RIDA®GENE Parasitic Stool Panel I

REF PG1715

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 02-20

1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Parasitic Stool Panel I is a multiplex real-time PCR assay for the direct, qualitative detection and differentiation of *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica* and *Dientamoeba fragilis* in human stool samples.

RIDA®GENE Parasitic Stool Panel I multiplex real-time PCR is intended for use as an aid in diagnosis of gastrointestinal infection caused by parasites.

2. Summary and explanation of the test

Giardia lamblia, *Cryptosporidium* spp. and *Entamoeba histolytica* and *Dientamoeba fragilis* are the most important diarrhea-causing protozoa.
Giardia lamblia (synonym *G. intestinalis* or *G. duodenales*) is one of the most important causes of diarrhea. According to the CDC (Center for Disease Control) about 2 % of all adults and 6 - 8 % of all children in developed countries and about a third of all people in developing countries are infected with giardiasis.[1] The CDC estimates about 77,000 cases of giardiasis each year in the U.S..[2] Infection with *G. lamblia* occurs by ingestion of cysts in contaminated food, drinking water or by faecal-oral route from person to person. The incubation time is between 1 to 3 weeks. The symptoms of giardiasis (lambliasis) are acute or chronic diarrhea, but asymptomatic cyst elimination is also occurring. Acute symptoms include watery diarrhea, loss of appetite, nausea, abdominal cramps and weight loss.[1]
Cryptosporidium parvum is one of several species of the genus *Cryptosporidium*. Besides *C. parvum*, also *C. hominis* most commonly causes cryptosporidiosis in humans.[4] However, also infections by other *Cryptosporidium* spp. such as *C. felis, C. meleagridis, C. canis,* and *C. muris may lead to clinical symptoms.*[3] *Cryptosporidiosis* was detected in up to 0.2 % of healthy individuals and about 2 % of patients with diarrhea in developed countries. In developing countries the prevalence elevated up to 9 %. In HIV patients with diarrhea *Cryptosporidium* spp. was detect up to 14 - 24 % while up to 5 % of asymptomatic HIV patients were infected.[5,6] During an outbreak in Milwaukee, USA in 1993 over 400,000 individuals were sickened.[4] Each year estimated 748,000 cases of cryptosporidiosis occur in the United States.[7] The infection occurs after the ingestion of oocysts in contaminated water and food, as well as by faecal-oral route from person to person. In immunocompetent persons the disease manifests itself after 2 to 10 days as a self-limiting watery diarrhea and may be accompanied by nausea, abdominal pain and weight loss. Immunocompromised individuals often develop serious, chronic, and sometimes fatal illness.[2,4]
Entamoeba histolytica is the only human pathogenic species of the genus *Entamoeba* and the causative agent of amoebiasis. Infection with *E. histolytica* occurs by ingestion of cysts in contaminated food, drinking water or by faecal-oral route from person to person. Most of the *E. histolytica* infections manifest as asymptomatic colonization. In 10 % of the cases the infection leads to amoebic colitis and on rare occasions to extraintestinal amoebiasis, mostly to the liver (amebic liver

abscess). Clinical symptoms associated with intestinal amoebiasis are stomach pain and severe diarrhea with bloody and slimy stools. The WHO estimates that about 50 million people worldwide suffer from amoebiasis each year, resulting in 100,000 deaths each year.[2,8]
Dientamoeba fragilis has a worldwide distribution and recent studies demonstrated the pathogenic potential and implicated it as a common cause of gastrointestinal disease. Infection with *D. fragilis* may be either symptomatic or asymptomatic. Symptoms of dientamoebiasis are abdominal pain and diarrhea. The prevalence of *D. fragilis* varies from 0.3 % to 52 % and often exceeds that of *Giardia lamblia*.[9,10]
Classically, diagnosis of *G. lamblia*, *Cryptosporidium* spp., *Entamoeba* spp. and *Dientamoeba fragilis* is achieved by microscopical examination of fecal samples, which require experienced personal. RIDA®GENE Parasitic Stool Panel I multiplex real-time PCR assay is a new and attractive alternative method for testing stool samples and has proven to be highly sensitive and specific for the simultaneous detection of the three most important diarrhea causing parasites (*Giarida lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica* and *Dientamoeba fragilis*).

3. Test principle

RIDA®GENE Parasitic Stool Panel I is a multiplex real-time PCR for the direct, qualitative detection and differentiation of *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica* and *Dientamoeba fragilis* in human stool samples.
After DNA isolation, amplification of gene fragments (ITS1-18S, if present) specific for *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica* and *Dientamoeba fragilis* occurs. The amplified targets for *Giardia lamblia*, *Cryptosporidium* spp., *Entamoeba histolytica* and *Dientamoeba fragilis* are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-Polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Parasitic Stool Panel I assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and to determine possible PCR inhibition.

