RIDA®UNITY Parasitic Stool Panel II

REF UN1725

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1. Intended use

For *in vitro* diagnostic use. The RIDA®UNITY Parasitic Stool Panel II test, performed on the RIDA® UNITY platform, is a multiplex real-time PCR for the direct qualitative detection of *Giardia lamblia*, *Cryptosporidium* spp*.*, and *Entamoeba histolytica* DNA in untreated human stool samples from persons with signs and symptoms of gastrointestinal infection*.*

The RIDA®UNITY Parasitic Stool Panel II test is intended to support the differential diagnosis of parasitic infections (*Giardia lamblia*, *Cryptosporidium* spp*.*, and *Entamoeba histolytica*) in patients with symptoms of gastrointestinal infection in connection with other clinical and laboratory findings.

Negative results do not rule out a parasitic infection (*Giardia lamblia*, *Cryptosporidium* spp*.*, and *Entamoeba histolytica*) and should not be used as the sole basis for diagnosis.

The product is intended for professional use.

2. Summary and explanation of the test

Giardia lamblia, *Cryptosporidium* spp., and *Entamoeba histolytica* are among the most important protozoa that cause diarrhea.
Globally, *Giardia lamblia*, also known as *G. intestinalis* or *G. duodenalis*, causes diarrheal diseases, also called giardiasis. About 500,000 new cases are reported every year(1). The prevalence of giardiasis ranges from 2% to 7% in industrialized countries, and from 20% to 30% in developing countries(2). Infection occurs through the ingestion of cysts in contaminated water or food, or through the fecal-oral route. Acute giardiasis develops after an incubation period of 15 to 30 days and usually lasts 1 to 3 weeks. Symptoms of acute infection are mushy, foul-smelling diarrhea, abdominal pain, bloating, nausea, and vomiting. There are, however, also chronic cases and even asymptomatic carriers(3).
Cryptosporidium parvum is one of several species of the genus *Cryptosporidium*. *C. parvum* and *C. hominis* are some of the most common causes of cryptosporidiosis in humans and are responsible for more than one million deaths a year. However, infections caused by other *Cryptosporidium* spp., such as *C. felis, C. meleagridis, C. canis,* and *C. muris*, can also lead to clinical symptoms(4). In the period from 2009 to 2017, a total of 444 outbreaks of cryptosporidiosis were reported in the USA, resulting in 7465 cases, 287 hospitalizations, and 1 death(5). Infection occurs primarily through the ingestion of oocytes containing sporozoites in contaminated water and food, and through fecal-oral transmission from human to human. Symptoms of cryptosporidiosis range from asymptomatic cases to serious watery diarrhea, as well as abdominal pain, nausea, and fever. The infection dose at which 50% of those exposed are infected, is low at 10-1,000 oocytes. In immunocompetent persons, the symptoms last for a total of about 1 to 2 weeks. Persons with immunodeficiencies, such as HIV patients, may experience a much more severe,

longer, and potentially life-threatening case of cryptosporidiosis[4]. In such cases, the course of the disease depends on the degree of weakness of the immune system[6,7].
Entamoeba histolytica is the only human-pathogenic species in the genus *Entamoeba* and causes infections of the gastrointestinal tract, which are also called amebiasis. Infection occurs via the fecal-oral route through the ingestion of cysts in contaminated water and food, but it can also be spread from person to person[8, 9].
Every year, about 50 million infections occur, resulting in 40,000 to 110,000 deaths[8].
About 90% of infections are asymptomatic. In symptomatic cases, symptoms can develop within 2 to 4 weeks and include diarrhea, abdominal pain, and fever, as well as severe diseases, such as amebic granuloma (ameboma), which is defined as a mass that formed in the colon[9].

