RIDA®GENE Parasitic Stool Panel

real-time PCR

Art. No.: PG1705

100 reactions

For *in vitro* diagnostic use.

-20 °C

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Parasitic Stool Panel is a multiplex real-time PCR for the direct, qualitative detection and differentiation of Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica and Dientamoeba fragilis in human stool samples. RIDA®GENE Parasitic Stool Panel real-time PCR is intended for use as an aid in diagnosis of gastrointestinal infection caused by parasites.

2. Explanation of the test

Giardia lamblia, Cryptosporidium parvum, Entamoena 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 that commonly causes cryptosporidiosis in human. Cryptosporidium 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 was detect up to 14-24% while up to 5% of asymptomatic HIV patients were infected.[3,4] During an outbreak in Milwaukee, USA in 1993 over 400,000 individuals were sickened.[5] Each year estimated 748,000 cases of cryptosporidiosis occur in the United States.[6] 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.[2,5]

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.[2,7]

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.[8,9]

Classically, diagnosis of G. lamblia, C. parvum, Entamoeba species und D. fragilis is achieved by microscopial examination of faecal samples, which require experienced personal. RIDA®GENE Parasitic Stool Panel multiplex real-time PCR 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 four most important diarrhea causing parasites (G. lamblia, C. parvum, E. histolytica und D. fragilis).

3. Test principle

The RIDA®GENE Parasitic Stool Panel is a multiplex real-time PCR for the direct, qualitative detection and differentiation of Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica and Dientamoeba fragilis in human stool samples.

After DNA-isolation, amplification of gene fragments (18s-ITS, if present) specific for Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica and Dientamoeba fragilis occurs.

The amplified targets for Giardia lamblia, Cryptosporidium parvum and Entamoeba histolytica 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 assay contains an Internal Control DNA (ICD) as an internal control of sample preparation procedure and to determine possible PCR-inhibition. The amplified target for Dientamoeba fragilis is evaluated by melting curve analysis.

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 1100 µl	yellow
2	Taq-Polymerase	1x 11 µl	red
D	Internal Control DNA	2x 1800 µl	orange
N	PCR Water	1x 500 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 5 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 equipment and materials required

- DNA-Extraction kit (e.g. RTP® Pathogen Kit, STRATEC Molecular)
  or
  DNA-Extraction system (e.g. Maxwell® 16 (Promega), NucliSENS easy®MAG™ (bioMérieux))
- Real-time PCR instrument:
  Roche: LightCycler® 480II
  Cepheid: SmartCycler®
  Applied Biosystems: ABI 7500
  Abbott: m2000rt
  Stratagene: Mx3000P, Mx3005P
  QIAGEN: Rotor-Gene Q
  Note: Use on the Rotor-Gene Q (QIAGEN) only 0.1 ml tubes.
- RIDA®GENE Color Compensation Kit I (PG0001) for run the LightCycler® 480II (Roche)
- 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

7. Precautions for users

- For *in vitro* diagnostic use only.
- DNA isolation, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- This test must only be performed by laboratory personnel trained in molecular biology methods.
- Strictly follow the working instructions.
- When handling samples, wear disposable gloves. After finishing the test, wash your hands.
- Do not smoke, eat or drink in areas where samples or test reagents are being used.
- 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.
- **Use on the Rotor-Gene Q (QIAGEN) only 0.1 ml tubes.**

8. Test procedure

8.1 Sample Preparation

8.1.1 DNA-Isolation from Stool Samples

Use for DNA-isolation of human stool samples a commercially available DNA isolation kit (e.g. RTP® Pathogen Kit (STRATEC Molecular)) or DNA extraction system (e.g. Maxwell® 16 (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 3,000 rpm for 30 sec. Use from the supernatant the appropriate volume according to the manufacturer's instruction.

The RIDA®GENE Parasitic Stool Panel assay contains 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.

If the ICD is used only as a PCR inhibition control, 1µl of the ICD should be added to the Master-Mix (see Tab.3).

If the ICD is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the ICD has to be added during extraction procedure. The ICD should always be added to the specimen-lysis buffer mixture and **not** direct to the specimen.

8.2 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.2, Tab.3). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Taq-Polymerase, the Positive Control, the PCR Water and the ICD before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab.2: 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	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 µl
	Total	**20.0 µl**	**220 µl**

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

Tab.3: 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	19.9 µl	218.9 µl
2	Taq-Polymerase	0.1 µl	1.1 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.

8.3 Preparation of the PCR-Mix

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

Negative control: Add 5 µl PCR Water as negative control to the pre-pipetted Master-Mix.

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

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

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

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

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument Set-up (see Tab.4, Tab. 5, Fig.1, Fig.2, Fig.3, Fig.4, Fig.5).

Note: Use on the Rotor-Gene Q (QIAGEN) only 0.1 ml tubes.

8.4 PCR Instrument Set-up

Tab.4: Real-time PCR profile for LightCycler® 480II, SmartCycler®, Rotor-Gene Q

Initial Denaturation Cycles PCR Denaturation Annealing/Extension	1 min, 95 °C 45 Cycles 10 sec, 95 °C 15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum
Melting Curve Tm-Analysis: Acquisitions	60 °C to 95 °C continuous 1 pro °C

Note: *Annealing and Extension occur in the same step.*

Tab.5: Real-time PCR profile for Mx3000P/Mx3005P, ABI7500 and m2000rt

Initial Denaturation Cycles PCR Denaturation Annealing/Extension	1 min, 95 °C 45 Cycles 15 sec, 95 °C 30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum
Melting Curve Tm-Analysis: Acquisitions	60 °C to 95 °C continuous 1 pro °C

Note: *Annealing and Extension occur in the same step.*

Note: Start the Melting Curve profile separate after the real-time PCR profile on the Mx3000P/Mx3005P (see Fig.4). For the Melting Curve profile select SYBR® Green (with Dissociation Curve) as experiment type and use only HEX as detection channel.

Note: Check that the Manual Tresh Flour Units for Channel 1 is set on 30.0 and for Channel 2 to 4 is set on 5.0 on the SmartCycler® (Cepheid).