4. Reagents provided

Tab. 1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µl	yellow
2	Taq-Polymerase	1x	80 µl	red
D	Internal Control DNA	2x	1700 µl	orange
N	No Template Control	1x	450 µl	white
P	Positive Control	1x	200 µl	blue

5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw and fully defrost reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 15 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all reagents should be stored cold in an appropriate way (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Parasitic Stool Panel I real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2: Necessary equipment

Extraction platform	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
Real-time PCR instrument:	
Roche	LightCycler® 480II
Agilent Technologies	Mx3005P

If you want to use other extraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (nuclease-free)

7. Precautions for users

For *in vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.
For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

8. Collection and storage of samples

8.1 Sample preparation from stool samples

For DNA isolation of human stool samples, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.

We recommend to dilute the stool samples before extraction 1:3 with water. Vortex the diluted stool sample intensely and centrifuge at 1000 x g for 30 sec. Use from the supernatant the appropriate volume according to the manufacturer's instruction.

The RIDA®GENE Parasitic stool panel I assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient.

If the Internal Control DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab. 4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA should always be added to the specimen-lysis buffer mixture and **not** directly to the specimen. We also recommend to add 1 µl of the Internal Control DNA to the negative control and positive control PCR Mix.

9. Test procedure

9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and negative control must be included in each assay run.

We recommend to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab. 3, Tab. 4). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the No Template Control and the Internal Control DNA before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 3: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD as extraction and PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

Mix the components of the Master-Mix gently and briefly spin down.

Tab. 4: Calculation and pipetting example for 10 reactions of the Master-Mix (ICD only as PCR inhibition control)

Kit code	Master-Mix components	Volume per reaction	10 reactions (10 % extra)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
D	Internal Control DNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the components of the Master-Mix gently and briefly spin down.

9.2 Preparation of the PCR-Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

Negative control: Add 5 µl No Template Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the negative control.

Sample: Add 5 µl eluate to the pre-pipetted Master-Mix.

Positive control: Add 5 µl Positive Control to the pre-pipetted Master-Mix.

Note: If the Internal Control DNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control DNA to the PCR-Mix of the positive control.

Cover tubes or plate. Spin down shortly and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument set-up (see Tab. 5, Tab. 6, Tab. 7, Tab. 8).

9.3 PCR Instrument set-up

9.3.1 DNA real-time PCR profile

Tab. 5: DNA real-time PCR profile for LightCycler® 480II

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	10 sec, 95 °C
Annealing/Extension	15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 6: DNA real-time PCR profile for Mx3005P

Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.3.2 Universal real-time PCR profile

Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RNA real-time PCR assays in one run.

Tab. 7: Universal real-time PCR profile for LightCycler® 480II

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

Tab. 8: Universal real-time PCR profile for Mx3005P

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step.

9.4 Detection channel set-up

Tab. 9: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Note
Roche LightCycler® 480II	*Dientamoeba fragilis*	440/488	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	Giardia lamblia	465/510	
	ICD	533/580	
	Entamoeba histolytica	533/610	
	Cryptosporidium spp.	618/660	
Agilent Techn. Mx3005P	*Dientamoeba fragilis*	ATTO	**Check that the reference dye is none The "Filter Set Gain Settings" for ATTO have to be set to 8**
	Giardia lamblia	FAM	
	ICD	HEX	
	Entamoeba histolytica	ROX	
	Cryptosporidium spp.	Cy5	

10. Quality control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer's instructions. Positive control and negative control have to show correct results (see Table 10, Fig. 1, Fig. 2, Fig. 3, Fig. 4) in order to determine a valid run.
The Positive Control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies, respectively.

Tab. 10: For a valid run, the following conditions must be met:

Sample	Assay result	ICD Ct	Target Ct
Positive control	Positive	NA *[1]	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	0

*[1]*No Ct value is required for the ICD to make a positive call for the positive control.*

If the positive control is not positive within the specified Ct range but the negative control is valid, prepare all new reactions including the controls.

If the negative control is not negative but the positive control is valid, prepare all new reactions including the controls.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

