# DNA Extraction Methods from *Mycobacterium leprae* and *M. lepraemurium*

TO THE EDITOR:

*Mycobacterium leprae* and *M. lepraemurium*, respectively, the etiologic agents of human and murine leprosy, are rich in lipids and have thick cell walls. These mycobacteria thus resist DNA extraction. Although some methods have been used to extract DNA from mycobacteria ([2, 4, 10]), they lack efficacy and are time consuming. There is a need for a rapid, effective and simple method for DNA extraction from *M. leprae* and *M. lepraemurium* which could be used for the diagnosis, epidemiological investigation, molecular biology research and identification of these mycobacteria grown *in vitro*. Studies were carried out to find a suitable method of DNA extraction from *in vivo*-grown *M. leprae* and *M. lepraemurium*.

The sources of *M. leprae* were foot pads of nude mice and armadillo liver tissues, infected earlier with human leprosy bacilli. Bacilli from foot pads of nude mice free from host material were purified by the method of Franzblau and Hastings ([5]). Density separation of *M. leprae* from nude mouse foot pads was accomplished by 30% Percoll gradient separation ([8]). *M. leprae* recovered from infected armadillo liver were purified by the method described by Clark-Curtiss, *et al.* ([3]). *M. lepraemurium* (Hawaiian strain) isolated from C3H mice lepromas were purified by differential centrifugation ([6]), and were further purified by the method of Franzblau and Hastings ([5]).

During these studies, five different methods were used to extract DNA from *M. leprae* and *M. lepraemurium* bacillary preparations. Bacilli (50 mg wet wt, about $5 \times 10^{10}$ bacilli) were suspended in 1 ml of buffer, consisting of 100 mM NaCl, 10 mM EDTA, and 10 mM Tris-HCl, pH 7.5. These preparations were used for DNA extraction in the following five methods: 1) the intensive enzymic digestion method (M1) used was the same as described by Visuvanathan, *et al.* ([11]). 2) 2-min mechanical glass-bead disruption method (M2) was carried out according to Via and Falkinham ([10]) and Jacobs, *et al.* ([7]). 3) thermal shock method (M3). In this method, 50 mg of cells were suspended in 400 μl of distilled water and subjected to repeated (six times) heat/cold shock; boiling for 5 min at 100°C and snap-freezing for 5 min at −196°C in liquid nitrogen. 4) modified conventional enzymic digestion method (M4). Briefly, to 1 ml of a bacillary suspension containing 50 mg of bacilli, 2 mg of lysozyme was added and incubated at 37°C for 1 hr, then 0.3 mg each of proteinase K and SDS (to a final concentration of 3%) were added and the reaction mixture was further incubated at 56°C for 1 hr. 5) manual disruption with modified conventional enzymic digestion method (M5): 50 mg of cells were triturated for 30 min in a mortar containing dry ice and 0.1 g of glass beads (0.11 mm), and the rest of the procedure was essentially the same as described in M4.

Estimation of DNA concentration was performed spectrophotometrically using the standard method of Sambrook, *et al.* ([9]). The highest yield of 2.82 μg DNA/mg wet wt of *M. lepraemurium* was obtained by M2; this represents a theoretical yield of 78% ([1, 11]). The lowest DNA yield of 0.01 μg DNA/mg wet wt of *M. lepraemurium* was obtained by using M3.

When *M. leprae* recovered from armadillo liver were used, DNA yields of 1.25, 1.37 and 1.66 μg DNA/mg wet wt of cells were obtained, respectively, by M2, M4 and M5. Very low yields of DNA were achieved by M1 and M3. In our experience, it was comparatively more difficult to extract the DNA of *M. leprae* from the foot pads of nude mice than *M. leprae* from armadillo liver by all of the methods used. Also, *M. leprae* isolated from nude mice gave comparatively lower DNA yields. For example, when *M. leprae* recovered from the foot pads of nude mice were used, yields of 0.66, 0.27 and 1.43 μg DNA/mg wet wt of cells were obtained, respectively, by the M2, M4 and M5 methods. These results could be attributed to the variations in cultural conditions and strain differences. M3 and M1 were the least effective for the extraction of DNA

from *M. leprae* and *M. lepraemurium*. Based on the yields of DNA extracted by all of the methods used, both M2 and M5 are the time-saving (less than 4 hr), effective and simple methods for DNA extraction from *in vivo*-grown *M. leprae* and *M. lepraemurium*.

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# Radiometric Procedure for Detecting a Cultivable *Mycobacterium* in *Mycobacterium leprae*-Infected Armadillo Tissue

To the Editor:

For the past two decades, experimentally-infected armadillo tissues have been the principal source of *Mycobacterium leprae* for microbiological investigations. The results of earlier studies have revealed the presence of armadillo-derived mycobacteria (ADM) in some armadillos infected with *M. leprae* ([4, 5]). Contamination of purified *M. leprae* suspensions with ADM would undoubtedly interfere with any research exploiting the unique characteristics of *M. leprae*. In this communication, we describe a rapid radiometric procedure for detecting a mycobacterial species other than *M. leprae* in armadillo tissues.

Earlier *in vitro* studies have demonstrated the ability of *M. leprae* to significantly oxidize $^{14}$C-palmitic acid when incubated in