Fig. 1: Melting Curve profile LightCycler® 480II

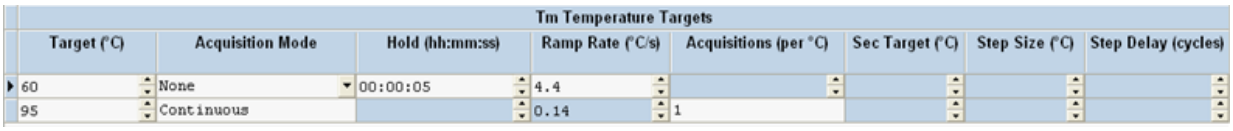


Fig. 2: Melting Curve profile SmartCycler®

Fig.3: Melting Curve profile Rotor-Gene Q

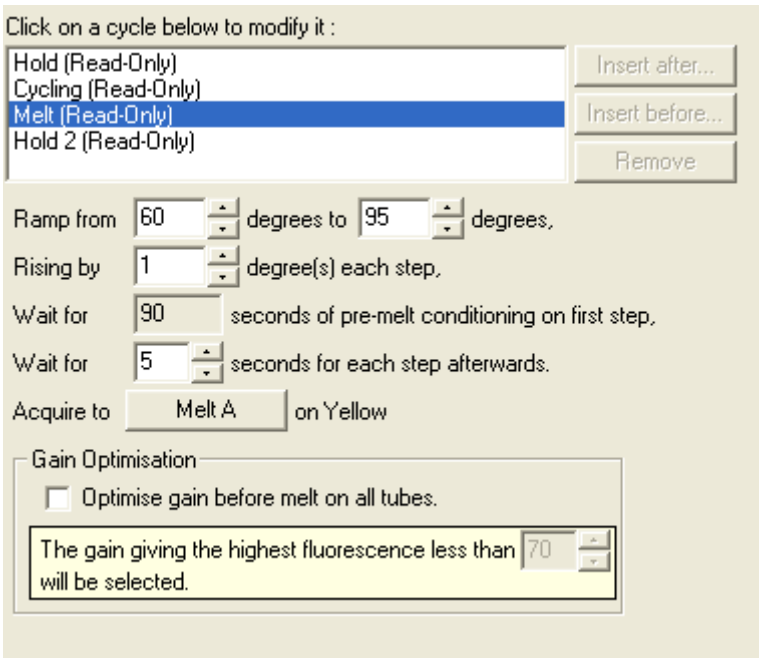


Fig. 4: Melting Curve profile Mx3000P and Mx3005P

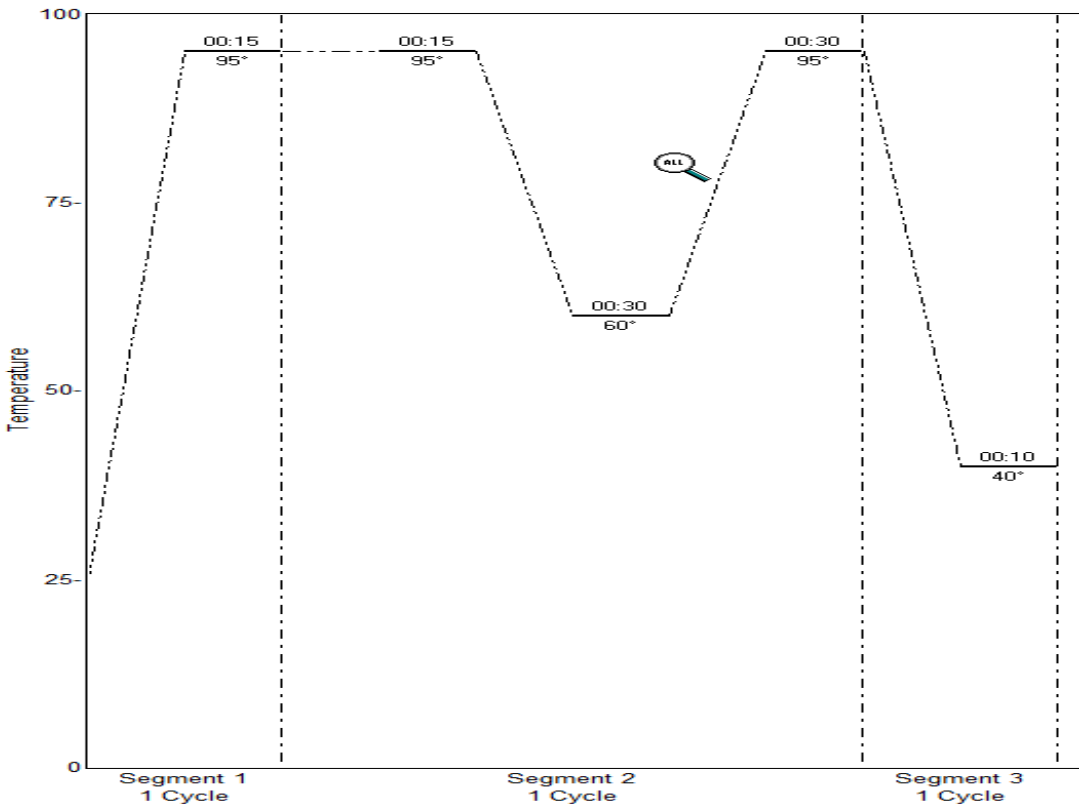
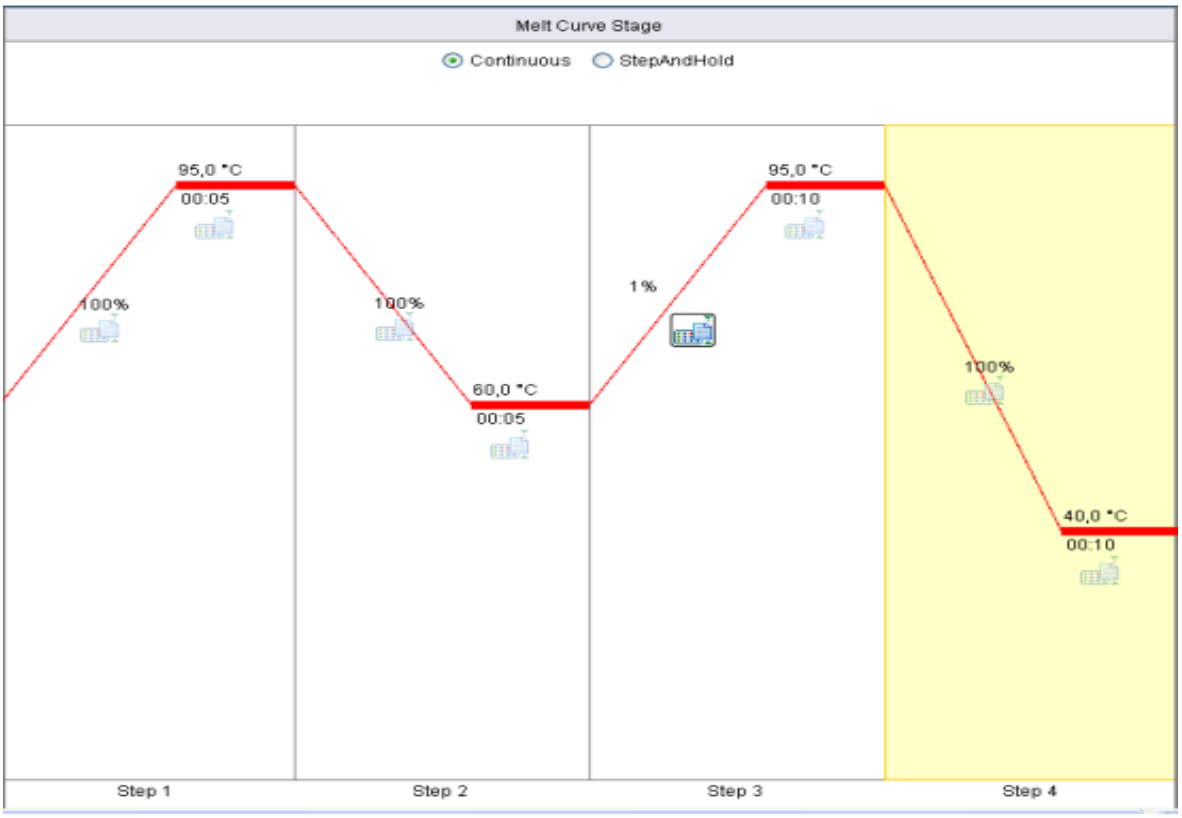