3. Test principle

The RIDA®UNITY Parasitic Stool Panel II test is a multiplex real-time PCR for the direct qualitative detection and differentiation of *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* DNA in *human stool samples.*
Processing is fully automated with the RIDA®UNITY System. First, the nucleic acids are extracted using the RIDA®UNITY Universal Extraction Kit and the Internal Control Kit.
The target sequence is detected always in a one-step real-time RT-PCR format (even with DNA assays), that is, reverse transcription (RT) and subsequent PCR are performed in one reaction vial. In the process, the isolated RNA (if present) is transcribed into cDNA with the help of reverse transcriptase. The specific gene fragments for *Giardia lamblia*, *Cryptosporidium* spp., and *Entamoeba histolytica* (ITS1-18S) are then amplified using real-time PCR.
The amplified target sequences are detected using hydrolysis probes that are labeled with a quencher at one end and a fluorescent reporter dye (fluorophore) at the other. The probes hybridize to the amplicon in the presence of a target sequence. During extension, Taq polymerase separates the reporter from the quencher. The reporter emits a fluorescent signal that is detected by the optical unit of a real-time PCR instrument. The fluorescent signal increases with the quantity of formed amplicons.
The RIDA®UNITY Internal Control Kit must be used at the same time to be able to check sample preparation and/or potential PCR inhibition.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.*

Tab. 1: Reagents provided

REF	Reagent	Amount		Lid color
UNZ1725RM	Reaction Mix	1 ×	1935 µL	yellow, ready for use
UNZ1725EM	Enzyme Mix	1 ×	350 µL	red, ready for use
UNZ1725PC	Positive Control	1 ×	200 µL	blue, ready for use
UNZ1725NC	Negative Control	1 ×	450 µL	white, ready for use

* With repeated use and in smaller series, the number of reactions may be reduced.

5. Storage instructions

- Please follow the handling guidelines in Table 2 and store the kit directly after use according to the information specified.
- All reagents must be stored away from light at -16 °C to -28 °C and, if unopened, can be used until the expiration date printed on the label. The quality guarantee is no longer valid after the expiration date.
- All reagents should be carefully thawed prior to use (e.g., in a refrigerator at 2 °C - 8 °C).
- Repeated freezing and thawing up to 8 times does not affect the test properties.

Tab. 2: Storage conditions and information

	Storage temperature	Maximum storage time
unopened	-16 °C to -28 °C	Can be used until the printed expiration date
opened	-16 °C to -28 °C	8 freeze-thaw cycles

6. Reagents required but not provided

The RIDA®UNITY Parasitic Stool Panel II multiplex real-time RT-PCR test is intended exclusively for use with the RIDA®UNITY system. The following products are absolutely required for correct use:

6.1 Reagents

The following reagents are needed to perform the RIDA®UNITY Parasitic Stool Panel II test:

Reagents	Item number
RIDA®UNITY Universal Extraction Kit (R-Biopharm AG)	UN0001
RIDA®UNITY Internal Control Kit (R-Biopharm AG)	UN0010

6.2 Laboratory equipment

The following equipment is needed to perform the RIDA®UNITY Parasitic Stool Panel II test:

Equipment
RIDA®UNITY System; item number: ZUNITY (R-Biopharm AG)
RIDA®UNITY consumables (tips, plates, reaction vials, films). See the instructions for use for the RIDA®UNITY System, ordering information for consumables.
Vortexer
Tabletop centrifuge
Powder-free disposable gloves

External cycler (possible system enhancement)
CFX96™ Dx (Bio-Rad)

The RIDA®UNITY Parasitic Stool Panel II kit can be used in conjunction with other compatible cyclers. Alternative real-time PCR instruments must be verified/validated by the user. Please contact R-Biopharm AG at pcr@r-biopharm.de to check the compatibility.

7. Warnings and precautions for the users

For *in vitro* diagnostic use.
This test must be carried out only by qualified laboratory personnel. The guidelines for working in medical laboratories must be observed.
Always adhere strictly to the instructions for use when carrying out this test.
Do not pipette samples or reagents using your mouth. Avoid contact with broken skin and mucous membranes.
Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test.
Do not smoke, eat, or drink in areas where samples are handled.
Avoid contaminating the samples and components of the kit with microbes and nucleases (DNase/RNase).
Clinical samples must be viewed as potentially infectious and must be disposed of appropriately, like all reagents and materials that come into contact with potentially infectious samples.
Do not exchange or mix the components (Reaction Mix, Enzyme Mix, Positive Control, Negative Control) of one kit lot with the components of another lot.
The test kit can be used for 8 weeks long after first opening (the kit can be reloaded up to 6 times). Do not use the test kit after the expiration date. These specifications are also checked by the RIDA®UNITY System.
Users are responsible for the proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

Further details on the Safety Data Sheet (SDS) can be found under the item number at https://clinical.r-biopharm.com/search/.

For users in the European Union: Report all serious adverse events associated with the product to R-Biopharm AG and the appropriate national authorities.

