SUMMARY
Summary

In the present study two enzyme immunoassays were developed for diagnosis of amoebiasis. The emphasis was on the development of field based, simple, rapid, highly sensitive and specific assays.

I. 10 Murine hybridoma clones (AC55, AB31, BB12, AC41, PB64, PB65, PB46, PD21, PA12 and PC14) were generated against *E. histolytica* NIH 200. All the MAbs were found to be reactive to axenic strains of *E. histolytica* NIH 200, HM 1 and SAW 1734.

Seven of the MAbs used for further characterisation, AC55, BB12, AB31, PB64, PB65, PD21 and PB46 were specific and did not react with *Giardia lamblia*, *E. coli* and *S. typhi*, the types of pathogens frequently encountered in dysentery. AC41 reacted not only with *E. histolytica* but also with *G. lamblia*. The reaction was half as much in ELISA. This antibody did not react with *E. coli* and *S. typhi*.

Six MAbs (AC55, BB12, PB64, PB65, PB46, PD21) recognized ten clinical isolates of *E. histolytica*. The antigen recognized by these antibodies were ranged between 14-21 kDa in Western blots. These antigenic determinants were carbohydrates as the reactivity was lost by treatment with periodate but not by incubation with proteinase k.

MAb AB31, which was also specific to *E. histolytica* reacted with high molecular weight antigen located in
the range 91-200 kDa. The reactivity of the antibodies with the antigen was lost by treatment with proteinase K and was unaffected by periodate indicating that determinants were protein in nature.

The MAb BB12, again specific for *E. histolytica* had an intense surface immunofluorescence. The antigenic determinants for MAb BB12 were localized on the membrane of *E. histolytica* as evident from indirect immunofluorescence. This antibody could agglutinate live *E. histolytica* trophozoites. Eight of the other MAbs showed cytoplasmic fluorescence and could not agglutinate trophozoites.

Purified AC55 after coupling with horse radish peroxidase was made to compete with rest of the MAbs to bind to *E. histolytica* antigens in competitive ELISA. It was observed that AC55 could compete with MAbs PB64, PB65, PB46 and PD21 but not with BB12, PC14 and AB31.

MAb AC55, carbohydrate reacting antibody with high reactivity in EIA with axenic *E. histolytica* strains NIH 200, HM1, SAW 1734 was selected for a potential sandwich EIA for detection of amoebic antigen. AC55 was used to capture the antigen followed by HRP labelled AC55 for revealing the antigen, which indicates the presence of repetitive epitopes on the antigen. The sensitivity of the sandwich ELISA was found to be 600 ng/ml of protein concentration of the soluble sonicate supernatant of
E. histolytica strain NIH 200 and ten thousand frozen and thawed trophozoites of clinical isolates of E. histolytica and about 100 trophozoites of E. histolytica strain HM 1.

Sandwich ELISA was performed using stool samples from patients suffering from a variety of intestinal syndromes. The results showed all eight samples positive by microscopy examination for E. histolytica cysts were found to be positive in sandwich ELISA. Nine samples out of 71 NAD samples and one from samples positive for parasites other than E. histolytica were found to be positive in sandwich ELISA.

Sandwich ELISA has the potential for use in pathological labs in addition to routine microscopic observation for diagnosis of amoebiasis.

II. Patients suffering from invasive amoebiasis have been shown to develop high levels of anti-E. histolytica antibodies in circulation. It is difficult to demonstrate parasite or antigen in the stool samples. Assays developed for antigen detection in liver pus or in serum are complicated and of low sensitivity. Tests for detection of for E. histolytica antibodies is routinely done in the clinics. Therefore a very simple, rapid and field based modality of dipstick dot-ELISA was developed for detection of antibodies. Important features of the assay include (i) 35 minutes to
complete, (ii) can be done by a para medical worker, (iii) can be done without aid of any sophisticated instrument in a primary health centre, (iv) the end point is appearance of blue coloured dot which can be seen with naked eye and does not require any ELISA reader, (v) the assay can be performed using finger prick blood which can be collected on a Whatman filter paper and stored at room temperature for three months without compromising sensitivity thereby simplifying collection and isolation and storage of the blood samples.

The validation of dipstick dot-ELISA was conducted at four different centres and the cumulative sensitivity and specificity was 97% and 98%. This assay is already commercialized.

III. A follow-up study of ALA patients was conducted to understand the relationship between recovery from clinical illness after successful treatment and the titres of different classes of anti-\textit{E. histolytica} antibodies up to a period of one year.

Following observations were made:
(i) No correlation was found between severity of disease (i.e. liver abscess size) and antibody titers at onset of the disease.

(ii) IgG was found to be major class of anti-\textit{E. histolytica} antibodies followed by IgM and IgA
classes of antibodies.

(iii) All the three classes of anti-\textit{E. histolytica} antibodies titres fall after successful treatment, IgA titres fall to baseline first followed by IgM and IgG,

(iv) The decrease in antibodies titres is accompanied by decrease in abscess size, i.e. there was a good correlation between fall in all classes of antibodies titres and decrease in abscess area.