Amoebiasis, an entero invasive and debilitating disease is caused by a protozoan Entamoeba histolytica. It is world wide in its distribution and is one of the major public health problems in developing and underdeveloped countries. According to the recommendations of World Health Organization Expert Committee (WHO, 1969), amoebiasis is defined as the condition of harbouring the parasite E. histolytica, with or without clinical manifestations. In a majority of individuals, E. histolytica remains as commensal in the gut, but in a small percentage of such individuals, the trophozoite invades the colonic mucosa resulting in inflammatory colitis or metastatic spread to other vital organs leading to the formation of an abscess (Juniper, 1971; Adams and Macleod, 1977; Ravdin and Jones, 1984; Salata and Ravdin, 1986). The most common site of dissemination of infection is liver where hepatic abscess is caused (Salata and Ravdin, 1986; Martinez-Palamo, 1987). Very rarely extra hepatic abscesses involving lung and brain are formed. According to an estimate, approximately 10% of the population of world carry the parasite in intestinal tract. Of more than 450 million individuals infected with the parasite, only 10% have their gut or liver invaded by the parasite. More than 75,000 deaths per year in the world have been attributed to amoebic infections (Guerrant, 1986; Walsh, 1986). Globally, amoebiasis ranks third among the deaths due to parasitic infections, being next to malaria and schistosomiasis (Walsh, 1986). The incidence of amoebiasis at a particular place depends upon socio-economic norms. In India, 5-58 percent of the individuals suffering with gastro-intestinal infection, harbour E. histolytica in their gut (Srivastava, 1953; Prakash and Tandon, 1966; Vinayak et al., 1967; Rao et al., 1971; Tandon et al., 1972; Arora et al., 1975, 1976; Veerman, 1977). Entamoeba histolytica is also an appreciable cause of acute diarrhoea in infants and children in India (Shetty et al., 1990). Keeping in view the wide distribution and huge loss of man-power associated with amoebiasis, effective control measures need to be employed. The indiscriminate mass chemotherapy cannot be the only solution to this
recognized problem of amoebiasis. An effective strategy for control of amoebiasis requires accurate diagnosis of amoebic infection and identification of carriers from active amoebic cases. The treatment of chronic or asymptomatic cases which remain practically undiagnosed is very important, because they serve as foci of dissemination of disease. With a view to develop better strategies for control/prevention of amoebic infection, the WHO expert group of amoebiasis (WHO, 1969) strongly recommended immunodiagnostic methods for both sero-epidemiological studies as well as for quick and reliable diagnosis of clinical cases.

LABORATORY DIAGNOSIS OF AMOEBIASIS

Conventional or morphological diagnostic method

The principle method of diagnosis of amoebiasis is by direct demonstration of *E. histolytica* cysts or trophozoites in the clinical specimens like stool, sigmoidoscopic material or pus. Intestinal amoebic infection is most commonly diagnosed by microscopic examination of fresh stool sample. Various fixatives, stains and concentration techniques are used for the direct identification of amoebae in stool specimen. Some of these methods are - saline/Iodine staining of the direct wet mount or staining with iron-hematoxylin, trichrome, methylene blue, Sargeants solution, chorazol Black E stain, merthiolate, iodine - formalin (MIF), Shaudinn's Fixative, polyvinyl alcohol fixative (PVA), formalin 4% and 10% Zinc-sulfate floatation, formalin ether sedimentation and formalin ethyl-acetate sedimentation (Vinayak *et al.*, 1967; Walsh 1986). Examination of at least three or more stool specimens on separate days is required to detect 90% of infections because of intermittent shedding of cysts (Knight *et al.*, 1974; Juniper, 1978). All the methods for stool examination are tedious, time consuming, expensive and dependent on a high degree of skill of the microscopists. In most of the clinical laboratories, false positive tests usually result from confusion of amoebic trophozoites.
with macrophages that have ingested red cells or misidentification of non-pathogenic protozoa for amoeba. The demonstration of actively motile *E. histolytica* trophozoites in the direct smear with ingested erythrocytes is the sole criterion of confirmation of amoebic dysentery cases. However, trophic or cystic form of *E. histolytica* in stool specimens from cases of amoebic liver abscess (ALA) can rarely be demonstrated (Spencer et al., 1976). Parasite can be demonstrated in the pus from the abscess cavity in not more than 10-15% of ALA cases, (Vinayak and Shandil, 1991).

