APPENDIX

1. Ringer's solution preparation

NaCl	90 g
$CaCl_2$	2 g
KCl	2 g

All salts were dissolved in distilled water and volume of the solution was adjusted to 1000 ml. The solution was then autoclaved and stored at 4°C until use. The solution must be diluted 1:10 with sterile distilled water before use.

2. Phosphate buffer saline (PBS) pH 7.4

a. 0.85% Normal saline solution (NSS)

NaCl	0.85 g
Distilled water	100 ml

b. M/15 KH_2PO_4

KH_2PO_4	9.07 g
Distilled water	1000 ml

c. M/15 Na_2HPO_4

Na_2HPO_4	9.46 g
Distilled water	1000 ml

All solutions, 0.85% NSS (500 ml), M/15 KH_2PO_4 (410 ml) and M/15 Na_2HPO_4 (90 ml) were mixed and pH was adjusted to 7.4. This solution was then autoclaved and stored at 4°C until use.

3. Sterile rice starch

Rice starch (500 mg) was placed in a 16- x 150-mm screw-capped tubes. The tube was then sterilized in hot air oven at 150°C for 3 hours and stored at 4°C until use.

4. Boeck-Drbohlav medium preparation

For the solid-phase preparation, 4 whole eggs were homogenized in 50 ml of Ringer's solution. The ovosuspension was dispensed in 3-ml

amounts in 16- × 150-mm crystal screw-capped tubes, which were inspissated at a 30° slant for 5 min in boiling water. Sterile PBS (pH 7.4) (2.4 ml) was added and stored at 4°C until use.

The liquid phase, which was prepared extemporaneously, was made up of inactivated (56°C, 30 min) calf bovine donor serum (0.5 ml), 200 U/ml penicillin G sodium (50 µl), 200 µg/ml streptomycin sulfate (50 µl). All the ingredients were added directly into PBS of the solid phase medium and mixed thoroughly. For *E. histolytica* cultivation a pinch of sterile rice starch (approximately 25 mg) was added into the liquid phase immediately before use.