CHAPTER I

INTRODUCTION
AND
REVIEW OF LITERATURE
Lymphatic filariasis is a major public health problem in the tropical and subtropical countries. About 1.1 billion people are exposed to infection worldwide, accounting for about 20% of the total world population (WHO, 1997). India alone contributes about 47% of global prevalence of chronic patients and 39% of the population is at the risk of filarial infection (NFCP, 1995; WHO, 1997). The disease results in severe morbidity and globally is one of the most important cause of permanent disability (WHO, 1995). While acute episodic adenolymphangitis causes severe physical suffering, chronic disease such as lymphoedema and hydrocoele causes permanent disfigurement and psychosocial problems (Pani et al., 1995; Gyapong et al., 1996; Ramaiah et al., 1996a), which ultimately results in loss of work, productivity, direct and indirect economic loss and functional impairment (Pani et al., 1995; Ramaiah et al., 1996b; Ramu et al., 1996).

The most prevalent filarial parasites that infect man and parasitise the lymphatics are *Wuchereria bancrofti*, *Brugia malayi*, and *B. trimori* (Sasa, 1976). In India about 90% of the infection is caused by *W. bancrofti*. Besides these, the filarial species infecting animals and birds are *Brugia pahangi* (cats), *Dirofilaria immitis* (dogs), *Setaria cervi*, *S. digitata* (cattles), *S. equina* (horse),
Fig. 1: Schematic life cycle of filarial parasite
Acanthocheilonema vitae (rodents), Litomosoides carinii (cotton rat) and Chandlerella hawkingii (Jungle crow).

The mosquito vectors that transmits lymphatic filariasis are Culex, Aedes and Anopheles, including Mansoni. These are also vectors of animal filariae, which are often sympatric with filariae of man (Sasa, 1976; Denham and McGreevy, 1977). Filarial nematodes have a biphasic life cycle comprising a period of larval development in a blood sucking arthropod vector and the maturation and sexual reproduction within a vertebrate host (Fig. 1). The adult filarial worms are fine filiform found in the lymphatic vessels and glands. The females (6.5-10 cm x 0.2-2.8 mm) are longer than the males (4 cm x 0.1 mm). The female has a tapering anterior end with a slight rounded swelling. The reproductive activity of the adults leads to the release of microfilariae (mf) which are characteristically present in circulation.

The microfilariae exhibits nocturnal periodicity, i.e. they display circadian rhythm with regards to the number of mf in circulation, which means that they are present in the peripheral blood in greater numbers during the night as compared to the day. The peak parasitemia is reached between 22.00 to 02.00 hour (hr). When the mosquito bites an infected individual, the salivary secretion causes
the concentration of microfilariae near the site of bite and the mosquito picks up the mf during the bite (Strong et al., 1934). In the vector, the microfilariae undergoes metamorphosis and reaches the mature infective larval stage. Now again, when this infected mosquito bites another healthy individual, the larvae breaks free from the labium and penetrates through the skin thus, causing infection. The infective larvae passes through the peripheral blood vessels to the lymphatics where they undergo development process and becomes mature in an estimated period of three months.

In most cases of filarial infection the parasites does not exercise any manifestation, therefore, a high but undefined proportion of infection, in endemic areas, remains undetectable until clinical pathology (lymphangitis and elephantiasis) develops. There are 70% microfilaremic cases which do not show any clinical or pathological sign of filarial infection. The pathological changes in W. bancrofti and B. malayi are related to the developmental and adult stages of the worms. The pathological changes in the lymphatics and lymph glands are the result of an immunological reaction by the host. The manifestations of the disease are the swelling of limbs, hydrocoele, elephantiasis, tropical eosinophilia, chyluria, etc or other subclinical abnormalities.
The effective control of filariasis depends on early and specific diagnosis of filariasis. The definitive diagnosis of filarial infections in humans is dependent solely on microscopic confirmation of the presence of microfilariae in the peripheral blood in addition to the patients’ clinical signs and symptoms (Sasa, 1978). But the phenomenon of nocturnal periodicity demonstrated by many strains dictates that collection of samples should be carried out between 22.00 and 02.00 hr when the peripheral microfilarial counts are at their highest. However, parasitological diagnosis cannot detect the prepatent phase of infection, obstructive lymphatic disease (elephantiasis) or the tropical pulmonary eosinophilia syndrome (Ottesen, 1980; Kagan, 1980; Piessens and Mackenzie, 1982). This method is also very inconvenient for both the patient as well as the investigator. Furthermore, the distribution of microfilariae in the bloodstream may be non-random (Eberhard et al., 1988) and thus, making this procedure is relatively insensitive (Das et al., 1990). Similarly, mf are not always detectable in the blood of asymptomatic individuals, living in endemic areas, showing specific immunological reactivity to filarial antigens (Ottesen et al., 1982).

For these reasons there has been considerable efforts focussed towards developing non-parasitological tests, in particular immunoassays for measuring antibody and circulating antigen (Kagan, 1981). Application of immunologic methods to the diagnosis of
lymphatic filariasis has earlier been focussed mainly on the detection of the host antibody response to the parasite antigens (Kagan, 1963; Ambroise-Thomas, 1974; Grover et al., 1977; Ottesen, 1980). Filaria specific antibody can be detected in the blood of individuals who have no apparent filarial infection as defined by clinical and parasitologic criteria. Furthermore, filaria-specific antibody can persist long after the clinically defined cure has been achieved, a fact that renders the measurement of antibody levels an unreliable method for discriminating between past and present infections. The anti-filarial antibodies are extensively cross-reactive (Ambroise-Thomas, 1974; Marcoullis and Grasbeck, 1976; Almond and Parkhouse, 1985; Cabrera and Parkhouse, 1987) with different filarial parasites and other helminths, thus the problems of specificity has always occurred with serologic assays employing the whole worm extracts.

The detection of soluble circulating parasite material has been suggested as the most likely approach for identifying the presence of active infection (Frank, 1946; Harinath, 1984). Several investigations of individuals suffering with either onchocerciasis (Steward et al., 1982; Des Moutis et al., 1983) or lymphatic filariasis (Au et al., 1981; Dissanayake et al., 1982; Kaliraj et al., 1981a) and jirds with B. pahangi infection (Karavodin and Ash, 1981) have demonstrated the presence of circulating antigen and immune complexes during the patent and post patent stages of infection. The development of
immunodiagnostic tests based on the detection of circulating filarial antigens would be particularly useful in epidemiological surveys of lymphatic filariasis as well as for monitoring the mass scale drug trails. Several groups of investigators have developed such assay by using polyclonal antibodies (Harinath, 1984; Kaliraj et al., 1979a; 1981b; Au et al., 1981; Ouaiissi et al., 1981; Hamilton et al., 1984; Dasgupta et al., 1984; Weil et al., 1984a; Weil, 1990) or monoclonal antibodies (Des Moutis et al., 1983; Dissanayake et al., 1984; Forsyth et al., 1985; Weil et al., 1984b; 1985) to detect filarial antigen in the sera and urine of the infected individuals and animals, but the problem of sensitivity and specificity of these assays still remains.