Because of the time, skill and expenses required for stool/pus examination, continuing efforts have been made to develop simple, less cumbersome and less time consuming techniques. Confirmation of etiological agent from clinical material can also be made by inoculating specimens in defined media, but cultivation procedures are cumbersome and not suitable as diagnostic tool (Vinayak, 1985, 1988). A large number of rich nutrient liquid media, both diphasic and monophasic have been developed by various workers. Culture medium like modified Boeck and Drbohlav medium (Boeck and Drbohlav, 1925) needs to be pre-conditioned by inoculating a bacterial associate so as to generate appropriate redox potential before the stool sample with mature cysts/trophozoites is inoculated (Jacobs, 1950; Harinasuta and Harinasuta, 1955). Most of these media are not suitable for isolation of *E. histolytica* from faecal samples for diagnosis of amoebiasis because they support the growth of undesirable mixed flora, blastocysts and trichomonads (Das and Singh, 1970).

**Immunological diagnosis of amoebiasis**

Because of the inherent limitations of direct demonstration of parasite in clinical specimens, subsequently more stress was laid on the use of immunodiagnostic tests. Immunodiagnosis is based on -
a) detection of anti-amoebic antibodies in the serum, stool eluates (copro-antibodies), saliva and milk of lactating women or demonstration of immunoglobulin bearing plasma cells in the mucosal/rectal biopsies,
b) detection of amoebic antigen(s) in the stool eluates, pus from amoebic liver abscess or detection of amoeba specific immune complexes in the serum of clinical subjects and
c) hypersensitivity reactions to the amoebic proteins (Vinayak and Shandil, 1991).

**Antibody detection based assays employing crude amoebic antigen:** The human host reacts to invasion by the parasites which in turn results in the development of anti-amoebic antibodies. The humoral immune response during a hepatic amoebic infection is more intense as compared to that in invasive intestinal amoebiasis. The detection of antibodies to sonicated extract of amoebae has been utilized for diagnosis of amoebic infections (Vinayak et al., 1974a, 1980b; Vinayak, 1975; Boss and Van den Eijk, 1976; Trissal, 1982; Thomas and Sinniah, 1982; Vinayak, 1989). The advent of axenic cultures of *E. histolytica* and use of axenic antigens markedly improved the sensitivity and specificity of immunodiagnostic tests.

A variety of serological tests developed for serodiagnosis of amoebiasis include, compliments fixation (CF), gel diffusion (GD), precipitin (tube and capillary), immunoelectrophoresis (IEP), counter immunoelectrophoresis (CIEP), cellulose acetate diffusion (CAD), indirect haemagglutination (IHA), latex agglutination (LA), bentonite flocculation (BF), indirect fluorescent antibody (IFAT) tests, enzyme linked immuno sorbent assay (ELISA), Dot-ELISA and western blotting (WB) (Garcia and Ash, 1979; Ackers, 1982; Melvin and Brook, 1982; Walsh, 1986; Vinayak and Shandil, 1991).
ELISA was found to be more specific and sensitive as compared to other immunological tests and hence despite of comparative high cost, is commonly used for serodiagnostic as well as sero-epidemiological studies in amoebiasis. Several workers have evaluated the suitability of ELISA system for routine serodiagnostic purposes in amoebiasis (Yang and Kennedy, 1979; Baveja et al., 1984; Kumar et al., 1985; Gandhi, 1986; Gandhi et al., 1987; Sathar et al., 1988; Torian et al., 1988). An economical and simple version i.e. Dot-ELISA has also been used in such studies (Kumar et al., 1985; Gandhi, 1986; Sharma et al., 1988; Kanwar and Vinayak, 1991). In Dot-ELISA, nitrocellulose paper is used as an immobilizing matrix in place of ELISA plate for coating amoebic antigen and reading can be taken visually, thus obviating the need of sophisticated ELISA reader. Though the DOT ELISA is as specific as plate ELISA, but is less sensitive (Kanwar and Vinayak, 1991). DOT ELISA has been reported to be comparable to IHA test (Mahajan et al., 1989). These authors speculated that as it is simple and easy to perform, the test appears to have great potential for specific diagnosis of amoebiasis in peripheral hospitals and also at field levels where sophisticated immunodiagnostic facilities are not available.

