SHORT COMMUNICATION

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Improved *Blastocystis* spp. detection method using swabs with Amies transport medium and charcoal

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Abstract

Blastocystis is one of the most frequently detected protozoa in the human large intestine. One of the most effective and cheap methods for detecting *Blastocystis* in faeces is culture on a special medium in anaerobic conditions. Sampling faeces using traditional containers and their transport to the laboratory has certain limitations: a sample taken in this way should reach the laboratory relatively quickly, moreover, some patients are uncomfortable during sampling and protection of material in this way. We propose utilizing a swab for sampling and transportation of the faeces samples to be examined for *Blastocystis* instead of using traditional containers. We believe this is an excellent method allowing the material to be transported over longer distances without additional, and sometimes expensive, safety measures, and at the same time permitting the possibility of obtaining living cells after a relatively long period of storage.

Key words
diagnostics, faeces, *Blastocystis*

INTRODUCTION

Blastocystis is one of the most frequently detected protozoa in the human large intestine. Since *Blastocystis* can be detected in healthy individuals as well as in those with gastrointestinal symptoms (such as abdominal pain, diarrhoea or constipation, nausea), the question of *Blastocystis*' pathogenicity remains open. Additionally, *Blastocystis* is being associated with the development of irritable bowel syndrome and cutaneous lesions (urticaria, pruritus) [1–4].

One of the most effective and cheap methods for detecting *Blastocystis* in faeces is culture on a special medium in anaerobic conditions [5, 6]. Taking samples in various places often quite distant from our laboratory we were wondering how to protect this organism, which is sensitive to oxygen and to variable temperature. Sampling faeces using traditional containers and their transport to the laboratory has certain limitations: a sample taken in this way should reach the laboratory relatively quickly, moreover, some patients are uncomfortable during sampling and the protection material in this way.

By trial and error, we have arrived at – in our opinion – an optimal and well-known (in microbiology) solution – that is, swabs with a transport medium. Such a set is sterile and is composed of a tube containing the transport medium with added charcoal, and a rod with a viscose head and a cork at the opposite end (e.g, Deltalab, Cat. No. 300285) (Fig. 1A). Amies medium is modified Stuart medium, the modification is substitution of buffers and charcoal for glycerol phosphate [7].

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This set is easy to use, and the material can be easily collected in exactly the same way as for microbiological purposes. The rod with the swab is immersed in the faeces sample, turning the rod several times, so that particles of faeces adhere to the fibres of the head. The rod is then placed in the tube with black medium and the tube tightly capped. Material taken in this way is protected from drying, from access to oxygen, which is lethal for *Blastocystis*, and can be conveniently and safely transported to the laboratory. The sample protected in this way can then be analysed by the method of *xenic in vitro culture* (XIVC) [8].

To start XIVC we use polyethylene tubes with tight caps (Fig. 1B), they are filled with 2.7 ml home-brew PBS (pH 7.2) with previously added 0.1% of yeast extract (e.g. Sigma, Cat. No. Y1625) and 0.3 ml horse (e.g. Sigma, Cat. No. H1270) or adult bovine (e.g. Sigma, Cat. No. B9433) serum. The swab taken out directly before initiating the culture is immersed in the tube and the swab is cut 2–3 cm from the viscose head with scissors passed through a burner flame (Fig. 1C).

The tightly-capped tube is placed in a thermostat (37 °C) (Fig. 1D). After 48–72 h approx. 20 µl of the sediment is placed on a microscope slide, a drop of Lugol fluid is added, the slide is covered and observed under a standard microscope.

If the result is negative, another passage into fresh medium should be performed in order to make sure that the sample is negative. If the result is positive, passaging to fresh medium can be performed to maintain the culture. In passaging to fresh medium, initially a large inoculum should be used gradually decreasing the volume appropriately depending on the growth. *Blastocystis* will be shaped like spheres 5–50 µm in size (Fig. 1E).

