007/A

Comparison of five DNA extraction methods from human blood for the detection of *Wuchereria bancrofti* by polymerase chain reaction assays

N D A D Wijegunawardana¹, Y I N Silva Gunawardane^{1*}, Aresha Manamperi¹, H A C Hapuarachchi², N K Gunawardene², S beysundara³, W beyewickreme^{1 & 2}

¹ Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Ragama

² Department of Parasitology, Faculty of Medicine, University of Kelaniya, Ragama

³ Department of Microbiology, Faculty of Medicine, University of Kelaniya, Ragama

Introduction: Lymphatic filariasis (Lf) is the second most common vector-borne disease globally. Approximately 90% of global burden of Lf is caused by Wuchereria bancrofti. W. bancrofti is routinely diagnosed by morphological identification of microfilariae (Mf) by microscopy which is a labour intense, low sensitive and time consuming method. Detection of W. bancrofti Deoxyribonucleic acid (DNA) using polymerase chain reaction (PCR) technique has become popular today, because of its high sensitivity and specificity. The overall success of the PCR strategy in detecting a filarial parasite in human blood varies between sample preparation methods. The objective of this study was to compare five DNA extraction methods (Lysis + centrifugation, Chelex method, Mf pellet method, Q1Aamp DNA Mini Kit commercial system, and Phenol-chloroform) with regard to duration of completion, labor involvement and PCR analytical sensitivity in-relation to DNA quality and quantity for the detection of W. bancrofti in human blood. Five blood samples positive for mf of W. bancrofti were tested for each DNA extraction method and were compared with respect to the sensitivity, time and quality/quantity of DNA and also by PCR analysis. Of the 5 methods tested. Mf pellet method was found to be the most simple and effective technique for the isolation of W. bancrofti Mf in human blood. This method was guick (15 min to complete), simple (5 min of manual labor), and very economical. It does not require any organic solvents, and the entire extraction procedure uses only two steps requiring supernatant transfer between tubes, hence minimizing the possibility of cross-contamination. Moreover, the PCR analytical sensitivity of the Mf pellet method was comparable to that of the commercial kit used. No PCR inhibitors were detected, independently of Mf count in the blood. Same method (optimal DNA extraction method) can be also used for the detection of parasite DNA from the field collected Mf positive mosquitoes using a PCR. Therefore, we recommend the Mf pellet method for processing large numbers of blood samples in community surveys aimed at determining the prevalence of W. bancrofti infection.

Financial assistance received from WHO/SEARO/TDR (grant no. SN1152) and University of Kelaniya (Grant no. RP/03/04/06/01/2006) are acknowledged.

*nilminis@graduate.hku.hk

Tel: 011-2960483