A GOOD CULTURE MEDIUM FOR ENDAMOEBA, BALANTIDIUM AND TRICHOMONAS

By Moses Yu and Teo Hock Kin
(Bacteriology Section, Dept. of Pathology, Singapore, 3)

INTRODUCTION

Since Boeck and Drbohlav (1925) described a method for culturing Endamoeba histolytica, a number of different culture media have been described. We have been using L. S. da Silva (1956) medium till 1962 when the present medium was introduced by Teo. The advantages of this medium are that it is simpler to prepare and that Balantidium and Trichomonas may also grow in it. The medium has been used in this laboratory for the last six years satisfactorily.

The correlation between the culture results and the direct microscopy examination has been more than satisfactory. The number of culture positives are more than twice the number of direct microscopy positives.

With this medium, we still require the presence of bacteria for the amoebae to grow. We have tried adding a particle-free bacterial culture filtrate. This did not support amoebic growth.

MATERIALS AND METHOD

1. *Buffer Saline pH 7.0 (according to Soerensen)*
   KH_2PO_4 (M/15 9.078 gm. to 1 litre distilled water) 185 ml.
   $Na_2HPO_4.2H_2O$ (M/15 11.876 gm. to 1 litre D.W.) 315 ml.
   add KCl — 0.2 gm.
   CaCl — 0.2 gm.
   NaCl — 4.0 gm.

2. *Liver Broth — 12.5%*
   Weigh 25 gms. Bacto Liver Powder
   Add 200mls. plain Buffer Saline 0.8%
   Steam for 2 hours
   Keep in refrigerator (+4°C) overnight
   Filter through 2 layers of lint
   Make up to 200 mls. with Buffer Saline 0.8%
   Autoclave at 15 p.s.i. for 30 minutes

3. *Rice-Charcoal Mixture*

Animal Charcoal	1	gm.
Bacto Rice Powder	14	gms.
Mix dry in hot air oven for	15	mins.
Autoclave at 20 p.s.i. for	30	mins.
Dry again in hot air oven for	10	mins.

PREPARATION OF MEDIUM

1. Buffer saline 0.8% pH 7.0	500	mls.
2. Penicillin, crystalline	100,000	units
3. Polymixin B	25,000	units
4. 12.5% Liver Broth	7.5	mls.
5. Horse serum (Evans)	35.0	mls.

Pass through Seitz filter then overlay 5 mls. of this liquid medium on to a nutrient agar slant.

Faecal Specimens: From the mucous part of the faecal specimen, a pea-size inoculum was taken with a wire loop and inoculated on to the side of the test tube in the liquid phase of the medium. A loopful (20-25 mgm.) of Rice Charcoal mixture was added and the test tube was incubated at 37°C. After an hour the test tube was shaken gently and reincubated for 48 hours at 37°C.

To examine for amoeba growth, the supernatant was gently decanted off and the deposit examined for Endamoeba or other protozoa under direct microscopy.

Pus from Liver Abscess and other Fluids: The same technique was used. It has been found that a loopful of normal faeces added after the inoculation of a pure pus specimen would enhance the growth of the amoebae in the pus specimen. In case of thick pus, it should be first emulsified with some of the liquid medium before inoculating.

INHIBITORY EFFECT OF HORSE SERUM

Some batches of horse sera have an inhibitory effect on the amoebae multiplying. Each batch of horse sera should be tested before use.

STORAGE OF SPECIMENS

Pus or faecal specimens should be inoculated as soon as possible. The vegetative amoebae disintegrate when left standing and unless there are cysts present, a negative culture would result. Specimens from patients who have been on amoebicidal drugs would not give a positive culture, even if in the direct microscopy, cysts were seen.

TABLE I

SHOWING RESULTS OF POSITIVE CULTURES COMPARED WITH DIRECT MICROSCOPY FOR SIX YEARS 1963-1968

Specimens	Total number of specimens	E. histolytica	E. histolytica small race
Faecal M.E.	13,705	709	—
Faecal Culture	13,705	1,289	329
Liver Pus M.E.	398	17	—
Liver Pus Culture	380	18	—
Post Mortem M.E.	25	1	—
Post Mortem Culture	25	1	—
Misc. M.E.	232	—	—
Misc. Culture	232	3	—

TABLE II

Specimens	Entamoeba coli	Endolimax nana	Dientamoeba fragilis	Trichomonas hominis	Balantidium coli
Faecal M.E.	—	—	—	128	9
Faecal Culture	69	132	21	624	11

AMOEBIC ANTIGEN FOR INDIRECT IMMUNOFLUORESCENT ANTIBODIES

The medium yields a good growth and it is suitable for maintaining a culture of amoeba and for the preparation of amoebic antigen. The stock culture should be subcultured every two days and a 48-hour culture would yield 3×10^6 amoebae per ml.

RESULTS AND COMMENTS

The results obtained from the culture of faecal and other specimens in the last six years are shown in the tables above. Out of a total of 13,705 specimens only 709 were found to be positive by direct microscopy whereas 1,289 gave a positive culture. Table II shows that in the course of culturing the 13,705 specimens for endamoebae, the other protozoa were isolated.

We have noticed that in the specimens of pus from liver abscesses less than 1 in 10 yielded a positive culture. There was no doubt that all these abscesses were caused by E. histolytica. We attribute the low positive results to the fact that the amoebae in the middle of an abscess are usually dead and disintegrated. The invading live-amoebae are in the abscess wall.

There are the normal E. histolytica and a small race E. histolytica. We have not been able to isolate the small race of E. histolytica from liver pus specimens. Among the cases of suspected amoebiasis, we have isolated from faecal specimens, 1,618 positive cultures of which 329 were of the small race. It would appear that if the small race E. histolytica were pathogenic, they were less pathogenic than the large race E. histolytica.

REFERENCES

1. Boeck, W.C. and Drbohlav, J. (1925): "The cultivation of E. histolytica." Amer. J. Hyg. vol. 5, pp. 371-407.
2. Hansen, E.L. and Anderson, H.H. (1948): "An essentially synthetic liquid medium for E. histolytica." Parasitology, vol. 39, pp. 69-72.
3. Jacobs, L. (1947): "The elimination of viable bacteria from cultures of E. histolytica and the subsequent maintenance of such cultures." Amer. J. Hyg. vol. 46, pp. 172-176.
4. L.S. da Silva (1956): "M.D. Thesis, Cultural examination for Amoeba in Singapore." pp. 15-16.
5. Swart, D.L. and Warren, L.G. (1962): "The origen of antigenic substances in E. histolytica and serologic manufestation of their antibody including properties." J. Parasitology, vol. 48, pp. 124-130.