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## A SIMPLE, RAPID AND RELIABLE SEROLOGICAL METHOD FOR DIAGNOSIS OF AMOEBIASIS

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COUNTERIMMUNOELECTROPHORESIS (CIEP) is a very simple, rapid, reliable, reproducible and economical method and does not require expensive apparatus or imported materials. A CIEP test has been standardized for quick diagnosis of amoebiasis using axenic *Entamoeba histolytica* antigen and specific hyperimmune serum raised in rabbits. The method detects specific *E. histolytica* antigen in serum samples of patients, and allows timely treatment and cure of the disease. Ganguly *et al*[1] have also found the CIEP test to be suitable for the detection of serum antigen and anti-*E. histolytica* antibodies in patients of amoebic liver abscess.

The antigen was prepared from an axenic culture of *E. histolytica* (strain 200:NIH). *E. histolytica* was mass cultured in modified monophasic Diamond's TPS-1 medium[2–4]. The antigen was prepared from the trophozoites by sonication following the procedure of Das *et al*[5]. Hyperimmune serum was obtained from rabbits immunized with antigen and complete Freund's adjuvant. The antibody was titrated by gel diffusion in 1% agar gel[6]. Blood samples of different categories of clinically diagnosed amoebiasis patients were collected from different local hospitals and King George's Medical College, Lucknow. For separation of serum, the samples were centrifuged at 5000 rpm at 4°C for 15 min. These samples were stored at −20°C until use.

CIEP was performed according to the method of Krupp[7] with slight modifications. The ionic strength of veronal buffer was 0.05 M, instead of 0.1 M. The 1% agarose slides were prepared in veronal buffer of ionic strength 0.01 M. The gels were examined after a run of 60–90 min (current 10 ma/slide) or 180 min (current 3–5 ma/slide) and overnight incubation at 4°C. Positive and negative controls were always run simultaneously.

The results for different categories of amoebiasis patients and normal subjects are shown in table 1. The patterns of bands are shown in figure 1. Serological detection of anti-*E. histolytica* antibodies in serum does not distinguish between presence and previous history of amoebiasis. Detection of specific

**Table 1** *Detection of E. histolytica antigen in sera of different amoebiasis patients*

| No. of samples | Clinical diagnosis | Serum antigen by CIEP | |
|---|---|---|---|
| | | Positive | Negative |
| 33 | Amoebic liver abscess | 31 (93.9%) | 2 |
| 8 | Amoebic hepatitis | 7 (87.5%) | 1 |
| 3 | Amoebic cyst passers | Nil (0%) | 3 |
| 13 | Amoebic colitis | 11 (84.6%) | 2 |
| 41 | Normal | 6 (14.6%) | 35 |

