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Author(s)	Chen, JHK; She, KKK; Kwong, TC; Wong, OY; Siu, GKH; Leung, CC; Chang, KC; Tam, CM; Ho, PL; Cheng, VCC; Yuen, KY; Yam, WC
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1 **Performance of the new automated Abbott RealTi*me* MTB assay for rapid detection of**

2 ***Mycobacterium tuberculosis* complex in respiratory specimens**

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4 Jonathan HK Chen[1], Kevin KK She[1], Tsz-Ching Kwong[1], Oi-Ying Wong[1], Gilman KH Siu[2], Chi-Chiu

5 Leung[4], Kwok-Chiu Chang[4], Cheuk-Ming Tam[4], Pak-Leung Ho[1,3], Vincent CC Cheng[1,3], Kwok-Yung

6 Yuen[1,3], Wing-Cheong Yam[1,3].

7

8 [1]Department of Microbiology, Queen Mary Hospital, The University of Hong Kong, Hong Kong Special

9 Administrative Region, China

10 [2]Department of Health Technology and Informatics, The Hong Kong Polytechnic University, Hong

11 Kong Special Administrative Region, China

12 [3]Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong Special Administrative

13 Region, China

14 [4] Tuberculosis and Chest Service, Centre for Health Protection, Department of Health, Hong Kong, China

15

16 Correspondence:

17 Wing-Cheong Yam, Department of Microbiology, Queen Mary Hospital, The University of Hong Kong,

18 Queen, Pokfulam, Hong Kong Special Administrative Region, China

19 (Tel: +852-22554821, Fax: +852-28551241, E-mail: wcyam@hkucc.hku.hk)

20

ABSTRACT

<u>Purpose</u>

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

<u>Methods</u>

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

<u>Results</u>

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

<u>Conclusion</u>

The new Abbott RealTi*me* MTB assay has good diagnostic performance which can be a useful diagnostic tool for rapid MTBC detection in clinical laboratories.

(Word count: 238 words)

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

INTRODUCTION

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

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

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

75 **METHODS AND MATERIALS**

76

77 **Specimen collection and sample processing**

78   A total of 535 sputum specimens were available from adult patients with chest symptoms and/or

79 chest radiographic infiltrates of undetermined origin between 4 December 2013 and 24 March 2014 in

80 Queen Mary Hospital and Chest Clinics. Clinical and treatment history of these patients were collected

81 for culture-PCR discrepancy analysis. All clinical specimens were first examined by fluorescence

82 Auramine O and Ziehl-Neelsen staining for AFB. The sputum specimens were then decontaminated and

83 concentrated as described previously [2]. The digested sediments were divided equally for mycobacterial

84 culture and subsequent PCR assays. Mycobacterial cultures were grown on Lowenstein-Jensen and

85 Stonebrink medium with incubation for 6 weeks. Positive AFB cultures were identified as described

86 previously [7, 11]. The species identification of non-tuberculosis mycobacteria (NTM) was determined

87 by 16S rRNA gene sequencing [12].

88   For the first 214 specimens collected during the study, parallel run on the Roche Cobas TaqMan

89 MTB assay and the Abbott RealTi*me* MTB assay would be performed. For the other 221 specimens

90 collected afterwards, only the Abbott assay would be performed. For specimens demonstrated to be PCR

91 positive, they were further confirmed by using the Cobas TaqMan MTB assay. On the other hand, the

92 limit of detection (LOD) for both assays was further evaluated by 8 serial dilutions of the *M. tuberculosis*

93 H37Rv control strain (0-100,000 AFB per ml) spiking into AFB negative sputum. The number of AFB

94 in each dilution was confirmed by actual count on bacterial culture colonies grown on Middlebrook 7H10

95 agar after 6 weeks incubation. This study protocol has been approved by the institutional review board

96 (IRB Reference Number: UW 12-309).