Most of the earlier detection systems were based on documentation of antibody in the sera of amoebic patients, but an inherent problem with the antibody detection based system is that antibodies are produced with variable frequency in non invasive and asymptomatic infection (Patterson et al., 1980; Vinayak et al., 1981; Thomas and Sinniah, 1982; Vinayak, 1989). Approximately 85-95 percent patients of ‘invasive’ amoebiasis have circulating antibodies detectable by either of the serological tests. The development of an intense serological response has often been found to be associated with colonization and invasion of the gut with pathogenic strain of *E. histolytica*, while mere colonisation with non-pathogenic strains of *E. histolytica* leads to development of rather low degree of humoral immune responses (Balamuth and Siddique, 1978; Vinayak, 1989). Even after
successful treatment of disease anti-amoebic IgG antibodies persist in circulation for years together (Juniper et al., 1972; Healy et al., 1974; Thomas and Sinniah, 1982). Moreover, rise or fall in antibody levels as observed in other microbial infections is not characteristic of amoebic infections (Healy et al., 1974). Thus, mere demonstration of anti-amoebic antibodies would indicate exposure to parasite only and are not able to differentiate the present from past, clinical or subclinical infections by the parasite, especially in endemic areas (Juniper et al., 1972; Healy et al., 1974; Vinayak et al., 1980a; Sharma et al., 1981; Thomas and Sinniah, 1982). Though gut invasion by *E. histolytica* is characterized by high anti-amoebic antibody responses, the levels of such antibodies have limited or no correlation with clinical status and severity of the disease (Vinayak et al., 1980; Ackers, 1982; Melvin and Brook, 1982).

Since the anti-amoebic antibodies in general cannot differentiate active ongoing infection from the previous silent/asymptomatic exposure, the specific measurement of immunoglobulin classes was considered to provide a better immunodiagnostic approach. Extensive studies from endemic areas have shown high levels of IgG immunoglobulin in sera of patients of amoebiasis (Cane et al., 1971; Vinayak et al., 1980a). The detection of IgM anti-amoebic antibodies in sera of patients employing IFAT test suggested them as possible reagent to differentiate active ongoing infection from past exposure (Jackson et al., 1984). The levels of IgM antibodies are known to last for six months only. The anti-amoebic IgM ELISA, though is highly specific, lacks required levels of sensitivity (Sathar et al., 1990). Therefore, in-depth investigations of assay systems demonstrating anti-amoebic IgM antibodies and their role in differentiating active ongoing amoebic disease require further evaluation.

The use of IgE antibodies in serodiagnosis of amoebiasis remain elusive due to the prevalence of concomitant helminthic infections in endemic areas which not only
stimulate high amounts of specific and non-specific IgE antibodies, but also potentiate the IgE response to the unrelated antigens (Turner et al., 1979). Immediate type of hypersensitivity occurs with variable frequencies in intestinal amoebic patients and invasive amoebic subjects (Savant et al., 1973; Vinayak et al., 1977).