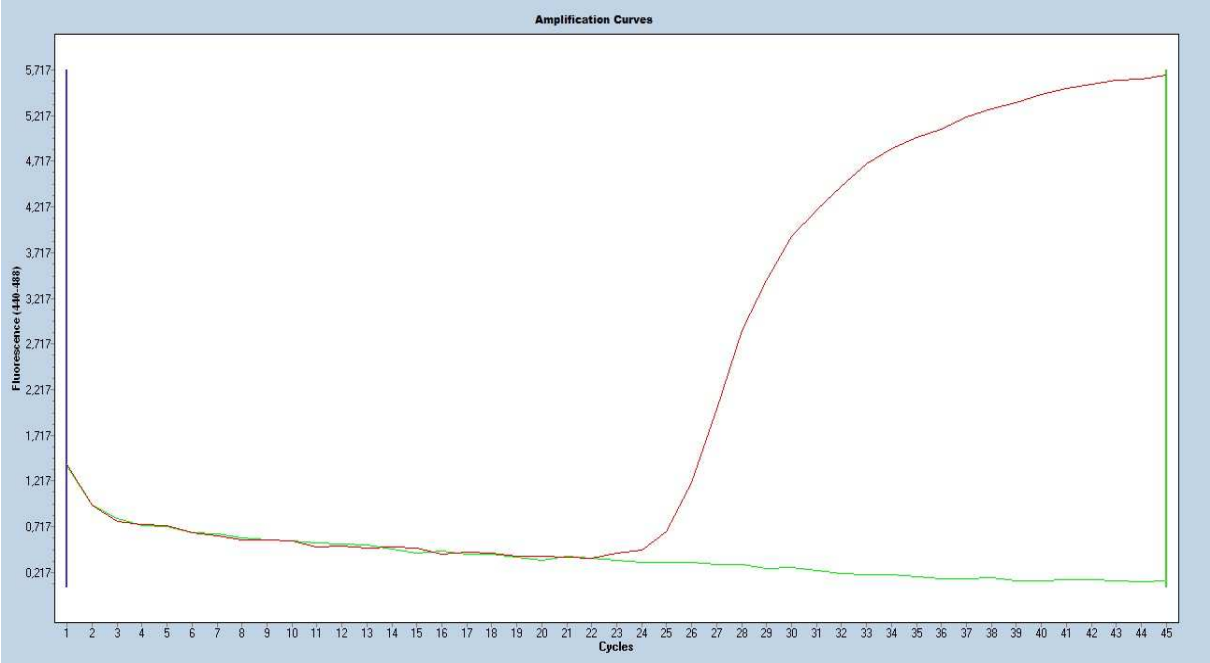

Fig. 1: Correct run of the positive control (red) and negative control (green) (*Dientamoeba fragilis*) on the LightCycler® 480II

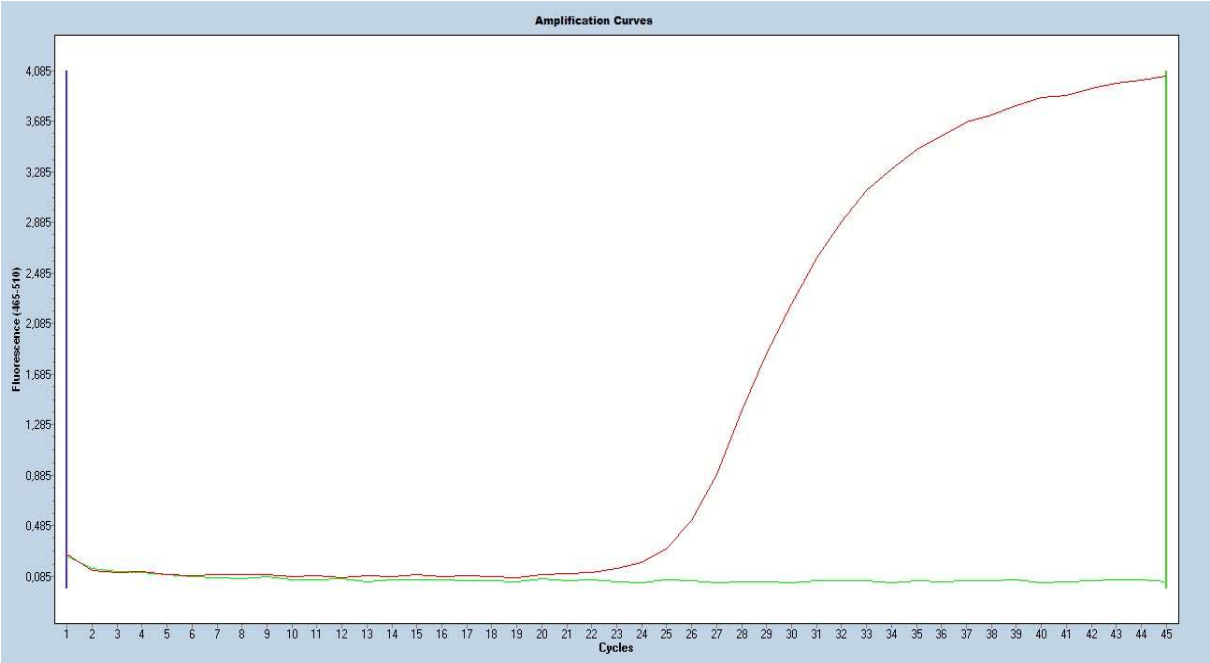

Fig. 2: Correct run of the positive control (red) and negative control (green) (*Giardia lamblia*) on the LightCycler® 480II