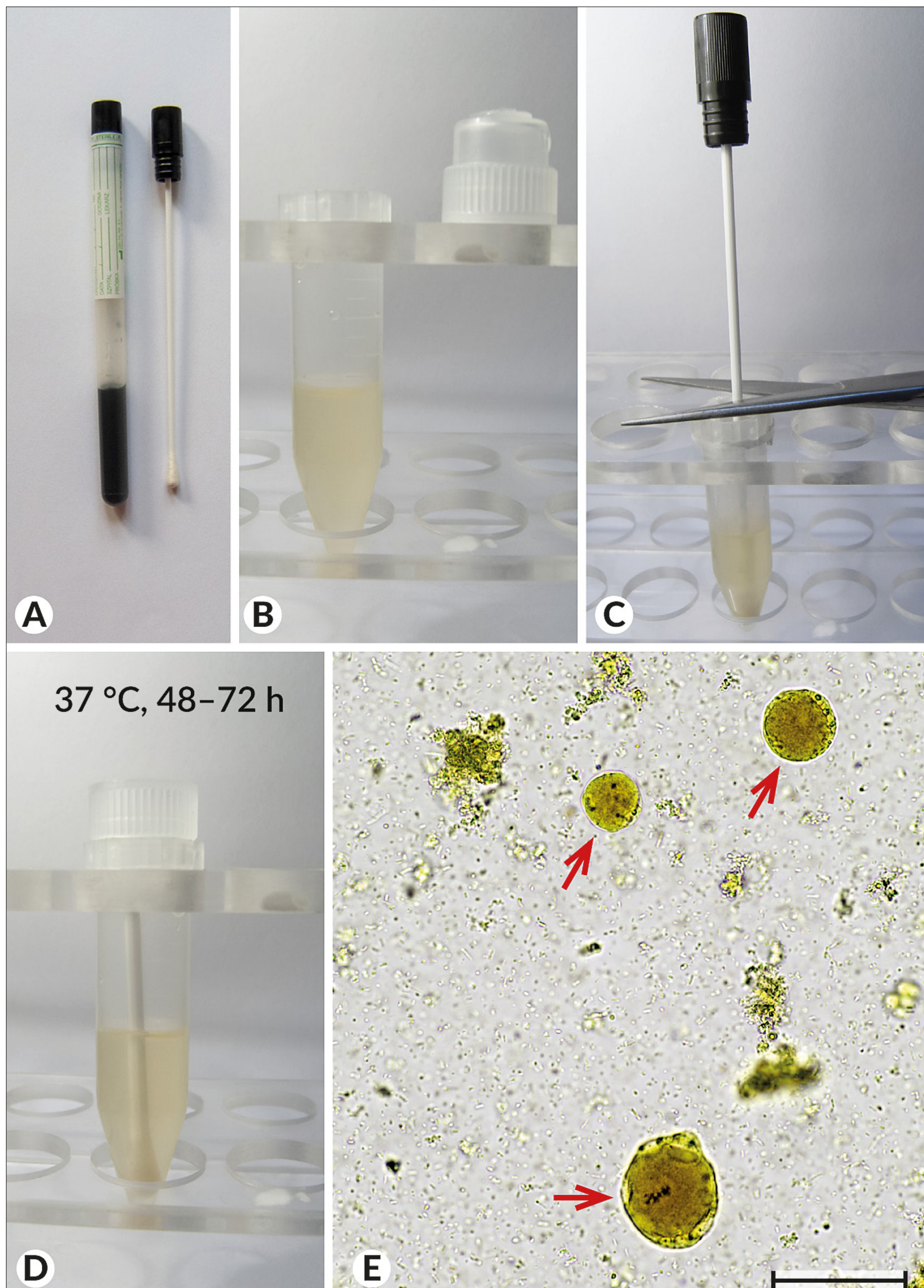

Figure 1. (A) Swab with Amies transport medium and charcoal. (B) Polyethylene tube with tightly-fitted cap, filled with PBS with addition of yeast extract and serum. (C) Cutting a swab with scissors. (D) Tube with the sample in a thermostat. (E) *Blastocystis* (arrows) under a microscope (scale bar: 50 µm).

We were able to detect *Blastocystis* in culture on modified Jones medium even after 7 days from material stored in a refrigerator at a temperature unfavourable for this protozoan (4–6 °C). Moreover, samples taken in this way are suitable for molecular diagnostics. We had no problems isolating *Blastocystis* DNA from swabs and also used it for PCR reactions.

We believe this is an excellent method allowing the transportation of material over longer distances without additional, sometimes expensive, safety measures and at the same time permitting the possibility of obtaining living cells after a relatively long period of storage.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The

authors have no relevant financial interest in the products or companies described in this article.

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