Given the potential advantage of secretory immune responses as an indicator of infectious diseases affecting mucosal sites, the detection of IgA antibodies provides an alternative to serum antibodies. Hence diagnosis of amoebiasis can be achieved by detecting copro-antibodies or IgA anti-amoebic antibodies in various secretions like milk and saliva. Since *E. histolytica* causes infection by mucosal colonization, high degree of IgA production at local sites is obvious (Vinayak et al., 1980a; Healy, 1986). Copro-anti-amoebic antibodies have been detected in stool eluates by various tests like CIEP and IHA (Shalan and Baker, 1970; Mahajan et al., 1972; Martinez-Cairos et al., 1979). The secretory IgA anti-amoebic antibodies in human milk have rarely been demonstrated (Grundy et al., 1983). Major limitation in detecting IgA anti-amoebic antibodies in milk is due to its utility in lactating women only. However, salivary IgA anti-amoebic antibodies are useful in confirming diagnosis of intestinal amoebiasis (Muro et al., 1990).

**Antibody detection based assay employing defined amoebic antigen(s):** The sensitivity and specificity of antibody based conventional assay can be improved by the use of defined antigens. The possibility of formulating a defined antigen based serodiagnostic tests has been indicated by various investigators by employing serologically reactive and defined antigen(s) of axenic *E. histolytica*. A metabolically labeled cytoplasmic antigen with 28 KD molecular mass (Bhattacharya et al., 1990) and other cytoplasmic antigens are recognized by sera from amoebic patients (Methews et al., 1986). Similarly gel filtered fraction F1 of soluble crude extract of *E. histolytica* has been isolated and characterized for diagnostic purposes as all patients with ALA and dysentery have shown high
reactivity to F1 in ELISA and IHAT (Jain et al., 1990). However, the evaluation of plasma membrane (PM) associated antigens as diagnostic reagents appears to be better than cytosolic antigens in serodiagnosing amoebiasis. It appears that potentially immunogenic antigens of PM may remain masked in whole extracts. The PM associated antigens have higher reactivity and lower non-specificity than cytosolic antigens with serum of patients (Methews et al., 1986). The IgG and IgM-ELISA with PM antigens had relative sensitivities of 95 and 91% respectively in demonstrating anti-PM antibodies in amoebic liver abscess. It has been suggested that the presence of antibodies to crude amoebic extract antigens and absence of anti-PM-IgM antibodies would indicate chronicity of infection with the absence of deep tissue invasion (Sathar et al., 1990). The elicitation of high degree of specific immune response to PM-associated antigens in human host are consistent with earlier observations on experimentally infected or immunized animals (Purnima et al., 1987; Vinayak et al., 1987).

