DNA Probes for Filariasis*

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Lymphatic filariasis is a debilitating disease with associated socio-economic effects in the developing countries. The disease shows a world wide distribution extending from tropical and sub-tropical Africa to Central and South America, Asia and Oceania. According to recent data compiled by the WHO, an estimated 751 million people are at risk in these countries, with 76.6 million people already infected. Of these, 72.8 million are infected with *Wuchereria bancrofti* and 5.8 million with *Brugia malayi* or *Brugia timori.*

The acute manifestations of lymphatic filariasis include sporadic attacks of adenolymphangitis, fever, acute epididymo-orchitis, inflammatory nodules in the breast, scrotum and skin. The chronic manifestations of the disease are hydrocele, lymphoedema and elephantiasis.

In contrast, in occult filariasis, classical clinical manifestations are absent, microfilariae are not found in peripheral blood but may be located in specific tissues as in tropical pulmonary eosinophilia, where hyper-eosinophilia (3000-50,000 cells per mm^3) is a common feature.

Two types of filariasis were present in Sri Lanka during the 1940s the urban type due to *W. bancrofti* and the rural type due to *B. malayi* (1). However, effective therapeutic measures and control programmes during 1940 to 1960, appears to have completely eradicated Brugian filariasis in Sri Lanka by the early 1960s. As a result of breakdown in monitoring systems and lack of control of vector population and due to rapid unplanned urbanization, by the late 1960s, bancroftian filariasis emerged as the major lymphatic filariasis in Sri Lanka (2). At present the endemic belt of bancroftian filaraisis extends from North Western coastal belt via Western to the South Western region, covering an approximate area of 560 square miles. An estimated population of 6 million out of a total population of 17 million are at risk today.

The parasitological and immunoassays are the two widely used diagnostic techniques. The parasitological technique requires night blood sampling between 2200h and 0200h due to the nocturnal periodicity of *W. bancrofti.* In this technique, a sample of capillary blood, 40-60ul is smeared on a microscopic slide. Following dehemoglobination and staining with Giemsa, the slide is examined under an ordinary light microscope.

Membrane filtration and Knott's concentration method both require venepuncture. In the so called DEC provocation test a dose of 100mg of diethylcarbamazine is given to induce microfilariae to appear in peripheral blood during day time. Even then it has been found that only 60 percent of the night blood positives can be detected.

Though the microscopic technique is simple, it is not suitable for large scale epidemiological surveys. Further, most microfilaraemics are asymptomatic and do not visit filaria clinics. Furthermore, in order to increase the sensitivity of the technique for the detection of very low microfilaraemia, the microscopist is required to spend 30-40 minutes per slide. However, both asymptomatic microfilaraemics as well as low microfilaraemics in an endemic area act as reservoirs for the transmission of the disease.

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Immunoassays do not require night blood sampling. These are directed towards detecting filarial antibodies or circulating filarial antigens. Presently available immunoassays, however, are not specific enough, positive results may be observed upto 3-4 months after the clinical cure of the disease. Also immunoassays show no relationship to the intensity of infection, and also cannot differentiate between a previous and current infection.

Thus it is apparent that more sensitive, practically feasible, less costly methods are required for the diagnosis of microfilaraemia and also for the determination of infection and infectivity in the vector mosquito.

In order to overcome some of these drawbacks in the diagnosis of lymphatic filariasis, the potential of newer technologies and analytical approaches of molecular biology and immunology have been successfully applied during the last decade. Particularly, the recombinant technology has made it possible for the identification of parasite specific DNA segments which can be used in a nucleic acid hybridization assay for the detection of the parasite.

In view of the global and national importance of bancroftian filariasis, and the urgent need for the development of alternative technologies we embarked on a project in 1988 to develop a species-specific DNA probe for the diagnosis of bancroftian filariasis. As a prerequisite to achieve this aim, a genomic library of *W. bancrofti* was constructed in the phage vector EMBL3. Following screening of this library for repetitive clones, 8 putative clones giving strong hybridization signals with *W. bancrofti* genomic DNA were isolated. Of these 8, a clone designated EMBL3Wb34 was selected for further characterization. Restriction analysis of the cloned fragment indicated that the cloned fragment was approximately 16kd in size. This cloned fragment detected 1 microfilariae in 60 ul of blood and 1 L3 larva in the mosquito when used as a radiolabelled probe.

At this stage of development the cloned fragment was subjected to an international evaluation along with the other then available DNA probes for *W. bancrofti*, sponsored by the World Health Organization and the New England Bio-Labs. This international evaluation showed that the 16kd fragment we have cloned was the only probe that did not show any appreciable hybridization with human DNA or with the vector mosquito DNA (3). However, the cloned fragment also showed small but appreciable hybridization with genomic DNA from *B. malayi*.

Subcloning of smaller fragments resulting from restriction enzyme, Sau 3A 1, cleavage of 16kd insert was therefore undertaken to increase the specificity. These small fragments were subcloned in the plasmid vector pUC 18 and one subclone designated pWb 12 was fully sequenced. This subclone had an insert of 950 bp and on evaluation of its specificity was found to be highly species specific. It was capable of detecting as little as 300 pg of *W. bancrofti* DNA or 1 microfilaria in 100 ul of human blood or 1 L3larva in the vector mosquito (4).

In view of the need to use radiolabelled detection methods in nucleic acid hybridization assays, which is a drawback in developing countries and in particular under field conditions, the potential of the polymerase chain reaction (PCR), was next explored with oligonucleotide primers derived from pWb12 sequence. These studies resulted in the development of a PCR technique where a pair of oligonucleotides amplified *W. bancrofti* specific fragment of approximately 490 bp (3). The sensitivity of the PCR assay was such that it detected as little as one microfilaria in 1ml of blood or one L3 larva in the presence of 20 uninfected mosquitoes or 0.4 pg of *W. bancrofti* DNA added to 100 ul of human blood (5).

In conclusion, the DNA probe we have developed is highly species-specific for *W. bancrofti*. It can detect very low levels of microfilaraemia in human blood samples and a single L_3 larva in the vector mosquito. The probe can therefore be used in diagnostic work, epidemiological surveys and for the detection of infection in the mosquito.

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