

Guideline

Subject: **Faecal pathogen testing by PCR and the detection of *Dientamoeba fragilis* and *Blastocystis* species**

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Summary:

The role of both *Dientamoeba fragilis* (a trichomonad protozoon) and *Blastocystis* species (a steramantopile closely related to algae) as gastrointestinal pathogens is highly controversial. *Dientamoeba fragilis* in particular has been difficult to identify by microscopy in the laboratory. It is only due to the recent introduction of DNA-based molecular methods (nucleic acid amplification testing including PCR) that we now know that these organisms are much more common than previously thought. Two genotypes of *Dientamoeba fragilis* and 17 genotypes (9 in humans) of *Blastocystis* (possibly separate species) have now been documented¹. Both these organisms are found in faeces of humans as well as in a number of animal species worldwide. Pathogenicity of *Blastocystis spp* and *Dientamoeba fragilis* has not been established in humans.

What has changed?

Since 2013, many laboratories in Australia have introduced a more sensitive and time saving technique (multiplex PCR), which detects *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium spp.*, *Blastocystis spp* and *Dientamoeba fragilis*, to screen for protozoa in faeces with potential to replace detection by microscopy, which is both subjective and time consuming². Following the introduction of PCR, the number of positives for these parasites has increased markedly – up to 20% of all faeces received in the laboratory. The increase has predominantly (approximately 75% of total) been due to *Blastocystis spp* and *Dientamoeba fragilis*. Positive results are predominantly in children³ and in formed or semi-formed faeces and rarely in loose faeces. Symptoms are often falsely attributed to the presence of these organisms leading to overtreatment. This can result in unnecessary anxiety for patients or their families and possible harm due to disruption of normal gut flora if antibiotics are prescribed. Laboratories are also being asked to ‘test for clearance’ in asymptomatic patients after treatment. This is clearly adding to the cost and time of pathology testing without evidence of clinical benefit.

The issues:

1. PCR is very sensitive: validated cut offs (Ct values) have not been established.
2. The various genotypes of *Blastocystis* cannot be differentiated in the diagnostic laboratory; nor can potential pathogenic types be identified. *Dientamoeba* PCR may cross react with other animal trichomonads and the current test cannot differentiate these.
3. The pathogenic potential of both organisms is not known.

4. The presence of these organisms in formed faeces, in asymptomatic individuals or people with vague symptoms (non-diarrhoea) is of unknown significance. Diarrhoea in the presence of these organisms is thought to be self-limiting in immune-competent individuals.
5. Optimal therapy is not established. Variability in response (both in vivo and in vitro) is noted with commonly used anti-parasitic drugs such as metronidazole ^{4,5}.
6. PCR will detect DNA from viable and non-viable forms and therefore has no value in post treatment testing.

Recommendations:

1. When testing for faecal pathogens, consider using a multiplex PCR without *Dientamoeba fragilis* and *Blastocystis* species. If there is a specific request to detect these organisms, microscopy (including stained smears) after discussion with a medical microbiologist, or PCR may be used. Where PCR is used, its diagnostic limitations in this context must be understood.
2. If a smear or PCR is positive, the report should contain a comment highlighting the questionable pathogenicity of these two organisms. Examples of comments suitable to include on pathology reports are as follows:
 - a. *Blastocystis*: The pathogenic role of *Blastocystis spp* has not been established. Most cases do not require treatment and antimicrobial treatment will often not clear the protozoa but may disrupt the normal gut flora. It can be acquired by contact with animals (eg pets) or contaminated water. Animal strains cannot be differentiated by the current available tests. If symptomatic, other causes should be excluded (eg other infections, Irritable Bowel Syndrome, food intolerances etc). Screening for clearance of the organism or testing of family members is not recommended.
 - b. *Dientamoeba*: The pathogenic role of *Dientamoeba fragilis* has not been established. Most cases do not require antimicrobial treatment and this will often not clear the protozoa but may disrupt the normal gut flora. If symptomatic, other causes should be excluded (eg other infections, Irritable Bowel Syndrome, food intolerances etc). Screening for clearance of the organism or testing of family members is not recommended.

References:

1. Stensvold C. Blastocystis: Genetic diversity and molecular methods for diagnosis and epidemiology. 2013. Tropical Parasitology Vol 3 (1); 26-34
2. Stark D et al. Evaluation of the EasyScreen™ Enteric Parasite Detection Kit for the detection of Blastocystis spp., Cryptosporidium spp., Dientamoeba fragilis, Entamoeba complex, and Giardia intestinalis from clinical stool samples. Diagnostic Microbiology and Infectious Disease 2014; 78: 149–152
3. Röser D et al. Dientamoeba fragilis in Denmark: epidemiological experience derived from four years of routine real-time PCR. 2013 Eur J Clin Microbiol Infect Dis (2013) 32:1303–1310. DOI 10.1007/s10096-013-1880-2
4. Röser D et al. Metronidazole Therapy for Treating Dientamoebiasis in Children Is Not Associated With Better Clinical Outcomes: A Randomized, Double-Blinded and Placebo-Controlled. Clin Infect Dis 2014 doi: 10.1093/cid/ciu188
5. Haris Mirza et al. A Rapid, High-Throughput Viability Assay for Blastocystis spp. Reveals Metronidazole Resistance and Extensive Subtype-Dependent Variations in Drug Susceptibilities. 2011. Antimicrobial Agents And Chemotherapy. Vol 55; p. 637–648