Recognition of three amoebic antigens of 37, 57 and 90 KD molecular masses by the serum of ALA patients in western immuno-blot assays of sonicated extract of amoebic trophozoites has been reported (Joyce and Ravdin, 1988). Another study has indicated that serum samples from patients of amoebiasis recognise six PM-associated proteins with molecular masses of 29, 38, 45, 67, 85 and 95 KD in western immuno-blots (Shandil and Vinayak, 1990). These molecules of the parasite are not recognized by serum of other clinical subjects like irritable bowel syndrome, idiopathic ulcerative colitis, giardiasis and apparently healthy subjects. These investigations, thus, indicated potential use of defined PM antigens in developing refined and specific sero-diagnostic procedures for ongoing amoebic infections (Shandil and Vinayak, 1990). One of the recent studies, has reported 56-65% recognition of 23, 24, 26, 51 and 62 KD amoebic antigens by sera from patients with acute phase intestinal amoebiasis (Ximenez et al., 1992). This is an important step towards the design of more accurate methods for
successful immunodiagnosis and epidemiology of acute intestinal amoebiasis. These authors in a separate study have compared serum antigenic recognition frequencies among various groups of invasive and non-invasive amoebic patients. The results of the study suggested that antigenic fractions 136, 132, 93, 70 and 62 KD were frequently recognized by sera of ALA patients and hence more important for use in serology for distinguishing recent from past invasive events (Ximenez et al., 1993). In addition, a 170 KD surface lectin of *E. histolytica* has consistently been shown to be recognized by immune sera from amoebic patients (Petri et al., 1987). In another investigation, a 170 KD purified amoebic lectin in an ELISA, has been recognized by 95% of amoebic patients who have antibodies to whole amoebic antigens. Approximately 99 percent of ALA patients and all those who have asymptomatic infection with pathogenic zymodeme of amoeba develop antibodies to this lectin, while healthy controls and patients infected with other parasites fail to do so (Ravdin et al., 1990). This 170 KD galactose inhibitable adherence protein (GIAP) from *E. histolytica* has also been used in ELISA for serodiagnosis of amoebic patients in Egypt. Ninety five per cent of the sera from Egyptian subjects with invasive amoebiasis were found to contain antibodies to GIAP of *E. histolytica*. (Abd-Alla et al., 1992). A 96 KD surface antigen has successfully been employed in IHA, ELISA and western immunoblot assays to achieve a high degree of sensitivity and specificity in sero-diagnosis of amoebiasis (Torian et al., 1988). Approximately 68 percent of amoebic patients have been shown to have antibodies to 96 KD surface molecule of *E. histolytica*, while patients infected with amoebae harbouring non-pathogenic zymodemes do not have them. Employing PM- antigens in an ELISA assay, detection of salivary IgA was reported to have a diagnostic accuracy of 91.5% (sensitivity 85% and specificity 98%). A 29 KD surface antigen of *E. histolytica* affinity purified by monoclonal antibodies has been employed to detect antibodies in clinical subjects of amoebiasis in an ELISA. All serum samples from patients of ALA and cyst
passers had anti 29 KD antibodies, though titres in cyst passers were significantly low (Shandil and Vinayak, 1992). Circulating antibodies to histolysin, the major cysteine proteinase of *E. histolytica*, have been detected in 72.7% cases of amoebic liver abscess and 18.17% of cyst passers, via a solid phase enzyme immuno assay (EIA). It suggests that a humoral immune response is induced by histolysin during amoebic liver abscess (Osoria *et al.*, Luacas, 1992). A 70 KD heat shock protein (HSP) of *E. histolytica* has recently been cloned and humoral response against this 70 KD HSP of *E. histolytica* has been studied in a group of patients with invasive amoebiasis. The study revealed detection of phages expressing 70 KD HSP by the IgG of only 25% patients with invasive amoebiasis (Ortner *et al.*, 1992). Recombinant surface antigens of *E. histolytica* have also been used for serodiagnosis of amoebiasis. A 52 KD recombinant serine rich *E. histolytica* protein has been used in western blotting for serodiagnosis of invasive amoebiasis and is highly specific with a high positive predictive value of 92%, though the sensitivity is 82% only (Stanley *et al.*, 1991). A western blot test using 170 KD surface antigen of *E. histolytica* is 90% sensitive and 98% specific (Zhang *et al.*, 1992).

A recombinant surface protein rec Eh P1 which represents part of an immuno dominant 125 KD surface protein of pathogenic *E. histolytica* has been used as antigen for the detection of anti-amoebic serum antibodies by immuno-blotting and ELISA. The ELISA using rec Eh P1 is 100% sensitive and specific for serodiagnosis of invasive amoebiasis (Lotter *et al.*, 1992). It has recently been indicated that antibody response to recombinant 29 KD peripheral membrane protein of pathogenic *E. histolytica* is similar to that of native 29 KD amoebic protein and hence supports the potential utility of a quantitative assay with defined recombinant antigen for serodiagnosis of invasive amoebiasis in non-endemic areas in conjunction with other diagnostic tools (Flores *et al.*, 1993). Thus it appears that serologic assays with defined antigens can provide greater
specificities than currently available assays, and further, the use of recombinant antigens will allow a large scale production of well defined antigens.