Fig. 5: Melting Curve profile ABI7500



8.5 Detection Channel Set-up

Tab.6: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection Channel	Dark-Quencher	Note
Roche LightCycler® 480II	Giardia lamblia	465/510	+	**RIDA®GENE Color Compensation Kit I (PG0001) is required**
	ICD	533/580	+	
	Entamoeba histolytica	533/610	+	
	Cryptosporidium parvum	618/660	+	
	Dientamoeba fragilis	Melting Curve 533/580	-	
Cepheid SmartCycler®	Giardia lamblia	Kanal 1	+	**Check that the Manual Tresh Flour Units for Channel 1 is set on 30.0 and for Channel 2 to 4 is set on 5.0**
	ICD	Kanal 2	+	
	Entamoeba histolytica	Kanal 3	+	
	Cryptosporidium parvum	Kanal 4	+	
	Dientamoeba fragilis	Melting Curve Kanal 2	-	
ABI 7500	Giardia lamblia	FAM	none	**Check that passive reference option ROX is none**
	ICD	VIC	none	
	Entamoeba histolytica	ROX	none	
	Cryptosporidium parvum	Cy5	none	
	Dientamoeba fragilis	Melting Curve VIC	-	
Abbott m2000rt	Giardia lamblia	FAM	none	**-**
	ICD	VIC	none	
	Entamoeba histolytica	ROX	none	
	Cryptosporidium parvum	Cy5	none	
	Dientamoeba fragilis	Melting Curve VIC	-	
Stratagene Mx3000P/ Mx3005P	Giardia lamblia	FAM	+	**Check that the reference dye is none**
	ICD	HEX	+	
	Entamoeba histolytica	ROX	+	**Start the Melting Curve profile separate after the real-time PCR profile**
	Cryptosporidium parvum	Cy5	+	
	Dientamoeba fragilis	Melting Curve HEX	-	
Qiagen Rotor-Gene Q	Giardia lamblia	Green	+	**-**
	ICD	Yellow	+	
	Entamoeba histolytica	Orange	+	
	Cryptosporidium parvum	Red	+	
	Dientamoeba fragilis	Melting Curve Yellow	-	

9. Result interpretation

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer`s instructions. Positive and negative controls have to show correct results (see Fig.6, Fig.7, Fig.8, Fig.9).

The positive control has for Giardia lamblia and Entamoeba histolytica a concentration of 10^4 copies / µl and for Cryptosporidium parvum and Dientamoeba fragilis 10^3 copies / µl. In each PCR run it is used in a total amount of 5 x 10^4 and 5 x 10^3 copies respectively.

Fig.6: Correct run of the positive and negative control (Giardia lamblia) on the LightCycler® 480II

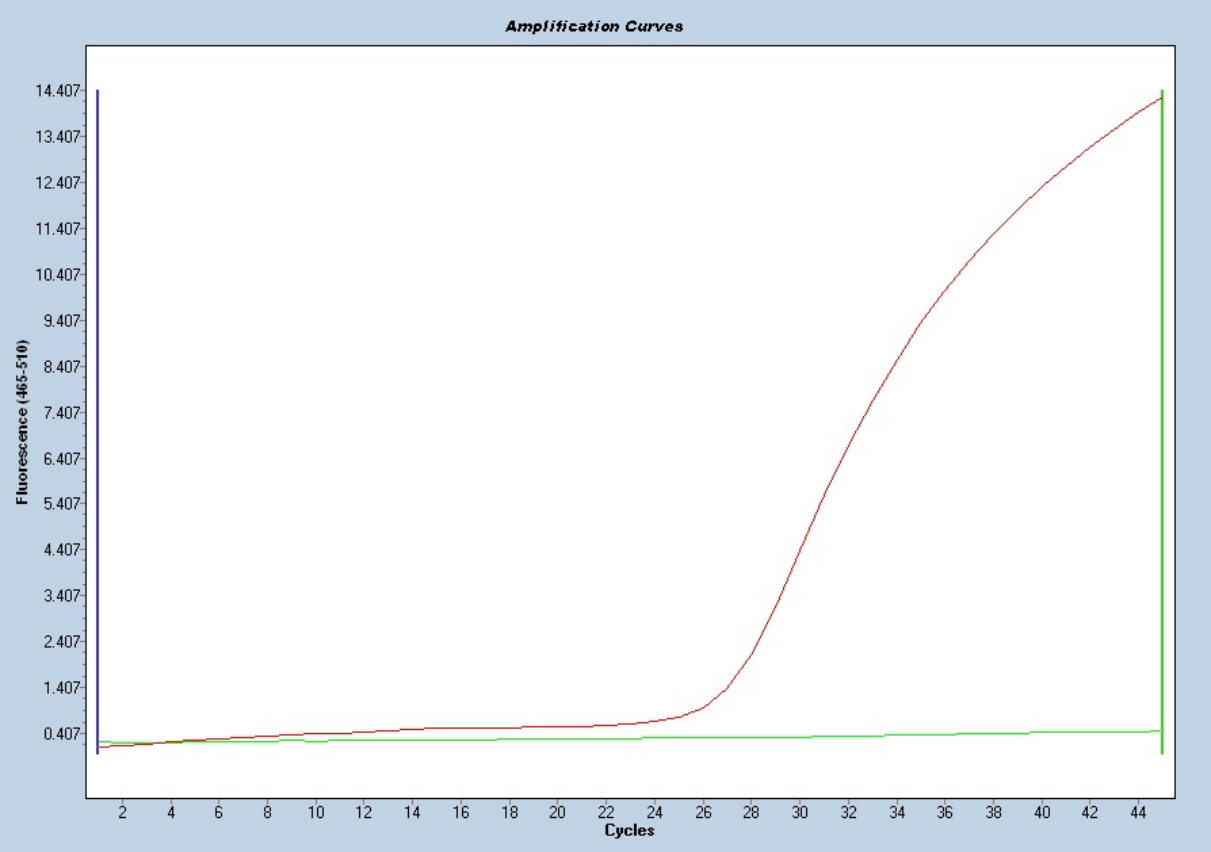


Fig.7: Correct run of the positive and negative control (Entamoeba histolytica) on the LightCycler® 480II

