A-10 Development of a non-radioactive oligonucleotide based hybridization assay for the detection of *Wuchereria bancrofti*

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Non-radioactive oligonucleotides possess complementary advantages over radioactive probes. They are stable, economical and problems encountered with regard to use of radioisotopes can be avoided. Also oligonucleotides make hybridization protocols rapid and simple. The aim of the study is to develop a non-radioactive oligonucleotide based hybridization assay, for the detection of *W. bancrofti*.

Five oligonucleotides based on a previously cloned diagnostic repetitive sequence from the genome of *W. bancrofti* were custom synthesized with a single biotin group at 5' hydroxyl end. DNA of *W. bancrofti*, microfilariae and infective stage larva (L_3) DNA was also extracted from mosquitoes (*Culex. quinquefasciatus)*, mosquitoes with L_3 larvae and microfilariae positive blood. Samples were then denatured and dot blotted. Optimal conditions for the hybridization assay were determined for the five pooled oligonucleotide probes. Hybridized probes were detected by chemiluminescence and the filters were thereafter, exposed to X-ray films. The optimal conditions for the hybridization assay were determined to be, prehybridization for 2 h at 42°C, hybridization in 50% formamide at 42°C for 2 h and post hybridization washing in 0.5 x SSC at 42°C for 3 x 15 min.

We have developed a non-radioactive oligonucleotide based hybridization assay for *W. bancrofti*, capable of detecting upto 100 pg *W. bancrofti* genomic DNA, a single L_3 larva, a single microfilaria. L_3 in the presence of mosquitoes can be detected at the DNA stage after passing through sephadex G-50. Assay also detects microfilariae positive blood samples at the stage of digestion with proteinase K.