Supplementary File 2

PCR methodology for 16S rRNA:

DNA extraction

DNA was extracted from CSF samples using DNAeasy blood and tissue kit (Qiagen) according to the manufacturer's instructions.

M. leprae strain Thai53 genomic DNA was kindly provided by Biodefence and Emerging Infections Research Resource Repository (BEI Resources), USA and was used in all PCR experiments as positive control. Nuclease free water was used as negative control.

Selection of gene target and primer design

The primer sequences for selected gene targets *16SrRNA* and *rlep* were acquired as described in earlier studies. The designed primers were obtained commercially by Sigma, USA. The primer sequences are mentioned in table 1.

Primer sequence of gene targets

Gene	Primer orientation	Sequence	Amplicon size
16SrRNA	Forward	5'-CGGAAAGGTCTCTAAAAAATCTT-3'	171 bp
16SrRNA	Reverse	5'-CATCCTGCACCGCAAAAAGCTT-3'	
rlep	Forward	5'-TGCATGTCATGGCCTTGAGG-3'	129 bp
rlep	Reverse	5'-CACCGATACCAGCGGCAGAA-3'	

PCR cycling parameters and reaction conditions

The entire clinical samples were tested using individual gene PCR targeting *16S rRNA* and *rlep* genes. PCR reaction mix for individual genes was prepared using 2x *Taq* PCR Master Mix (Cat. No. 201443, QIAGEN, Germany), forward and reverse primers (10 µM) and 2µl of template DNA. The final concentration of Master Mix was made 1x and 0.2µM for each primer in reaction mix of final volume 20µl made up with nuclease free water.

PCR amplifications were performed using thermal cycler (Corbett Research, Australia). The reaction conditions for individual gene PCR for *rlep* was one cycle of initial denaturation at 95^{o} C for 15 min followed by 40 repeats of cycling at 94^{o} C for 30 sec, 58 ^{o} C for 30 sec and 72^{o} C for 1 min. Termination of reaction was done after final extension at 72^{o} C for 10 min. Similarly, *16SrRNA* gene was amplified with reaction condition of one cycle of initial denaturation at 95^{o} C for 15 min followed by 40 repeats of cycling at 94^{o} C for 2 min, 57^{o} C for 2 min and 72^{o} C for 3 min. Termination was done after final extension at 72^{o} C for 10 min. The amplified products were electrophoresed on 2% agarose gel (Tris-Borate-EDTA) at

constant voltage (100V). The gel image was captured using gel documentation system (AlphaImager EC).