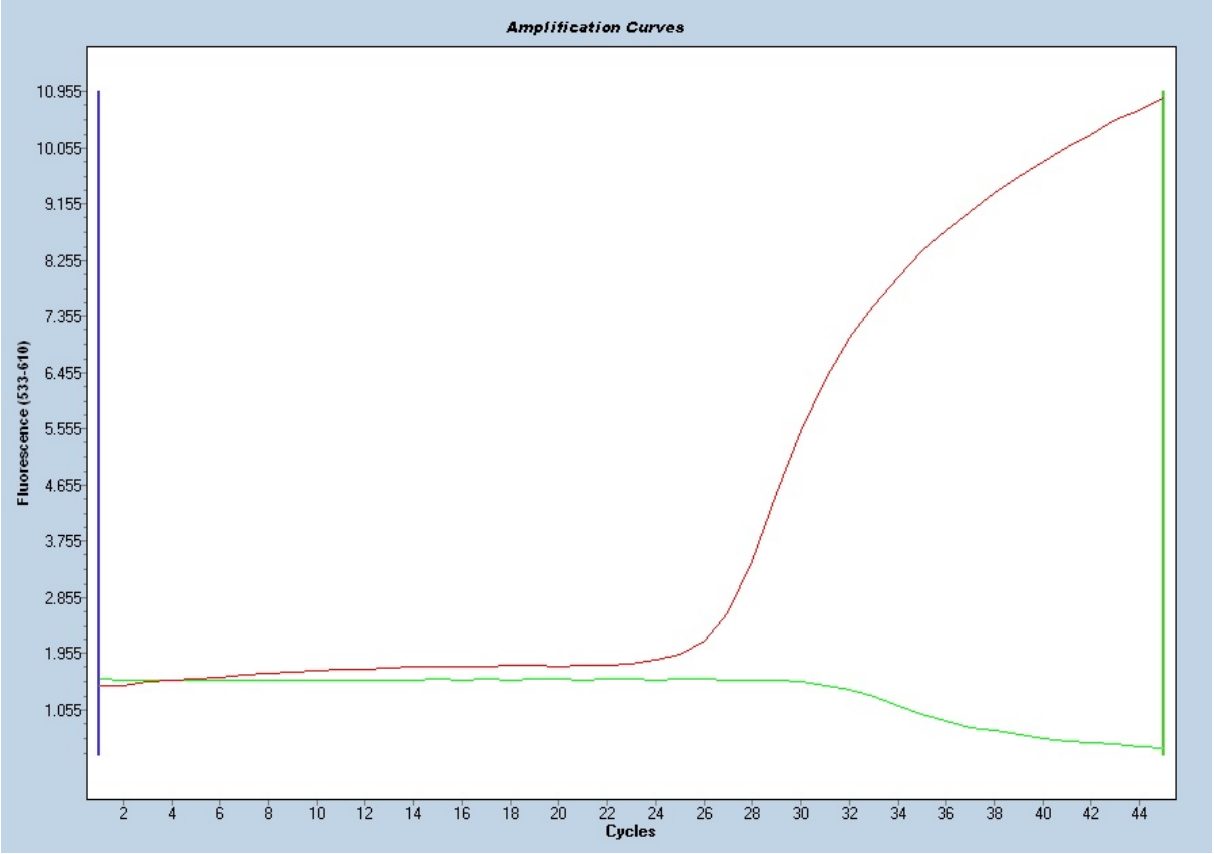


Fig.8: Correct run of the positive and negative control (Cryptosporidium parvum) on the LightCycler® 480II