8. Collection and storage of samples

It is recommended to use fresh sample material to achieve the best performance of the RIDA®UNITY Parasitic Stool Panel II assay.

Avoid repeated freezing and thawing of the sample.

Do not collect the stool samples in transport containers that contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since such substances can interfere with the RIDA®UNITY tests.

It is recommended to produce aliquots of the specimens to avoid repeated thawing and freezing. Frozen specimens should be thawed immediately prior to extraction to prevent degradation of the nucleic acids.

Follow the sample storage instructions in Tables 3 to 5.

Tab. 3: Sample storage - detection of *Giardia lamblia*

Native samples - stool		
20 °C - 25 °C	2 °C - 8 °C	-20 °C / -80 °C
≤ 7 days	≤ 7 days	≤ 6 months

In eluate		
30 °C	2 °C - 8 °C	-20 °C
≤ 24 hours	≤ 36 hours	≤ 7 days

At a storage temperature of -20 °C / -80 °C, repeated freezing/thawing of the samples up to 5 times does not affect the test properties.

At a storage temperature of -20 °C, repeated freezing/thawing of the eluate up to 3 times does not affect the test properties.

Tab. 4: Sample storage - detection of *Entamoeba histolytica*

Native samples - stool			
20 °C - 25 °C	2 °C - 8 °C	-20 °C	-80 °C
-	≤ 7 days	≤ 3 months	≤ 6 months

In eluate		
30 °C	2 °C - 8 °C	-20 °C
≤ 24 hours	≤ 36 hours	≤ 7 days

At a storage temperature of -80 °C, repeated freezing/thawing of the sample up to 3 times does not affect the test properties. Repeated freezing and thawing of samples at -20 °C should be avoided for these analytes.
At a storage temperature of -20 °C, repeated freezing/thawing of the eluate up to 3 times does not affect the test properties.

Tab. 5: Sample storage - detection of *Cryptosporidium* spp.

Native samples - stool			
20 °C - 25 °C	2 °C - 8 °C	-20 °C	-80 °C
< 1 day	≤ 7 days	≤ 3 months	≤ 6 months

In eluate		
30 °C	2 °C - 8 °C	-20 °C
≤ 24 hours	≤ 36 hours	≤ 7 days

The analyte *Cryptosporidium* spp. is stable after repeated freezing/thawing of the stool sample at -80 °C for five freeze-thaw cycles and at -20 °C for three freeze-thaw cycles.
At a storage temperature of -20 °C, repeated freezing/thawing of the eluate up to 3 times does not affect the test properties.

8.1 DNA preparation from stool samples

To isolate DNA from stool samples, use the RIDA®UNITY Universal Extraction Kit. Follow the correct procedures in the instructions for use for the RIDA®UNITY Universal Extraction Kit (Section: Nucleic acid preparation from stool samples; Section: Nucleic acid preparation from culture samples).

9. Test procedure

Place both the samples and the reagents of RIDA®UNITY Parasitic Stool Panel II on the RIDA®UNITY system at the beginning of use.
Beforehand, adequately mix the Reaction Mix, Negative Control, and Positive Control using a vortexer. Do not vortex the Enzyme Mix. Afterward, briefly centrifuge all components.
The PCR tubes for the samples to be examined must be positioned beforehand in the integrated PCR cycler.
Carriers are available for correctly loading the system with reagents and consumables. For the loading process, follow the instructions of the RIDA®UNITY System. Observe the relevant sections in the manual of the RIDA®UNITY System (Section: Performing a run).

The RIDA®UNITY Parasitic Stool Panel II test may be used only in combination with the RIDA®UNITY Internal Control Kit. This allows for early recognition of potential PCR inhibition, verification of reagent integrity, and confirmation of successful nucleic acid extraction. The procedure is described in the instructions for use of the RIDA®UNITY Internal Control Kit (Section: Test procedure).

Automated processing is described in the RIDA®UNITY System manual (Section: Performing a run).

9.1 Device settings

9.1.1 Universal real-time PCR profile

To harmonize the RIDA®UNITY assays, the RIDA®UNITY Parasitic Stool Panel II assay was exclusively verified in the universal profile. This makes it possible to combine DNA and RNA assays with each other. Generally speaking, reverse transcription therefore comes first in the universal profile.

Tab. 6: Universal real-time PCR profile for RIDA®UNITY

Reverse transcription	10 min, 58 °C
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 take place in the same step.

