Evaluation of the rapid molecular diagnostic test for the New Zealand Mycobacterium tuberculosis Rangipo strain in a clinical setting

Claire V Mulholland, Duncan Thorpe, Ray T Cursons, Noel Karalus, Yang Fong, Vickery L Arcus, Gregory M Cook, Htin Lin Aung

Tuberculosis (TB) is a curable disease but claims over 1.7 million lives annually, and there were estimated to be 10.4 million new cases of TB worldwide in 2016. Despite New Zealand being a low-TB burden country, there are disproportionately high rates of TB in socioeconomically disadvantaged populations of New Zealand. Māori, the indigenous people of New Zealand, have an approximately nine-fold higher rate of TB compared to New Zealand Europeans. Molecular typing of Mycobacterium tuberculosis (M. tb) isolates using the Mycobacterium interspersed repetitive units (MIRU) system has shown that approximately two-thirds of New Zealand-born TB notifications can be assigned to clusters of infection. The largest M. tb cluster is known as the Rangipo cluster and has been the cause of ongoing outbreaks for at least the last 25 years. This cluster is strongly associated with Māori, with nearly 90% of Rangipo TB cases reported in Māori in the last 10 years (personal communication with Dr Sherwood, ESR (The Institute of Environmental Science and Research)). Anecdotal evidence and reports from previous outbreaks suggest the Rangipo strain may be highly transmissible and has high rates of progression to active disease relative to other circulating strains. If this strain is indeed more virulent, close supervision to ensure treatment adherence and the broadening contact tracing networks may be necessary to ensure secondary cases are completely and quickly detected to prevent potential later reactivation and further spread. Therefore, it is of the utmost importance to timely diagnose this strain to control further transmission.

Recently, we developed a whole genome sequencing-directed, single nucleotide polymorphism (SNP)-based, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) diagnostic for the rapid identification of Rangipo strain using DNA from cultured M. tb. Our diagnostic was also shown to offer greater discriminatory power and higher resolution over conventional MIRU typing. Here, we have applied this PCR-RFLP diagnostic in a clinical setting using culture-independent M. tb DNA from sputum as proof of concept that Rangipo can be differentiated from other M. tb strains directly from sputum for rapid diagnosis upon patient presentation.

In this study, four clinical sputum specimens received at the Waikato DHB Laboratory were subjected to the Rangipo PCR-RFLP diagnostic. Briefly, decontaminated sputum samples were heat inactivated at 95°C for 20 minutes and then spun down at 4,500rpm for 10 minutes. A PCR reaction was performed directly on 0.5µl of the supernatant to amplify a 455bp region of the M. tb Rv1821 gene (Figure 1A, lane 2 and...
3). Using the G1380A SNP in Rv1821, which is only present in Rangipo as a molecular marker, digestion of amplified products with the MboI restriction enzyme produces cut fragments of 386 and 69 bp for Rangipo, and 215, 171 and 69bp for non-Rangipo samples (Figure 1A, lane 4–7). Results were produced within 24 hours and the banding patterns obtained from the diagnostic identified Sample 1 and 2 as Rangipo (Figure 1A, lane 4 and 5) and Sample 3 and 4 as non-Rangipo (Figure 1A, lane 6 and 7). The sputum samples were also sent to the Tuberculosis Reference Laboratory (LabPlus) in Auckland for routine strain identification, which involves a total of 3–4 weeks culturing and MIRU typing. Consistent with our diagnostic results, the MIRU typing identified Sample 1 and 2 as Rangipo (MIRU code 233325153324341444223362) and Sample 3 and 4 as non-Rangipo (MIRU code 223325173533445643423382). We have shown that our diagnostic produces the correct results from sputum specimens within 24 hours, substantially reducing the turnaround time of 3–4 weeks for strain typing (Figure 1B). Hence, this Rangipo diagnostic can be used as a standard and timely test upon patients’ presentation in a clinical setting for effective intervention to prevent further transmission.

In short, this affordable, rapid and reliable diagnostic will serve as a valuable tool for the district health boards in New Zealand to control the spread of the Rangipo strain, the cause of a prolonged and sustained TB outbreak in New Zealand.

Figure 1: Rangipo PCR-RFLP diagnostic.
Competing interests:
Nil.

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Author information:
Claire V Mulholland, PhD Candidate, School of Science, University of Waikato, Hamilton; Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland; Duncan Thorpe, Medical Lab Scientist, Waikato Hospital, Waikato District Health Board, Hamilton; Ray T Cursons, Adjunct Senior Lecturer, School of Science, University of Waikato, Hamilton; Noel Karalus, Emeritus Respiratory Clinician, Waikato Hospital, Waikato District Health Board, Hamilton; Yang Fong, PhD Candidate, Institute of Fundamental Sciences, Massey University, Palmerston North; Vickery L Arcus, School of Science, University of Waikato, Hamilton; Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland; Gregory M Cook, Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland; Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago, Dunedin; Htin Lin Aung, Sir Charles Hercus Fellow, Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Auckland; Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago, Dunedin.

Corresponding author:
Dr Htin Lin Aung, Sir Charles Hercus Fellow, Department of Microbiology and Immunology, University of Otago, Dunedin.
htin.aung@otago.ac.nz

URL:

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