Specific antibodies against defined antigens are required for developing antigen detection assays. In earlier studies, the antibodies against the somatic antigens/extracts were employed for the detection of circulating antigen in filarial patient sera (Weil, 1990; Ouaiissi et al., 1981; Hamilton et al., 1984; Weil et al., 1986). The excretory-secretory (E-S) products, released by the living parasites in the host, are found to be less complex in nature and more specific in defining infection as compared to the somatic antigens/extracts. The fractionation and characterization of E-S products is of immense interest for exploration of their utility in immunodiagnosis and for the development of specific antibodies and diagnostic reagent.
REVIEW OF LITERATURE

The accurate diagnosis of filarial infection is a major requirement in the management of this disease. The identification and characterization of parasite-derived antigenic components appears to be especially important for understanding the functional immunity to the parasite (Thorson, 1961; Poulain et al., 1976; Ogilvie et al., 1973) and for establishing specific diagnostic techniques in filarial infection. Various filarial antigenic preparations such as somatic extracts, surface antigens and excretory-secretory products, from both human as well as animals, have been analysed and characterized by a number of workers (Kwan-Lim et al., 1989; Kaushal et al., 1982; Morgan et al., 1986; Egwang and Kazura, 1987; Malhotra et al., 1987; Devaney, 1988; Srivastava et al., 1995).

I. CHARACTERIZATION OF ANTIGENS

The antigens of greater practical importance in filariasis are those related to immunodiagnosis, immunopathology and protective immunity. Since these are the molecules that are defined functionally (by eliciting an immune response), their analysis should also be closely integrated with functional studies. The characterization of
parasitic-derived antigen appears to be especially important for identifying the antigenic molecules having protective and diagnostic potential (Thorson, 1951, Poulain et al., 1976) Filarial antigens such as somatic extracts, surface antigens along with the excretory-secretory (E-S) antigens were analysed by different workers, using a number of biochemical, immunochemical and radiolabelling techniques (Forsyth et al., 1981a; Maizels et al., 1982; Kaushal et al., 1982; Malhotra et al., 1987).

Several investigators have analysed the crude somatic extracts/antigens of the adult filarial parasites with the aim of identifying and characterizing these antigens (Neilson 1975; Dissanayake and Ismail, 1980b; 1983; Ho et al., 1986; Morgan et al., 1986; Das et al., 1987; Kaushal et al., 1987; Maizels et al., 1987; Lal and Ottesen, 1988; Lammie et al., 1990; Lobos et al., 1992; Bradley et al., 1993a; b). In view of the non-availability of human filarial parasites in sufficient quantities, antigens from related animal filarial parasites including Setaria digitata (Dissanayake and Ismail, 1980b), Onchocerca gibsoni (Forsyth et al., 1981a; Catmull et al., 1994), Brugia pahangi (Maizels et al., 1982), Dirofilaria immitis (Weil et al., 1984; 1985; 1987) and S. cervi (Malhotra et al., 1986; Kaushal et al., 1987; Srivastava et al., 1996) have been employed in different studies for the characterization of somatic extracts/antigens.
Several techniques were employed for the characterization of somatic antigens such as, gel diffusion, immunoprecipitation, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), enzyme linked immunosorbent assay (ELISA), western blotting/immunoblotting. The immunoelectrophoresis (IEP) and crossed immunoelectrophoresis (CIE) were used to analyse the antigenic pattern of somatic extracts of bovine filarial parasites, *S. cervi* (Malhotra et al., 1987; Srivastava et al., 1996) and *Onchocerca* species (Lobos and Weiss, 1986) were analysed by immunoelectrophoretic techniques. The immunoelectrophoretic analysis of the *S. cervi* somatic antigens revealed the presence of 9-10 antigenic components, whereas, 22-24 antigens were observed on further analysis of the *S. cervi* somatic antigen by crossed immunoelectrophoresis (CIE) showed 22-24 antigens (Malhotra et al., 1986; Srivastava et al., 1996). Lobos and Wiess (1986) have also employed the IEP technique for analysing the antigenic components of *O. volvulus*. The IEP analysis revealed 10-11 antigenic proteins in *O. volvulus*.

The SDS-polyacrylamide gel electrophoresis (SDS-PAGE), has been employed by a number of workers to analyse the protein pattern of the somatic antigens/extracts. The SDS-PAGE analysis of somatic extracts of the *B. malayi* adult worms showed 30-35 protein bands, in the molecular weight range of 10-200 kD (Kaushal et al., 1982). The same technique was used to analyse the protein pattern of adult and mf
stages of bovine filarial parasite, *S. cervi* and (*S. cervi* adult somatic extracts showed the presence of 35-40 protein bands molecular weight range of 10-200 kD), while 25-29 protein bands in the microfilarial antigenic extracts (molecular weight range of 10-70 kD) (Malhotra et al., 1986; Kaushal et al., 1987; Srivastava et al., 1996). Farrar and others (1991) showed the presence of 42 protein bands in the molecular weight range of 12-160 kD by the western blot analysis of the *B. pahangi* adult somatic extracts.

Considerable efforts have been made to analyse the surface antigens of filarial parasites as they appear to play a role in protective immunity as well as in immunodiagnosis (Maizels et al., 1982; Sutanto et al., 1985; Selkirk et al., 1986; Philipp and Davis, 1986; Theodore and Kaliraj, 1990; Devaney et al., 1990; Kwan-Lim and Maizels, 1990; Petralanda and Piessens, 1991). Analysis of the surface proteins by radiolabelling has suggested them to be antigenically simple and distinct nature of nematodes surface (Philipp et al., 1984; Storey and Philipp, 1990). The surface radioiodination and immunoprecipitation studies of *O. volvulus* revealed a 22 kD antigen which showed reactivity with sera from *W. bancrofti* infected patients (Philipp et al., 1984). Morgan and others (1986) have analysed the surface antigens of adult *W. bancrofti* using Iodogen radiolabelling techniques and Bolton-Hunter procedures, which revealed molecules in the molecular weight range of 15-67 kD on the surface of *W. bancrofti*. 
The 15, 20 and 29 kD protein bands showed resemblance with the pattern obtained by Iodogen labelling of both *B. pahangi* (Sutanto *et al.*, 1985) and *B. malayi* (Maizels *et al.*, 1985a). A 30 kD surface antigen of *B. pahangi* was studied by radioiodination method (Devaney, 1987; 1988).

The presence of carbohydrate and glycoprotein moieties on the surfaces of filarial parasites was analysed by lectin binding studies. No appreciable carbohydrate moieties exposed on the surfaces of either adult or third stage larval (L3) forms of *B. malayi* could be detected (Kaushal *et al.*, 1984; 1989). These studies have indicated that there is stage specificity of the carbohydrate-containing antigens on the parasite surface. The dynamic change of the antigens on surface molecules may be either masked by host proteins or actual loss of surface antigens, as microfilariae matures *in vivo* (Kaushal *et al.*, 1984; Ottesen, 1984). A major surface antigen of 35 KD was observed by 2-dimensional gel electrophoresis on the surface of *D. immitis* L3 (Philipp and Davis, 1986).

