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International Journal of Mycobacteriology 4 (2015) 13–14

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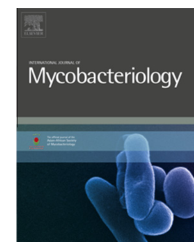

An "all-in-one" solution for simultaneous spoligotyping and drug resistance gene analysis of *Mycobacterium tuberculosis*: TB-SPRINT and TB-SPRINT*plus*

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ARTICLE INFO

Article history:
Received 4 November 2014
Accepted 8 November 2014
Available online 7 January 2015

Keywords:
Multi-drug resistance
Tuberculosis
Molecular diagnostics
Multiplexing
Personalized medicine
Spoligotyping

ABSTRACT

The aim of this study is to develop an innovative and alternative nucleic acid-based method for multidrug-resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) diagnostics, such as the Cepheid GeneXPert or the Hain–Line Probe Assay methods, to simultaneously identify MDR-TB (patient benefit) and to provide clues as to MDR-TB transmission (community benefit) at a reasonable cost to the national public health programs.

The DPO (Dual-Priming Oligonucleotide) principle was used to initially develop a 4-Plex multiplexed polymerase chain reaction (PCR) that simultaneously amplifies: (1) the CRISPR; (2) the *rpoB* hotspot, the *katG* and the *inhA* genes. The current maximal version of the assay allows the characterization of up to 68 markers, 59 of which are used routinely in one step (for the Luminex 200) or two-step methods (for the MagPix). Spoligotyping requires 43 markers, and *rpoB*516, 526, 531, *katG*315, *inhA*-8, -15, *gyrA*94, *rrs*1401, 1402, 1484 requires 25 markers. For each primer couple, one is biotinylated. Hybridization is performed after a PCR reaction on a microbead-based suspension array device with individually detectable oligonucleotide-coupled beads (Luminex 200 or MagPix systems) and detection proceeds through Streptavidin–Phycoerythrin labelling of the biotinylated-hybridized PCR products.

This study shows that this technique provides 100% specificity and sensitivity for *rpoB* and 100% specificity and 90% sensitivity for isoniazid resistance on DNA extracted from cultures, compared with phenotypic DST. The method was fully validated against sequencing. The method is currently in the experimental validation phase on DNA extracted from biological material and preliminary results will be shown. This technique was recently upgraded to detect fluoroquinolone and aminoglycoside resistance by including 2 new PCR primer couples and 9 more probes on the *rrs* and *gyrA* genes. It is suggested that

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http://dx.doi.org/10.1016/j.ijmyco.2014.11.042

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population studies using this laboratory method could provide a simpler and cheaper way to perform national TB-resistance surveys and would also allow to delineate more precisely, without further enquiries, MDR-TB transmission risks.

In conclusion, these methods appear to be both economically and technically very innovative. The extension from a Research Use Only to a CE-marked In-Vitro Diagnostics assay is in progress.

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