Research Note

A simple method to axenize *Blastocystis hominis*

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Blastocystis hominis, a protozoan parasite, continues to attract attention from clinicians and researchers as it can cause diarrhoea in man. Many aspects of the parasite remain an enigma as contamination of *Blastocystis* culture with bacteria, hamper research into molecular, immunology and biochemical aspects of the organism.

Several methods on axenic culture of *B. hominis* have been described (Zierdt & Williams, 1974; Kukoschke & Muller, 1991; Ho *et al.*, 1993; Teow *et al.*, 1992; Lanuza *et al.*, 1997), but these are time consuming and complicated.

There is a need to develop simpler protocols to achieve axenization of *B. hominis* cultures. Such axenic cultures would be useful for the study of parasite pathogenicity. The present paper describes a technique that combines the centrifugation method of Lanuza *et al.* (1997) with the use of antibiotics by the method of Teow *et al.* (1992). This modification was found to be effective in reducing most of the bacteria and ultimately eliminating them from culture.

A *B. hominis* isolate obtained from a faecal sample of a Bangladeshi patient attending the out-patient clinic at the University Hospital, Kuala Lumpur, was subsequently cultured in Jones' medium (Suresh *et al.*, 1997). Cultures were maintained in Bijou bottles and the contents of each bottle were then pooled. The sediment with about 2 x 10^6 parasites was layered onto 8 ml of Lymphoprep solution (Nycomed™) in white-capped long tubes, using sterile pipettes. The tubes were then centrifuged at 500g for 10 minutes.

A distinct layer was formed 0-1 cm from the meniscus (U) while another layer (L) banded just below it. A sterile Pasteur pipette with a sharp pointed tip was used to suck the contents of the two layers into separate centrifuge tubes. The parasite counts for layers U and L were 6.96 x 10^5 and 1.48 x 10^6 per ml respectively. On microscopic examination, layer L was found contaminated with bacteria. This contamination was more obvious after culture in Jones' medium for 3 days. Layer U parasites were only found to have bacteria after day 5 of culture, showing that there was less bacterial contamination than layer L.

Parasites from layer U were again re-layered onto fresh Lymphoprep and centrifuged at 500 g for 10 minutes. Two layers were formed. Parasites from the upper layer were re-suspended in culture tubes with 10 ml PBS, pH 7.2, containing ampicillin (4000 µg/ml) and streptomycin (1000 µg/ml). The tubes were then centrifuged at 500 g for 10 minutes. The supernatant was discarded and the pelleted cells were again re-suspended in 5 ml PBS with the same concentration of antibiotics as above. The washing process was repeated twice. Finally, the pelleted cells were placed in universal bottles and re-suspended in 10 ml Jones' medium supplemented with 10% horse serum.

Parasites from day 2 cultures were sub-cultured into fresh universal bottles containing 10 ml Jones' medium with 10% horse serum. The parasites were maintained in continuous cultures, and sub-cultured into fresh medium every second day.

In the present study, Lymphoprep (Nycomed) was used instead of Ficoll-metrizoic acid because it was cheaper and easier to obtain. Lanuza *et al.* (1997) used 10 ml of Ficoll-metrizoic acid and centrifugation at 500 g for 30 minutes in their protocol, while we used Lymphoprep (8 ml) and centrifugation at 500 g for 10 minutes. Shortening the centrifugation time reduced damage of the fragile parasites, thus increasing the yield of viable parasites that will multiply and outgrow bacteria in the culture. This protocol also differed from that of Teow *et al.* (1992) in that the parasites in the present study were subjected to density gradient centrifugation prior to washing with PBS containing antibiotics. Teow (1991) reported that Percoll and Ficol-Paque could not be used to separate the parasites from bacteria. Lanuza *et al.* (1997) used Ficoll-metrizoic acid and showed that parasites were found in a band that formed at the top of the tube after centrifugation, but were silent on whether the parasites isolated were free from bacteria. In the present study with Lymphoprep, the parasites were banded into distinct upper (U) and lower (L) layers. The parasites from the upper layer had low or microscopically undetectable bacteria. The present protocol exploited this advantage of a large parasite yield with low contamination of bacteria for further re-centrifugation and treatment with antibiotics to eliminate any persisting bacteria.

The key factors in effective axenization appear to be the initial large parasite yield, minimal centrifugation time, and the critical dose of the appropriate antibiotic. This protocol took these into account for axenization and provides a simple method to reduce most of the

bacteria accompanying the parasite.

References

Ho LC, Singh M, Suresh K, Ng GC & Yap EH (1993). Axenic culture of *Blastocystis hominis* in Iscove's modified Dulbecco's medium. *Parasitology Research* 79, 614 – 616.

Kukoschke KE & Muller HE (1991). SDS Page and immunological analysis of different axenic *Blastocystis hominis* strains. *Journal of Medical Microbiology* 35, 35-39.

Lanuza MD, Carbajal JA, Villar J & Borras R (1997). Description of an improved method for *Blastocystis hominis* culture and cultivation. *Parasitology Research* 83, 60-63.

Suresh K, Khairul Anuar A, Saminathan R, Ng KP & Init Ithoi (1997). *In vitro* culture technique: a better diagnostic tool for *Blastocystis hominis*. *International Medical Research Journal* 1, 5-7.

Teow WL, Ho LC, Ng GC, Chan EH, Yap EH, Chan PP, Howe J, Zaman V & Singh M (1992). Viruslike particles in *Blastocystis* specis from the sea-snake, *Lapemis hardwickii*. *International Journal for Parasitology* 22, 1029-1032.

Teow WL, Zaman V, Ng GC, Chan YC, Yap EH, Howe J, Gopalkrishnakone P & Singh M (1991). A *Blastocystis* species from the sea snake, *Lapemis hardwicki* (Serpentes: hydrophiidae). *International Journal for Parasitology* 21, 723-726.

Zierdt CH & Williams RL (1974). *Blastocystis hominis*: axenic cultivation. *Experimental Parasitology* 36, 233-243.

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