The filarial parasites reside in lymphatics where they eat, excrete and procreate therefore, the parasite products are detectable in the blood of infected individuals. Such materials have been collectively termed as "excretory-secretory (E-S) products". Consequently, the E-S products come in close contact with the host
immune system, tending to provoke an immune response. The nature and properties of the E-S products is one of the most intriguing and highly speculated area of research on parasitic nematodes (Kaushal et al., 1984; Piessens et al., 1982; Maizels et al., 1986; 1987b; Sugunan and Raj, 1990). In filariasis, these materials have been reported to play a significant role in the survival of the parasites, immunopathological reactions in the host as well as in immunodiagnosis (Sasa, 1976; Kaushal et al., 1984; Maizels et al., 1986; 1987a; Piessens et al., 1982; Mak et al., 1989; Parkhe et al., 1990).

The excretory-secretory (E-S) antigens of the filarial parasites were found to be less complex in nature and more specific in defining infection as compared to the somatic antigens (Desavigney and Tizard, 1977; Kaushal et al., 1982; 1984; Malhotra et al., 1987). The importance of filarial excretory-secretory antigens to diagnosis and immunization has also been shown in onchocerciasis (Schiller et al., 1980) and bancroftian filariasis (Kharat et al., 1982; Malhotra et al., 1982; Reddy et al., 1984c; Kaushal et al., 1984; Harinath et al., 1984; Kaushal and Ottesen, 1987; Malhotra et al., 1987; Dumenigo et al., 1993; Espino and Finlay, 1994). However, not much efforts were made to identify and characterize the excretory-secretory products of human filarial parasites. The main reason is the practical difficulties in obtaining sufficient quantities of parasitic
materials for characterizing the antigens and their use in the available diagnostic procedures. First studies in this direction were done with that of *B. malayi* E-S products (Kaushal *et al.*, 1982). Subsequently, E-S products from a number of human (Maizels *et al.*, 1986; 1987; Kwan-Lim *et al.*, 1989) and animal (Parkhouse *et al.*, 1985; Maizels *et al.*, 1985a; Malhotra *et al.*, 1987; Harnett *et al.*, 1989; Thilagavathy *et al.*, 1990; Kaneko *et al.*, 1990) filarial parasites were analysed and characterized by immunochemical techniques.

The characterization of the antigenic components of the E-S products of filarial parasites is essential not only to identify suitable antigen target but for the development of sensitive and specific immunodiagnostic tests (Morgan *et al.*, 1986; Ottesen, 1984; Selkirk *et al.*, 1986; Harinath, 1984; 1986). A variety of techniques have been used by several workers for the characterization of E-S antigens of the adult filarial parasites (Kaushal *et al.*, 1982; Malhotra *et al.*, 1987; Reddy *et al.*, 1984c; Parkhouse *et al.*, 1985; Maizels *et al.*, 1985a; 1987b; Thilagavathy *et al.*, 1990; Wisrewshi *et al.*, 1990; Mizuno *et al.*, 1991; Zarnowska and Jastrzebka, 1994). The in vitro released E-S products from *Brugia* (Kaushal *et al.*, 1982; Maizels *et al.*, 1985a; Weil, 1988b) and *Wuchereria* (Kaushal *et al.*, 1982) have been assayed for diagnostic specificities as have those for both adults (Daveau and Ambroise-Thomas, 1982) and
microfilariae (Kharat et al., 1980) and also of *O. volvulus* (Schiller et al., 1980; Ngue/./., 1981).

The E-S products from adult *B. malayi* have been identified and characterized by using $^{125}$I radiolabelling method (Kaushal et al., 1982; Kaushal and Ottesen, 1987). The autoradiography revealed the presence of 11 radioactive protein bands in the *B. malayi* adult E-S products in the molecular weight range of 10 000 to 70 000 D. Studies of Kaushal et al. (1982; 1984) on *B. malayi* E-S products as well as studies by various workers with different filarial parasites have indicated that the E-S products mainly comprised of low mol.wt. proteins ranging between 10 to 200 kD (Kaushal et al., 1982; Maizels et al., 1986; Weil, 1988; Kwan-Lim et al., 1989; Parkhe et al., 1990; Lobos et al., 1992). Subsequent studies have also demonstrated major protein bands in low molecular weight regions and a few high molecular weight protein bands in E-S products of filarial parasites. Low molecular weight (15-82 kD) protein bands were observed in *L. carinii* E-S products (Rajasekhariah et al., 1986a). Egwang and Kazura, (1987) have analysed the *B. malayi* E-S products by Iodobead radioiodination method and have identified major protein bands of the mol. wt. 30, 55 and 150 kD. Mainly low mol. wt antigens of 10-22 kD along with few high molecular weight proteins of upto 120 kD in *B. malayi* E-S products have also been identified by the studies conducted by Kwan-Lim et al. (1989) Kwan-Lim et al. (1989). The 29 kD
protein band identified as one of the major surface antigen in *B. pahangi* (Lai and Ottesen, 1988; Devaney, 1987; 1988; Maizels *et al*., 1985; Flecher and Wu, 1992). The E-S products of adult *D. immitis* male and female worms revealed the presence of 16 and 21 protein bands respectively, while 7 and 10 protein bands were found in both male and female *D. immitis* E-S products when immunoblotting was employed (Kaneko *et al*., 1990).

The E-S antigens are known to be formed in the uterus during embryonic development (Decruize and Raj, 1988) and are released during moulting as secreted or excreted products (Maizels and Selkirk, 1988a; b; Kaushal *et al*., 1982; Sugunan and Raj, 1986; Dhas *et al*., 1993). Sugunan and Raj (1986) have also shown to have a direct relationship between E-S materials and the number of mf released. Besides, the E-S products are amongst the first parasite molecules to encounter the host immune system and continue to do so during the course of parasite’s life. They may therefore, influence parasite survival and indeed, having a role in protective immunity against filariasis (Mimori *et al*., 1987; Ey, 1988). The E-S products may contribute to parasite pathology (Sisley *et al*., 1987), including that arising during drug treatment (Greene *et al*., 1983).
Presence of host serum proteins in the filarial E-S products

The filarial parasites reside in the lymphatics of the host. To protect themselves from the immunologically hostile environment of the host and to evoke the immune response of the host, the filarial parasites are known to adsorb some of the host serum proteins on their surfaces. Several workers have reported the presence of albumin on the surface of the parasites such as the infective larvae of *Trichenella spiralis* (Parkhouse et al., 1981), *O. gibsoni* (Mitchell et al., 1982), mf of *W. bancrofti* (Maizels et al., 1984a; b; Kar et al., 1993; Mania and Kar, 1994), infective larvae of *W. bancrofti* (Maizels et al., 1986) and *L. carinii* (Phillip et al., 1984a). The evidence for the presence of host serum proteins has also been shown in the E-S preparation of *B. malayi* (Kaushal et al., 1982), *B. pahangi* (Parkhouse et al., 1985) and a bovine filarial parasite *S. cervi* (Malhotra et al., 1987).

