RIDA®UNITY Viral Stool Panel II

REF UN1325

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1. Intended use

For *in vitro* diagnostic use. The RIDA®UNITY Viral Stool Panel II test, performed on the RIDA®UNITY platform, is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of rotavirus RNA, astrovirus RNA, and adenovirus 40/41 DNA in untreated stool samples from persons with signs and symptoms of acute gastroenteritis.

The RIDA®UNITY Viral Stool Panel II test is intended to support the differential diagnosis of viral infections (rotavirus, adenovirus 40/41, and astrovirus) in patients with symptoms of gastroenteritis in connection with other clinical and laboratory findings.
Negative results do not rule out infection with rotavirus, adenovirus 40/41 or astrovirus, 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

Diarrheal diseases are a significant health problem and cause approximately 1.7 billion cases per year in children worldwide. According to the World Health Organization (WHO), these diseases are the second leading cause of death with approximately 525,000 deaths per year in children under 5 years, especially in developing countries[1]. Rotaviruses, adenoviruses, and astroviruses number among the causes of diarrheal disease [2, 7, 9].

Rotaviruses are one of the leading causes of severe gastroenteritis, especially among children under 5 years. Even though a rotavirus vaccine is available, rotaviruses still cause more than 200,000 deaths a year worldwide.[2, 3] Rotaviruses are nonenveloped, double-stranded RNA viruses with complex architecture consisting of three concentric capsids, and belong to the family *Reoviridae*. Rotaviruses are divided into a total of 10 different species (A-J) based on the sequence and antigen differences of viral protein 6 (VP6). Rotavirus A has proven to be the most common group responsible for severe acute gastroenteritis in toddlers.[4] Rotaviruses are transmitted primarily via the fecal-oral route, but can also be transmitted through contaminated hands and less frequently through food and water. Typical symptoms of rotavirus infections are diarrhea and vomiting, which usually result in dehydration. These symptoms can result in hospitalizations and, if not treated, in death.[2]

Adenoviruses are non-enveloped, icosahedral, double-stranded DNA (dsDNA) viruses and belong to the family *Adenoviridae.*[5, 6] Currently, more than 84 genotypes, including all previously characterized the serotypes, have been identified and categorized into seven different species (A-G)[5]. In particular, species A to F circulate worldwide and can cause periodic outbreaks of infections[7]. Adenoviruses A, F and G cause infections of the gastrointestinal tract. Serotypes 40 and 41 of species F are most widespread and are clearly associated with diarrhea

in children[5, 6, 8]. The skin transmission path of adenoviruses is through indirect contact with surfaces.[8].

Astroviruses are one of the primary causes of acute gastroenteritis with a high burden of disease among children, older persons and the immunosuppressed. They are small, single-strand RNA (ssRNA) viruses that belong to the *Astroviridae* family. Eight different genotypes have currently been identified, and infections caused by astrovirus 1 are the most widespread worldwide. The symptoms of an astrovirus infection last around 2 to 4 days and are characterized by watery diarrhea, headaches, stomach aches, loss of appetite, and infrequently fever. In healthy children and adults, such infections are frequently free of symptoms, although infections in immunosuppressed individuals are clinically relevant[9].

3. Test principle

RIDA®UNITY Viral Stool Panel II Assay is a multiplex real-time PCR for the direct qualitative detection of rotavirus RNA, astrovirus RNA, and adenovirus 40/41 DNA from untreated 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 always detected 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 rotavirus (NSP3), astrovirus (capsid protein; CAP), and adenovirus 40/41 (hexon) 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
UNZ1325RM	Reaction Mix	1 ×	1935 µL	yellow, ready for use
UNZ1325EM	Enzyme Mix	1 ×	350 µL	red, ready for use
UNZ1325PC	Positive Control	1 ×	200 µL	blue, ready for use
UNZ1325NC	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 thawing/freezing cycles, however only useful through the printed expiration date

6. Reagents required but not provided

The RIDA®UNITY Viral 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 Viral 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 Viral 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 Viral 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 compatibility.

7. Warnings and precautions for 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 Viral Stool Panel II assay.

Avoid repeated freezing and thawing 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 rotavirus

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

In eluate (from stool)		
30°C	2°C - 8°C	-20°C
≤ 24 hours	≤ 36 hours	< 1 month

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

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

Tab. 4: Sample storage - detection of astrovirus

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

In eluate (from stool)		
30°C	2°C - 8°C	-20°C
≤ 24 hours	≤ 36 hours	< 1 month

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

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

Tab. 5: Sample storage - detection of adenovirus 40/41

Native samples - stool			
20°C - 25°C	2°C - 8°C	-20°C	-80 °C
≤ 6 days	≤ 6 days	≤ 5 months	≤ 6 months

In eluate (from stool)		
30°C	2°C - 8°C	-20°C
≤ 24 hours	≤ 36 hours	< 1 month

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

At a storage temperature of - 80°C, repeated freezing/thawing of the stool sample up to 3 times does not affect the test properties.