97

98 **Cobas TaqMan MTB assay**

99   The Cobas TaqMan MTB assay which included 2 major steps, the DNA extraction and PCR

100 amplification was conducted according to the manufacturer's instructions [13]. Briefly, 0.5 ml of each

101 digested sediment was mixed with same volume of wash solution and centrifuged. The supernatant was

102 discarded and the pellet was lysed. Finally, the mixture was incubated and neutralized. MTB DNA was

103 then extracted by using the Cobas Amplicor Respiratory Specimen Preparation Kit (Roche Diagnostics,

104 Switzerland). Fifty microliters of DNA extract were used for each PCR reaction by using the Cobas

105　TaqMan 48 Analyzer (Roche Diagnostics, Switzerland) with positive and negative controls. A maximum

106　of 48 samples could be performed per run. One negative control and 1 positive control were included in

107　each run, therefore allowing a maximum of 46 specimens to be processed per run.

108

109　**Abbott RealTi*me* MTB automated assay**

110　　　The RealTi*me* MTB automated assay included a simple manual sample inactivation step and

111　the automated sample preparation and amplification steps. A maximum of 96 samples could be

112　performed per run. One negative control and 1 positive control were included in each run, therefore

113　allowing a maximum of 94 specimens to be processed per run. For sample inactivation, 0.5ml of each

114　digested sediment was manually mixed with 1.5ml inactivation reagent (0.4M NaOH, 60% Isopropanol

115　and 0.18% Tween-20). The mixtures were incubated for at least 1 hour but no more than 24 hours before

116　loading into the Abbott *m*2000*sp* instrument for nucleic acid extraction. Sample preparation step was

117　then performed automatically in the Abbott *m*2000*sp* instrument by using the *m*Sample Preparation

118　System DNA kit. Twenty-five microliters of extraction elutate were then mixed with 25 μl amplification

119　reagent mix from the RealTi*me* MTB Amplification Reagent Kit. The sample amplification step was

120　then performed in the Abbott *m*2000*rt* real-time PCR thermocycler with the "RealTi*me* MTB assay

121　application" protocol.

122

123　**Statistical analysis**

124　　　The diagnostic performance of PCR was evaluated against that of bacterial culture plus medical

125　history including chest X-ray and clinical coughing symptoms as gold standard. The sensitivities,

126　specificities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated

127　based on the results of concurrently performed mycobacterial cultures. The area under curve (AUC)

128　calculated from the receiver operating characteristic (ROC) curve that generated by the MedCalc

129　software version 14.12.0 (MedCalc Software, USA) were compared between the two methods for

130　performance evaluation. The correlation of the two assays was also compared by the McNemar's test.

131

132 **RESULTS**

133

134 **Limit of detection for the Abbott RealTi*me* MTB assay**

135   Serial dilutions of the MTB standard strain H37Rv were used to compare the LOD of the Abbott

136 and Cobas assays. The average LOD for each assay was determined by the average of 2 serial dilution

137 sets of H37Rv (Table 1). The Cobas TaqMan MTB assay was found to have an average LOD at 167.5

138 AFB/ml (C_p value = 44.7) while the Abbott assay demonstrated to have an average LOD at 22.5 AFB/ml

139 (C_t value = 39.0).

140

141 **Comparative analysis of the Abbott RealTi*me* MTB and Roche Cobas TaqMan MTB assays**

142   The first 214 clinical sputum specimens received from the chest clinic were subjected in parallel

143 to the Cobas TaqMan MTB and Abbott assays. No PCR inhibition was observed in the Abbott RealTi*me*

144 MTB assay, while there were 23 specimens demonstrated PCR inhibition in the Cobas TaqMan MTB

145 assay and they were removed from the comparative analysis. The other 191 non-inhibited specimens

146 were eligible for the analysis of diagnostic performance of the two assays (Table 2).

147   The overall diagnostic sensitivity was 100% for the Abbott assay and 76.1% for the Cobas

148 assay, while the overall diagnostic specificity for the Abbott and Cobas assay was both 99.3% (Table 2).

149 The McNemar's test results (P=0.0034) showed that the two assays had significant difference in

150 diagnostic performance for clinical respiratory specimens. The ROC curve for the two assays showed

151 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

152 Cobas assay at optimal C_p cut-off value at 46.7 (Figure 1). Significant difference could be observed

153 between the AUC of the two assays (P=0.0002).

