A-02: Development of a rapid oligonucleotide based hybridization assay for the detection of *Setaria digitata*

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The cloning characterization of a diagnostic repetitive DNA sequence from the genome of *Setaria digitata* was previously reported. Compared to cloned sequences oligonucleotide probes have several advantages. They are well defined and could be non enzymatically labelled during their synthesis.

In addition the use of oligonucleotides allow simple and rapid hybridization protocols. In this report the assessment of optimal hybridization and post hybridization washing conditions for a biotin labelled oligonucleotide for the detection of *S.digitata*, are described.

An oligonucleotide (5 CGATTGAATGTCAAGATAGAGGTCAAGCAC3') based on a previously cloned tandem repeat was custom synthesized with a single biotin group at the 5' hydroxyl end.

DNA of *S. digitata*, infective stage larvae (L_3) and microfilariae were isolated using standard DNA isolation methods. The DNA samples (50 μl) were denatured and dot blotted on nylon membranes using a dot blot apparatus. All blots were prehybridized (2h) in prehybridization solution at room temperature (30°C). Hybridization conditions were varied in each experiment to determine the optimal formamide concentration, temperature and duration of hybridization for the oligonucleotide, The hybridization solution was identical to the prehybridization solution except for the inclusion of formamide and the biotinylated oligonucleotide (2 pmol/ml). The blots were then washed at 42°C and 30°C with varying monovalent salt ion concentrations. Bound probe was detected with avidin - alkaline phosphatase conjugate using the chemiluminescent substrate, adamanty1 - 1,2. dioxetane phosphate (AMPPD) according to the manufacturer's instructions. The filters were then exposed to X-ray films.

The critical parameters to be determined with regard to optimal hybridization conditions are the formamide concentration, monovalent salt ion concentration during hybridization and post hybridization washes, hybridization temperature and the washing temperature. In the experiments carried out to determine the optimal formamide concentration, non specific binding to host DNA was observed with formamide concentrations up to 40%. Non specific binding was not seen at 50% formamide concentration and furthermore it had no effect on the signal intensity. Optimal washing conditions were found to be 0.5 x SSC at 42°C. The signal intensity was not affected even when the probe concentration was reduced to 1 pmol/ml and hybridization time to 1 h. Studies carried out using the optimal hybridization conditions indicate that the probe is capable of detecting 250 pg *S.digitata* genomic DNA a single L_3 larva and a single microfilaria in dot blot assays after 15 min exposure to X-ray films. It was also found to be specific as it did not cross hybridize with DNA of any the hosts, vector or other filarial parasites, but detects both *S.digitata* and *S.labiato papiliosa* DNA.

The optimal hybridization conditions for an oligonucleotide based diagnostic assay for *S.digitata* have been evaluated and established. The probe appears to be very sensitive and capable of detecting a single microfilaria and a single L_3 larva of *S.digitata* with the chemiluminescent substrate AMPPD.

The rapid and simple hybridization protocol makes it well suited for the development of a diagnostic kit for field use.