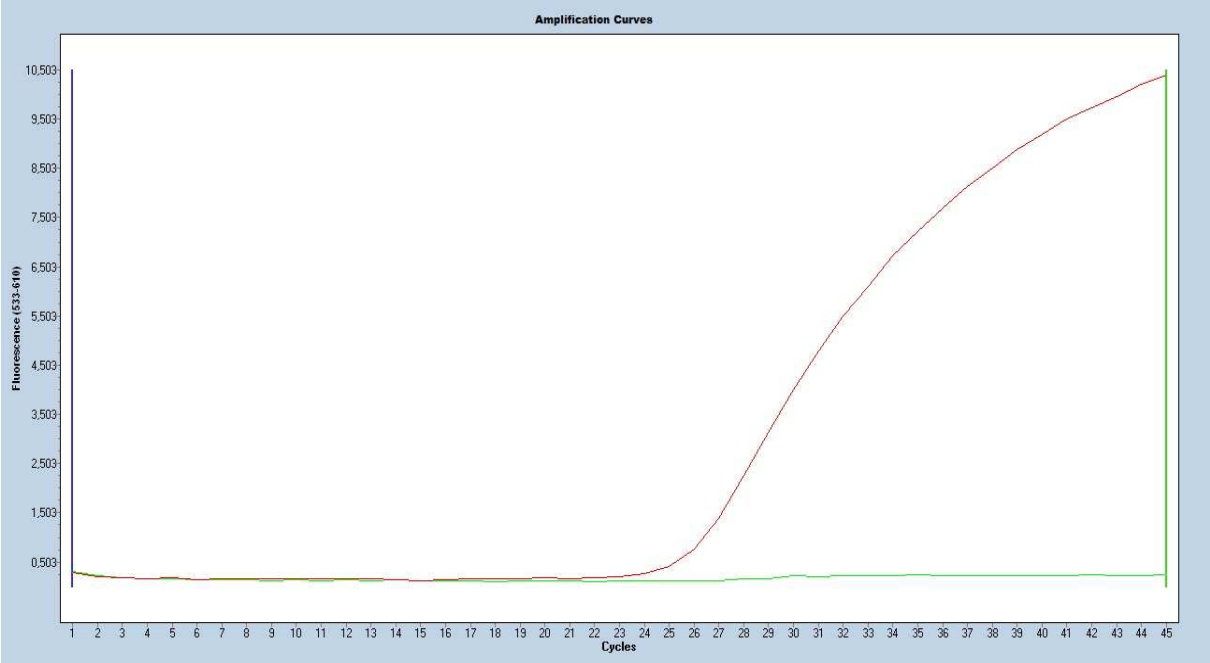

Fig. 3: Correct run of the positive control (red) and negative control (green) (*Entamoeba histolytica*) on the LightCycler® 480II

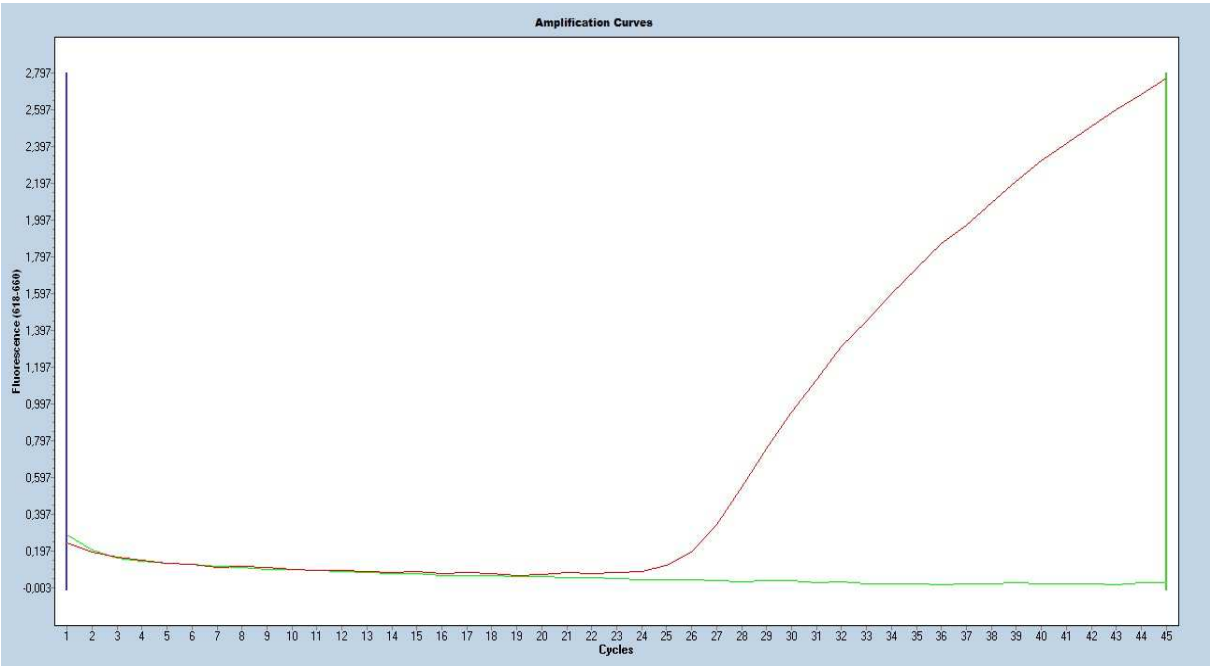

Fig. 4: Correct run of the positive control (red) and negative control (green) (*Cryptosporidium* spp.) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 11.