FRACTIONATION OF THE EXCRETORY-SECRETORY PRODUCTS

The fractionation of antigenic preparations may lead to the identification of the relevant antigen of diagnostic significance and thus, for developing immunodiagnostic measures for human filariasis. Relatively, little efforts has been made for the fractionation of E-S
products of the filarial parasites. The non-availability of a convenient and suitable animal model for *W. bancrofti* makes it difficult to obtain sufficient parasite material for immunodiagnosis of human filariasis. Hence, antigens shared by different filarial species have been explored by several workers for isolating the diagnostic important antigens (Hamilton *et al.*, 1984; Ottesen *et al.*, 1985; Weil *et al.*, 1987; Cheirmaraj *et al.*, 1990). *W. bancrofti* microfilarial E-S products has been found to be identical to some of the active circulating filarial antigen fractions (CFA2-1, 9, 11 and 12) of microfilaremic cases (Parkhe *et al.*, 1990). Chenthamarakshan and colleagues (1996b) fractionated the *B. malayi* microfilarial E-S products using DEAE cellulose and obtained two fractions BmE DE1 and BmE DE2. The BmE DE1 was more active in binding to the immunoglobulin G fraction of filarial serum immunoglobulin. This fraction has a sensitivity and specificity of 83%. Similarly, E-S proteins of *S. digitata* was fractionated on Sephadex G150 and resolved into three protein components of mol. wt. 70 kD (ES F1), 16.5 kD (ES F2) and 11 kD (ES F3) (Sugunan and Raj, 1990). The 16.5 kD (ES F2) fraction was found to be the major components. Later these fractions (ES F2 and ES F3) showed 100% sensitivity in detecting human filarial antibodies in patients’ sera.

Homologous filarial parasite antigen isolated from blood (Reddy *et al.*, 1986), hydrocoele fluids (Malhotra *et al.*, 1985; Ramaprasad and
Harinath, 1989) and urine (Ramaprasad and Harinath, 1987) have been fractionated and used as diagnostic reagent by Harinath and colleagues. The albumin absorbed UFA-C2 fraction has shown high sensitivity in detecting filarial antibody (Ramaprasad and Harinath, 1987). The same group of workers showed positive correlation between UFA C2-A and mfES antigen in detection of filarial antibody. Ramaprasad and Harinath (1995) have found that large scale isolation of UFA C2-A fraction from filarial urine samples or production of monoclonal antibodies to UFA C2-A overcomes the lack of parasite material and removes the difficulty in collection of mfES antigen. Chenthamarakshan and others (1995) fractionated the *B. malayi* adult SDS soluble antigen and evaluated the diagnostic utility of active antigen fraction in bancroftian filariasis. The antigenic analysis of BmA SDS S Ag revealed two fractions BmA-6 (37-45 kD) and BmA-9 (20-25 kD) with high antigenic activity. Antibody were produced against these antigen fractions in order to evaluate their utility in detection of circulating filarial antigen in bancroftian filariasis.

III. IMMUNODIAGNOSIS

Diagnosis of parasite infectious forms an important element is the identification of infected individuals and for studies on
epidemiology, protective immunity, immunopathology and control of disease. Highly specific tests based on detection of parasites/parasite antigens and/or antibody requirement for specific and accurate diagnosis of infection. Ideally diagnostic test should be simple, sensitive, quantifiable and consistently reproducible. In addition, such test should be inexpensive and applicable for large scale field use. Immunodiagnosis of filariasis is one of the important area in filarial immunology (Kagan, 1963; 1981; Ottesen, 1984; Taylor and Denham, 1986). A number of immunodiagnostic tests based on antibody and antigen detection have been developed for human and animal filarial infections (Ambroise-Thomas, 1974). Immunodiagnostic techniques with ‘low or moderate sensitivity’ (Kagan, 1974) such as complement fixation, gel diffusion, latex agglutination, indirect haemagglutination and indirect immunofluorescence have largely been replaced by those of ‘high sensitivity’, such as radio immunoassay, immunofluorescence assays, enzyme linked immunosorbent assay, luminescence immunoassay, immunoradiometric assay etc., have been explored for the diagnosis of filariasis. A practical and sensitive assay would improve understanding of the epidemiology of the disease and could be useful for monitoring the success of vector control efforts and drug trials (WHO, 1984).
A. Parasitological Diagnosis

The diagnosis of filarial infections in humans still largely depends on an assessment of the patients' clinical signs and symptoms in addition to microscopic conformation for the presence of microfilariae (mf) circulating in peripheral blood (*W. bancrofti, B. malayi*) or dwelling in the skin (*Onchocerca volvulus*) (Sasa, 1978). However, there are technical, practical and biological factors that limit the usefulness of microfilaria detections as a diagnostic test for filarial infection. The technical limitation is that the sensitivity of such test depends on microfilariae counts, the volume of blood (or the number of skin snips in case of onchocerciasis) examined, and the skill of the microscopist. The microfilariae of *W. bancrofti* and *B. malayi* shows nocturnal periodicity and this imposes a practical limitation on the effective use of microfilarial detection. Moreover, this method is inconvenient for both the investigator as well as the patient. The biological limitation of microfilaria detection is that it fails to detect the disease when mf are sparse or sequestered inside the tissues or in inaccessible sites. This method is also insensitive because of the well recognized phenomenon of filariasis without microfilariae, i.e., amicrofilaremic stage (Beaver, 1970). The parasitological detection of microfilariae is found to be relatively insensitive, giving emphasis to the development of immunodiagnostic techniques, based on antibody and antigen detection, for filariasis.
B. Antibody Detection