Tab. 7: Universal real-time PCR profile for CFX96™ Dx

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation	15 sec, 95 °C
Annealing/extension	30 sec, 60 °C
Temperature transition rate / ramp rate	Maximum

Note: Annealing and extension take place in the same step.

9.2 Detection channel setting

Tab. 8: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Note
R-Biopharm RIDA®UNITY	*Giardia lamblia*	FAM	SEEK channel Giardia
	Internal Control	HEX	SEEK channel ICD
	Entamoeba histolytica	ROX	SEEK channel Enta
	Cryptosporidium spp.	Cy5	SEEK channel Crypto
Bio-Rad CFX96™ Dx	*Giardia lamblia*	FAM	SEEK channel Giardia
	Internal Control	VIC	SEEK channel ICD
	Entamoeba histolytica	ROX	SEEK channel Enta
	Cryptosporidium spp.	Cy5	SEEK channel Crypto

10. Quality control - indication of instability or expiration of reagents

The samples are evaluated using the RIDA®SEEK analytical software of the RIDA®UNITY System. The Negative Control and Positive Control must show the correct results (see Tab. 9).

The Positive Control is present in a concentration of 10^3 copies/µL. It is used in a total quantity of 5×10^3 copies in every PCR run.

The Negative Control already contains the RIDA®UNITY Internal Control. Since the controls do not contain a template, no signals are to be anticipated in the target channels. Positive signals in the IC channel with which the internal control is detected are essential (see Tab. 9).

Tab.9: A valid PCR run must meet the following conditions:

Sample	Result	IC Ct	Target gene Ct
Positive control	+	N/A*	See Certificate of Analysis
Negative control	-	Ct > 20	0

*In certain circumstances, the IC channel can have a positive signal in the positive control and therefore should not be evaluated.

If the positive control is not within the specified Ct range, but the negative control is valid, all reactions, including the controls, need to be reanalyzed in the PCR.

If the negative control is not negative, but the positive control is valid, all reactions, including the controls, need to be reanalyzed in the PCR.

If the specified values are not met, check the following items before repeating the test:

- Expiration date of the reagents used
- Functionality of the equipment being used
- Correct test procedure

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

Sample evaluation and interpretation are done using the RIDA®UNITY System analytical software, RIDA®SEEK.

There is no current internationally recognized reference method or reference material for standardization. The control materials are metrologically traceable to internal R-Biopharm AG standards based on specific DNA amplicons.

For further information on metrological traceability, please contact R-Biopharm AG.

The specified values, ranges, and further details can be found in the Certificate of Analysis (CoA).

Tab.10: Result interpretation*

Detection of				
Giardia lamblia	***Entamoeba histolytica***	***Cryptosporidium spp.***	**ICD**	**Result**
+	-	-	**+/-**	***Giardia lamblia* detectable**
-	**+**	-	**+/-**	***Entamoeba histolytica* detectable**
-	-	**+**	**+/-**	***Cryptosporidium* spp. detectable**
+	**+**	-	**+/-**	***Giardia lamblia, Entamoeba histolytica* detectable**
+	-	**+**	**+/-**	***Giardia lamblia*, *Cryptosporidium* spp. detectable**
-	**+**	**+**	**+/-**	***Entamoeba histolytica, Cryptosporidium* spp. detectable**
+	**+**	**+**	**+/-**	***Giardia lamblia, Entamoeba histolytica*, and *Cryptosporidium* spp. detectable**
-	-	-	**+**	**Target genes not detectable**
-	-	-	**-**	**Invalid**

*+ = positive
- = negative

A sample is positive if the sample DNA and the Internal Control show an amplification signal in the detection system.

A sample is also positive if the sample DNA shows an amplification signal, but no amplification signal can be seen for the Internal Control in the detection system. Detecting the Internal Control is not necessary in this case because high amplicon concentrations can result in a weak or absent signal of the Internal Control.

A sample is negative if the sample DNA does not show an amplification signal, but an amplification signal is visible for the Internal Control in the detection system. Inhibition of the PCR reaction and prior extraction can be ruled out by the detection of the Internal Control.

A sample is invalid if the sample DNA and the Internal Control do not show an amplification signal in the detection system. There are inhibitors in the sample, or an error occurred during the extraction process.