**Antigen detection based assays:** Since tests based on the demonstration of anti-amoebic antibodies have limitations in differentiating ongoing amoebic infection from the past exposure, alternate procedures are required to be developed and evaluated. The World Health Organization has recommended the development and evaluation of a diagnostic test based on the detection of parasite product in clinical specimen of amoebic patients. The *E. histolytica* copro-antigen has been detected by ELISA and such a system appears to be a promising one (Palacioso *et al.*, 1978). The usefulness of the detection of the amoebic antigen in stool specimens has been confirmed by many workers (Root *et al.*, 1978; Grundy *et al.*, 1987; Wonsit *et al.*, 1992). An anti-adhesin MoAb based ELISA to detect pathogen specific amoebic antigen in stool specimens with 97% specificity and 100% sensitivity has been reported (Haque *et al.*, 1993). Attempts have also been made to demonstrate the amoebic antigen in rectal biopsy specimen. The amoebic antigen has been demonstrated in 90% of the lysate of biopsied material from patients with amoebic colitis, however, 55% of the patients with ulcerative colitis also have detectable level of amoebic antigen in lysate of biopsied material (Kaur *et al.*, 1982). Promising results have been obtained by detecting intact *E. histolytica* trophozoites in faeces by polyclonal/monoclonal antibodies in ELISA system (Ungar *et al.*, 1985). The ELISA system detected *E. histolytica* trophozoites in the stool of 82% of patients (with demonstrable *E. histolytica* on microscopy). One of the recent studies indicated that electron dense granules (EDG) of *E. histolytica* are shed by parasite and the same can be detected by micro - ELISA system (Munoz *et al.*, 1990) in symptomatic intestinal amoebic infection. An immuno-enzymatic test for stool has been developed by Luaces *et al.* (1992). The demonstration of *E. histolytica* or parasite specific free antigen by immunological procedures in stool appear to be more promising than direct stool
examination. Moreover, single specimen would be sufficient to detect parasite or parasite specific antigen(s) and avoids inconvenience to both patient and diagnostician in examining repeated stool samples. The demonstration of amoebic antigen in the stool indicates undoubtedly the *E. histolytica* infection. However, tests are needed to be developed which may differentiate symptomatic amoebic patients from asymptomatic intestinal infection. Such a differentiation of chronic cases would be possible, provided the nature of *E. histolytica* copro-antigen is known and several monoclonal antibodies to relevant antigenic epitopes of copro-antigen are employed.

The amoebae in pus obtained from abscess cavity cannot be demonstrated in a substantial number of amoebic liver abscess cases. The amoebic antigen in the pus has been demonstrated by employing counter immuno-electrophoresis (Mahajan *et al.*, 1974) and ELISA (Agarwal *et al.*, 1988).

*Intact E. histolytica* trophozoites in liver pus (aspirated from the liver abscess) were identified in 91.3% cases of ALA by IFAT (Yang *et al.*, 1991). A recent study has indicated the presence of 66 KD major surface antigen in liver abscess pus detected by using monoclonal antibody in western immunoblotting (Kaur *et al.*, 1993).

