

Letter to the Editor

MPT64 gene mutations leading to non-reactivity on a rapid immunochromatographic assay

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Dear Editor,

Tuberculosis is an infectious disease that remains one of the biggest killers in the world. Despite technological advances in terms of diagnostic and susceptibility testing, it still disproportionately afflicts communities in developing countries. Limited resources in developing countries further compound the difficulties faced when managing this illness; cost-effectiveness is a major factor to consider in determining the most suitable diagnostic tests [1]. Where resources are lacking, laboratory diagnosis may be limited to the identification of acid-fast bacilli (AFB) on direct staining. However, the sensitivity and specificity of AFB smears is limited. Ideally, facilities to culture, identify, and perform susceptibility testing of members of the *Mycobacterium tuberculosis* complex (MTC) are required to direct appropriate therapy. In addition, with the increase in multi-drug-resistant-tuberculosis (MDRTB) and extreme-drug-resistant-tuberculosis (XDRTB), performance of culture and susceptibility testing is becoming increasingly essential to guide treatment.

Nucleic acid based testing for tuberculosis provides a high-level of diagnostic accuracy [2]. Yet the approach is often technically complex and requires specialized equipment to perform. The high cost of such molecular assays precludes their use in resource-limited areas. Simpler, easy-to-use, rapid test alternatives are the current MPT64-based immunochromatographic (ICT) assays that are used to detect MTC from cultures. MPT64 is a MTC specific antigen secreted during bacterial growth, which has been shown to be an excellent target for differentiating MTC from

mycobacteria other than tuberculosis (MOTT) [3,4]. Some *M. bovis* Calmette-Guérin (BCG) strains may however exhibit variable expression due to absence of the *mpt64* gene [5]. Polymorphisms in the MPT64 gene have also appeared in other MTC [6]. Three commercially available MPT64-based ICT tests are: SD BIOLINE TB Ag MPT64 (Standard Diagnostics, Seoul, South Korea), BD MGIT TBc Identification Test (Becton Dickinson, Franklin Lakes, USA), and Capilia TB-Neo (Tauns, Izunokuni, Japan). Validations of these assays indicate very high sensitivity and specificity [7-11].

Our diagnostic microbiology laboratory uses the BACTEC MGIT 960 Mycobacterial Detection System (BD Diagnostics, Franklin Lakes, USA) for mycobacterial cultures. For cultures that flag positive by the MGIT system, Ziehl-Neelsen staining is performed to determine the presence of AFB with serpentine cords. When AFB with cording was identified, the SD BIOLINE TB Ag MPT64 assay was carried out according to manufacturer's instructions. This provided a rapid identification of MTC. Between the years 2012 and 2016, eight isolates were found to be negative by the SD BIOLINE TB Ag MPT64, despite phenotypic features consistent with MTC. These isolates were later confirmed as MTC using molecular methods (Amplified MTD test (Hologic, Inc. San Diego, USA) or the Xpert TB/RIF (Cepheid, Sunnyvale, USA)), as well as an in-house PCR assay differentiating members of MTC [12]. PCR amplification and full-length gene sequencing of MPT64, including 527-base pairs upstream of the translational start site of MPT64 was performed. For six

isolates, a 63-base pair deletion between nucleotides 197 to 259 resulted in the truncation of the MPT64 protein. One isolate had a 2-base pair insertion at nucleotide 347 resulting in a frame shift mutation and another isolate had a missense mutation at nucleotide 257 (Table 1).

The 63-base pair deletion in MPT64 appears to be the most prevalent mutation followed by non-synonymous substitutions [6,13,14]. These mutations affect the structural topology and subsequently the antibody-based recognition of the MPT64 antigen by the immunochromatographic strip. The SD BIOLINE TB Ag MPT64 negative isolates in this study were not genotyped, but previous reports did not demonstrate any association between MPT64 mutations and spoligotypes [6].

Although the true prevalence of such strains is unknown, current data suggests that they are rare. Nevertheless, for laboratories that perform culture, other methods for confirmation of identification still need to be preserved. A negative MPT64 assay cannot

in itself exclude the diagnosis of tuberculosis. Laboratory staff must be aware that other features such as colony morphology also need to be considered before a culture of acid-fast-bacilli can be labelled as MOTT.

References

- Molicotti P, Bua A, Zanetti S (2014) Cost-effectiveness in the diagnosis of tuberculosis: choices in developing countries. J Infect Dev Ctries 8: 24-38.doi:10.3855/jidc.3295
- Hughes R, Wonderling D, Li B, Higgins B (2012) The cost effectiveness of nucleic acid amplification techniques for the diagnosis of tuberculosis. Respir Med 106:300-307.
- Abe C, Hirano K, Tomiyama T (1999) Simple and rapid identification of the Mycobacterium tuberculosis complex by immunochromatographic assay using anti-MPB64 monoclonal antibodies. J Clin Microbiol 37: 3693-3697.
- Marzouk M, Kahla IB, Hannachi N, Ferjeni A, Salma WB, Ghezal S, Boukadida J (2011) Evaluation of an immunochromatographic assay for rapid identification of Mycobacterium tuberculosis complex in clinical isolates. Diagn Microbiol Infect Dis 69: 396-399.
- Li H, Ulstrup JC, Jonassen TO, Melby K, Nagai S, Harboe M (1993) Evidence for absence of the MPB64 gene in some substrains of *Mycobacterium bovis* BCG. Infect Immun 61: 1730-1734.