12. Limitations of the method

1. The RIDA®UNITY Parasitic Stool Panel II test detects *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* DNA in untreated human stool samples. A connection between the level of the determined Ct value and the occurrence of severe clinical symptoms cannot be derived from this. The results obtained must always be interpreted in combination with the complete clinical symptoms.
2. The diagnosis should not be based on the result of the molecular biological analysis alone, but should always take the patient's medical history and symptoms into account.
3. This test is approved only for automated processing using the RIDA®UNITY System.
4. This test is verified only for stool samples.
5. When using the culture matrix, do not transfer the agar medium into the PCR reaction because this can lead to potential interference.
6. Improper sampling, transport, storage, and handling, or a pathogen load below the test's analytical sensitivity can lead to false-negative results.
7. The presence of PCR inhibitors can lead to false-negative or invalid results.
8. As with all PCR-based *in vitro* diagnostic tests, extremely low concentrations of the target sequences, which are under the limit of detection (LoD 95%), can be detected. The results obtained are not always reproducible.
9. Mutations or polymorphisms in the primer or probe binding sites can interfere with the detection of new or unknown variants and can lead to false negative results using RIDA®UNITY Parasitic Stool Panel II.
10. A positive test result does not necessarily indicate the presence of viable organisms. A positive test indicates that the presence of specific gene fragments for *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica* (ITS1-18S).
11. This assay should be performed in compliance with the regulation on good laboratory practice (GLP). Users must precisely follow the manufacturer's instructions when performing the test.

13. Performance characteristics

13.1 Clinical performance characteristics

The RIDA®UNITY Parasitic Stool Panel II multiplex real-time PCR was compared in an external laboratory with a CE-marked reference test based on 278 stool samples from patients with symptoms of gastrointestinal infection.

The results show high sensitivity and specificity in the detection of *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidum spp.* using the RIDA®UNITY Parasitic Stool Panel II kit.

Tab. 11: Detection of *Giardia lamblia*

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Parasitic Stool Panel II-*Giardia lamblia*	Positive	77	1	78
	Negative	8	192	200
	Total	85	193	278

Relative sensitivity (95% CI)	90.6% (82.3% - 95.8%)
Relative specificity (95% CI)	99.5% (97.1% - 100%)

Tab. 12: Detection of *Entamoeba histolytica*

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Parasitic Stool Panel II-*Entamoeba histolytica*	Positive	70	1	71
	Negative	7	200	207
	Total	77	201	278

Relative sensitivity (95% CI)	90.9% (82.2% - 96.3%)
Relative specificity (95% CI)	99.5% (97.3% - 100%)

Tab. 13: Detection of *Cryptosporidium spp.*

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Parasitic Stool Panel II-*Cryptosporidium spp.*	Positive	74	1	75
	Negative	1	202	203
	Total	75	203	278

Relative sensitivity (95% CI)	98.7% (92.8% - 100%)
Relative specificity (95% CI)	99.5% (97.3% - 100%)

13.2 Analytical performance characteristics

13.2.1 Detection limit (LoD 95%)

A positive control sample (negative stool samples, spiked, or using a culture sample) was measured in five dilution steps (in 0.25-log steps) for each target with 20 replicates per step in one lot to determine the LoD (limit of detection). This was followed by a probit analysis. Next, the calculated LoD was confirmed with 20 replicates per target for the calculated dilution step/concentration.
The following strains were used for testing:

- *Cryptosporidium* spp. from pooled positive clinical stool samples (MF171865 and MF172060); initial concentration not known; Ct-range 24-25
- *Giardia lamblia* from pooled positive clinical stool samples (34544905 and 34504696); initial concentration not known; Ct-range 29-30
- *Entamoeba histolytica* CF (ATCC® 30015™); initial concentration 2.7 x 10^6 cells/mL

The following detection limit were determined for the detection of *Cryptosporidium* spp., *Giardia lamblia* and *Entamoeba histolytica* DNA using the RIDA®UNITY Parasitic Stool Panel II assay on the UNITY system
The results of these measurements are shown in Table 14.

Tab. 14: Limit of detection results of the RIDA®UNITY Parasitic Stool Panel II test for the parameters *Cryptosporidium* spp., *Giardia lamblia*, and *Entamoeba histolytica*.