Since *E. histolytica* is associated with invasion and metastasis to various other organs, it usually sheds large quantities of amoebic antigens in circulation. Amoebic antigen - antibody complexes in circulation are frequently found in the serum of ALA patients (Pillai and Mohimen, 1982; Vinayak *et al.*, 1986, 1990; Gandhi *et al.*, 1988; Mohimen *et al.*, 1989). Circulating immune complexes (CICs) have also been documented in many other parasitic infections such as malaria (Soothill and Hendrickse, 1967; Allison *et al.*, 1969; Houba *et al.*, 1970; Ziegler, 1973; Fakunle *et al.*, 1978;), trypanosomiasis (Franklin *et al.*, 1957, Wyler and Oppenbein, 1974; Greenwood and Vick, 1975; Rosenberg, 1978; Lobayakawa *et al.*, 1979), schistosomiasis (Madwar and
Voller, 1975; Phillips and Draper, 1975; Smith et al., 1975; Natalai and Cioli, 1976; Bout et al., 1977; Lawley et al., 1979), onchocerciasis (Ngu and Blackett, 1977; Lambert et al., 1978) and toxoplasmosis (Ginsburg et al., 1974; Shahin et al., 1974) and many studies have indicated their significant role in the pathogenesis of all these parasitic diseases (WHO, 1977). Immune complex activity in 58% ALA patients has been reported in amoebiasis (Onyemelukwe and Onyewotu, 1981). The presence of increased serum circulating immune complexes in patients with amoebic liver abscess and in chronic cyst passers have been employed for the diagnosis of amoebiasis (Nuti et al., 1982). Immune complex glomerulonephritides associated with an amoebic liver abscess which disappeared after treatment of the abscess has also been reported (Margolis et al., 1971; Westendorp et al., 1990). Another study has indicated the presence of amoebic antigen in CIC with a solid phase radioimmunoassay in the serum of 100% of the patients with ALA and colonic amoebic infection (Pillai and Mohimen, 1982). The use of double antibody sandwich ELISA as a diagnostic tool for demonstration of amoebic antigen specific CIC has been designed and evaluated (Vinayak et al., 1986, 1990). An amoebic antigen capture ELISA with a titre of 160 in PEG precipitated serum samples has been considered to be of clinical significance. These ELISA based immuno assays detected CIC in 93-95% of confirmed ALA patients and 55-57% of suspected cases of ALA. The diagnostic potential of amoeba specific CIC has been confirmed by Mohimen et al. (1989). These authors reported the presence of amoebic antigen bound in the immune complex. Attempts have now been made to convert antigen detection based tests into much simpler and economical test procedures like DOT-ELISA (Gandhi et al., 1988). These workers have developed a simple spot test for detecting circulating E. histolytica antigen - antibody complexes in patients suffering from amoebiasis and have found that demonstration of amoebic antigens in blood in the form of circulating immune complex of recently infected individuals may be a reliable method to distinguish the present from
the past infection. Comparison of results of DOT with plate ELISA indicated DOT immuno binding (DIB) assay to be less sensitive than plate ELISA in detecting amoebic antigen in patients of ALA (Baveja et al., 1991).

A recent study has reported the successful detection of *E. histolytica* galactose inhibitible adherence protein (GIAP) heavy subunit antigen in serum and feaces of amoebic patients by ELISA using epitope specific monoclonal antibodies, indicating this antigen to be a highly specific marker for infection by pathogenic *E. histolytica* (Abd-Alla et al., 1993). This GIAP antigen has further been characterized by immuno-blotting PEG-precipitated serum immune complexes with anti-GIAP monoclonal antibodies (Abd-Alla et al., 1993).

Several investigations relating to the detection and characterization of circulating immune complexes in various other diseases have been reported. A 65 KD mycobacterial antigen was identified in CIC from leprosy patients and it was found that the patients with a relatively recent and massive infection were more frequently positive for antigen than the others (Sinha et al., 1992). Another report has indicated the presence of a 46 KD heat stable antigen in the CIC of *Onchocerca volvulus* patients, but no association between the level of parasite specific CIC and clinical disease has been observed (Thambiah et al., 1992). In another study, *Echinococcus granulosus* specific 50 and 60 KD antigens have been detected from the FPLC fraction 25 of the CIC in cystic hydatid patients (Bonifacino et al., 1993). Detection and characterization of immune complex components has also been carried out in patients with gastrointestinal cancer (Bartoloni et al., 1993), tuberculosis (Kansal et al., 1992), versiniosis (Lehesmaa-Rantala et al., 1987) and hydatid disease (D' Amelio et al., 1983; Craig and Nelson 1984; Kanwar and Vinayak, 1992).
It is, thus, quite clear that the demonstration of the parasite product in clinical specimens could provide direct evidence of etiology of an ongoing amoebic infection. Moreover, demonstration of parasite product may be useful in infected individuals where anti-amoebic antibodies are produced at low levels due to immuno-suppression or other underlying factors simultaneously. Another useful feature of demonstration of amoebae specific antigens is that their titres correlate with clinical status of the patients and frequently decline or disappear within 3-6 months after successful treatment of amoebic disease. The later observation may in turn help in monitoring efficacy of chemotherapy and response to amoebicidal drugs (Vinayak et al., 1990). However, immuno assays based on the detection of amoebic antigen needs to be evaluated in the community to assess its potential to identify active invasive amoebiasis cases and in order to develop a simple diagnostic assay for distinguishing a present infection from the past amoebic exposure, the precise nature of the antigen in immune complexes must be looked into.