Application of immunological methods to the diagnosis of lymphatic filariasis has earlier been focussed on the detection of the antibody response to the parasite (Kagan, 1963; Ambroise-Thomas, 1974; Grover et al., 1977). Antibodies have been detected readily in sera of bancroftian filarial patient using heterologous or homologous antigens from adults, larvae and microfilariae (mf) stages, as well as excretory-secretory products from filarial parasites (Ambroise-Thomas, 1974; 1980; Chandra et al., 1974; Gonsaga dos Santos et al., 1976; Grover and Davies, 1978; Dissanayake and Ismail, 1981; Au et al., 1982; Kaushal et al., 1982). A number of immunological methods such as gel diffusion (Petithory et al., 1972; Khatoon et al., 1987), immunoelectrophoresis (Capron et al., 1968; 1970), counter current immunoelectrophoresis (Desowitz and Una, 1976; Weil et al., 1984; 1986; Dasgupta et al., 1980), indirect haemagglutination test (Takahashi and Sato, 1976; Kaliraj et al., 1981a; b; Das et al., 1987), indirect immunofluorescent antibody test (Diesfeld et al., 1973; Forsyth et al., 1985; Kaliraj, et al., 1981c; Das et al., 1987; Dissanayake et al., 1984) and the skin test (Swada et al., 1969; 1975; Swada and Sato, 1969; Gupta and Ansari, 1989) have been used for detecting filarial antibodies employing crude antigenic extracts from the homologous and heterologous filarial parasites (Ottesen et al., 1982).
The skin test used previously for the diagnosis of filarial infection by detecting antibodies in filarial patients’ sera. The test was carried out by subcutaneous injection of the filarial antigen in the arm of an individual, the response is of immediate type and can be read usually within 15 to 30 min, but the delayed hypersensitivity reaction i.e. after twenty four hour has also been noted. Wharton (1947) has reported that delayed reaction, due to the use of antigen in very high concentrations, was actually of immediate type skin reaction which persisted and produced exacerbation of symptoms in the infected individuals. The false positive results obtained with skin test may be attributed to cross-reaction with intestinal helminths (Bozicevich and Hutter, 1944; King, 1944; Hunter, 1958). The skin test showed cross-reactivity with patients infected with hookworms and strongyloides (Woodruff et al., 1958). It has also been observed that in some cases of elephantiasis, chyluria and hydrocoele skin test was found negative (Frank et al., 1947). Chandra and coworkers (1974) developed a filarial skin test kit using B. malayi infective larval antigen for the detection of antibody in filarial patient sera. This test has been evaluated for its sensitivity and specificity in the immunodiagnosis of human filariasis covering over 2500 cases from filaria endemic and non-endemic regions of India (Katiyar et al., 1985 and Sircar et al., 1990). The major limitation of the kit was that the crude L3 antigen was used, which was prepared from the mosquito and it showed cross-reactivity with other helminth parasites. Acton and Rao (1933) used
hydrocoele fluid as antigen in the skin test for the diagnosis of human filariasis.

Another widely employed diagnostic test in sixties-seventies, the complement fixation, was found to be more sensitive than skin test for the diagnosis of human filariasis (Kagan, 1963). The complement fixation test showed fairly high sensitivity with sera of patients infected Loa loa whereas, from W. bancrofti infected patients and those having other filarial infections showed comparatively less sensitivity (Scofield, 1957). This test also showed cross-reactivity against anchylostoma, schistosomiasis and stronglyoidiasis and infections with other intestinal parasites (Stemplen, 1944).

The presence of filarial antibody in the serum samples of infected humans and animals has been reported by several investigators using the precipitation reaction (Biguet et al., 1962; Desowitz and Una, 1976; Khatoon et al., 1987). The precipitin test has limited use in the diagnosis of filarial infection. The antigens used in the test were the saline extracts of the whole worms and the results varied considerably. The precipitin test was found to be less sensitive than the skin test for the diagnosis of Mansonella ozzardi (Biagi, 1956). Ellsworth and Johnson (1973) used diffusion capillary tubes for the precipitate formation using the sera of Dirofilaria immitis.
infected dogs. The immunodiffusion method was used by Khatoon, et al. (1987) for the diagnosis of *Setaria cervi* infection and this method was found to be comparatively simple and reliable. The sensitivity and reactivity of the precipitin test are so much lower than other conventional serological tests that its use for routine diagnosis can be hardly recommended.

Another precipitation technique i.e. immunoelectrophoresis (IEP) has been used by Wheeling and Hutchinson (1971) for the diagnosis of human filariasis employing antigens prepared from *D. immitis* microfilariae and adults. Kaeuffer and coworkers (1974), however, did not show any false positive reactions when the sera of 14 Tahitians infected with *W. bancrofti* were tested in IEP using adult *D. immitis* antigen. IEP has also successfully been employed for the diagnosis of onchocerciasis with antigen extracted from the adult worms of *Onchocerca* (Biguet et al., 1962; 1964; Capron et al., 1968; 1970). Using the above technique (i.e. IEP) Gentilini et al. (1973) have found that 8 out of 9 patients, suffering from bancroftian filariasis were positive for the infection, whereas, no precipitin bands were observed when sera of 3 patients were tested against *O. volvulus* adult antigen. The number of arcs produced have been shown to be related to the clinical severity of the filarial infection (D'Haussy et al., 1972). The degree of certainty for the specificity of these precipitin test is not known. Wheeling and Hutchinson (1971) obtained positive
reaction with *D. immitis* antigen in case of human filariasis. On the other hand Kauffer and others (1974) tested a number of *W. bancrofti* microfilareamic patients with the same antigen (*D. immitis* adult antigen) and found entirely negative results. The investigation carried out by Niel *et al.* (1972) have indicated the possibility of broad spectrum of antigenic specificity, and have also found that the antigens prepared from *Setaria labiato-papillosa* and *Dipetalonema vitae* produced precipitin lines in immunodiffusion and IEP against the sera of patients with loiasis, onchocerciasis, bancroftian filariasis and dracunculiasis. The above workers also reported the cross-reactivity of the sera of filarial patients with antigens of *Ascaris suum* using the same technique. Counter current immunoelectrophoresis (CIEP) was used by Desowitz and Una (1976) for the detection of filarial antibodies using *D. immitis* adult antigen and was found to be fairly sensitive. CIEP has the advantage of simplicity of performance, rapidity and the capability of processing relatively large numbers of serum samples at the same time.

The indirect haemagglutination test (IHAT) has also been employed for the diagnosis of human filariasis. The haemagglutination test was used by Jung and Harris (1960) for the detection of human filarial infection using somatic antigen from *D. immitis* adult worms. The IHAT employing heterologus somatic antigen extracts (*D. immitis*) showed apparent sensitivity in the
diagnosis of filariasis due to *Acanthocheilonema perstans* (Kagan *et al.*, 1963) and *W. bancrofti* (Fujita *et al.*, 1970) and also gave false positive reactions with sera from normal individuals as well as with patient sera harbouring other helminth infections.

The indirect fluorescent antibody test (IFAT) has been used for immunodiagnosis of filariasis in limited number of studies. The adult worm extracts of *A. vitae* (Ambroise-Thomas and Kein Trong, 1974), *S. digitata* (Tan *et al.*, 1988) or sonicated *W. bancrofti* microfilarial and larval antigens (Hedge and Ridley, 1977; Kaliraj *et al.*, 1979b; 1981c; Das *et al.*, 1987; 1988c) have been used in IFAT for the detection of filarial antibody. However, the use of immunofluorescence test for large scale testing of patient samples is tedious and requires special equipments.