154   About the processing time, the Abbott RealTi*me* MTB assay required 6 hours for the detection

155 of 46 specimens plus 2 controls in each batch. The sample inactivation, nucleic acid extraction and PCR

156 amplification mixture preparation steps took 4 hours while the amplification needed 2 hours. The

157 automated extraction and detection design of the Abbott RealTi*me* MTB assay could minimize the actual

158 manual handling time to 0.5 hour. For handling the same number of specimens by using the Cobas

159 TaqMan MTB assay, 1.5 hour was required for nucleic acid extraction and another 0.5 hour was required

160 for PCR amplification mixture preparation. The PCR amplification step required another 4 hours for

161 completion, which made the total processing time to 6 hours. Since the extraction and PCR amplification

162 mix preparation for the Cobas assay required manual processing, the actual labor handling time for this

163 assay could be up to 2 hours for handling every 46 specimens.

164

165 **Prospective analysis of diagnostic performance for Abbott RealTi*me* MTB assay**

166   In order to further evaluate the clinical performance of the Abbott assay, a total of 532 sputum

167 samples were collected within the study period. All specimens were tested by culture, smear staining and

168 the Abbott assay (Table 3). After excluding 12 culture contaminated specimens, results of 520 specimens

169 were included for diagnostic performance analysis. Among the 520 specimens, 42 of them were

170 confirmed to be NTM including *M. avium* complex, *M. abscessus/chelonae*, *M. celatum*, *M. kansasii*, *M.*

171 *gordonae*, *M. neoaurum* and *M. xenopi* by 16S rRNA gene sequencing. All these 42 NTM were

172 determined as PCR negative in the Abbott RealTi*me* MTB assay.

173

174 <u>Smear positive specimens</u>

175   A total of 63 out of 520 specimens (12.1%) were AFB smear positive. All specimens showed

176 concordant results (48 positives and 15 negatives) between the Abbott RealTi*me* MTB assay and MTB

177 culture after reviewing the medical history of the discrepant cases. The Abbott assay was found to have

178 100% sensitivity and 100% specificity among the smear positive specimens after discrepancies had been

179 resolved.

180

181 <u>Smear negative specimens</u>

182   For smear negative specimens, a total of 457 out of 520 specimens (87.9%) were collected.

183 There were 425 specimens showing concordant results (78 positives and 377 negatives) between the

184 Abbott RealTi*me* assay and culture after reviewing the clinical symptoms and medical history of the

185 discrepant cases. There were still 2 PCR positive but culture negative cases remaining unresolved. The

186 Abbott assay showed a 100% sensitivity and 99.5% specificity among the smear negative specimens

187 after resolving the discrepancies.

188

189 <u>Overall performance of Abbott RealTi*me* MTB assay</u>

190   Overall, the RealTi*me* MTB assay yielded 520 (99.6%) concordant results (126 positives and

191 394 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 RealTi*me* MTB assay showed that the area under curve was 0.999 (95% CI: 0.991-

194   1.000).

195   **DISCUSSION**

196

197   Early diagnosis and initiating treatment are vital steps for tuberculosis infection control and

198   patient care. Due to the low sensitivity of smear staining and the long incubation time of MTB bacterial

199   culture, PCR-based tests with high sensitivity and short turn-a-round time had been widely used in

200   clinical mycobacteriology laboratories. In the present study, we evaluated the diagnostic performance

201   for the new Abbott RealTi*me* MTB automated high-throughput real-time PCR assay (maximum 94

202   clinical specimens per batch) on clinical respiratory specimens. The performance of this new assay was

203   also compared with the Roche Cobas TaqMan MTB assay.

204   The new Abbott assay was demonstrated to be nearly 10-fold more sensitive than the Cobas

205   assay. This could be due to duo-targets (multiple copies IS6110 gene and the MTBC specific PAB gene)

206   used in Abbott assay, whereas the Cobas TaqMan assay targets solely the single copy 16S rRNA gene

207   in MTBC. The high sensitivity of the Abbott assay could benefit the detection of low MTBC DNA load

208   in clinical respiratory specimens. The McNemar's test results of the two assays demonstrated the

209   significant difference in diagnostic sensitivity of the two assays. The significantly higher AUC in Abbott

210   assay showed that the new Abbott assay has better diagnostic performance in comparing to the widely

211   used Roche Cobas TaqMan MTB assay.

