# The Development of a non-radoactive oligonucleotide based hybridization assy for the Detection of Wucheraria bancrotifti

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Abstract:

Five biotin labelled oligonucleotides (30-45 nucleotides) were custom synthesized based on a previously cloned and characterized repetitive sequence specific for Wuchereria bancrofti. Hybridization studies carried out with a single oligonucleotide (40 nucleotides) using chemiluminescence, was able to detect 5 ng of W. bancrofti DNA. The biotin tailing with terminal deoxynucleotidyl transferase, of the same probe increased the detection limit to 1 ng of W. bancrofti DNA. To increase the sensitivity of detection further, all five oligonucleotides were used. Optimal prehybridization, hybridization and post hybridization washing conditions were determined for the oligonucleotide based hybridization assay. Under the optimal conditions (prehybridization for 2 h at 42 0C, hybridization in 50 percent formamide, with a probe concentration of 5 pmol/ml of each probe, at 42 0C for 2 h and post hybridization washing in 0.5 x SSc at 42 0C for 3 x15 min) the oligonucleotide mix (containing all five probes) was capable of detecting as little as 100 pg of purified W. bancrofti microfilarial DNA or a single infective stage larva or a single microfilaria in 50 ul blood sample. However, the oligonucleotides were observed, to cross hybridize with DNA of Brugia malayi, at high concentrations (10 ng). It was also revealed, that the number of steps involved in the preparation of blood samples for the dot blot assay can be reduced. The blood samples lysed with sterile distilled water and treated with proteinase K in the presence of a detergent; can be directly used for the hybridization assay, obviating the DNA precipitation step. The assay also detected DNA extracted from microfilariae positive blood samples stored at room temperature for 5 days without the addition of any preservative. In the detection of infective stage larvae with mosquitoes; the samples should be processed to the DNA precipitation step and passed through sephadex G-50 mini columns, as mosquito DNA extracts resulted in false-positive signals. These biotinylated oligonucleotides, due to their rapid, sensitive and simple hybridization protocols, forms the basis for the future development of a specific and sensitive method for the identification of W.bancrofti in field specimens.

Key Words : Wuchereria bancrofti / Wuchereria bancrofti-isolation and purification / Filariasis