Tab. 11: Sample interpretation

Target genes					
D. fragilis	***G. lamblia***	***E. histolytica***	***Crypto.* spp.**	**ICD**	**Result**
positive	negative	negative	negative	**positive/negative**	***D. fragilis* detected**
negative	**positive**	negative	negative	**positive/negative**	***G. lamblia* detected**
negative	negative	**positive**	negative	**positive/negative**	***E. histolytica* detected**
negative	negative	negative	**positive**	**positive/negative**	***Cryptosporidium* spp. detected**
positive	**positive**	negative	negative	**positive/negative**	***D. fragilis* and *G. lamblia* detected**
positive	negative	**positive**	negative	**positive/negative**	***D. fragilis* and *E. histolytica* detected**
positive	negative	negative	**positive**	**positive/negative**	***D. fragilis* and *Cryptosporidium* spp. detected**
negative	**positive**	**positive**	negative	**positive/negative**	***G. lamblia* and *E. histolytica* detected**
negative	**positive**	negative	**positive**	**positive/negative**	***G. lamblia* and *Crypto*sporidium spp. detected**
negative	negative	**positive**	**positive**	**positive/negative**	***E. histolytica* and *Cryptosporidium* spp. detected**
positive	**positive**	**positive**	negative	**positive/negative**	***D. fragilis*, *G. lamblia* and *E. histolytica* detected**
positive	**positive**	negative	**positive**	**positive/negative**	***D. fragilis*, *G. lamblia* and *Cryptosporidium* spp. detected**
positive	negative	**positive**	**positive**	**positive/negative**	***D. fragilis*, *E. histolytica* and *Cryptosporidium* spp. detected**
negative	**positive**	**positive**	**positive**	**positive/negative**	***G. lamblia*, *E. histolytica* and *Cryptosporidium* spp. detected**
positive	**positive**	**positive**	**positive**	**positive/negative**	***D. fragilis*, *G. lamblia*, *E. histolytica* and *Cryptosporidium* spp. detected**
negative	negative	negative	negative	**positive**	**Target genes not detected**
negative	negative	negative	negative	**negative**	**Invalid**

A sample is evaluated positive, if the sample DNA shows an amplification signal and an amplification signal for the Internal Control DNA is observed in the detection system.

A sample is also evaluated positive, if the sample DNA shows an amplification signal, but no amplification signal for the Internal Control DNA in the detection system. The detection of the Internal Control DNA is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the internal amplification control.

A sample is evaluated negative, if the sample DNA shows no amplification signal and an amplification signal for the Internal Control DNA is observed in the detection system. An inhibition of the PCR reaction or a failure in the extraction procedure can be excluded by the detection of Internal Control DNA.

A sample is evaluated invalid, if both, the sample and the Internal Control DNA show no amplification signal in the detection system. The sample contained a PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for stool samples.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Parasitic Stool Panel I assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target genes (ITS1-18S).

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE Parasitic Stool Panel I multiplex real-time PCR assay has a detection limit of ≥ 50 DNA copies per reaction for *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica*, respectively.

The following figures 5, 6, 7 and 8 show dilution series of *Dientamoeba fragilis, Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* (each 10^5 - 10^2 DNA copies per µl) on the LightCycler® 480II.

