Amoebiasis, an enteroinvasive disease, is caused by a protozoan parasite, *Entamoeba histolytica*. The *E. histolytica*, ubiquitous in distribution, is one of the most common intestinal parasites of human. Infection with this organism continues to be a serious public health concern in developing countries, affecting 10% of the world's population. The incidence of amoebiasis, varies from place to place depending upon socio-economic norms. In India, 5-58% of the individuals suffering with gastrointestinal infections harbour *E. histolytica* in their gut (Vinayak *et al.*, 1967; Tandon *et al.*, 1971, 1972; Arora *et al.*, 1975; Sharma *et al.*, 1984). *E. histolytica* has also been shown to be a cause of acute diarrhoea in infants and children in India (Shetty *et al.*, 1990). Most amoebic infections remain restricted to colon causing debilitating colitis, but severe invasion may lead to metastasis to other vital organs of the body (Trissal, 1982; Ravdin, 1986, 1989). The most common site of metastasis is the liver leading to development of 'amoebic liver abscess' (ALA). In moderate cases, ALA has been regarded as amenable to medication with low mortality, but in patients with severe abscesses, fatal outcome has occasionally been observed.

Amoebiasis includes a wide spectrum of clinical manifestations and it is difficult to contain the disease. The mass chemotherapy may not be sole and effective measure for containment of amoebiasis. Provision of clean drinking water, improvement in sanitation and enhancing literacy rate have been suggested to be effective measures in the control of amoebiasis (WHO, 1985). Moreover, an effective strategy for control of amoebiasis requires accurate diagnosis of amoebic infection and identification of carriers from active invasive amoebic cases.

The conventional method for laboratory diagnosis of amoebiasis is based on direct demonstration of *E. histolytica* trophozoites or cysts or both in the clinical specimens like stool, sigmoidoscopic material or pus. Repeated examinations of the stool
specimens are required before confirming or ruling out amoebic infection. The diagnostic accuracy can be enhanced significantly by employing staining and concentration methods for the identification and detection of *E. histolytica* cysts in stool samples (Vinayak et al., 1967; Taylor et al., 1980). This method is time consuming and impracticable for field use owing to the requirement of high degree of microscopic skill and need to examine at least three stool specimens on consecutive days.

Alternatively, demonstration of anti-amoebic antibodies in the serum, stool eluates (copro-antibodies), saliva and milk of lactating women has been widely used for diagnosing amoebic infections (Vinayak et al., 1974a; Vinayak, 1975; Boss and Vanden Eijk, 1976; Vinayak et al., 1980a; Thomas and Sinniah, 1982; Trissal, 1982; Vinayak, 1989). The availability of antigen from polyxenic cultures with that from axenic cultures of *E. histolytica* markedly improved the sensitivity and specificity of immunodiagnostic tests. Rationale of preferring immunodiagnostic tests over the conventional methods lies in the ability of the former in providing lead to clinicians especially in situations where direct demonstration of the etiological agent is difficult. Although the anti-amoebic antibodies have been demonstrated in circulation, they have no or limited use in confirming ongoing intestinal amoebic infection. This is because of the facts that (a) not more than 60% of infective individuals have demonstrable level of anti-amoebic antibodies and (b) anti-amoebic IgG antibodies are known to persist for years even after the eradication of the parasite (Juniper et al., 1972; Healy et al., 1974; Thomas and Sinniah, 1982). Although gut invasion of *E. histolytica* is characterized with high degree of anti-amoebic antibody responses, the level of such antibodies has limited or no correlation with clinical status and severity of the disease (Vinayank et al., 1980a; Melvin and Brook, 1982; Vinayak, 1989).
In order to improve the sensitivity and specificity of antibody based conventional assays, several investigators have used defined antigens of axenic *E. histolytica*. These include 170 KD amoebic lectin (Ravdin et al., 1990), 96 KD surface molecule (Torian et al., 1988) and 29 KD surface antigen (Shandil and Vinayak, 1992). Recently recombinant surface antigens of *E. histolytica* have also been used for serodiagnosis of amoebiasis. These include a 52 KD recombinant serine rich protein (Stanley et al., 1991), 170 KD antigen (Zhang et al., 1992), rec Eh P1 (Lotter et al., 1992) and 29 KD peripheral membrane protein (Flores et al., 1993).

Serologic assay with such defined recombinant antigens seems to be potentially more specific, but such assays require further evaluation since tests based on the demonstration of anti-amoebic antibodies have limitations in differentiating ongoing amoebic infection from the past exposure. An expert committee of WHO has recommended the development and evaluation of assay systems to demonstrate the products of parasites in clinical specimen of amoebic patients (WHO, 1984). Several workers have demonstrated the copro-antigen in stool samples of patients with intestinal amoebiasis (Palacios et al., 1978; Root et al., 1978; Grundy et al., 1983; Ungar et al., 1985; Munoz et al., 1990; Luaces et al., 1992; Wonsit et al., 1992; Haque et al. 1993). Even the amoebic antigen or intact trophozoites have been demonstrated in pus using asays like counter immunoelectrophoresis (CIEP), enzyme linked immunosorbent assay (ELISA) or immuno fluorescent antibody test (IFAT) (Mahajan et al., 1974; Agarwal et al. 1988; Yang et al., 1991; Kaur et al., 1993).

Amoebic antigen entrapped in the immune complexes has been reported in the circulation of patients with amoebic liver abscess (Pillai and Mohimen, 1982; Vinayak et al., 1986; Gandhi et al., 1988; Mohimen et al., 1989; Vinayak et al., 1990; Baveja et al., 1991). Detection of such circulating immune complexes in the blood of recently infected
individuals is a reliable method to distinguish the present from the past infection (Gandhi et al., 1988). In order to detect circulating antigen in sera of patients, several immune assays have been tried over the years. These include solid phase radioimmunoassay to detect circulating antigens (Pillai and Mohimen, 1982) and an enzyme linked immunosorbent assay to detect amoebic antigen in polyethylene glycol (PEG) precipitable circulating immune complexes of *E. histolytica* in sera of patients with amoebic liver abscess (Vinayak et al., 1986; Vinayak et al., 1990). Amoebic antigen has been seen in immune complex form by Mohimen et al. (1989). Gandhi et al. (1989) have developed a simple spot test for detecting circulating *E. histolytica* antigen antibody complexes in patients. Dot immunobinding (DIB) assay developed by Baveja et al. (1991) is less sensitive than plate ELISA. Abd-Alla et al. (1993) have reported the detection of *E. histolytica* galactose inhibitable adherence protein in serum and faeces of amoebic patients.

Though, it is evident that demonstration of circulating immune complexes in the serum samples of ALA patients provides a direct evidence of an ongoing amoebic infection, it is felt that in order to develop a simple diagnostic assay for distinguishing a present from a past amoebic exposure, the precise nature of the antigen in immune complexes must be looked into.

It is in this context, that the present study to identify the amoebic antigen in circulating immune complexes of ALA patients and to develop a simple and specific antigen detection based immunoassay using monoclonal antibodies to the most immunodominant amoebic antigen present in circulation has been carried out.