	Giardia lamblia	***Entamoeba histolytica***	***Cryptosporidium* spp.**
LoD	1.68E-2 dilution factor** (Ct 35.34 ± 0.72)	9.53 cells/mL	1.98E-2 dilution factor* (Ct 36.18 ± 2.05)

* Relative dilution of the stock concentration. Clinically positive sample with initial concentration Ct 24-25

** Relative dilution of the stock concentration. Clinically positive sample with initial concentration Ct 29-30

The LoD for *Giardia lamblia* in stool samples was identified at a dilution factor of 1.68E-2.

The LoD for *Entamoeba histolytica* in stool samples was identified at 9.53 cells/mL.

The LoD for *Cryptosporidium* spp in stool samples was identified at a dilution factor of 1.98E-2.

For the enhanced workflow using the CFX96™ Dx, these LoD values were confirmed under the assumption that we stay in a 2-3-fold LoD range.

13.2.2 Analytical specificity

Interfering substances

The presence of PCR inhibitors and interfering substances can lead to false-negative or invalid results. Therefore, the effects of various substances that may exist given their widespread use for gastrointestinal infections or widespread occurrence in the corresponding specimens were investigated.
Substances that could potentially significantly influence the test results were examined initially at high concentrations (triple the daily dose or simulation of the worst case) in an interference screen.
No interference was found for the substances listed in Table 15.

Tab. 15: Potentially interfering substances

Potentially interfering substance	Concentration
Azithromycin-ratiopharm® 500 mg film-coated tablets (azithromycin)	0.75% [w/v]
Cologran® liquid sweetener (saccharin + cyclamate)	1.3% [w/v]
Human blood	5% [v/v]
Charcoal tablets 250mg (charcoal)	6% [w/v]
Mucins	5% [w/v]
Stearic/palmitic acid	40% [w/v]

Cross-reactions

Various organisms (bacteria, parasites, fungi, and viruses) that can be commonly found in the stool matrix were investigated. The microorganisms to be investigated for this assay were chosen because they either naturally occur in stool samples, or they cause corresponding symptoms as gastrointestinal pathogens. Bacterial cultures (between 10^6 and 10^9 CFU/mL), fungal or viral cultures, supernatants of viral cultures, isolates, and LGC standards of the respective organism were used for analyses.

The RIDA®UNITY Parasitic Stool Panel II multiplex real-time PCR is specific for *Cryptosporidium spp., Giardia lamblia* and *Entamoeba histolytica.*
No cross-reactivities with the following species were detected (see Tab. 16):

Tab. 16: Potentially cross-reactive organisms.

Organism	Test result*		
	Giardia lamblia	***Entamoeba histolytica***	***Cryptosporidium* spp.**
Adenovirus 40, human, strain Dugan	-	-	-
Adenovirus 41, human, strain Tak	-	-	-
Astrovirus	-	-	-
Bacillus cereus	-	-	-
Bacteroides fragilis	-	-	-
Campylobacter coli	-	-	-
Campylobacter fetus subsp. *fetus*	-	-	-
Campylobacter jejuni	-	-	-
Campylobacter lari subsp. *lari*	-	-	-
Campylobacter upsaliensis	-	-	-
Candida albicans	-	-	-
Citrobacter freundii	-	-	-
Clostridium bifermentans	-	-	-
Clostridium difficile	-	-	-
Clostridium novyi	-	-	-
Clostridium perfringens	-	-	-
Clostridium septicum	-	-	-
Clostridium sordellii	-	-	-
Clostridium sporogenes	-	-	-
Coxsackievirus B4	-	-	-

E. coli (O157:H7)	-	-	-
E. coli (O26:H-)	-	-	-
E. coli (O6)	-	-	-
Echovirus type 11	-	-	-
Entamoeba dispar	-	-	-
Enterobacter cloacae	-	-	-
Enterococcus faecalis	-	-	-
Enterovirus type 71	-	-	-
Klebsiella oxytoca	-	-	-
Norovirus GI	-	-	-
Norovirus GII	-	-	-
Proteus vulgaris	-	-	-
Pseudomonas aeruginosa	-	-	-
Rotavirus	-	-	-
Salmonella enteritidis	-	-	-
Salmonella typhimurium	-	-	-
Serratia liquefaciens	-	-	-
Shigella flexneri	-	-	-
Staphylococcus aureus	-	-	-
Staphylococcus epidermidis	-	-	-
Trichomonas vaginalis	-	-	-
Trichomonas vaginalis	-	-	-
Trichomonas vaginalis	-	-	-
Vibrio parahaemolyticus	-	-	-
Yersinia enterocolitica	-	-	-

* - = negative

13.2.3 Precision

The precision of the RIDA®UNITY Parasitic Stool Panel II real-time PCR test was determined for the following levels of consideration.

