

A-03: Polymerase chain reaction (PCR) technique for the detection of *Wuchereria bancrofti*

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The most sensitive *Wuchereria bancrofti* specific DNA probe so far reported is the probe pWB 12, reported by us previously. We showed that pWB 12 can detect as little as 300 pg of genomic DNA or DNA from a single infective larva or one microfilaria when nick translated, ³²P-labelled probe was used.

However, procedures that require radioactive materials are impractical and often impossible to apply in developing countries. Thus, the PCR using oligonucleotide primers derived from pWB 12 nucleotide sequence was developed as an approach to exclude the radioactive detection methods. The primers we selected amplify a 490 bp fragment specific for *W. bancrofti* and can be visualized by agarose gel electrophoresis and ethidium bromide staining. This was used to detect a single L3 larva in a pool of 20 uninfected mosquitoes, a single microfilaria in 100µl of blood or hydrocele fluid, and 0.4 pg of *W. bancrofti* genomic DNA added to human blood or serum. Parasite DNA in mosquito extracts was isolated free of inhibitors by gel filtration through Sephadex G-50 columns, and in blood and hydrocele fluid by lysis followed by Proteinase K digestion and boiling. The sensitivity of detection was greater than radioactive probes and the assay is suitable for field application in endemic countries.

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