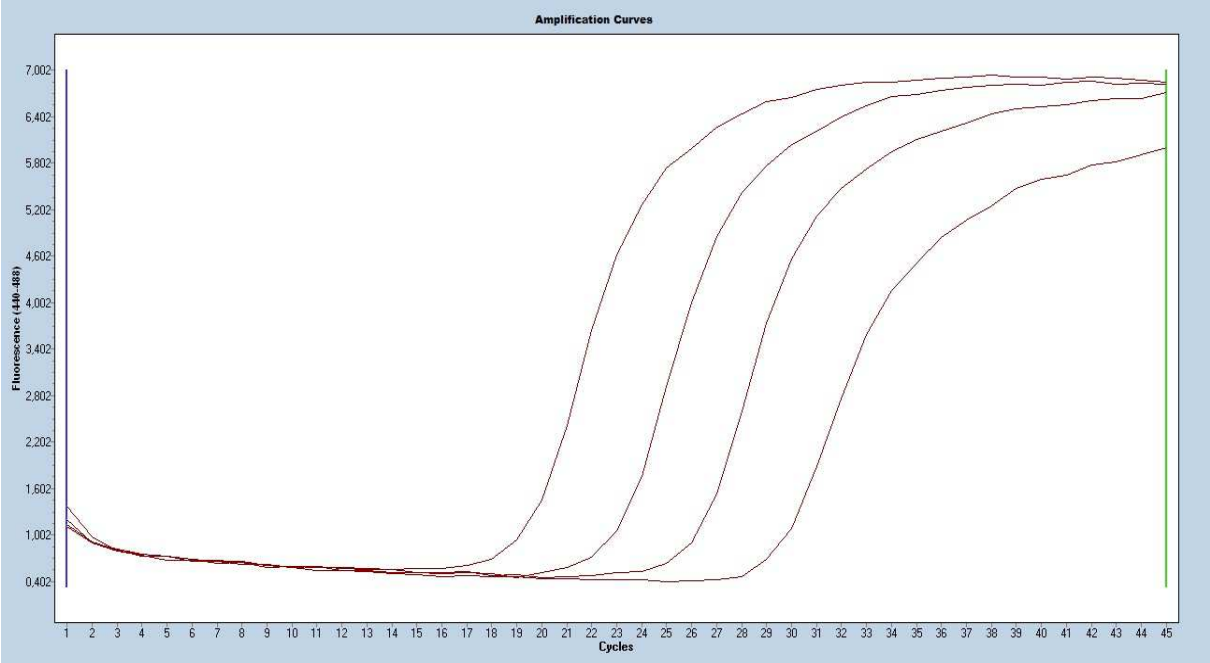

Fig. 5: Dilution series *Dientamoeba fragilis* (10^5 – 10^2 DNA copies/µl) on the LightCycler® 480II

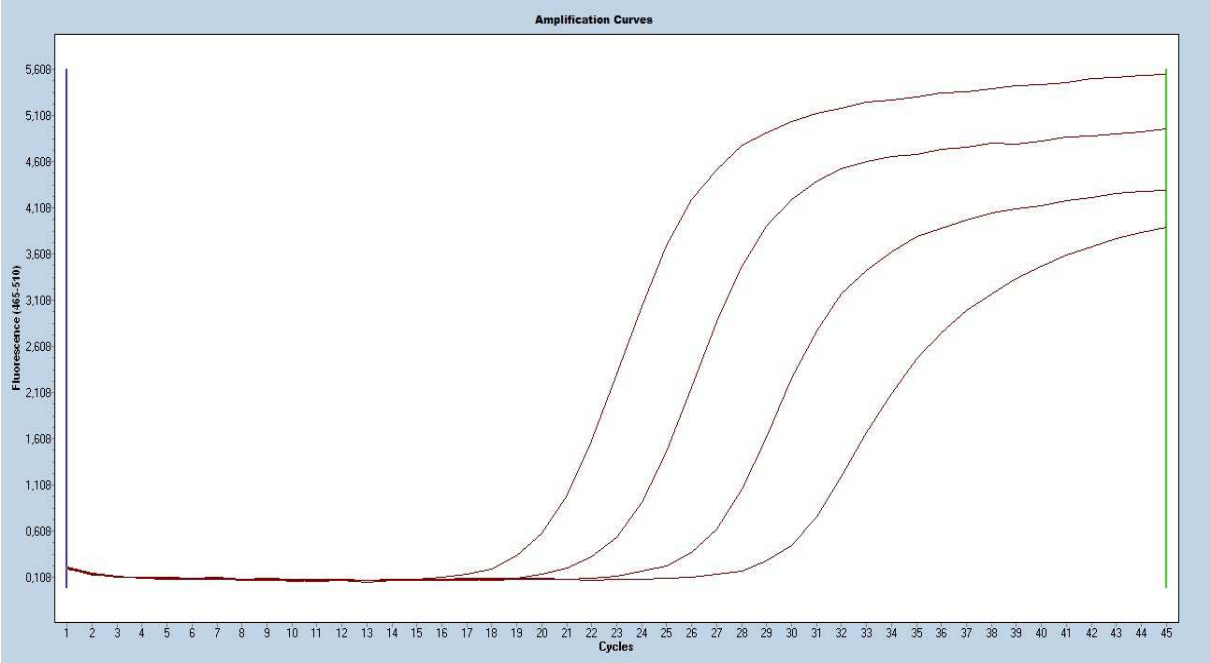

Fig. 6: Dilution series *Giardia lamblia* (10^5 – 10^2 DNA copies/µl) on the LightCycler® 480II

