R-Biopharm AG

Evaluation of a novel RIDA®GENE Parasitic Stool Panel assay for the detection of *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Dientamoeba fragilis* in stool specimen

D. Martens[1], *H. von Thien*[2], *E. Tannich*[2]
[1] R-Biopharm AG, Darmstadt, Germany
[2] Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany

Objectives

Diarrhoeal disease is a major health care problem and is the 2nd most common cause of child deaths among children under 5 years globally, particularly in developing countries. More than 5000 children are dying every day as a result of diarrheal disease.[1,2] *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Dientamoeba fragilis* are the most important diarrhoea-causing protozoa. Traditionally intestinal parasites are identified by microscopy but it is time consuming, labor intensive, requires multiple samples as well as species-specific concentration and staining methods to improve performance.
We evaluated the RIDA®GENE Parasitic Stool Panel multiplex real-time PCR assay for the qualitative, direct detection and differentiation of *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Dientamoeba fragilis* in human stool specimens.

Picture 1: RIDA®GENE Parasitic Stool Panel multiplex real-time PCR

Methods

The RIDA®GENE Parasitic Stool Panel is a qualitative multiplex real-time PCR assay targeting the 18s-ITS for *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica* with fluorogenic target-specific hydrolysis probes and *Dientamoeba fragilis* with an intercalating dye. An Internal Control DNA (ICD), which can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as a PCR inhibition control ensures reliable results. RIDA®GENE Parasitic Stool Panel can be run on commonly used real-time PCR instruments such as LightCycler®480II, SmartCycler®, ABI 7500, Mx3005P or Rotor-Gene Q.

Fig. 1: Analyte target gene and detection channel overview

Analyte	Target gene	Detection Channel Rotor-Gene Q
Giardia lamblia	18s-ITS	Green
Cryptosporidium parvum	18s-ITS	Red
Entamoeba histolytica	18s-ITS	Orange
Dientameoba fragilis	18s-ITS	Melting Curve Yellow
ICD	synthetic target DNA sequence	Yellow

Evaluation of the assay was performed on 218 DNA samples previously characterized by microscopy and in-house PCR at the Bernhard-Nocht-Institute (BNI). 40 samples were positive for *Giardia lamblia*, 28 were positive for *Cryptosporidium parvum*, 29 were positive for *Entamoeba histolytica* and 38 were positive for *Dientamoeba fragilis*. 32 DNA samples of the sample pool were also positive for Entamoeba dispar by BNI in-house PCR. Multiplex real-time PCR was carried out on the Rotor-Gene Q (Qiagen).

Results

Using the BNI in-house PCR as gold standard the sensitivity, specificity, PPV, NPV of the RIDA®GENE Parasitic Stool Panel were 95 %, 100 %, 100 % and 98.9 % for *Giardia lamblia*, 96.4 %, 99.5 %, 96.4 % and 99.5 % for *Cryptosporidium parvum*, 100 %, 100 %, 100 % and 100 % for *Entamoeba histolytica* and 97.4 %, 100 %, 100 % and 98 % for *Dientamoeba fragilis*.

Compared to RIDA®GENE Parasitic Stool Panel the sensitivity, specificity, PPV, NPV of microscopy were 65.8 %, 99.5 %, 96.2 % and 93.2 % for *Giardia lamblia*, 60.7 %, 100 %, 100 % and 94.5 % for *Cryptosporidium parvum*, 62.1 %, 75.1 %, 27.7 % and 92.8 % for *Entamoeba histolytica* and 45.9 %, 99.4 %, 94.4 % and 90 % for *Dientamoeba fragilis*.

Fig. 2: RIDA®GENE Parasitic Stool Panel vs. BNI in-house PCR for *Giardia lamblia*

		BNI PCR +	BNI PCR -	Total
RIDA®GENE Parasitic Stool Panel	+	38	0	38
	-	2	178	180
	Total	40	178	218

Fig. 3: RIDA®GENE Parasitic Stool Panel vs. BNI in-house PCR for *Cryptosporidium parvum*

		BNI PCR +	BNI PCR -	Total
RIDA®GENE Parasitic Stool Panel	+	27	1	28
	-	1	189	190
	Total	28	190	218

Fig. 4: RIDA®GENE Parasitic Stool Panel vs. BNI in-house PCR for *Entamoeba histolytica*

		BNI PCR +	BNI PCR -	Total
RIDA®GENE Parasitic Stool Panel	+	29	0	29
	-	0	189	189
	Total	29	189	218

Fig. 5: RIDA®GENE Parasitic Stool Panel vs. BNI in-house PCR for *Dientamoeba fragilis*

		BNI PCR +	BNI PCR -	Total
RIDA®GENE Parasitic Stool Panel	+	37	0	37
	-	1	57	58
	Total	38	57	95

Fig. 6: Microscopy vs. RIDA®GENE Parasitic Stool Panel for *Giardia lamblia*

		Microscopy +	Microscopy -	Total
RIDA®GENE Parasitic Stool Panel	+	25	13	38
	-	1	179	180
	Total	26	192	218

Fig. 7: Microscopy vs. RIDA®GENE Parasitic Stool Panel for *Cryptosporidium parvum*

		Microscopy +	Microscopy -	Total
RIDA®GENE Parasitic Stool Panel	+	17	11	28
	-	0	190	190
	Total	17	201	218

Fig. 8: Microscopy vs. RIDA®GENE Parasitic Stool Panel for *Entamoeba histolytica*

		Microscopy +	Microscopy -	Total
RIDA®GENE Parasitic Stool Panel	+	18	11	29
	-	47	142	189
	Total	65	153	218

Fig. 9: Microscopy vs. RIDA®GENE Parasitic Stool Panel for *Dientamoeba fragilis*

		Microscopy +	Microscopy -	Total
RIDA®GENE Parasitic Stool Panel	+	17	20	37
	-	1	180	181
	Total	18	200	218

The analytical sensitivity was determined with ≤ 5 DNA copies per reaction. No cross-reaction was found with a wide range of common pathogenic organisms of the intestinal tract.

References:
[1] World Gastroenterology Organisation Global Guidelines: Acute diarrhea in adults and children: a global perspective.
[2] UNICEF/WHO, Diarrhoea: Why children are still dying and what can be done, 2009.

Conclusion

The RIDA®GENE Parasitic Stool Panel is a rapid, high sensitive and specific multiplex real-time PCR assay for the diagnosis of *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica* and *Dientamoeba fragilis*.

Results are available in less than 2 hours.

The multiplex real-time PCR assay is a diagnostic alternative in the clinical laboratory for the detection of intestinal parasites.

R-Biopharm AG
An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Phone: +49 (0) 61 51 - 81 02-0, Fax: +49 (0) 61 51 - 81 02-40
www.r-biopharm.com, E-mail: info@r-biopharm.de