Challenge PA2310-2

September 2023

Stool: *Dientamoeba fragilis, Blastocystis* species

CMPT QA/QC/ Statistics

This sample was verified by two reference laboratories. Laboratories were expected to report the presence of *Dientamoeba fragilis* and *Blastocystis* species

All challenge components are confirmed before shipping by the reference laboratories. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories: both laboratories reported *Dientamoeba fragilis* and *Blastocystis* species

Participants: 10/11 (91%) reporting laboratories reported *D. fragilis*; 11/11 (100%) labs reported *Blastocystis* species; 3 labs also reported *Endolimax nana* (Table 1).

Suitability for Grading

A challenge component is considered suitable for grading if agreement is reached by both (100%) reference laboratories and at least 70 percent of the participants.

Parasite identification was correctly performed by both reference laboratories and greater than 70 percent of all laboratories and was thus, determined to be suitable for grading.

IDENTIFICATION

(This critique will focus on *D. fragils; Blastocystis* species has been discussed in PA2210-3)

Diagnosis of *D. fragilis* infection depends on proper feces sample collection and processing techniques. Immediate fixation of feces is necessary to preserve the morphology of *D. fragilis* as the trophozoites degenerate rapidly in unpreserved stools.[1]

As daily shedding of *D. fragilis* trophozoites is highly variable, multiple samples may be required to maximize the chances of detection.

Additional stool examinations have shown to increase the percentage of positive results by 31.1% for *D. fragilis*. [2]

Identification of trophozoites in wet mounts is difficult as they may be encountered as refractile, rounded forms, varying in size. [1] As the nuclear structure can be challenging to see in saline or iodine preparations, the trophozoites may be dismissed as artifacts. [3]

D. fragilis trophozoite identification requires examination of a permanent stained smear using an oil immersion lens (total magnification of 1,000X) as the stained smear is the only method that ensures maintaining the morphology of the organism.

Grading

Reporting the presence of *D. fragilis* and *Blastocystis* species was graded Acceptable.

Table 1. Results reported

Reported	Labs	Grade
Dientamoeba fragilis, ***Blastocystis* species,** +/- few RBC	8	Acceptable
***Dientamoeba fragilis*, *Blastocystis* species,** *Endolimax nana*	1	Acceptable
***Dientamoeba fragilis*, *Blastocystis* species,** *Endolimax nana*, CLC	1	Acceptable
***Blastocystis* species,** *Endolimax nana*	1	Not Acceptable
No report	1	Not Acceptable
Total	**12**	

Trophozoites measure 5-15 µm (range, 4-30 µm) in diameter and contain 1-2 nuclei. The most common form is binucleated, but approximately 20-30% are uninucleated. [4] The diameter of the nuclei varies from 1 to 3 µm, but depends largely on the size of the trophozoite. Internally, the nuclei appear fragmented, usually containing four to eight granules, without peripheral chromatin. [3]

Permanent stain smears need to be examined carefully because the trophozoites may be pale-staining and can be easily missed.[1]

Other diagnostic methods

Interpretation of stained slides requires experienced personnel to distinguish *D. fragilis* from other protozoa.

Some commercial and laboratory-developed molecular Gastrointestinal (GI) multiplex panels now include *D. fragilis* as target, and can be used in the clinical setting for enhanced sensitivity.

While not routinely performed in the clinical laboratory setting, *D. fragilis* can be detected by culture techniques. Apart from being complex and needing fresh stool samples, culture techniques are highly influenced by the time elapsed from collection and refrigeration, therefore, only fresh unrefrigerated samples should be cultured which is impractical for clinical use. [5]

Stark et. al [5] compared microscopy, parasite culture using different media, and the molecular techniques (NAT and PCR) for the detection of *D. fragilis* in 650 samples.

PCR showed the highest sensitivity (assigned 100%), detecting 35 positive samples, while conventional NAT detected 15 cases (43%), culture detected 14 positive samples (40%), and microscopy detected *D. fragilis* in 12 stools (34%). These techniques are more sensitive than microscopy and labs are beginning to perform in clinical settings. Choice of molecular panel is an important consideration as only select targets are included in each panel; thus, frequently there is still the necessity to have the ability to perform conventional Ova & Parasites examination.

Differential diagnosis

Organisms with one nucleus can easily be confused with *Endolimax nana* or *Entamoeba hartmanni.* [2,3] *E. nana* trophozoites may appear delicate and similar to *D. fragilis*. (add to comments on results)

The nucleus of *E. nana* has a large flat karyosome, however, in some trophozoites, the karyosome may be divided into several parts. [6]

In some trophozoites of *D. fragilis*, the nuclear chromatin tends to mimic that of *E. nana* or *E. hartmanni*, particularly if the organisms are overstained. [6]

CLINICAL RELEVANCE

Intestinal infection with *D. fragilis* can be asymptomatic or cause a wide range of symptoms. [3] Intermittent diarrhea, abdominal pain, nausea, anorexia, malaise, fatigue, and poor weight gain, have been associated with *D. fragilis* infection.

The presence of eosinophilia [11,12] in approximately 50% of patients has prompted experts to recommend that *D. fragilis* be included in the differential diagnosis of chronic diarrhea and eosinophilic colitis. [10]

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