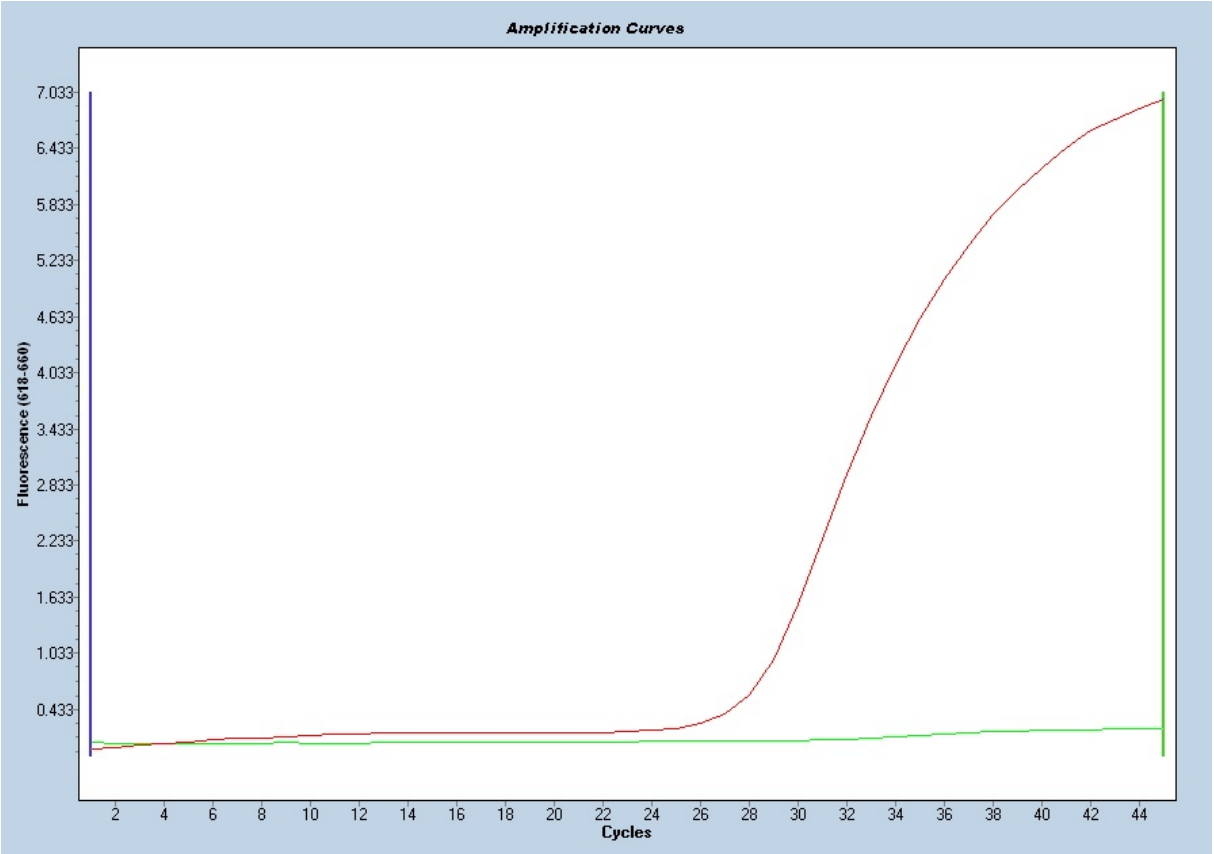
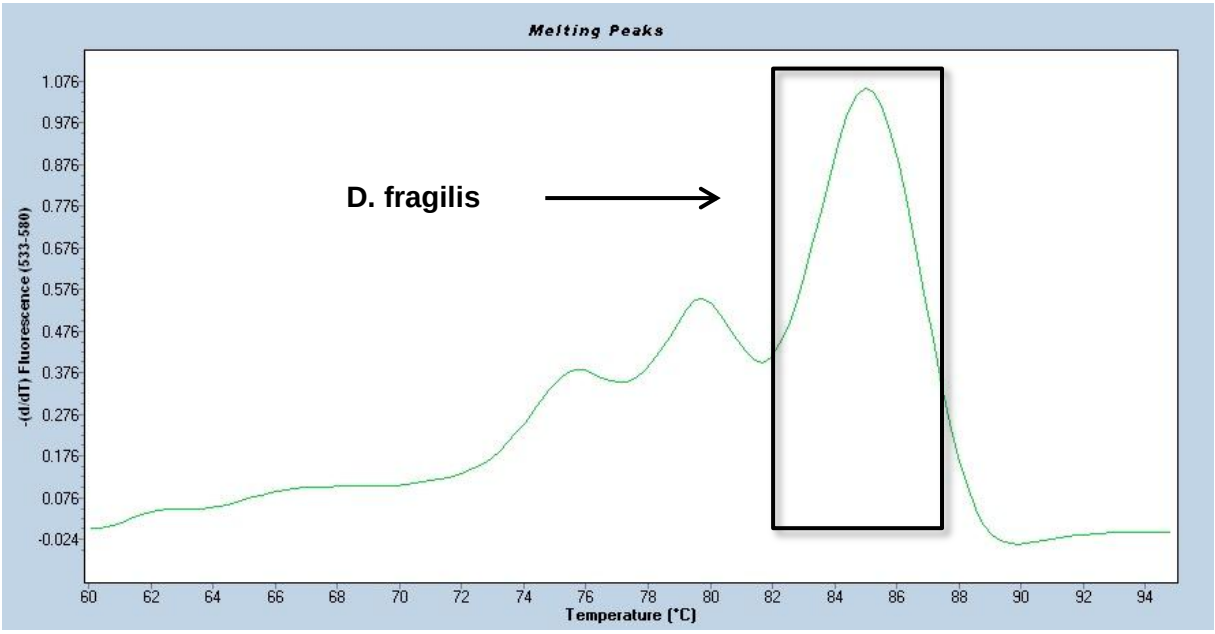


Fig.9: Correct run of the melting curve analysis (Dientamoeba fragilis) on the LightCycler® 480II



The result interpretation is done according to Table 7 and 8.

Tab.7: Interpretation of the Melting Curve analysis D. fragilis

Real-time PCR instrument	Melting Curve temperature D. fragilis
Roche LightCycler® 480II	85 °C (+/- 1 °C)
Cepheid SmartCycler®	85 °C (+/- 1 °C)
ABI 7500	83.5 °C (+/- 1 °C)
Abbott m2000rt	84 °C (+/- 1 °C)
Stratagene Mx3000P/ Mx3005P	85 °C (+/- 1 °C)
Qiagen Rotor-Gene Q	85.5 °C (+/- 1 °C)

Tab.8: Sample interpretation

	Target genes					
	Giardia lamblia	**E. histolytica**	**C. parvum**	**D. fragilis**	**ICD**	**Result**
Detection Channel/ Tm-Peak	**positive**	negative	negative	**-**	**positive/negative**	**G. lamblia detected**
Detection Channel/ Tm-Peak	negative	**positive**	negative	**-**	**positive/negative**	**E. histolytica detected**
Detection Channel/ Tm-Peak	negative	negative	**positive**	**-**	**positive/negative**	**C. parvum detected**
Detection Channel/ Tm-Peak	negative	negative	negative	**positive**	**positive/negative**	**D. fragilis detected**
Detection Channel/ Tm-Peak	**positive**	**positive**	negative	**-**	**positive/negative**	**G. lamblia and E. histolytica detected**
Detection Channel/ Tm-Peak	**positive**	negative	**positive**	**-**	**positive/negative**	**G. lamblia and C. parvum detected**
Detection Channel/ Tm-Peak	**positive**	negative	negative	**positive**	**positive/negative**	**G. lamblia and D. fragilis detected**

	Target genes					
	Giardia lamblia	**E. histolytica**	**C. parvum**	**D. fragilis**	**ICD**	**Result**
Detection Channel/ Tm-Peak	negative	**positive**	**positive**	**-**	**positive/negative**	**E. histolytica and C. parvum detected**
Detection Channel/ Tm-Peak	negative	**positive**	negative	**positive**	**positive/negative**	**E. histolytica and D. fragilis detected**
Detection Channel/ Tm-Peak	negative	negative	**positive**	**positive**	**positive/negative**	**C. parvum and D. fragilis detected**
Detection Channel/ Tm-Peak	**positive**	**positive**	**positive**	**-**	**positive/negative**	**G. lamblia, E. histolytica and C. parvum detected**
Detection Channel/ Tm-Peak	**positive**	**positive**	**positive**	**positive**	**positive/negative**	**G. lamblia, E. histolytica, C. parvum and D. fragilis detected**
Detection Channel/ Tm-Peak	negative	negative	negative	**-**	**positive**	**Target genes are not detected**
Detection Channel/ Tm-Peak	negative	negative	negative	**-**	**negative**	**Invalid**

Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and Dientamoeba fragilis is detected, if the sample DNA shows an amplification signal or a specific peak in the melting curve analysis (see Tab. 7) and an amplification signal for the Internal Control DNA (ICD) in the detection system.

Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and Dientamoeba fragilis is also detected, if the sample DNA shows an amplification signal or a specific peak in the melting curve analysis (see Tab. 7), but no amplification signal for the Internal Control DNA (ICD) in the detection system. The detection of the Internal Control DNA (ICD) is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the internal amplification control.

Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and Dientamoeba fragilis are not detected, if the sample DNA shows no amplification signal or no specific peak in the melting curve analysis (see Tab. 7) and an amplification signal for the Internal Control DNA (ICD) in the detection system. An inhibition of the PCR reaction or a failure in the extraction procedure can be excluded by the detection of the Internal Control DNA (ICD).

A sample is invalid, if the sample DNA show no amplification signal for Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and the Internal Control DNA (ICD) as well as no peak in the melting curve analysis for D. fragilis (see Tab. 7) in the detection system. The sample contains 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.

10. Performance characteristics

10.1 Clinical Performance

In a validation study at the Bernhard-Nocht-Institute (BNI) for tropical medicine in Hamburg, Germany, 218 DNA samples from symptomatic patients were tested through real-time PCR with the RIDA®GENE Parasitic Stool Panel and the results were compared with those of in-house PCR testing. The results are summarized in the following tables:

Tab.9: Correlation of the Giardia lamblia results with the RIDA®GENE Parasitic Stool Panel real-time PCR and BNI in-house PCR

		BNI PCR	
		positive	negative
RIDA®GENE Parasitic Stool Panel	positive	38	0
	negative	2	178

Sensitivity	95.0 %
Specificity	100.0 %
PPV	100.0 %
NPV	98.9 %

Tab.10: Correlation of the Entamoeba histolytica results with the RIDA®GENE Parasitic Stool Panel real-time PCR and BNI in-house PCR

		BNI PCR	
		positive	negative
RIDA®GENE Parasitic Stool Panel	positive	29	0
	negative	0	189

Sensitivity	100.0 %
Specificity	100.0 %
PPV	100.0 %
NPV	100.0 %

Tab.11: Correlation of the Cryptosporidium parvum results with the RIDA®GENE Parasitic Stool Panel real-time PCR and BNI in-house PCR

		BNI PCR	
		positive	negative
RIDA®GENE Parasitic Stool Panel	positive	27	1
	negative	1	189

Sensitivity	96.4 %
Specificity	99.5 %
PPV	96.4 %
NPV	99.5 %

Tab.12: Correlation of the Dientamoeba fragilis results with the RIDA®GENE Parasitic Stool Panel real-time PCR and BNI in-house PCR

		BNI PCR	
		positive	negative
RIDA®GENE Parasitic Stool Panel	positive	37	0
	negative	1	57

Sensitivity	97.4 %
Specificity	100.0 %
PPV	100.0 %
NPV	98.3 %

10.2 Analytical sensitivity

The RIDA®GENE Parasitic Stool Panel multiplex real-time PCR has a detection limit of ≤ 5 DNA copies per reaction for Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica and Dientamoeba fragilis respectively (see Fig.10, Fig.11, Fig.12, Fig.13, Fig.14).

Fig.10: Dilution series Giardia lamblia (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

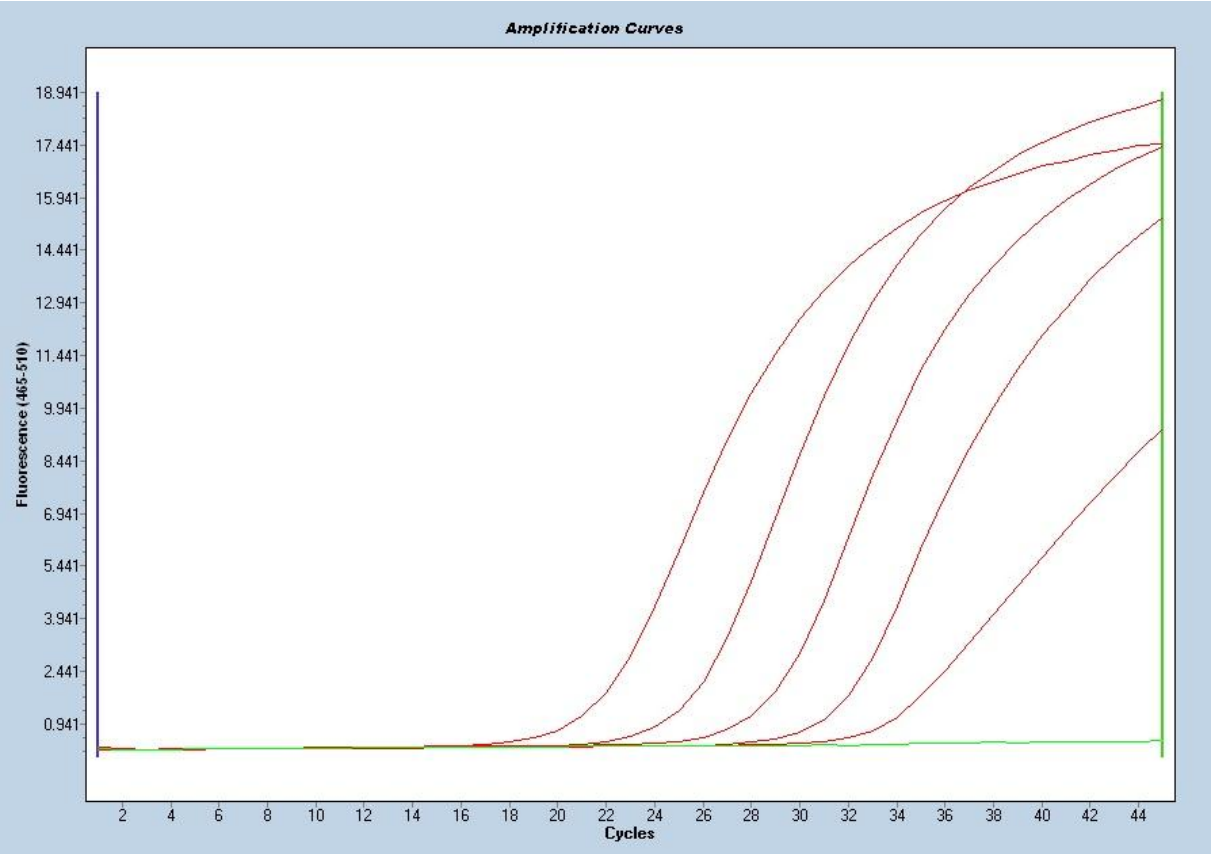


Fig.11: Dilution series Entamoeba histolytica (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

