Clinical evaluation of the RIDA®GENE Dientamoeba fragilis real-time PCR assay in human stool samples

A. Fruth[†], *L. Kastl*[‡], *E. Glocker*[†]
[†] *Department of Medical Microbiology and Hygiene, University Hospital Freiburg*, Freiburg, Germany
[‡] *R-Biopharm AG*, Darmstadt, Germany

Objectives

Dientamoeba fragilis is considered to be an important diarrhea-causing protozoa and is distributed worldwide. Due to different reports on the human pathogenic potential of this protozoan, the clinical diagnosis of Dientamoeba fragilis is diverse, dependent on the respective countries. Hence, the reported prevalence of Dientamoeba fragilis varies from 0.3 % to 52 %.[1] Infection with Dientamoeba fragilis may be either asymptomatic or symptomatic. Symptoms of dientamoebiasis are abdominal pain and diarrhea.[2]
Classically, diagnosis of Dientamoeba fragilis is achieved by microscopic examination of faecal samples, which requires experienced staff. Parasitic detection by real-time PCR analysis has become an alternative for the detection of major protozoans including Dientamoeba fragilis. This study aimed to evaluate the clinical performance of the RIDA®GENE Dientamoeba fragilis real-time PCR assay for the detection of Dientamoeba fragilis in human stool samples.

Picture 1: RIDA®GENE Dientamoeba fragilis Art. No. PG1745

Methods

The RIDA®GENE Dientamoeba fragilis real-time PCR assay qualitatively detects Dientamoeba fragilis in human stool samples by targeting the 18s-ITS region with fluorogenic target-specific hydrolysis probes. An included Internal Control DNA (ICD) detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient and hence ensures reliable results.

Overall, DNA from 200 human stool samples was manually extracted with the RIDA® Xtract Kit (R-Biopharm AG). Subsequently, extracted DNA was analysed on the LightCycler® 2.0 (Roche) using the RIDA®GENE Dientamoeba fragilis real-time PCR assay (Figure 1). Results were compared to a routine in-house real-time PCR assay which targets the SSU rRNA gene (Figure 1).

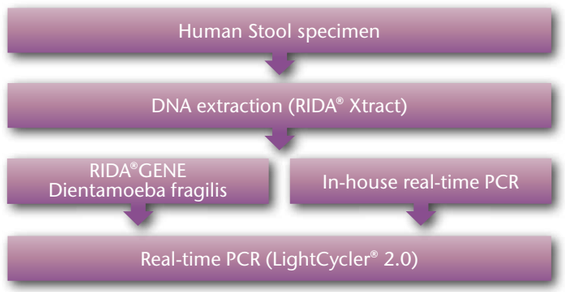

Figure 1: Study design

Results

Of the overall 200 stool samples tested, 46 samples (23 %) were positive for Dientamoeba fragilis by the in-house real-time PCR method (Table 1). All 46 positive samples were correctly identified by the RIDA®GENE Dientamoeba fragilis real-time PCR assay (Table 1). 154 stool samples (77 %) were negative for Dientamoeba fragilis by the RIDA®GENE Dientamoeba fragilis real-time PCR assay which concurred with the results from the in-house real-time PCR method (Table 1). Compared to the in-house real-time PCR, positive, negative and overall agreement was 100 %, respectively (Table 2).

Table 1: In-house real-time PCR vs. RIDA®GENE Dientamoeba fragilis real-time PCR

		In-house real-time PCR +	In-house real-time PCR -	Total
RIDA®GENE Dientamoeba fragilis	+	46	0	46
	-	0	154	154
	Total	46	154	200

Table 2: Clinical performance of the RIDA®GENE Dientamoeba fragilis real-time PCR assay

Positive agreement: 100 %
Negative agreement: 100 %
Overall agreement: 100 %

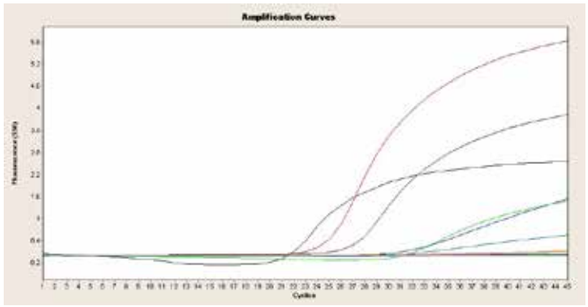

Figure 2: Example of Dientamoeba fragilis run on the LightCycler® LC2.0

[1] *Baratt JLN et al.* A review of Dientamoeba fragilis carriage in humans: several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes. 2011, 2(1):3-12.
[2] *Stark D et al.* A review of the clinical presentation of dientamoebiasis. Am J Trop Med Hyg. 2010, 82(4):614-619.

Conclusion

The RIDA®GENE Dientamoeba fragilis real-time PCR assay shows good correlation with an established in-house real-time PCR method. Results are available in less than 2 hours. An included Internal Control DNA (ICD) detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient and hence ensures reliable results. The RIDA®GENE Dientamoeba fragilis assay proved to be a sensitive and specific real-time PCR assay for the diagnosis of Dientamoeba fragilis infections.

03/2015