The advancement of immunological techniques led to the development of sensitive assays for detecting parasitic infections. The enzyme linked immunosorbent assay (ELISA) was found to be simple, sensitive and suitable for mass screening of most parasitic infections (Voller *et al.*, 1974; 1975a; b; 1976a; b; Kaliraj *et al.*, 1981b; c). Since its development (Engvall and Pearlman, 1972) ELISA has been successfully used for detecting antibodies and antigens of a variety of organisms (Sever and Madden, 1977). In recent years, ELISA had been found very useful in the diagnosis of
many parasitic diseases including filariasis (Voller et al., 1976a; Bartlett et al., 1975; Bartlett and Bidwell, 1976; Barakat et al., 1983; Gueglio et al., 1995). Both homologous and heterologous antigens were used for measuring antibody response in filarial infection (Ruitenberg et al., 1975; Spencer et al., 1981). A microtitre plate ELISA was used by Bartlett et al. (1975; 1976) for the detection of antibody in sera of *Onchocerca volvulus* infected patients’ sera but the use of homologous antigen was not possible as contaminants of host origin reacted non-specifically with the secondary antibody conjugate. No such problem was encountered when the same antigen was used after purification (Marcoullis et al., 1978). The *W. bancrofti* mf antigen and mf excretory-secretory (mf ES) antigen was used for the detection of filariasis by a number of workers (Kaliraj et al., 1981b; c; Kharat et al., 1982; Malhotra et al., 1982; Malhotra and Harinath, 1984; Harinath et al., 1984; 1986; Dissanayake and Ismail, 1980a). In all these cases ELISA was found to have better sensitivity than night blood examination. Isolation and characterization of filarial antigens from urine and hydrocoele fluids have shown fair diagnostic potential (Ramaprasad and Harinath, 1989). The purified antigens showed high sensitivity and specificity when tested in sandwich ELISA (sELISA) (Malhotra et al., 1985a; b; Singh et al., 1993; Ramaprasad and Harinath, 1989; 1995) and stick enzyme immunoassay (Parkhe et al., 1988; Ramaprasad and Harinath, 1989) for the detection of filarial antibodies in filarial patient sera. Besides, the use of homologous
antigens, heterologous antigens were also used by many workers for the diagnosis of human filariasis, i.e., *B. malayi* (Spencer *et al*., 1981; Cheirmaraj *et al*., 1990; Kumar and Santhanam, 1990; Chanteau *et al*., 1991; Yuan *et al*., 1992; Li *et al*., 1993), *D. immitis* (Weil *et al*., 1985; 1987) and *S. digitata* (John *et al*., 1995; Dhas and Raj, 1995).

The serodiagnostic tests for filariasis based on antibody detection were useful for testing sera from people visiting endemic areas, but they were not very suitable for testing sera from endemic areas because of extensive antigenic cross-reactivity among different nematode antigens (Oliver-Gonzales and Morales, 1945; Kagan, 1963; Ambroise-Thomas, 1980). In addition, people who have been exposed to filarial parasites, so called ‘endemic normals’, often have antiparasite antibody titres that are at least as high as those people with proven infections (Ambroise-Thomas, 1980; Ottesen *et al*., 1982). The next level of refinement in antibody testing improved specificity by using subsets of antigens purified from crude extracts (Weiss and Karam, 1989) or by measuring antibodies of specific isotypes (Weiss *et al*., 1982; Lal and Ottesen, 1988a; Kwan-Lim *et al*., 1990; Weil *et al*., 1990). The IgG₄ is normally a minor component of total circulating immunoglobulins in lymphatic filarial infection but shows prominent response (Ottesen *et al*., 1985). Lal and Ottesen (1988a) demonstrated that IgG₄ antibody detection produce increased specificity for detecting
filarial infection, as compared to total IgG, even when crude parasite antigen was used. These assays were more specific than assays those measures total antibodies to crude antigen-mixture, but they did not achieve full species-specificity and also do not correlate the active infection. The lack of acceptable specificity and sensitivity of antibody detection assays in the serodiagnosis of filarial infections was one of the major reasons for the development of antigen detection assays (Dissanayake and Ismail, 1987).

C. Antigen Detection

The parasite antigen detection is more sensitive and specific for diagnosing active filarial infection than parasitological examination or antibody detection (Harinath et al., 1984; Forsyth et al., 1985; Weil et al., 1985; 1987; Lal et al., 1987; Anon, 1989). Considerable emphasis has been given to the detection of circulating antigen in the blood and other body fluids of filarial patients (WHO, 1992; Weil, 1990). The antibodies raised against the filarial antigen obtained from infected patients have been employed by some investigators for the detection of circulating filarial antigen in patients’ sera (Kaliraj et al., 1979; Forsyth et al., 1985; Weil et al., 1986; 1988; Weil, 1990; Mustafa et al., 1996). Several groups of investigators recently have developed antigen
detection assays using polyclonal (Harinath, 1984; Kaliraj et al., 1979; 1981; Au et al., 1981; Ouaissi et al., 1981; Hamilton et al., 1984; Dasgupta et al., 1984; Weil et al., 1986; Mustafa et al., 1995; 1997) and monoclonal (DesMoutis et al., 1983; Dissanayake et al., 1984; Forsyth et al., 1985; Weil et al., 1985; More and Copeman, 1990; Kaushal et al., 1994a; b; Weil et al., 1997) antibodies (MoAb) to detect filarial circulating antigen. The earlier studies were mainly focussed on the antisera raised against somatic extracts of the heterologous filarial parasites, i.e. *L. carinii* (Dasgupta and Bala, 1978; Kaliraj et al., 1981a) and *D. immitis* (Tanabe, 1959; Weil et al., 1984a; 1986).

Circulating filarial antigen was first demonstrated in bancroftian filariasis in 1946, using the technique of passive cutaneous anaphylaxis (Frank, 1946). The soluble circulating parasite materials has been detected by number of workers in *W. bancrofti* infected individuals (Reddy et al., 1984a; b; Zheng et al., 1987b; Lutsch et al., 1987; 1988; Ramaprasad and Harinath, 1995) and also in patient infected with onchocerciasis (Ouaissi et al., 1981; Des Moutis et al., 1983; Petralanda et al., 1988; Schlie-Guzman and Rivas Alcala, 1989; Chandrashekhar et al., 1990) and thus indicating the active infection of filarial parasites.
Circulating parasite antigen in patients with bancroftian filariasis has been demonstrated using techniques of 'moderate sensitivity' such as counter current immunoelectrophoresis and indirect haemagglutination test (Kaliraj et al., 1981c; Au et al., 1981) but positive results were obtained only in sera of microfilaraemic positive individuals. The CIEP was used for the detection of circulating antigen using rabbit anti-filarial sera (Kaliraj et al., 1979a; Dasgupta et al., 1980; 1984; Das et al., 1988c; Kumar et al., 1991; Dumenigo et al., 1993). The antibodies raised in rabbits against L. carinii somatic antigen were used for the detection of filarial antigen in human patients' sera and it was observed that 5.7% cases diagnosed by parasitological examination can be increased to 62.8% by CIEP (Dasgupta et al., 1980). The CIEP used to detect the D. immitis antigens represents a significant improvement over previously available diagnostic techniques because it is more sensitive than microfilarial test, and can be related to the active infection and can be used as practical diagnostic test for active W. bancrofti infection ((Kaliraj et al., 1981a; Weil et al., 1984; 1986). The CIEP is simple and rapid as compared to IHAT, with increased sensitivity over immunodiffusion for the detection of circulating antigen have been established in various studies (Krupp, 1974; Dasgupta and Bala, 1978; 1980; Shariff and Parija, 1991). Hamilton et al., (1984) have used antibodies specific for B. malayi adult worms to detect cross-reactive antigens in bancroftian filarial patient by immunoradiometric assay.
(IRMA). Using polyclonal antibodies against adult *S. digitata*, Dissanayake and others (1982) detected antigens in the immune complex from bancroftian filariasis patients. The above workers detected the antigens in the circulating immune complex (CIC) reactive with polyclonal antibodies, but did not assay the amount of each antigen. Zheng *et al.* (1987a), Using polyclonal and monoclonal antibodies (E34 and HC 11), have shown filarial antigen in 95% of sera from mf donors with bancroftian or brugian filariasis and approximately 60% of sera from mf donors and 60% of clinical cases by inhibition ELISA using E-S antigen conjugated to penicillinase. However, filarial antigen was detected in all the 38 sera from mf patients by CIEP with rabbit antibodies to *D. immitis* and *B. malayi* (Weil *et al.*, 1986). Recently, 100% positive results were reported with mf positive sera by Cheirmaraj *et al.* (1992) using polyclonal antibodies raised in mouse ascites.

