ABSTRACT

Two "freezing-thawing" methods, published by Woods and Cole (1989) and Jamil *et al.* (1994), were used to extract genomic DNA from *Mycobacterium leprae* and compared. Although both methods were equally efficient for the extraction of *M. leprae* genomic DNA, the extraction method of Jamil *et al.* (1994) was preferred over the method of Woods and Cole (1991) which uses mineral oil that affects the accuracy in pipetting the DNA extracts.

The amplification parameters of the polymerase chain reaction (PCR) published by Woods and Cole (1991) were followed. Two primer combinations were tried out with a view to increase the sensitivity of detecting *M. leprae*. The primer combination of R1 & R2 and C6 & C7 was chosen for subsequent amplification as the annealing temperatures of these primers were similar and gave better result than the primer combination of R5 & R6 and C6 & C7.

PCR reaction volume was reduced proportionally to 50, 20, and 10 µl from the original volume of 100 µl (Woods and Cole, 1991). All reaction volumes gave the same result with DNA extracted from human skin biopsy samples, while the 20 µl of total reaction volume gave the highest number of PCR-positive result with DNA extracted from mouse foot-pad homogenates.

Two PCR amplification methods (Woods and Cole, 1991; Jamil *et al.*, 1994) were compared by using the purified *M. leprae* genomic DNA. The method of Woods and Cole (1991) had a detection sensitivity of 0.5 pg (i.e., 100 genome equivalents), whereas the colorimetric one-tube nested (OTN) PCR method of Jamil *et al.* (1994) had a detection sensitivity of 5 fg (i.e., 1 genome equivalent).

The two PCR amplification methods were later compared by using human skin punch biopsy samples and mouse foot-pad homogenates. Both methods were able to detect *M. leprae* in human skin punch biopsy samples with at least BI (bacteriological or bacterial index) = 3.0 and MI (morphological index) = 0.2, microscopic density count = 2+, and viability count = 1/85. Both methods had also detected *M. leprae* DNA in human skin punch biopsy samples that were kept at -20°C for 7.5 years.

When applied to mouse foot-pad homogenates, the colorimetric OTN PCR method of Jamil *et al.* (1994) gave the most sensitive and reliable result compared to the methods of Woods and Cole (1991) and microscopic viability count.

Of 46 biopsy samples, collected all over Malaysia and sent by the National Leprosy Control Centre, Sungai Buloh, 45 (97.83%) were tested positive by the method of colorimetric OTN PCR. They had BI from 0.7 to 5.6 and MI from 0 to 24.3; and their homogenates had microscopic density counts from 0 to 5+ and viability counts of 0/100 to 9/91.

Furthermore, of 33 biopsy samples collected from Sabah and Sarawak by a research team carrying out surveillance study on household contacts of leprosy patients, 19 (57.58%) were positive by colorimetric OTN PCR.

This study showed that the bacterial suspension from human skin punch biopsy could be used as an alternative material in colorimetric OTN PCR, if the skin biopsy sample was not available.

Finally, no correlation was found between the colorimetric OTN PCR and IgM-phenolic glycolipid (PGL) ELISA.