

# A simplified culture method for *Blastocystis hominis*

**Key words:** *Blastocystis hominis* culture.

**Palabras clave:** *Blastocystis hominis* cultivo.

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## Abstract

A simplified method of culturing *Blastocystis hominis* is described. Two hundred fresh stool samples were inoculated in tubes containing a modified Pavlova's medium. The tightened tubes were incubated at 36° C without any additional conventional system of anaerobiosis. Cultures were examined after 24, 48 and 72 hours. Overall, 140 (70%) cultures were positive for *B. hominis*, whereas wet mount examination was positive only in 42 (21%) samples. The different forms of parasite described with the standard method were visualized. This method is proposed as an alternative to the conventional one.

**B***lastocystis hominis* is a strict anaerobic protozoan that occurs in the intestine of humans and other mammals (Zierdt, 1991).

Recently, phylogenetic analyses of ribosomal RNAs place *B. hominis* within the stramenopiles (Silberman et al., 1996). Its pathogenic role in human beings is still disputed by some investigators (Markell and Udkow, 1986; Miller, 1988). The mechanism by which it can lead to diarrhea is not understood. Diagnosis of clinical stool samples is generally based on finding of typical forms described in textbooks (Healy, 1991; Pessoa, 1988). However, these references do not mention the different forms of *Blastocystis hominis* such as those