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

8.1 RNA/DNA preparation from stool samples

To isolate RNA/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).

9. Test procedure

Place both the samples and the reagents of RIDA®UNITY Viral 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 Viral 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 A Viral 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 Annealing/extension	10 sec, 95°C 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 Annealing/extension	15 sec, 95°C 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	Rotavirus	FAM	SEEK channel Rota
	Internal Control	HEX	SEEK channel ICR
	Astrovirus	ROX	SEEK channel Astro
	Adenovirus 40/41	Cy5	SEEK channel Adeno
Bio-Rad CFX96™ Dx	Rotavirus	FAM	SEEK channel Rota
	Internal Control	VIC	SEEK channel ICR
	Astrovirus	ROX	SEEK channel Astro
	Adenovirus 40/41	Cy5	SEEK channel Adeno

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. 8).

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 RNA- amplicons and 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				
Rotavirus	**Astrovirus**	**Adenovirus 40/41**	**ICR**	**Result**
+	-	-	**+/-**	**Rotavirus detectable**
-	**+**	-	**+/-**	**Astrovirus detectable**
-	-	**+**	**+/-**	**Adenovirus 40/41 detectable**
+	**+**	-	**+/-**	**Rotavirus and astrovirus detectable**
+	-	**+**	**+/-**	**Rotavirus and adenovirus 40/41 detectable**
	+	**+**	**+/-**	**Astrovirus and adenovirus 40/41 detectable**
+	**+**	**+**	**+/-**	**Rotavirus, astrovirus, and adenovirus 40/41 detectable**
-	-	-	**+**	**Target genes not detectable**
-	-	-	**-**	**Invalid**

*+= positive
- = negative

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

A sample is also positive if the sample DNA/RNA 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/RNA 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/RNA and the Internal Control do not show amplification 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 Viral Stool Panel II Test detects rotavirus RNA, astrovirus RNA and adenovirus 40/41 DNA from untreated human stool samples. No connection between the level of the determined Ct value and the occurrence of severe clinical symptoms can 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. Improper sampling, transport, storage, and handling, or a pathogen load below the test's analytical sensitivity can lead to false negative results.
6. The presence of PCR inhibitors can lead to false-negative or invalid results.
7. 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.
8. 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 Viral Stool Panel II.
9. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target genes for rotavirus (NSP3), astrovirus (capsid protein, CAP), and adenovirus 40/41 (hexon) are present.
10. 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 Viral Stool Panel II multiplex real-time PCR was compared in an external laboratory with a CE-marked reference test using 356 stool samples. Given the high rate of co-infections in the reference test, a second CE-marked reference test was used to determine the final data.

The results indicate a high sensitivity and specificity in detecting rotavirus RNA, astrovirus RNA and adenovirus 40/41 DNA using the RIDA®UNITY Viral Stool Panel II Kits.

Tab. 11: Detection of rotavirus

		Reference Tests		
		Positive	Negative	Total
RIDA®UNITY Viral Stool Panel II - Rotavirus	Positive	98	7	105
	Negative	12	215	227
	Total	110	222	332

Relative sensitivity (95% CI)	89.1% (81.7% - 94.2%)
Relative specificity (95% CI)	96.8 % (93.6% - 98.7%)

Tab. 12: Detection of astrovirus

		Reference Tests		
		Positive	Negative	Total
RIDA®UNITY Viral Stool Panel II - Astrovirus	Positive	75	24	99
	Negative	2	231	233
	Total	77	255	332

Relative sensitivity (95% CI)	97.4 % (91.0 % - 99.7%)
Relative specificity (95% CI)	90.6 % (86.3 % - 93.9%)

Tab. 13: Detection of adenovirus 40/41

		Reference Tests		
		Positive	Negative	Total
RIDA®UNITY Viral Stool Panel II - Adenovirus 40/41	Positive	99	27	126
	Negative	7	199	206
	Total	106	226	332

Relative sensitivity (95% CI)	93.4 % (86.9% - 97.3%)
Relative specificity (95% CI)	88.1 % (83.1 % - 92.0 %)

13.2 Analytical performance characteristics

13.2.1 Detection limit (LoD 95 %)

A positive control sample (negative stool samples, spiked) 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:

- Rotavirus Infectious Culture Fluid by ZeptoMetrix (#0810041CF), Initial concentration 1.70 x 10^5 $TCID_{50}$/ml
- Adenovirus Type 40 Infectious Culture Fluid by ZeptoMetrix (#0810084CF), Initial concentration 1.26 x 10^6 $TCID_{50}$/ml
- Adenovirus Type 41 Infectious Culture Fluid by ZeptoMetrix (#0810085CF) Initial concentration 4.57 x 10^6 $TCID_{50}$/ml
- Astrovirus: clinical stool sample; initial concentration unknown (Ct value: 13)

The following detection limits were determined for detecting rotavirus RNA, astrovirus RNA and adenovirus 40/41 DNA using RIDA®UNITY Viral Stool Panel II Assays with the RIDA®UNITY System.
The results of these measurements are shown in Table 14.

