

DEVELOPMENT AND EVALUATION OF THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX ON THE BD MAX™ SYSTEM IN A EUROPEAN MULTICENTER STUDY



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Objectives

The ability to rapidly diagnose *Mycobacterium tuberculosis* complex (MTB) is a priority for healthcare worldwide. Furthermore, there is a need for automated tests which can be done in non-specialised settings. The purpose of this study was to evaluate an in-house qPCR for the detection of MTB on the BD MAX system (Becton Dickinson, figure 1). To demonstrate multi-user acceptability custom made BD MAX sealed snap-in tubes were made containing dried primers and probes which were evaluated for MTB assay performance in 3 laboratories.



Figure 1. BD MAX™ system.

Methods

The MTB2012 QCMD panel was tested on the BD MAX at three laboratory sites. This panel of ten specimens with 0-10,000 cells/sample of MTB, was treated with proteinase K and inactivated for 20 minutes at 100°C. The total sample volume was transferred to a Sample Buffer Tube and loaded onto the BD MAX instrument. The extraction was performed using the ExK DNA-1 extraction kit followed by qPCR with BD MMK (SPC) mastermix and dried down primers and probes for MTB IS6110 detection.

The analytical limit of detection (LoD) of the assay was determined by testing several concentrations of the target sequence, which was cloned into a plasmid. Each concentration was tested in replicas and the LoD was defined as the lowest concentration at which all replicates tested positive. A retrospective evaluation of 278 BAL and sputum samples was performed. Specimens were treated according to the customary pre-processing protocol at the specific laboratory sites and tested in the MTB assay. Pre-treatment for laboratories 1, 2 and 3 were, respectively, proteinase K treatment (93 samples); 1:1 sputasol treatment followed by decontamination with a 2% NaOH-NALC solution and phosphate buffer (91 samples) and BD Mycoprep decontamination treatment (94 samples). qPCR results were compared to culture using Mycobacteria Growth Indicator Tube (BD) which was performed at all sites. Each laboratory used their routine MTB PCR setting to analyse discrepant results.

Table 1: Results BD MAX qPCR with culture as a gold standard for *M.tuberculosis* complex positivity

		Culture		
		Positive	Negative	
qPCR	Positive	79	3	PPV: 96.3%
	Negative	3	193	NPV: 98.5%
		Sens: 96.3%	Spec: 98.5%	N= 278

Results

Eight QCMD specimens containing 100-10,000 cells/sample were positive and 2 with no cells were negative in the MTB assay at all laboratory sites. This is in complete concordance with the final QCMD report. All plasmid-dilutions with a concentration of 16 target copies in the PCR provided a MTB-positive result in the assay. In addition, 1 target copy per PCR was negative in all replicas. Thus, this study shows a 100% LoD for 16 target copies per PCR reaction. Furthermore, the retrospective clinical evaluation has shown an overall sensitivity, specificity, positive and negative predictive values of 96.3%, 98.5%, 96.3% and 98.5%, respectively, using different pre-treatment methods (table 1). Discrepancy analysis on PCR positive, culture negative samples shows 100% concordance with the routine MTB PCR setting, this results in a true positive predictive value of 100%. Additionally, neither of the PCR negative, culture positive samples could be confirmed positive with the use of another PCR method.

CONCLUSIONS

- A sensitive assay has been developed for the detection of *Mycobacterium tuberculosis* complex on the BD MAX.
- The utilisation of the dried down primer/probes on the automated BD MAX offered considerable practical advantages in terms of walk away functionality, minimal hands on time and ease-of-use.
- This type of assay set up on BD MAX could enable a wider use of PCR for MTB diagnosis.

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