

## Differentiation of Species within *Mycobacterium tuberculosis* complex by Real-time PCR-Based Genomic Deletion Analysis

**J. I. Pounder**<sup>1</sup>, K. V. Voelkerding<sup>1,2</sup>, C. M. Anderson<sup>1</sup>, D. Storts<sup>3</sup>, C. A. Petti<sup>1,2</sup>

<sup>1</sup>ARUP Laboratories, Salt Lake City, UT, <sup>2</sup> University of Utah, Salt Lake City, UT, <sup>3</sup> Promega Corp., Madison, WI

**Background:** Differentiation of members within *Mycobacterium tuberculosis* complex (MTC) is essential for patient management and infection control. MTC is comprised of *M. tuberculosis* (Mtb), *M. bovis* (Mb), *M. bovis* BCG (vaccine strain; Mbcg), *M. africanum* (Ma), and *M. microti* (Mm). Because MTC have highly similar nucleotide sequences, a rapid, reliable method to distinguish species within MTC is not widely available. Genomic deletion analysis has been used, but is labor-intensive. We developed a genomic deletion assay based on real-time PCR with melting temperature ( $T_m$ ) analysis to differentiate species within MTC. **Methods:** Genomic regions of difference (RD) 1, 9, and 10 were selected for initial screening of MTC. DNA was extracted from ATCC strains of Mtb, Mb, Mbcg, Ma, and Mm using an IDI lysis kit. Two primers that were complementary to sequences flanking a deletion region, and one internal primer were created for each RD. Different sized amplicons were generated depending on presence or absence of genomic deletions. Amplifications were performed using Plexor chemistry on a Rotorgene 3000, and amplicon  $T_m$  were recorded. **Results:** Expected RD were observed for each ATCC strain within MTC and findings were confirmed on repeated runs. For RD1, a 2.1°C difference in  $T_m$  between 150 bp (no deletion expected for Mtb, Ma, Mm) and 200 bp (deletion expected for Mbcg) amplicons was observed ( $86.12^\circ\text{C} \pm 0.13$  and  $88.22^\circ\text{C} \pm 0.24$ , respectively). For RD9, a 3.9°C difference in  $T_m$  between 206 bp (deletion expected for Mb, Mbcg, Ma, Mm) and 306 bp (no deletion expected for Mtb) amplicons was observed ( $89.29^\circ\text{C} \pm 0.11$  and  $93.22^\circ\text{C} \pm 0.11$ , respectively). For RD 10, a 5.0°C difference in  $T_m$  between 202 bp (deletion expected for Mb, Mbcg, Ma, Mm) and 308 bp (no deletion expected for Mtb) amplicons was observed ( $88.67^\circ\text{C} \pm 0.03$  and  $93.7^\circ\text{C} \pm 0.28$ , respectively). The size of amplicons generated was confirmed by agarose gel electrophoresis. **Conclusions:** Melting analysis of RD 1, 9, and 10 can identify Mtb, Mbcg, and Ma. Development of multiplex real-time PCR with  $T_m$  analysis and additional primers to differentiate other species within MTC warrants further study and is ongoing.

## Research: Pre-Submission Approval Form

Approval is required **before information is presented outside of ARUP and enters the public domain** to ensure that HIPAA and IRB protocols have been followed. Please ensure that this document is signed and appropriate documents are attached before submitting any information for publication/presentation outside of ARUP.

Attach copy of (please indicate) manuscript, poster, **abstract**, or other presentation

Presentation/Poster presented at (specify meeting or conference): \_\_\_\_\_

ARUP Cited: Yes  No  If no, state reason: \_\_\_\_\_

Global IRB #7275 applies, and PRCS-0020, *Internal Sample Request: De-Identification of Samples* has been followed **OR**

Independent Institutional Review Board (IRB) approval, IRB# \_\_\_\_\_  
Attach copy of approved IRB protocol.

**OR**

IRB is not applicable. Please explain: \_\_\_\_\_

Scientist/Researcher: \_\_\_\_\_ Date: \_\_\_\_\_  
*Signature*

### Approval Signatures and Dates:

Medical Director: \_\_\_\_\_ Date: \_\_\_\_\_

R&D Group Manager or  
ARUP Privacy Officer: \_\_\_\_\_ Date: \_\_\_\_\_

CONFIDENTIAL: This material is prepared pursuant to Utah Code Annotated, 26-25-1, et seq., for the purpose of evaluating health care rendered by hospitals or physicians and it NOT PART of the medical record.