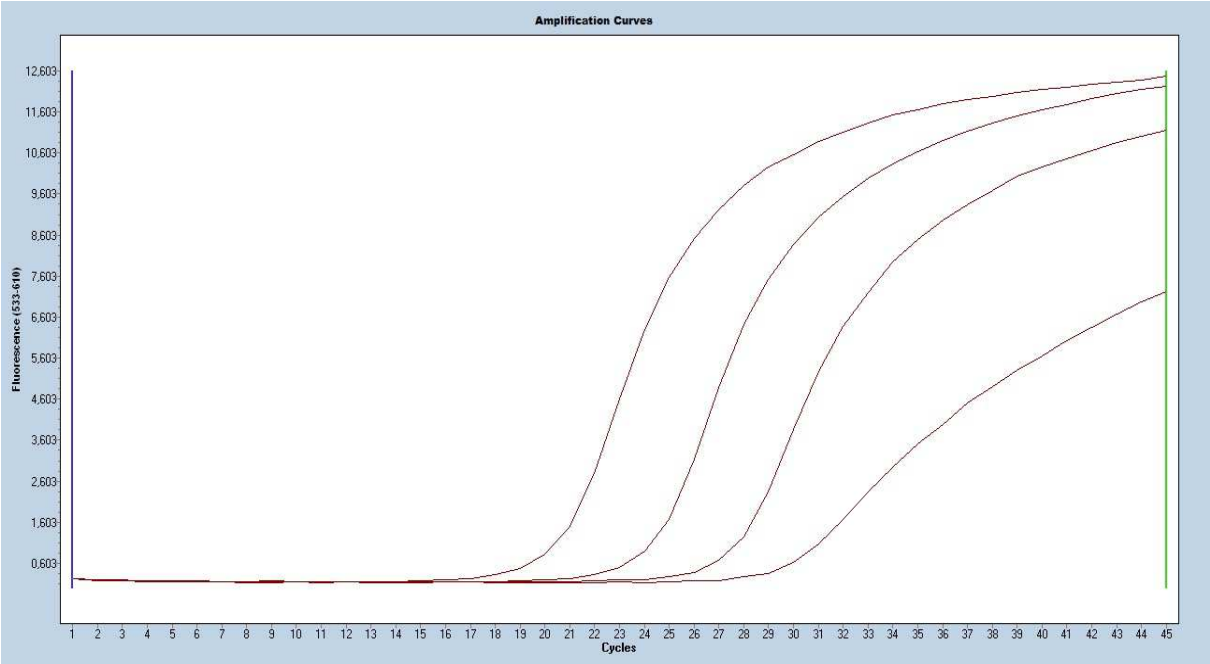

Fig. 7: Dilution series *Entamoeba histolytica* (10^5 - 10^2 DNA copies/µl) on the LightCycler® 480II

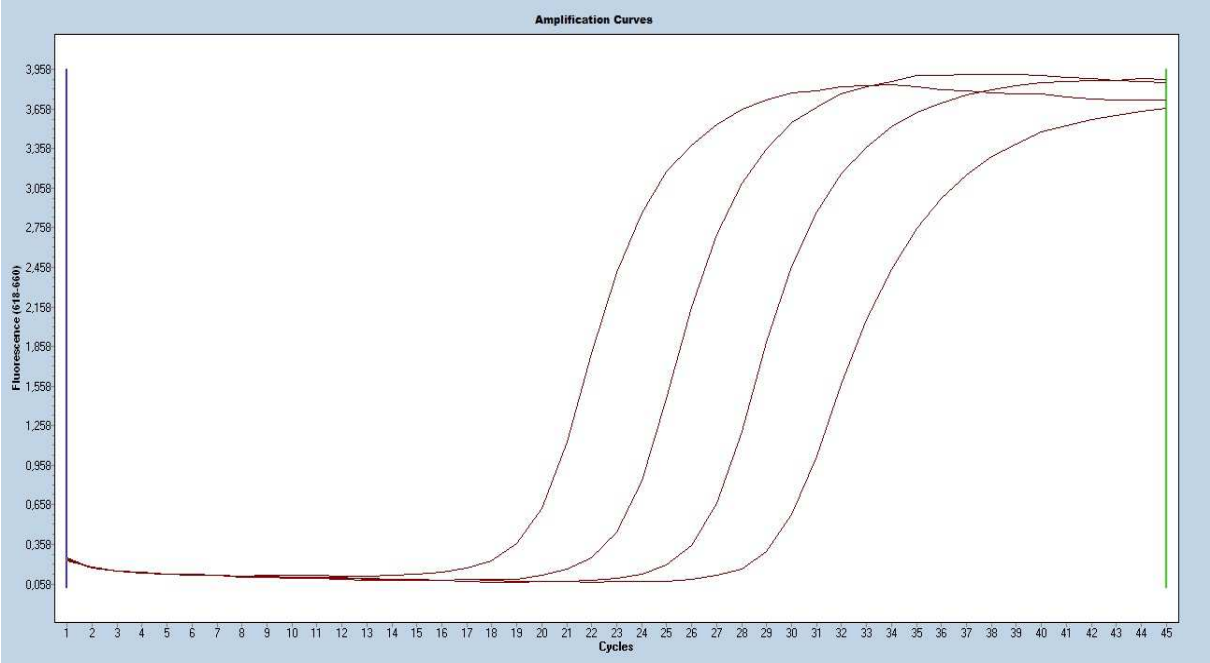

Fig. 8: Dilution series *Cryptosporidium* spp. (10^5 – 10^2 DNA copies/µl) on the LightCycler® 480II

The detection limit of the whole procedure depends on the sample matrix, DNA extraction and DNA concentration