212   Through this evaluation, around 10% of PCR inhibition could be found by using the Cobas

213   assay, whereas the Abbott assay did not show any inhibition among the respiratory specimens. The low

214   PCR inhibition rate of the Abbott system could be explained by the use of multiple silica beads washing

215   steps during the DNA extraction. These washes could significantly minimize the amount of PCR

216   inhibitors present in the nucleic acid elutates. On the other hand, the Cobas assay used crude DNA

217   alkaline lysis method to extract nucleic acid. The cell debris and other crude proteins in the DNA extract

218   could become inhibitors during PCR amplification. Among these 23 Cobas PCR inhibited specimens

219   found in this evaluation, 6 of them were actually MTB culture positive and MTB DNA could be detected

220   in all 6 specimens by the Abbott assay. The Abbott assay seems to have advantage in handling clinical

221   specimens with PCR inhibitors.

222   Other than the clinical performance, the ease of handling was also compared. Although both the

223   Abbott and Cobas assays required 6 hours for processing, we found that the actual manual handling time

224   for the Abbott system (0.5 hours) is much less than that of the Cobas TaqMan MTB system (2 hours).

225 The difference is mainly due to the fully automated extraction steps in the Abbott assay versus the manual

226 alkaline lysis extraction steps in the Cobas assay. The shortening of labor manual handling time can

227 benefit the human resources management in clinical laboratories.

228 From the prospective analysis in this study, the Abbott assay demonstrated high sensitivity

229 (100%) and specificity (99.5%) on respiratory specimens. These performance values of the Abbott

230 system were better than those reported in various studies for the Cobas TaqMan MTB assay [8, 14-17].

231 The high sensitivity and specificity indicated that the chance of production of false positives and

232 negatives in the Abbott system was low. The high overall PPV (98.4%) and NPV (98.4%) of the Abbott

233 assay indicated that the new system has high precision and specificity to MTBC and it is reliable for

234 excluding NTM in clinical specimens.

235 For the smear positive specimens, the Abbott assay showed perfect sensitivity and specificity

236 on both culture positive and negative specimens. This indicates that the Abbott system has superior

237 performance in detection of MTBC in AFB smear positive specimens. Among the smear negative

238 specimens, high sensitivity (100%) PPV (97.5%) was also observed for the Abbott assay.

239 In conclusion, the diagnostic performance of the CE-IVD Abbott RealTi*me* MTB assay was

240 comparable to the widely using Roche Cobas TaqMan MTB assay. The new Abbott assay has both high

241 sensitivity and specificity to either AFB smear positive and negative respiratory specimens. The

242 automated extraction steps of the Abbot system can significantly shorten the labor manual handling time

243 and this can benefit the laboratory management. Therefore, our study results suggest that this new Abbott

244 RealTi*me* MTB assay can be a good candidate for routine *M. tuberculosis* complex detection in clinical

245 laboratories.

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290

FIGURE AND TABLE LEGENDS

TABLE 1

Limit of detection of the Abbott RealTi*me* MTB and Roche Cobas TaqMan MTB assays

TABLE 2

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

TABLE 3

Diagnostic performance of the Abbott RealTi*me* MTB assay on 520 clinical specimens

FIGURE 1

ROC curve of the Cobas TaqMan MTB and Abbott RealTi*me* 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 RealTi*me* MTB assays

Serial dilutions	Average actual concentration (AFB/ml)	Abbott RealTi*me* MTB		Roche Cobas Taqman MTB	
		Interpretation	Target C_t value ($\pm$SD)	Interpretation	Target C_p value ($\pm$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 RealTi*me* 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)		
RealTi*me*	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 [#]		% (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 [a]	(100-100)	(100-100)	(100-100)	(100-100)
Smear negative (457)	Positive	78	2	100	99.5	97.5	100
	Negative	0	377 [b]	(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

[a] 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

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

[#] The results were adjusted after discrepancy analysis.

Figure1