Electronic Supplementary Material (ESI) for RSC Advances

Sensitive detection of the IS*6110* sequence of *Mycobacterium tuberculosis* complex based on PCR-magnetic bead ELISA

Soe Paing Kyaw[a,b], Jariya Hanthamrongwit[a], Kulachart Jangpatarapongsa[c], Prasong Khaenam[d], Chaniya Leepiasakulchai[*a]

[a] *Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, 73170*
[b] *Clinical Pathology Laboratory, (1000) Bedded General Hospital, Nay Pyi Taw, Myanmar, 15011*
[c] *Center for Research and Innovation, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, 73170*
[d] *Center for Standardization and Product Validation, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, 73170*

* Corresponding author: Chaniya Leepiasakulchai, email: chaniya.lee@mahidol.edu

Optimization of PCR primer

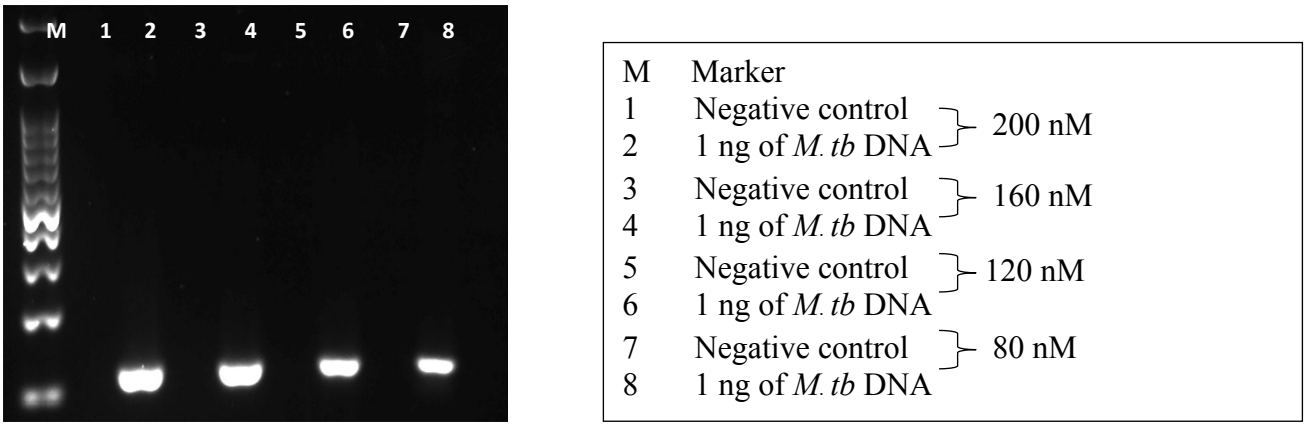
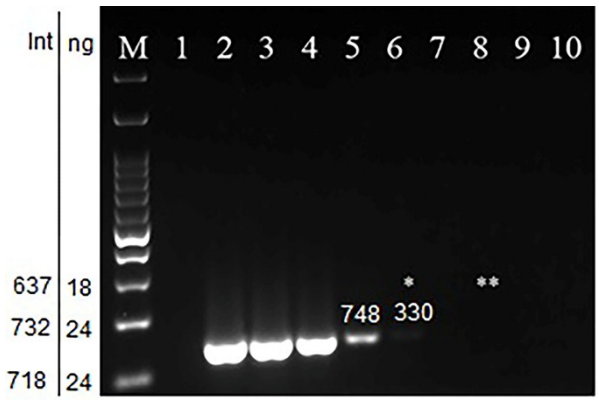


Figure S1 Optimization of PCR primer concentration. The optimal concentration of each DIG and biotin-labelled forward and reverse primer was determined by varying the concentration of primers from 80 nM to 200 nM. One nanogram of *M. tuberculosis* (H37Rv) DNA was used as the template in PCR amplification. The optimal concentration of each forward and reverse primer was selected as 160 nM since the amplified product obtained from this primer set is comparable to that obtained from 200 nM.

Estimation of PCR product concentration



M	Marker
1	Negative control
2	0.5 ng of *M. tb* DNA
3	50 pg of *M. tb* DNA
4	5 pg of *M. tb* DNA
5	0.5 pg of *M. tb* DNA
6	50 fg of *M. tb* DNA
7	5 fg of *M. tb* DNA
8	0.5 fg of *M. tb* DNA
9	50 ag of *M. tb* DNA
10	5 ag of *M. tb* DNA

Figure S2 Agarose gel electrophoresis of PCR products obtained from 10-fold serial dilution of H37Rv DNA starting from 0.5 ng to 5 ag. The estimation of PCR products concentration was done by comparing the band intensity of each PCR product obtained from 10-fold serial dilution of H37Rv DNA with the concentration of 100 bp DNA ladder as suggested by manufacturer (Biotechrabbit, Berlin, Germany). Mean intensity of DNA ladder and each PCR product were measured with the volume tool of Image Lab software, version 5.2.1 (Hercules, CA, USA). Due to the possible PCR saturation, the concentration estimation was done for lane 5 and 6 only. The estimated quantity of 100 bp DNA ladder was 24 ng (in 3 µl) and the concentration of amplicons obtained from the 0.5 pg and 50 fg DNA template concentration were 4 ng/µl and 2 ng/µl respectively. (*) Indicated the lowest detection limit of agarose gel electrophoresis whereas (**) indicated the lowest detection limit of magnetic bead ELISA. (Int = mean intensity, ng = the estimated quantity of DNA in nanogram).