Quantitative PCR Assays for Molecular Diagnosis of *M. leprae* and *M. lepromatosis*

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Objective: To develop molecular diagnostic assays for leprosy (Hansen's Disease) caused by *Mycobacterium leprae* and *M. lepromatosis.*

Design: *M. leprae* was believed to be the exclusive causative agent of leprosy until another species, *M. lepromatosis*, was identified in 2008. We isolated and cultured the first strain of *M. lepromatosis* in the mouse footpad (MFP), re-sequenced the genomes, and developed a *M. lepromatosis* specific Q-PCR assay. We characterized this assay, and a previously designed *M. leprae* specific Q-PCR assay, for molecular diagnosis of leprosy according to the Clinical Laboratory Improvement Amendments (CLIA) guidelines.

Materials and Methods: *M. lepromatosis* was harvested from MFP, and host DNA was depleted by 0.1 N NaOH treatment of the infected MFP homogenate prior to DNA extraction. A genomic library was prepared using a NextEra XT kit and sequenced using a V3 kit (2X300 bp reads) on a MiSeq instrument (Illumina). All the reads were first aligned with mouse genomes, and only ~24% of the reads non-homologous to mouse genomes were aligned with a previously sequenced *M. lepromatosis* genome. A genomic sequence (~200 bp) which was at much higher coverage (>2000X) compared to the average genome coverage (~136X) was identified which we hypothesized must represent multi-copy regions in the genome, and thus the potential target for highly sensitive PCR assays. A *M. lepromatosis* specific Q-PCR assay (LPM) targeting this sequence was designed. This LPM assay, along with our earlier published RLEP assay for molecular detection of *M. leprae*, were CLIA validated.

Results: Polynomial regression analysis of standard curves (n=5) for both assays have excellent linearity (RLEP: $r^2<0.9946$; LPM: $r^2<0.9995$), and efficiency of the RLEP and LPM assays is 94.82 ± 3.27 and 93.05 ± 6.78, respectively. The Limit of Detection (LOD) of the LPM and RLEP assays is 3.05 ($Ct\leq 34.184 \pm 0.395$) and 0.76 30 ($Ct\leq 34.839 \pm 0.846$) bacilli in the Q-PCR reaction, respectively. The assays were tested on clinical specimens and samples from wild armadillos. Only 1.4% (3 / 206) of samples from the United States and 31% (15/47) of specimens from Mexico were positive for *M. lepromatosis. A*ll 180 patient samples from the Philippines and 96 samples from US armadillos were negative for *M. lepromatosis*.

Conclusions: We have developed the first CLIA-validated PCR diagnostic assays for leprosy and have implemented them into the point of care at NHDP.

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