealTime MTB assay			
			Positive	Negative	Sensitivity	Specificity	PPV	NPV
Smear positive (63)	Positive		48	0	100	100	100	100
	Negative		0	15 <sup>a</sup>	(100-100)	(100-100)	(100-100)	(100-100)
Smear negative (457)	Positive		78	2	100	99.5	97.5	100
	Negative		0	377 <sup>b</sup>	(100-100)	(98.7-100)	(94.1-100)	(100-100)
Overall (520)	Positive		126	2	100	99.5	98.4	100
	Negative		0	392	(100-100)	(98.8-100)	(96.3-100)	(100-100)

CI, Confidence Intervals; PPV, Positive predictive value; NPV, Negative predictive value

<sup>a</sup> 15 samples were confirmed non-tuberculosis mycobacteria (7 *M. abscessus/chelonae*, 4 *M. avium* complex, 3 *M. kansasii* and 1 *M. xenopi*) by 16S rRNA gene sequencing

<sup>b</sup> 27 samples were confirmed non-tuberculosis mycobacteria (15 *M. avium* complex, 7 *M. abscessus/chelonae*, 2 *M. celatum*, 1 *M. brumae*, 1 *M. goodii* and 1 *M. neoaurum*) by 16S rRNA gene sequencing

<sup>#</sup> The results were adjusted after discrepancy analysis.





Figure1

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