The non-availability of adult *W. bancrofti* for antigenic material, demands the use of antigens from heterologous filarial parasites to raise polyclonal antibodies in rabbits and monoclonal antibodies for the detection of circulating antigens in filarial infections has been employed by several workers (Dasgupta *et al.*, 1980; 1984; More and Copeman, 1990; 1991; Kumar *et al.*, 1991; Jaoko, 1995; Forsyth *et al.*, 1985; Weil *et al.*, 1984; 1985; 1986; 1987; Maizels *et al.*, 1983; 1985b; Kazura *et al.*, 1986; Freedman *et al.*, 1989; Parab *et
Besides these, materials from the microfilariae and infective larvae of *W. bancrofti* and of related filarial parasites were also used by a number of researchers to produce antisera for the detection of filarial circulating antigens in patient sera (Almeida *et al.*, 1990; Reddy *et al.*, 1984b; c; Lutsch *et al.*, 1987; Cheirmaraj *et al.*, 1990; 1992; More and Copeman, 1991; Dumenigo *et al.*, 1993; Zheng *et al.*, 1987; Lal 1991; Li *et al.*, 1993; Chenthamarakshan *et al.*, 1995; 1996).

Polyclonal antibodies were raised against somatic extracts of *W. bancrofti* microfilariae, and could detect the filarial circulating antigen in microfilaremic positive patient sera (Kaliraj *et al.*, 1979a). Due to the use of polyclonal antibodies raised against crude somatic antigens, for the detection of circulating filarial antigen, problems of specificity and sensitivity remain (Weil, 1990). Therefore, monoclonal antibodies have been employed by several investigators for the detection of circulating antigen in the filarial infected individuals (Dissanayake *et al.*, 1984; Weil *et al.*, 1985; 1987; Forsyth *et al.*, 1985; Lal *et al.*, 1987; Weil and Liftis, 1987; Zheng *et al.*, 1987; Santhanam *et al.*, 1989; More and Copeman, 1990; Ramzy *et al.*, 1991; Li *et al.*, 1993; Kaushal *et al.*, 1994; Ramaprasad and Harinath, 1995; Gyapong *et al.*, 1998; Lalitha *et al.*, 1998). Assays describing detection of
Circulating antigen in onchocerciasis patients were first reported in the early 1980s (Ouaissi et al., 1981; Des Moutis et al., 1983) and was found to be highly sensitive and specific (Cabrera and Parkhouse, 1987; More and Copeman, 1990; 1991). Phosphorylcholine (PC) was the first truly defined molecules to be targeted in the serum of onchocerciasis patients (Weiss, 1985; Maizels et al., 1990). An improvement in sensitivity (92.3%) was obtained by Schlie-Guzman and Rivas Alcala (1989).

An immunoradiometric assay (IRMA) for the detection of filarial antigen was developed using an IgM monoclonal antibody (Gib-13) to *O. gibsoni* egg antigen (Dissanayake et al., 1984; Forsyth et al., 1985). The assay detected the parasite circulating antigen in 93% and 75% of the sera from microfilaremic subjects with *W. bancrofti*, however, it takes two days to perform the Gib-13 IRMA. Moreover, as it is an radioimmunoassay, it could not be used in field conditions. The Gib-13 shows phosphorylcholine (PC) specificity detecting molecules of 140, 52, 56 and 62 kD molecular weight and recognizes a carbohydrate moieties. Zheng et al. (1987a; b; 1990) used monoclonal antibody based sandwich ELISA (sELISA) for the detection of circulating filarial antigen using rabbit anti-microfilarial immunoglobulin as first antibody and monoclonal antibodies, against *W. bancrofti* mf E-S antigen and *B. malayi* L3 antigen as second antibody, the positivity rates were 94.5% and 89.0%
respectively in microfilaremic patients. Most of the earlier attempts to produce monoclonal antibodies against filarial circulating antigen have resulted in monoclonals showing reactivity with phosphorylcholine epitope and to other, more specific determinants (Forsyth et al., 1985; Lal et al., 1987; Weil et al., 1987; Zheng et al., 1987a; More and Copeman, 1990). The sensitivity of PC assays for *W. bancrofti* infection have ranged from 85 to 93% for sera from microfilaremic patients depending upon the population surveyed. PC antigenemia has also been detected in sera from patients infected with *B. malayi* (Maizels et al., 1985a). However, the sensitivity of PC antigen detection for human *B. malayi* infections has been very poor. In addition, studies on PC antigenemia in filariasis have been reported conflicting specificity results. The PC is a widely distributed determinant, particularly among the nematode parasites and its presence on the filarial parasite antigens may account for much of the cross-reactivity observed with other helminth parasites (Maizels et al., 1987c; Pery et al., 1974; Zheng et al., 1987). Therefore, assays detecting the PC determinant is not suitable for the specific diagnosis of human filariasis (Forsyth et al., 1985; Lal et al., 1987; Sutanto et al., 1985). Lal and others (1987) detected a 200 KD antigen in the sera of 93% of patients with microfilaremia, 46% of those with lymphatic obstruction, and 56% of those with tropical pulmonary eosinophilia syndrome using a monoclonal antibody (CA₂ or CA₁₀₁) raised against circulating antigen from *W. bancrofti*. This
A monoclonal antibody could detect parasite antigen concentration as low as 30 to 4000 ng/ml in patients living in an area endemic for bancroftian filariasis and it also shows specificity for PC determinants. The CA101 monoclonal antibody is an IgMk with the T15 idotype.

