Detection of *Dientamoeba fragilis* and *Blastocystis hominis* using a simple staining method

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Dientamoeba fragilis and *Blastocystis hominis* pose considerable diagnostic challenges for the microbiology laboratory. Despite reports linking these parasites with symptoms,[1,2] relatively few laboratories in the UK regularly detect *D. fragilis* and *B. hominis*. Permanent staining methods[3,4] or culture[5] are essential for the accurate detection of *D. fragilis* and *B. hominis*. However, both parasites can be seen on occasion in direct faecal preparations, and it is important that laboratories are at least able to confirm putative positives with a simple staining method.

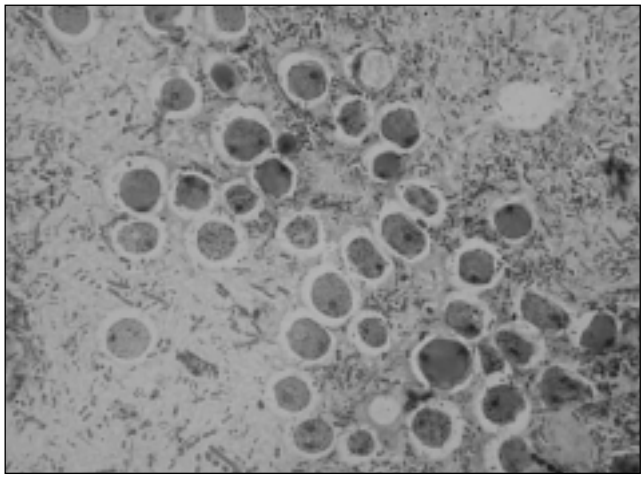

Fig. 1. *Blastocystis hominis* showing characteristic pleomorphic morphology (original magnification x500).

D. fragilis trophozoites can be seen in direct faecal preparations as spherical refractile bodies measuring 5–15μm.[6] The nuclei are not visible in either saline or iodine, making confirmation impossible without staining. *B. hominis* can also be found in unstained preparations, although its morphological diversity can cause diagnostic difficulties for laboratory staff.[7] Both parasites are pleomorphic and thus it can be extremely difficult to distinguish *D. fragilis* and *B. hominis* in unstained faecal smears.

Parasitology staining methods for faecal protozoa vary from the simple (industrial methylated spirit (IMS)-fixed smears stained with Giemsa or Field's stain) to the more complex, which uses a specialised fixative and suitable stain.[1] In North America, sodium acetate/acetic acid/formalin (SAF) is usually used in combination with iron haematoxylin, or polyvinyl-alcohol (PVA) in combination with a trichrome stain.[1] As many of these fixatives and stains are not available in some diagnostic laboratories, this study investigated the use of a commercial rapid differential kit to stain *D. fragilis* and *B. hominis*.

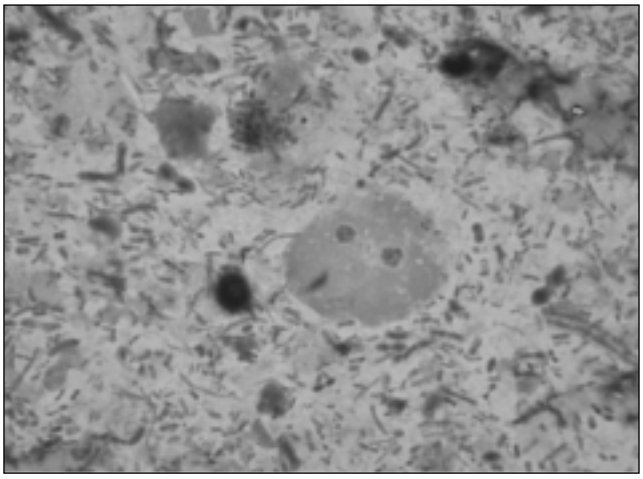

Fig. 2. Bi-nucleated trophozoite of *Dientamoeba fragilis*, showing a fine vacuolated cytoplasm (original magnification x1000).

Smears were made from faecal samples in which round refractile bodies were seen on direct saline microscopy. After air-drying, the smears were stained using the Diff-3 kit (GCC Diagnostics, Sandycroft, Flintshire). The smears were fixed in IMS for 10 sec and then stained in solution 1 (eosin) for 2–3 sec. After a rinse in the wash buffer, the slides were stained in solution 2 (polychrome methylene blue) for 3–4 sec, rinsed with wash buffer and air-dried. Results proved comparable with those obtained with Giemsa or Field's stain (Figs. 1 and 2). In theory, any commercial differential kit could be used, and excellent results have also been obtained using the Hemacolor kit (Merck, Darmstadt, Germany). *D. fragilis* and *B. hominis* were detected using the Diff-3 kit and confirmed using Robinson's xenic parasite culture.[5] This staining method was also used to confirm *Giardia lamblia* trophozoites from a clinical sample (Fig. 3).

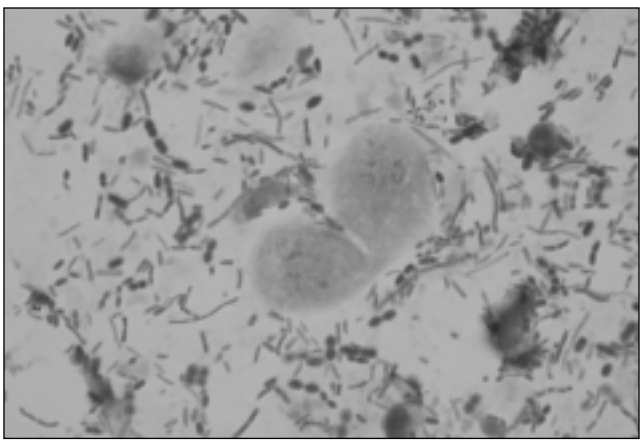

Fig. 3. Trophozoites of *Giardia lamblia* (original magnification x1000).

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D. fragilis and *B. hominis* have been associated with irritable bowel syndrome (IBS)[8,9] and with IBS-like symptoms;[10] therefore, it is important that laboratories are able to identify these parasites with confidence from patients that present with gastrointestinal symptoms.

Rapid differential kits are used routinely in many microbiology laboratories to differentiate white blood cells found in cerebrospinal and other fluids. This study demonstrates that this method can also be used to stain protozoan trophozoites in faecal samples. Thus, the use of a rapid differential stain is recommended to confirm putative *D. fragilis*- and *B. hominis*-positive specimens when unstructured refractile bodies are seen on direct microscopy. □

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