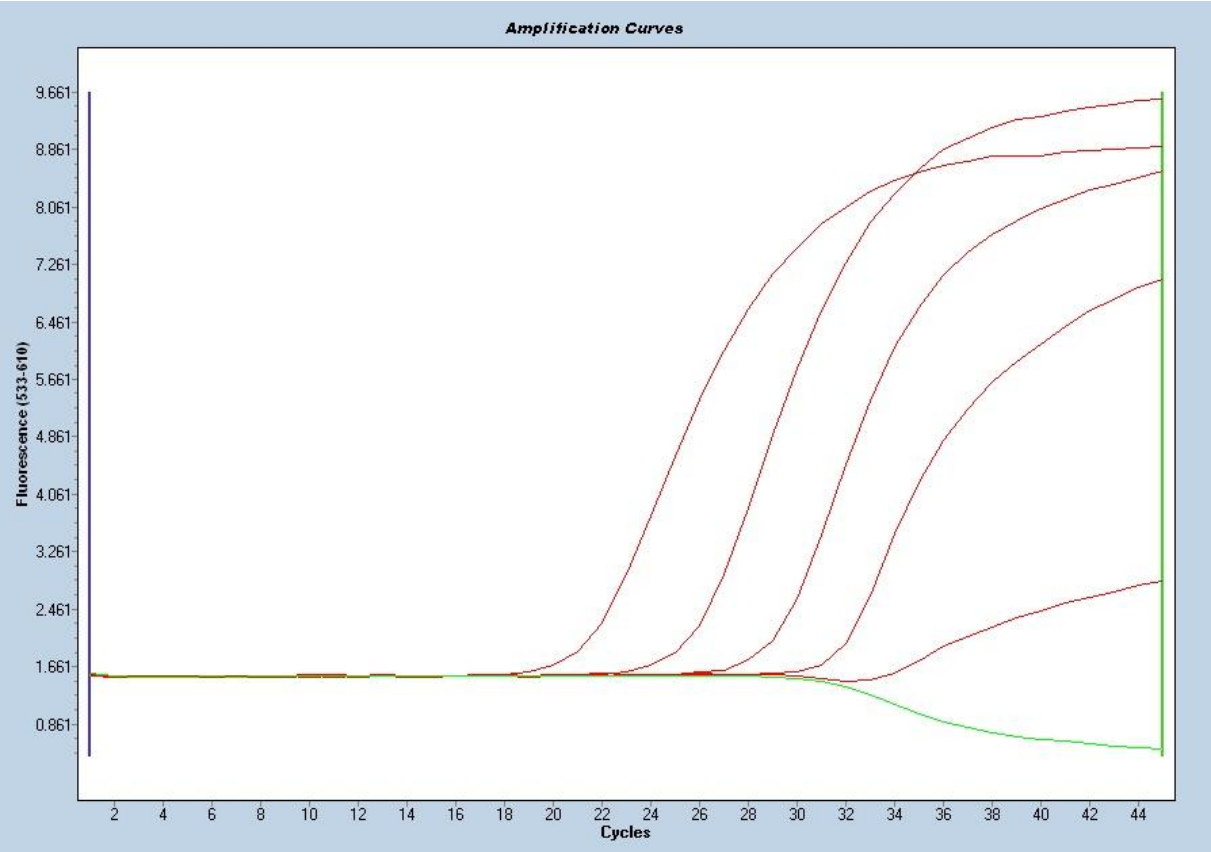


Fig.12: Dilution series Cryptosporidium parvum (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

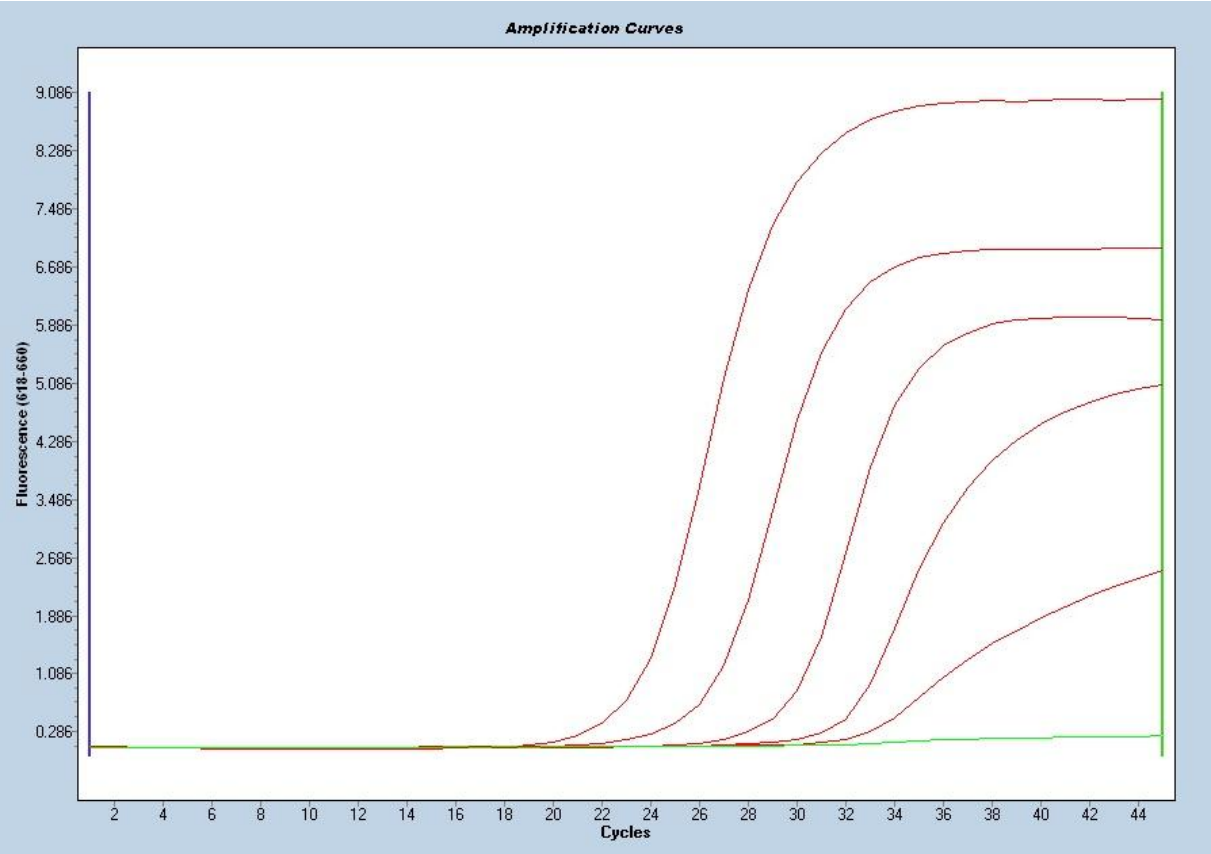


Fig.13: Dilution series Dientamoeba fragilis (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