Table 1. Molecular characterization of *mpt64* of SD BIOLINE TB Ag MPT64-negative clinical *Mycobacterium tuberculosis*.

Isolate	Date of isolation	Description			SD BIOLINE TB Ag MPT64 result	MTB-BCG PCR assay*
		<i>mpt64</i>	<i>mpt64</i> regulatory region	Effect on MPT64		
TB272	7/1/2016	63bp deletion	WT	Loss of amino acids 66 to 86, leading to truncated MPT64 of 207 amino acids	Negative	TB
TB243	30/11/2015	63bp deletion	WT	Loss of amino acids 66 to 86, leading to truncated MPT64 of 207 amino acids	Negative	TB
TB402	22/07/2015	63bp deletion	WT	Loss of amino acids 66 to 86, leading to truncated MPT64 of 207 amino acids	Negative	TB
TB359	19 /01/2015	63bp deletion	WT	Loss of amino acids 66 to 86, leading to truncated MPT64 of 207 amino acids	Negative	TB
TB102	11/11/2014	2 bp (TC) insertion at nucleotide position 347	WT	Truncated MPT64 of 166 amino acids	Negative	TB
TB662	18/11/2013	63bp deletion	WT	Loss of amino acids 66 to 86, leading to truncated MPT64 of 207 amino acids	Negative	TB
T278	10/5/2013	63bp deletion	WT	Loss of amino acids 66 to 86, leading to truncated MPT64 of 207 amino acids	Negative	TB
T73	15/1/2012	Missense mutation, 257 C→G	WT	PRO86ARG	Negative	TB
<i>M. tuberculosis</i> H37Ra #	-	WT	WT	WT	Positive	TB
<i>M. bovis</i> BCG Pasteur #	-	No amplification	No amplification	None	Negative	BCG

WT; wild type sequence; *PCR assay for identification of *M. tuberculosis* complex members. (Parsons *et al.*, 2002); #*M. tuberculosis*H37Ra and *M. bovis* BCG Pasteur were included as positive control.

6. Jiang Y, Lui H, Wang H, Dou X, Zho X, Bai Y, Wan L, Li G, Zhang W, Chen C, Wan K (2013) Polymorphism of antigen MPT64 in *Mycobacterium tuberculosis* strains. *J Clin Microbiol* 51: 1558-1562.
7. Brent AJ, Mugo D, Musyimi R, Mutiso A, Morpeth S, Levin M, Scott JAG (2011) Performance of the MGIT TBc Identification test and meta-analysis of MPT64 assays for identification of the *Mycobacterium tuberculosis* complex in liquid culture. *J Clin Microbiol* 49: 4343-4346.
8. Hasegawa N, Miura T, Ishii K, Yamaguchi K, Lindner TH, Merritt S, Matthews JD, Siddiqi SH (2002) New simple and rapid test for culture confirmation of *Mycobacterium tuberculosis* complex: a multicenter study. *J Clin Microbiol* 40: 908-912.
9. Kumar VGS, Urs TA, Ranganath RR (2011) MPT64 antigen detection for rapid confirmation of *M. tuberculosis* isolates. *BMC Research Notes* 4: 79.
10. Ngamlert K, Sinthuwattanawibool C, McCarthy KD, Sohn H, Starks A, Kanjanamongkolsiri P, Anek-vorapong R, Tasaneeyapan T, Monkongdee P, Diem L, Varma JK (2009). Diagnostic performance and costs of Capilia TB for *Mycobacterium tuberculosis* complex identification from broth-based culture in Bangkok, Thailand. *Trop Med Int Health* 14: 748-753.
11. Yu M-C, Chen H-Y, Wu M-H, Huang W-L, Kuo Y-M, Yu F-L, Jou R (2011). Evaluation of the rapid MGIT TBc Identification test for culture confirmation of *Mycobacterium tuberculosis* complex strain detection. *J Clin Microbiol* 49: 802-807.
12. Parsons LM, Brosch R, Cole ST, Somoskövi A, Loder A, Bretzel G, Van Soolingen D, Hale YM, Salfinger M (2002) Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. *J Clin Microbiol* 40:2339-2345.
13. Basu I, Bower JE, Henderson GK, Lowe O, Newton S, Vaughan R, Roberts SA (2015) False-negative BD MGIT™ TBc Identification Test results in routine tuberculosis diagnosis: a New Zealand perspective. *Int J Tuberc Lung Dis* 19:1073-1075.
14. Qiu Y, Wan L, Liu H, Wan K, Guan C, Jiang Y (2015) Impact of 63-bp deletion and single-base mutation in *mpt64* gene on *M.tb* diagnosis. *Int J Clin Exp Pathol* 8: 3210-3214.

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