13.2 Analytical specificity

The analytical specificity of the RIDA®GENE Parasitic Stool Panel I multiplex real-time PCR assay is specific for *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium* spp. und *Entamoeba histolytica*. No cross-reaction could be detected for the following species (see Tab. 12):

Tab. 12: Cross-reactivity testing

Adenovirus 40, human, strain Dugan	-	*Citrobacter freundii*	-	*E. coli* (O6)	-	Rotavirus	-
Adenovirus 41, human, strain Tak	-	*Clostridium bifermentans*	-	Echovirus Type 11	-	*Salmonella enteritidis*	-
Astrovirus	-	*Clostridium difficile*	-	*Entamoeba dispar*	-	*Salmonella typhimurium*	-
Bacillus cereus	-	*Clostridium novyi*	-	*Enterobacter cloacae*	-	*Serratia liquefaciens*	-
Bacteroides fragilis	-	*Clostridium perfringens*	-	*Enterococcus faecalis*	-	*Shigella flexneri*	-
Campylobacter coli	-	*Clostridium septicum*	-	Enterovirus Type 71	-	*Staphylococcus aureus*	-
Campylobacter fetus subsp. *fetus*	-	*Clostridium sporogenes*	-	*Klebsiella oxytoca*	-	*Staphylococcus epidermidis*	-
Campylobacter jejuni	-	*Clostridium sordellii*	-	Norovirus GGI	-	*Trichomonas vaginalis*	-
Campylobacter lari subsp. *lari*	-	Coxsackievirus B4	-	Norovirus GGII	-	*Vibrio parahaemolyticus*	-
Campylobacter upsaliensis	-	*E. coli* (O157:H7)	-	*Proteus vulgaris*	-	*Yersinia enterocolitica*	-
Candida albicans	-	*E. coli* (O26:H-)	-	*Pseudomonas aeruginosa*	-		

13.3 Analytical reactivity

The reactivity of the RIDA®GENE Parasitic Stool Panel I multiplex real-time PCR assay was tested with *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium* spp. (see Tab. 13). All *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium* spp. strains tested were detected by the RIDA®GENE Parasitic Stool Panel I multiplex real-time PCR assay or by sequence alignment (*).

Tab. 13: Analytical reactivity testing

Giardia lamblia					
Giardia lamblia	+	*G. intestinales* Portland 1	+	*G. intestinales* WB Clone 6	+
Entamoeba histolytica					
E. histolytica	+				
***Cryptosporidium* spp.**					
*C. baileyi**	+	*C. hominis*	+	*C. sp skunk**	+
*C. bovis**	+	*C. muris*	+	*C. ubiquitum**	+
*C. canis**	+	*C. parvum*	+	*C. viatorum**	+
*C. cuniculus**	+	*C. sp horse**	+	*C. xiaoi**	+
*C. felis**	+				

14. Version history

Version number	Chapter and designation
2019-04-09	General revision 4. Reagents provided 5. Storage instructions 6. Additional necessary reagents and necessary equipment 7. Precautions for users 8. Collection and storage of samples 9. Test procedure 10. Quality control 11. Result interpretation 13. Performance characteristics 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

	For *in vitro* diagnostic use
	Consult instructions for use
	Lot number
	Expiry
	Store at
	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Testspecific symbols

Not applicable

16. Literature

1. Centers for Disease Control and Prevention 2011. Giardia Epidemiology & Risk Factors, http://www.cdc.gov/parasites/giardia/epi.html. Aufgerufen am 10.07.2012.
2. Food and Drug Administration (FDA) 2011. Bad Bug Book 2nd Edition. http://www.fda.gov/food/foodsafety/foodborneillness/foodborneillnessfoodbornepathogensnaturaltoxins/badbugbook/default.htm. Aufgerufen am 10.07.2012.
3. Centers for Disease Control and Prevention. http://www.cdc.gov/parasites/crypto/biology.html. Aufgerufen am 07.03.2014.
4. Leitch GJ und Qing He. Cryptosporidosis - an overview. J Biomed Res. 2012, 25(1): 1-16.
5. Lee JK *et al.* Prevalence of diarrhea caused by Cryptosporidium parvum in non-HIV patients in Jeollanam-do, Korea . Korean J Parasitol. 2005, 43(3):111-114.
6. Robert Koch Institut 2010. Kryptosporidiose (Cryptosporidium parvum). RKI-Ratgeber für Ärzte 2004. Aufgerufen am 24.07.2012.
7. Scallan E *et al.* Foodborne Illness Acquired in the United States - Major Pathogens. Emerg Infect Dis. 2011, 17(1): 7-15.
8. Fotedar R *et al.* Laboratory diagnostic techniques for Entamoeba species. Clin Microbiol Rev. 2007, 20(3):511-532.
9. Stark D *et al.* A review of the clinical presentation of dientamoebiasis. Am J Trop Med Hyg. 2010, 82(4):614-9.
10. 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.