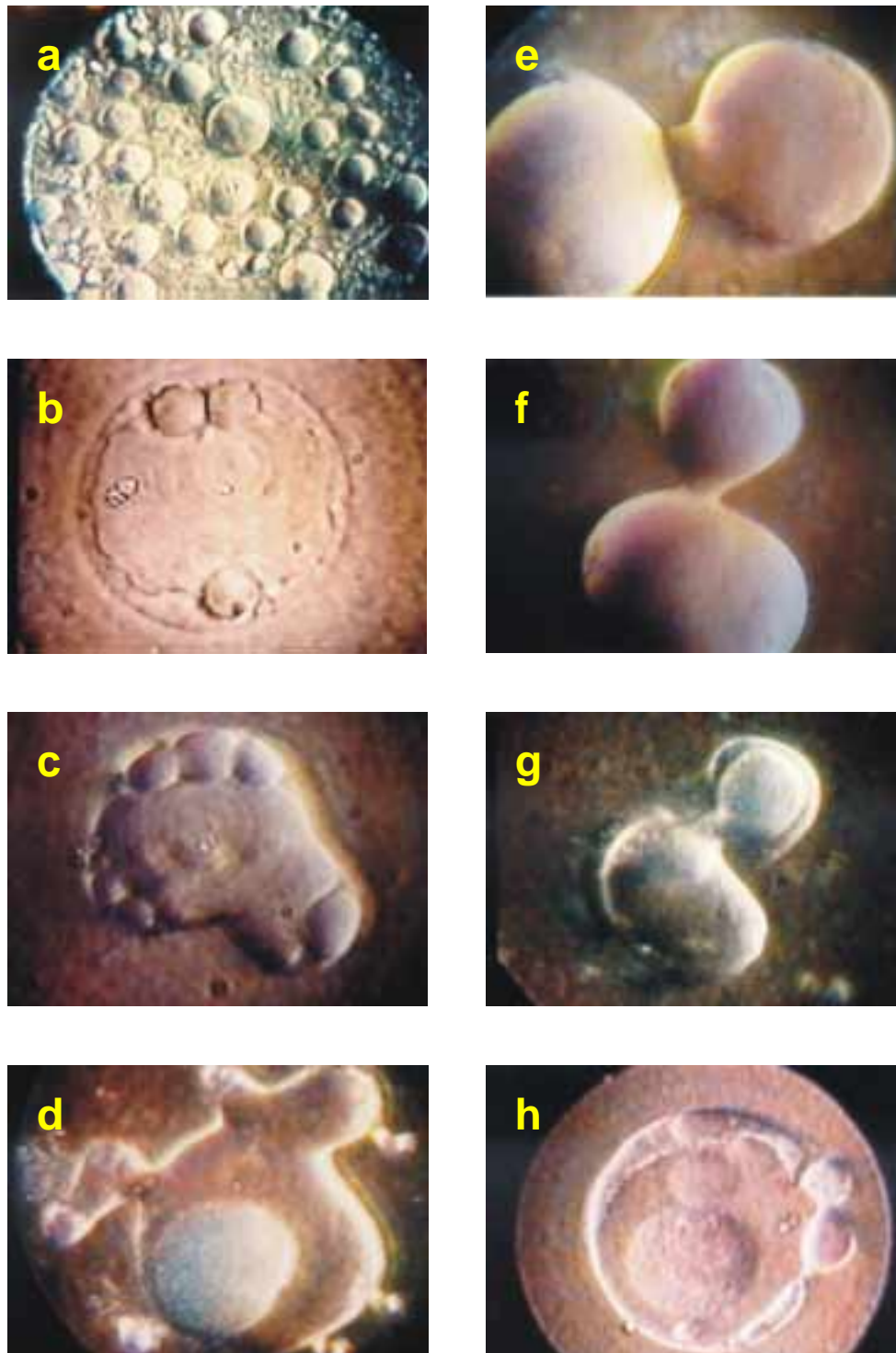
## Resumen

Aquí es descrito un método simplificado de cultivo de *Blastocystis hominis*. Se inocularon doscientas muestras fecales frescas en tubos que contenían un medio de Pavlova modificado. Los tubos herméticos fueron incubados a 36°C sin ningún sistema adicional de anaerobiosis convencional. Los cultivos fueron examinados después de 24, 48 y 72 horas. En conjunto, 140 (70%) de los cultivos fueron positivos para *B. hominis*, mientras que el monto del examen microscópico en fresco fue positivo sólo en 42 (21 %) muestras. Se observaron las diferentes formas del parásito descritas con el método estándar. Este método es propuesto como una alternativa al convencional.

described in a recent comprehensive review (Zierdt, 1991) thus resulting most probably in misdiagnosis of numerous cases.

The medium of choice for *in vitro* culture is a modified whole-egg slant medium with Locke solution overlay that requires addition of 30% horse serum (Zierdt, 1991). Medium in screw-capped tubes needs reduction by means of incubation under anaerobic conditions for 3 days or longer. The caps should be loosened to permit gas exchange and tightened afterwards when removed from the anaerobic atmosphere.

Inoculated tubes with stool samples should also be anaerobically incubated. With this method



**Figure 1.** Different forms of *Blastocystis hominis* obtained from positive cultures are shown. Panel **a**, spheric forms with different size. Panels **b** and **c**, forms with central body. Panel **d**, ameboid forms. Panel **e**, binary fission. Panel **f**, budding forms. Panel **g**, endodyogeny.

cultures become positive quickly and identification after 24 hours is feasible. Stock cultures need to be transferred every 3 to 4 days. A simplified method of culturing *B. hominis* is described here.

A total of 200 fresh stool samples received for parasitological examination were studied. The specimens were inoculated in a slightly modified Pavlova's medium, using human plasma instead of horse serum.

The medium used contains sodium acid phosphate 12 H<sub>2</sub>O, 8.95 g; potassium phosphate, 1.15 g; chloride sodium, 20 g; yeast extract, 4 g; and distilled water, csp 2,750 mL. Sodium hydroxide (1 N) was used to adjust the pH to 7.2-7.4. In addition, 5% human plasma was added.

Then 2.75 g of sterile rice starch was added, potasic G penicillin (Squibb) 1,000 IU/mL and streptomycin 50-100 µg/mL were added after sterilization with a Seitz filter. Then the medium was distributed in sterile glass tubes, 10 mL each (Barral de Martínez, 1993). The amount of inoculum consisted from stool fractions similar to those used for microscopical examination, except that they were first inoculated in the tubes containing the culture medium. The tubes were tightened and incubated without any additional conventional system of anaerobiosis at 36°C. Examinations were performed after 24, 48 and 72 h. Similar inocula were used for initial microscopic examination of wet mount preparations to compare them with culture results. The wet mounts of all specimens were microscopically observed using saline and parasitological lugol solutions.

Of a total of 200 stool samples studied, 140 (70%) were positive for *B. hominis* by the modified culture. By contrast, wet mount examination was positive only in 42 (21%) samples.

The different forms of *B. hominis* obtained from positive cultures are shown in *figure 1* (panels a to h). They are similar to those described with the choice medium (Zierdt, 1991).

*Blastocystis hominis* has been described as a fastidious strict anaerobic organism for culture (Sten-

zel and Boreham, 1996). Studies of this parasite which include culture are rarely found in the literature (Healy, 1991; Pessoa, 1988).

The College of American Pathologists has prompted reporting of *B. hominis* from clinical samples. Clinical diagnosis is facilitated through culture when microscopic examination is uncertain.

An easily available culture method such as the one described here may make feasible the study of several aspects about *B. hominis* in the laboratory, even in those with scarce resources. The different forms of the organism such as those that we show in the *figure 1* can be observed, e.g. central body forms, ameboid forms. Also, the different division modes (binary fission, budding, plasmotomy, and endodyogeny) can be noticed.

This method is cost-effective because it does not require horse serum, reduction during its preparation is not necessary, and it does not need an additional anaerobic system. Stock cultures need to be transferred every 3 to 4 days in order to maintain the organisms viable. We propose the use of this method as an alternative to the standard methods presently known.

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