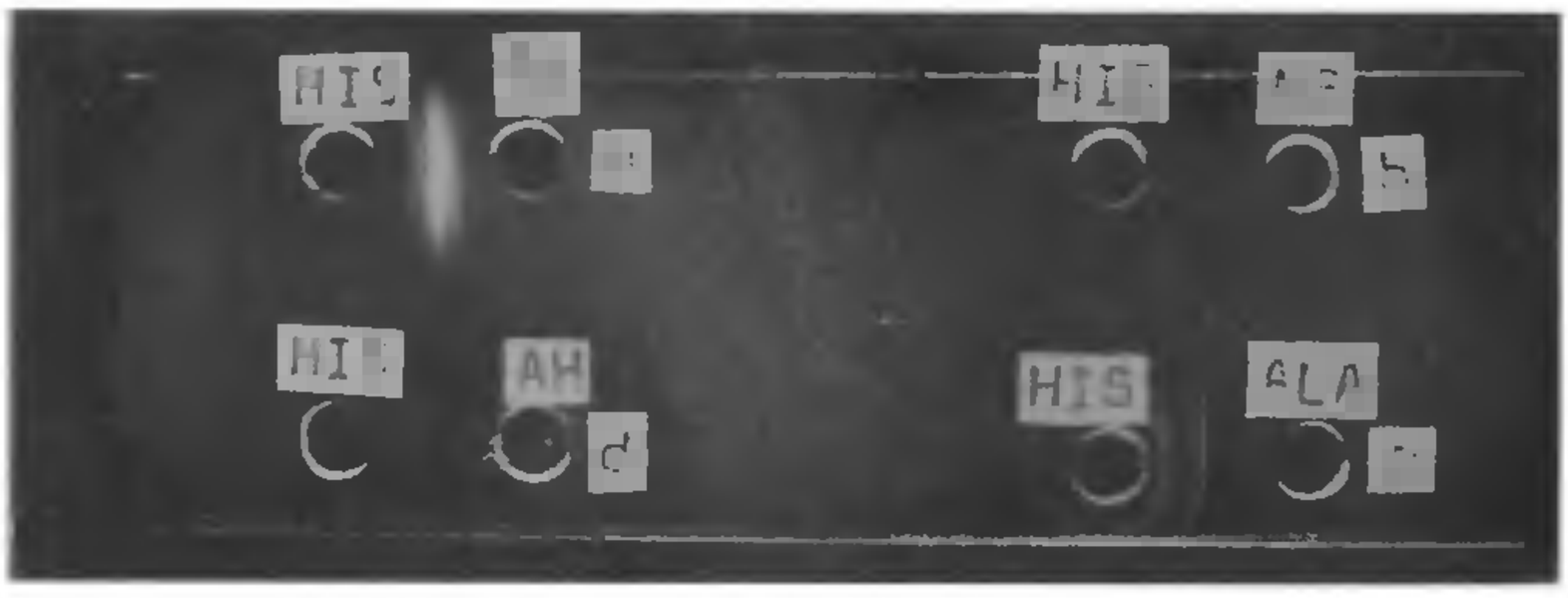


**Figure 1.** Counterimmunoelectrophoresis patterns of different serum samples. **a.** Positive control, **b.** Negative control, **c.** Amoebic liver abscess (ALA), and **d.** Amoebic hepatitis (AH). [HIS, Hyperimmune serum; Ag, Amoebic antigen; NS, Normal serum.]

*E. histolytica* antigen in serum or in faeces (coproantigen) is a positive indication of infection. ELISA and RIA for antigen detection are expensive, time-consuming, and require sophisticated apparatus and imported reagents. CIEP, although slightly less sensitive than ELISA and RIA, can be performed with easily available materials even by paramedical personnel. Ganguly *et al*[1] have reported 26.8% positive cases among amoebic liver abscess patients and no positivity in non-amoebic controls. Our results are different: a much higher positivity (93.9%) was recorded in cases of amoebic liver abscess. The earlier workers have also not detected serum antigen in the other categories of amoebiasis patients, viz. amoebic hepatitis, amoebic cyst passers and amoebic colitis, whereas we have reported 87.5%, 0% and 84.6% positivity respectively for these categories.

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## SIMPLE COLORIMETRIC ESTIMATION OF FUROSEMIDE IN DOSAGE FORMS—PART I

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FUROSEMIDE (4-chloro-*N*-furfuryl-5-sulphamoylanthranilic acid) is a well-known diuretic. It is estimated officially by titrimetry and UV spectrophotometry[1]. Other methods include colorimetry[2–6], potentiometry[7], iodometry[8], gas chromatography[9] and HPLC[10]. None of the reported methods involves complexation. As anthranilic acid is supposed to undergo complexation with metals, it was thought worthwhile to exploit this property for the estimation of Furosemide in marketed formulations. The present communication deals with the application of the formation of a green-coloured complex of copper with Furosemide to estimate the latter in marketed formulations. The complex was found to have absorbance maximum at 742 and 730 nm in methanol and ethyl acetate respectively.

*Chemicals and reagents*

All the chemicals used were of AR and GR grade, of BDH or Sarabhai, unless otherwise specified. Furosemide, received as gift sample from M/s Hoechst India Ltd, Bombay, was used without further purification.

A 1% (w/v) cupric acetate solution was prepared in methanol:water mixture (3:2) and the pH was adjusted to between 3 and 4 with dilute acetic acid for the estimation in methanol. For the estimation in ethyl acetate, an aqueous solution of cupric acetate (1% w/v, pH adjusted to 3–4 with acetic acid) was used. All spectral measurements were made on a Shimadzu UV-150 double-beam spectrophotometer.

*Estimation in methanol*

The standard solution of Furosemide (10 mg/ml) was prepared in methanol. Aliquots (10–50 mg Furosemide) were transferred to 10 ml volumetric flasks, 2 ml of cupric acetate solution were added, and the volume made up with methanol. The absorbances were measured at 742 nm against the reagent blank. The reagent blank was prepared without adding the drug solution. The calibration curve was plotted in the range 1–5 mg/ml of the drug.

*Estimation in ethyl acetate*

The standard solution of Furosemide (10 mg/ml) was prepared in ethyl acetate. As above, aliquots (10–50 mg Furosemide) were transferred to 10 ml volumetric flasks and volume made up with ethyl acetate. The contents were transferred to a separating funnel containing 5 ml of cupric acetate prepared for the purpose, and the funnel was shaken. The layers were allowed to separate and the ethyl acetate layer was collected. Absorbances were measured at 730 nm and the calibration curve was plotted as in the earlier case.