

DETECTION, CHARACTERISATION AND QUANTIFICATION OF *MYCOBACTERIUM LEPRAE* DNA FROM ARCHAEOLOGICAL MATERIAL

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Demonstration of pathogen DNA in human remains is valuable in confirming paleopathological diagnosis and expanding our understanding of the host/pathogen relationship. This study examined how additional molecular techniques, such as Real-Time(RT)-PCR increase the information that is recoverable from the rare specimens with evidence of infectious disease.

The skeleton of a 12-14 year-old individual from the medieval (9th-10th century) Prusánky burial ground showed signs of periostitis consistent with leprosy or treponemal disease. DNA was extracted from nasal scrapings, radial epiphysis, fibula and rib. PCR was performed using primers for the *Mycobacterium leprae* repetitive element RLEP (37 copies/cell) and the single-copy 18 k-Da antigen locus¹. RT-PCR² and a specific probe for the 18 k-Da antigen locus were used to quantify the amount of *M. leprae* DNA, and genotyping was also performed³.

The probe and RT-PCR demonstrated *M. leprae* DNA in all specimens, with a significantly greater quantity in the fibula and nasal scrapings. In the DNA extraction, pre-incubation with *N*-phenacylthiazolium bromide (PTB) facilitated DNA strand separation and increased *M. leprae* DNA recovery. RT-PCR is a convenient rapid technique and also enables PCR inhibition to be assessed. We confirmed the diagnosis of leprosy in the skeletal remains examined; demonstrated that the individual had disseminated, therefore lepromatous leprosy; and showed differential localisation of *M. leprae* DNA within the body.

¹ Donoghue HD, Holton, J, Spigelman M. PCR primers that can detect low levels of *Mycobacterium leprae* DNA. *Journal of Medical Microbiology* **50**: 177-182, 2001.

² Taylor GM, Watson CL, Bouwman AS, Lockwood DNJ, Mays SA. 2006. Variable number tandem repeat (VNTR) typing of two palaeopathological cases of lepromatous leprosy from Mediaeval England. *Journal of Archaeological Science* **33**:1569-1579, 2006.

³ Monot M, Honoré N, Garnier T, Araoz R, Coppée JY, Lacroix C, Sow S, Spencer JS, Truman RW, Williams DL, Gelber R, Virmond M, Flageul B, Cho SN, Ji B, Paniz-Mondolfi A, Convit J, Young S, Fine PE, Rasolofoa V, Brennan PJ, Cole ST. On the origin of leprosy. *Science* **308**:1040-1042, 2005.