Intra-assay precision: Determination of 5 control samples using 20 replicates each on the RIDA®UNITY under identical conditions.

Inter-assay precision: Determination of 5 control samples in 20 runs in duplicate on 10 workdays (2 runs per day) performed by different technicians under reproducible conditions.

Testing for *intra*- and *inter*-assay precision was carried out using three different lots.

The coefficients of variation obtained for each measurement using the RIDA®UNITY Parasitic Stool Panel II real-time PCR test on the RIDA®UNITY and the CFX96™ Dx were 5.06 %.

Tab. 17: Results of the precision of the RIDA®UNITY Parasitic Stool Panel II test for *Giardia lamblia* from stool samples (RIDA®UNITY system).

Ct mean value/CV		***Intra*-assay**			***Inter*-assay**			***Inter*-lot**
		Kit lot 1	**Kit lot 2**	**Kit lot 3**	**Kit lot 1**	**Kit lot 2**	**Kit lot 3**	**Kit lots 1 - 3**
1	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	Ct	34,3	34,5	34,0	35,0	34,8	35,2	35,0
	CV (%)	2,40 %	1,73 %	1,60 %	2,38 %	2,22 %	2,23 %	2,31 %
3	Ct	34,9	35,2	34,2	35,5	35,2	35,3	35,3
	CV (%)	2,14 %	1,88 %	2,28 %	3,05 %	2,29 %	2,50 %	2,63 %
4	Ct	33,1	33,4	33,2	33,0	33,0	33,0	33,0
	CV (%)	1,67 %	1,60 %	1,45 %	1,96 %	1,93 %	2,01 %	1,97 %
5	Ct	33,3	33,7	33,6	33,8	33,6	33,6	33,0
	CV (%)	1,73 %	1,74 %	1,46 %	1,82 %	1,91 %	1,81 %	1,97 %

Tab. 18: Results of the precision of the RIDA®UNITY Parasitic Stool Panel II test for *Giardia lamblia* from stool samples (CFX96™ Dx).

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1 - 3
1	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	Ct	35,0	35,5	34,9	35,3	35,1	35,2	35,2
	CV (%)	2,76 %	1,86 %	2,51 %	1,97 %	2,26 %	2,27 %	2,22 %
3	Ct	35,8	35,8	35,3	35,5	35,3	35,2	35,3
	CV (%)	2,83 %	2,52 %	2,32 %	2,56 %	2,41 %	2,57 %	2,47 %
4	Ct	33,2	33,2	33,3	32,9	32,9	32,8	32,9
	CV (%)	2,08 %	2,18 %	2,16 %	2,10 %	1,54 %	1,64 %	1,78 %
5	Ct	34,1	34,7	33,7	33,7	33,6	33,6	33,6
	CV (%)	1,81 %	1,88 %	2,39 %	1,77 %	1,90 %	1,79 %	1,82 %

Tab. 19: Results of the precision of the RIDA®UNITY Parasitic Stool Panel II test for *Entamoeba histolytica* from stool samples (RIDA®UNITY system).

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1 - 3
1	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	Ct	31,9	32,2	32,1	34,5	34,3	34,5	34,4
	CV (%)	5,46 %	1,30 %	1,29 %	3,33 %	2,86 %	3,68 %	3,31 %
3	Ct	31,5	31,9	30,4	33,6	33,5	33,6	33,6
	CV (%)	2,14 %	1,89 %	3,16 %	2,81 %	3,24 %	3,53 %	3,21 %
4	Ct	29,7	30,4	30,0	30,7	30,7	30,8	30,7
	CV (%)	0,85 %	0,87 %	1,34 %	1,94 %	1,69 %	2,02 %	1,89 %
5	Ct	31,3	32,8	32,6	31,9	31,8	31,9	31,9
	CV (%)	3,21 %	4,73 %	2,47 %	2,60 %	2,23 %	2,71 %	2,52 %

Tab. 20: Results of the precision of the RIDA®UNITY Parasitic Stool Panel II test for *Entamoeba histolytica* from stool samples (CFX96™ Dx).

