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AN AUTOMATED IS*6110*-BASED FINGERPRINTING METHOD FOR ACCURATE ESTIMATION OF RECENT TUBERCULOSIS TRANSMISSION

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Several limitations have restricted the use of IS*6110* restriction fragment length polymorphism (IS*6110* RFLP), the method that served for long as a gold standard for detecting recent tuberculosis (TB) transmission events. This has prompted us to conceive an alternative modality, IS*6110*-5'3'FP, a plasmid-based cloning approach coupled to a single PCR amplification of differentially labeled 5' and 3' IS*6110* polymorphic ends and their automated fractionation on a capillary sequencer. The potential of IS*6110*-5'3'FP to be used as an alternative to IS*6110* RFLP has been previously demonstrated, yet further technical improvements are still required for optimal discriminatory power and versatility. Here we introduced critical amendments to the original IS*6110*-5'3'FP protocol and compared its performance to that of 24-loci multiple interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR), the current standard method for TB transmission analyses. IS*6110*-5'3'FP protocol modifications involved: (i) the generation of smaller-sized polymorphic fragments, (ii) omission of the plasmid amplification step in *E. coli*, (iii) the use of more stable fluorophores, (iv) automated subtraction of background fluorescent signals, and (v) the automated conversion of fluorescent peaks into binary data. In doing so, the overall turnaround time of IS*6110*-5'3'FP was reduced to 4 hours. The new protocol allowed detecting almost all 5' and 3' IS*6110* polymorphic fragments of any given strain, including IS*6110* high-copy number Beijing strains. IS*6110*-5'3'FP proved much more discriminative than 24-loci MIRU-VNTR, particularly with strains of the *M. tuberculosis* lineage 4. In conclusion, the IS*6110*-5'3'FP protocol described herein reached the optimal discriminatory potential of IS*6110* fingerprinting and proved more accurate than 24-loci MIRU-VNTR in estimating recent TB transmission. The method, which is highly cost-effective, was rendered versatile enough to prompt its evaluation as an automatized solution for a TB integrated molecular surveillance.

Biography

Helmi M, Doctor in Veterinary Medicine (DVM), has carried out his PhD in Virology Immunology at the Institut Armand-Frappier, University of Quebec. He is head of a research laboratory of the Institut Pasteur de Tunis. Currently, Dr Mardassi is leading a research group focusing on the delineation of the molecular mechanisms underlying emergence of TB outbreaks, the success of particular *M. tuberculosis* clones, and the impact of drug resistance on fitness. He has over 30 publications with an H-index of 19.

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