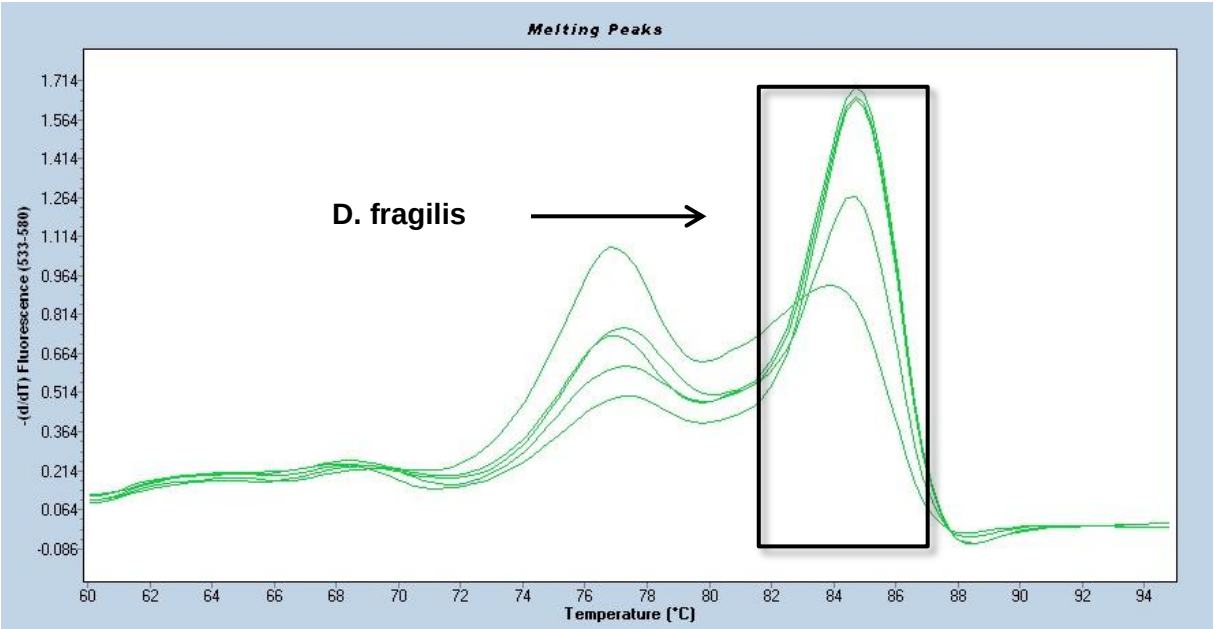
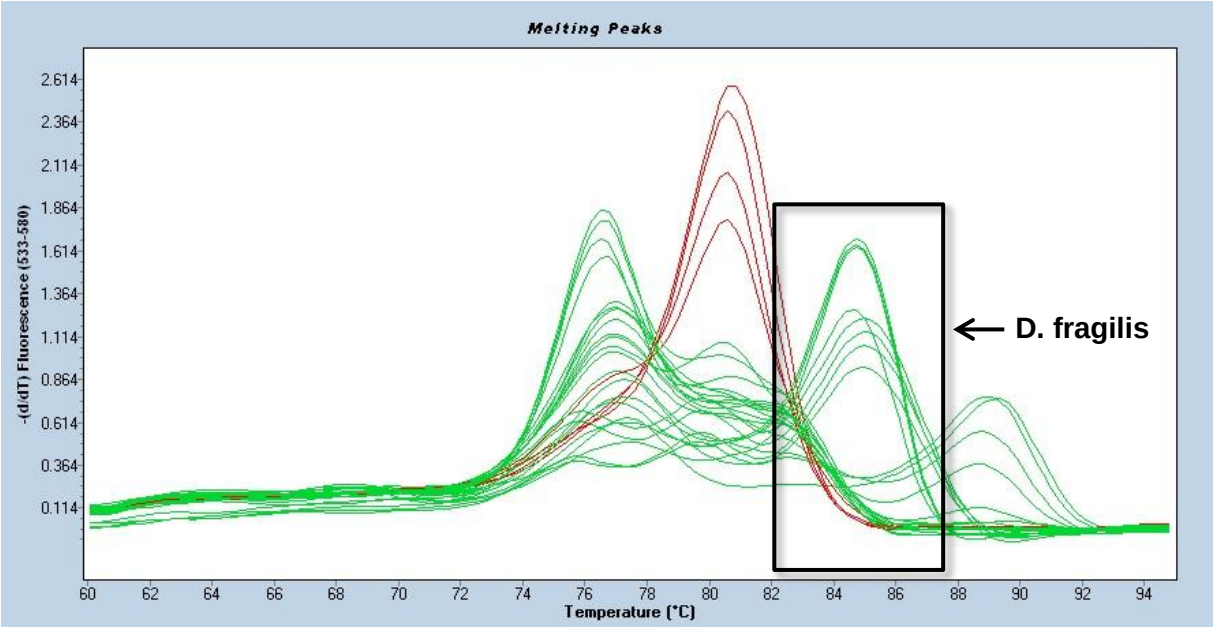


Fig.14: Exemplary melting curve analysis of Dientamoeba fragilis on the LightCycler® 480II



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

10.3 Analytical specificity

The analytical specificity of the RIDA®GENE Parasitic Stool Panel multiplex real-time PCR is specific for Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and Dientamoeba fragilis. No cross-reaction could be detected for the following species (see Tab.13):

Tab.13 Cross-reactivity testing

Arcobacter butzleri	-	*Clostridium perfringens*	-	*Pseudomonas aeruginosa*	-
Adenovirus (Type 40, 41)	-	*Clostridium sordellii*	-	*Salmonella enteritidis*	-
Aeromonas hydrophila	-	Enteropathogenic *E. coli*	-	*Salmonella typhimurium*	-
Bacillus cereus	-	Enterotoxigenic *E. coli*	-	*Serratia liquefaciens*	-
Bacteroides fragilis	-	Enterohemorrhagic *E. coli*	-	*Shigella flexneri*	-
Campylobacter coli	-	*Enterobacter cloacae*	-	*Staphylococcus aureus*	-
Campylobacter jejuni	-	*Enterococcus faecalis*	-	*Staphylococcus epidermidis*	-
Candida albicans	-	*Klebsiella oxytoca*	-	*Vibrio parahaemolyticus*	-
Citrobacter freundii	-	Norovirus	-	*Yersinia enterocolitica*	-
Clostridium difficile	-	*Proteus vulgaris*	-		

11. 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 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 (18s-ITS).

12. Literature

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