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1 - 3
1	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	Ct	32,4	32,8	32,2	33,9	33,8	33,7	33,8
	CV (%)	2,12 %	2,21 %	2,12 %	2,63 %	3,02 %	3,00 %	2,89 %
3	Ct	31,7	31,7	31,3	33,0	32,9	32,7	32,9
	CV (%)	2,02 %	1,71 %	1,95 %	3,16 %	2,55 %	2,71 %	2,82 %
4	Ct	30,0	29,6	29,5	30,4	30,5	30,4	30,5
	CV (%)	0,60 %	0,76 %	0,72 %	1,98 %	1,63 %	1,79 %	1,81 %
5	Ct	32,6	33,3	32,0	31,3	31,4	31,3	31,3
	CV (%)	2,09 %	3,68 %	2,23 %	2,01 %	1,78 %	1,58 %	1,80 %

Tab. 21: Results of the precision of the RIDA®UNITY Parasitic Stool Panel II test for *Cryptosporidium* spp. from stool samples (RIDA®UNITY system).

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1 - 3
1	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	Ct	31,5	32,9	33,3	32,0	34,7	34,3	33,7
	CV (%)	2,25 %	1,61 %	1,41 %	1,83 %	3,15 %	2,74 %	5,06 %
3	Ct	33,8	35,0	34,4	32,8	35,4	35,0	34,4
	CV (%)	2,92 %	2,31 %	1,95 %	2,42 %	3,27 %	3,40 %	5,03 %
4	Ct	27,3	28,1	28,3	26,6	28,8	28,6	28,0
	CV (%)	0,70 %	1,15 %	0,90 %	1,41 %	2,86 %	2,60 %	4,94 %
5	Ct	28,5	29,7	29,5	27,2	29,4	29,3	28,6
	CV (%)	0,70 %	1,04 %	0,94 %	1,57 %	2,82 %	2,47 %	4,84 %

Tab. 22: Results of the precision of the RIDA®UNITY Parasitic Stool Panel II test for *Cryptosporidium spp.* from stool samples (CFX96™ Dx).

Ct mean value/CV		*Intra*-assay			*Inter*-assay			*Inter*-lot
		Kit lot 1	Kit lot 2	Kit lot 3	Kit lot 1	Kit lot 2	Kit lot 3	Kit lots 1 - 3
1	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	Ct	30,5	32,3	31,8	32,4	34,2	34,3	33,6
	CV (%)	1,81 %	2,07 %	1,23 %	2,93 %	2,97 %	2,58 %	4,16 %
3	Ct	31,8	32,9	34,6	33,5	34,6	34,7	34,3
	CV (%)	3,68 %	1,53 %	1,84 %	3,38 %	2,90 %	2,75 %	3,55 %
4	Ct	27,0	28,1	28,2	27,2	28,6	28,6	28,1
	CV (%)	0,78 %	1,02 %	0,93 %	2,20 %	2,87 %	2,21 %	3,74 %
5	Ct	28,3	29,4	29,4	27,7	29,1	29,0	28,6
	CV (%)	0,83 %	0,72 %	0,91 %	1,64 %	2,75 %	1,91 %	3,59 %

13.2.4 Analytical reactivity

The reactivity of the RIDA®UNITY Parasitic Stool Panel II multiplex real-time PCR test was examined on a defined panel of parasites (see Table 23).

Tab. 23: Analytical reactivity testing

Strain	Result*		
	Giardia lamblia	***Entamoeba histolytica***	***Cryptosporidium* spp.**
*Giardia lamblia***	**+**	**-**	-
*Giardia intestinalis*** Portland 1	**+**	**-**	-
*Giardia intestinalis*** WB Clone C6	**+**	-	-
Entamoeba histolytica	-	**+**	-
Cryptosporidium parvum (isolate)	-	-	**+**
Cryptosporidium parvum (artificial DNA)	-	-	**+**
Cryptosporidium muris	-	-	**+**
Cryptosporidium hominis	-	-	**+**

*+ = positive (at least 2 of 3 replicates positive)
- = negative
** *Giardia intestinalis* and *Giardia lamblia* are the same organism.

14. Version history

Version number	Section and designation
2022-09-13	Release version

15. Explanation of symbols

General symbols

	For *in vitro* diagnostic use
	Follow instructions for use
	Batch number
	Use before
	Storage temperature
	Item number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Reaction Mix	Reaction Mix
Enzyme Mix	Enzyme Mix
Negative Control	Negative control
Positive Control	Positive control

16. References

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