There are few monoclonal antibodies which are directed against non-phosphorylcholine epitopes and are also capable of detecting filarial circulating antigen in bancroftian filariasis with minimal or no cross-reactivity (Weil and Liftis, 1987; Weil et al., 1987; More and Copeman, 1990; Zheng et al., 1990; Kaushal et al., 1994). These assays have been successfully used for detecting the filarial circulating antigen in patients’ sera (Weil et al., 1988; 1991). Another monoclonal antibody (ES 34) which is against *W. bancrofti* mf ES antigen (55-63 kD) has shown promising results in field studies conducted in India and China (Zheng et al., 1987; Reddy et al., 1989). But this monoclonal could not detect antigen in clinical cases and specificity data was insufficient. A specific assay, which is currently undergoing commercial development, is based on the monoclonal antibody AD12.1 (Weil et al., 1987) which recognises a 200 kD antigen in the circulation of individuals infected with *W. bancrofti* (Weil and Liftis, 1987) which again is of ES products of adult worm (Weil et al., 1996) and is specific for *W. bancrofti* only (Weil, 1990; Weil et al., 1987; Ramzy et al., 1991). The monoclonal antibody is of IgG1 isotype and recognizes the carbohydrate epitopes and detects the absolute
amount of protein by a factor of at least 2.4. The sensitivity of AD12.1 assay for sera from microfilaremic patients was approximately 95%. The filarial antigen was detected neither in sera from non-endemic areas nor from patients with other parasitic infections (Reddy et al., 1989). The ICT Filariasis card test is a new, rapid-format filarial antigen test based on monoclonal antibody AD12.1, was developed by ICT diagnostics (Balgowlah, New South Wales, Australia). This assay takes only 5-15 min to complete, requires no specialized equipment and gives comparable results (Weil et al., 1997).

Another assay that could successfully detect the circulating antigen in ELISA is commercially available for detection of *W. bancrofti* infection (Trop-Ag *W. bancrofti* ELISA kit, JCU Tropical Biotechnology Pty Ltd, Queensland, Australia). The assay is based on a monoclonal antibody, Og4C3, which curiously, in spite of its being raised against antigens of the bovine parasite *O. gibsoni*, detects circulating antigen in serum from *W. bancrofti* infected patients, but not *O. vovulus* (More and Copeman, 1990) using sandwich ELISA. Negative results were also obtained with sera from individuals harbouring *B. malayi*, *B. trimori* and *Loa loa* infections. The antigen detected in the circulation of bancroftian filariasis patients is of adult origin (Chanteau et al., 1994). The monoclonal antibody Og4C3 has IgM antibodies. Og4C3 binds to a range of filarial and non-filarial
nematodes (O. gibsoni, O. vovulus, D. immitis, A. caninum and Toxocara canis) but not to phosphrylcholine. It recognizes antigens of M W > 130 kD and 50-60 kD from Og male antigen, with epitopes having both protein and carbohydrate moieties. The target antigens of Og4C3 are located at the junction of the cuticle, hypodermis, in cells of the gut, in intrauterine embryos and extra-uterine microfilariae of O. gibsoni. Both assays have been evaluated as diagnostic tools in a number of studies (Nicholas, 1997) and can detect circulating antigen in virtually all (94-100%) microfilarial carriers (Weil et al., 1987; 1997; Turner et al., 1993; Chanteau et al., 1994a; b; Rocha et al., 1996; Nicholas, 1997). However, most of these MoAbs are of IgM isotypes and not very suitable for use in the field test. Kaushal and others (1994a) produced MoAb against antigenic epitopes common between the bovine (Setaria cervi) and human filarial parasites. The two monoclonal antibodies (13B4 and 15D6) that showed reactivity with filarial circulating antigen of IgG1 isotype and are directed to the protein epitope. The target antigen have been cloned using these monoclonal antibodies (Kaushal et al., unpublished data). These monoclonals showed high reactivity with filarial antigen while very little or no reactivity was observed with the non-filarial antigen (Kaushal et al., 1994; Kaushal and Kaushal, 1995). This assay has high sensitivity but lacks sufficient sensitivity. Ramzy and others (1991) evaluated the performance of antigen detection in the sera from an endemic area of W. bancrofti in Egypt using MoAb raised against
*Dirofilaria immitis*. They reported that 97% of microfilaremic subjects were antigen positive and antigen levels were significantly correlated with mf counts. Those methods for detecting circulating antigens using MoAbs were reported to have high sensitivities correlating with mf counts.
SCOPE AND PLAN OF WORK

Improved methods for diagnosing active filarial infections are needed to monitor control efforts and to evaluate new drugs. The most commonly used method for the diagnosis of filariasis, is based on demonstration of mf in the night blood smears of the patient. This test is inconvenient for both the patients as well as the investigator and fails to detect the amicrofilaremic stage of the disease or when mf are present in very small numbers or sequestered in the tissues. This has put impetus for developing alternate means for filaria diagnosis such as immunodiagnostic test. Earlier studies on immunodiagnosis of filariasis were focussed mainly on the detection of antibodies, but the major shortcoming is their inability to distinguish past exposure from current infection. Therefore, in the recent past considerable emphasis has been given to the detection of circulating antigen in blood or other body fluids of the infected individuals. The demonstration of the parasite antigen in circulation of the infected individual appears to be an useful indicator of active filarial infection. For establishing specific diagnostic techniques identification and characterization of parasite-derived antigen is important. Due to the complex nature and extensive cross-reactivity of the crude somatic antigens with the other nematode parasites, antigens present in the excretory-secretory (E-S) products (obtained by the in vitro
maintenance of filarial parasites) of these parasites were analysed and evaluated for the immunodiagnosis of filariasis.

Due to the non-availability of human filarial parasite, antigens from *Setaria cervi* (a bovine filarial parasite) have been used in the present study. The E-S products were obtained by maintaining *S. cervi* adult worm by short-term *in vitro* incubation in culture. An effort was made to isolate the *S. cervi* E-S products, raise polyclonal antibodies against E-S antigens and this anti E-S antibodies could detect the circulating antigen in human filarial patient sera. Therefore, the E-S products were fractionated in order to identify the relevant antigen (equivalent to the filarial circulating antigen), produce polyclonal and monoclonal antibodies and the potential of these monoclonal antibodies were evaluated for the detection of circulating antigen in human filariasis.

The present study deals with the following aspects:

I. Preparation and immunochemical characterization of excretory-secretory (E-S) products from *S. cervi* adult worms.
II. Raising of polyvalent hyperimmune antibodies against *S. cervi* E-S products in rabbits and its evaluation for the detection of circulating antigen in filarial patient sera.

III. Fractionation of *S. cervi* E-S products and immunochemical characterization of fractionated E-S products and identification of antigen equivalent to filarial circulating antigen.

IV. Production and characterization of polyclonal/monoclonal antibodies against the relevant E-S antigens.

V. Evaluation of the antibodies for the detection of circulating antigen in filarial patient sera.