Title: NON TUBERCULOSIS MYCOBACTERIA NUCLEIC ACID DIAGNOSTIC DEVELOPMENT USING smpB GENE SEQUENCES

Authors: Leotério, E.S.[1 2], Finnegan, A.[2], O´Shea, D.[2], Barry, T.[2].

Institution: [1] UFCSPA **-** Federal University of Health Sciences of Porto Alegre (Rua Sarmento Leite, 245 - Centro Histórico, Porto Alegre - RS; Brazil, 90050-170); [2] NUIG - National University of Ireland, Galway (University Road, Galway, Ireland).

Abstract:

Nowadays the amount of new pathogens has increased suddenly and the Non Tuberculosis Mycobacteria (NTM) is a good example about this fact. The clinical relevance of NTMs has been a matter of crescent concern due to their association with human infections, mainly in immunocompromised patients. It is extremely imperative perform the accurate diagnostic of NTM and the strains responsible for causing the infection as quickly as possible. With this in mind, this study seeks to develop an accurate and rapid molecular based diagnostic assay to identify some of the most clinically relevant NTMs responsible for cause human diseases around the world, using the *smpB* gene sequences. To achieve this goal *in silico* analysis of possible nucleic acid targets from *Mycobacterium avium* complex, *M. fortuitum*, *M. xenopi* e *M. gordonae* were performed. The most suitable diagnosis targets were identified. These *in silico* identification of nucleic acid sequences were used to develop NTM specie-specific conventional and real-time polymerase chain reaction (PCR) diagnostics assays. NTMs were cultured on specific media and the genomic deoxyribonucleic acid (DNA) were then isolated, purified and quantified. The genomic DNA were used to test NTM specific PCR assays. Despite any amplification had been achieved with the designed primers, can be very hard design an appropriate assay due to NTM genetic sequence characteristics. However, several improvements and changes in the protocols were taken to optimize the conventional PCR, including increase the deoxyribonucleotide triphosphate (dNTP) concentration, reduce the Taq Polymerase enzyme concentration, vary the annealing temperature for a gradient and add DMSO in the reactions. Even without succeeded positive results, the use of the *smpB* sequence can not be discarded. Alternative approaches using this gene can be tried in the future to claim if it can be useful as a marker to detect NTMs as smoothly as to detect other infectious pathogens.

Keywords: non tuberculosis mycobacteria (NTM); *smpB* gene; *in silico* analysis; nucleic acid diagnostic.

Development agency: none.