Tab. 14: Results of the detection limit of the RIDA®UNITY Viral Stool Panel II Tests for the targets rotavirus, astrovirus and adenovirus 40/41.

	Rotavirus	Astrovirus	Adenovirus 40	Adenovirus 41
LoD	454 TCID*$_{50}$/mL	3.94E-08 dilution factor** (Ct range 35.72 ± 0.54)	6.5 $TCID_{50}$/mL	27.0 $TCID_{50}$/mL

*TCID: Tissue culture infectious dose

** Relative dilution of the stock concentration. Clinically positive sample with initial concentration Ct 13

The LoD for Rotavirus in stool samples was determined at454 $TCID_{50}$/mL.

The LoD for astrovirus in stool samples was identified at a dilution factor of 3.94E-08.

The LoD for adenovirus 40 in stool samples was determined at 6.5 $TCID_{50}$/mL.

The LoD for adenovirus 41 in stool samples was determined at 27.0 $TCID_{50}$/mL.

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 first examined 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]
Barium sulphate	18.5% [v/v]
Cologran® liquid sweetener (saccharin + cyclamate)	1.3% [w/v]
Human blood	5% [v/v]
Charcoal tablets 250 mg (charcoal)	6 % [w/v]
Mucins	5 % [w/v]
Stearic/palmitic acid	40 % [w/v]

Cross-reactions

Various organisms (bacteria, parasites, 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 Viral Stool Panel II multiplex real-time PCR is specific for rotavirus, astrovirus and adenovirus 40/41. No cross-reactivities with the following species were detected (see Tab. 16):

Tab. 16: Potentially cross-reactive organisms.

Organism	Test result*		
	Rotavirus	Astrovirus	Adenovirus 40/41
Adenovirus type: 11 cell line: KB	-	-	-
Adenovirus type: 31 cell line: KB	-	-	-
Adenovirus type: 37 cell line: KB	-	-	-
Adenovirus type: 4 cell line: KB	-	-	-
Adenovirus type: 5 stock strain: N/A	-	-	-
Adenovirus type: 7A cell line: KB	-	-	-
Aeromonas hydrophila	-	-	-
Arcobacter butzleri	-	-	-
Bacillus cereus	-	-	-
Bacteroides fragilis	-	-	-
Campylobacter fetus subsp. *fetus*	-	-	-
Campylobacter lari subsp. *lari*	-	-	-
Campylobacter upsaliensis	-	-	-
Campylobacter coli	-	-	-
Campylobacter jejuni	-	-	-
Candida albicans	-	-	-
Citrobacter freundii	-	-	-
Clostridium bifermentans	-	-	-
Clostridium difficile	-	-	-
Clostridium novyi	-	-	-
Clostridium perfringens	-	-	-
Clostridium septicum	-	-	-

Clostridium sordellii	-	-	-
Clostridium sporogenes	-	-	-
Cryptosporidium muris	-	-	-
Cryptosporidium parvum	-	-	-
E. coli (O157:H7)	-	-	-
E. coli (O26:H-)	-	-	-
E. coli (O6)	-	-	-
Entamoeba histolytica	-	-	-
Enterobacter cloacae	-	-	-
Enterococcus faecalis	-	-	-
*Giardia intestinalis*** Portland 1	-	-	-
*Giardia intestinalis*** WB Clone C6	-	-	-
*Giardia lamblia***	-	-	-
Klebsiella oxytoca	-	-	-
Norovirus GII	-	-	-
Norovirus GI (633)	-	-	-
Norovirus GI (636)	-	-	-
Norovirus GI (637)	-	-	-
Norovirus GI (639)	-	-	-
Proteus vulgaris	-	-	-
Pseudomonas aeruginosa	-	-	-
Salmonella enterica (serovar Enteritidis)	-	-	-
Salmonella enterica (serovar Typhimurium)	-	-	-
Serratia liquefaciens	-	-	-
Shigella flexneri	-	-	-
Staphylococcus aureus	-	-	-
Staphylococcus epidermidis	-	-	-
Vibrio parahaemolyticus	-	-	-
Yersinia enterocolitica	-	-	-

* - = negative

** *Giardia intestinalis* and *Giardia lamblia* are the same organism.

13.2.3 Precision

The precision of the RIDA®UNITY Viral 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 Viral Stool Panel II real-time PCR test on the RIDA®UNITY and the CFX96™ Dx were 5.82%.

Tab. 17: Results of the precision of the RIDA®UNITY Viral Stool Panel II test for rotavirus 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	33.3	30.3	29.8	32.9	30.1	29.8	30.9
	CV (%)	1.06%	0.94%	0.59%	1.82%	1.30%	1.10%	5.82%
3	Ct	31.8	29.0	28.8	31.2	28.8	28.7	29.5
	CV (%)	0.88%	1.01%	0.74%	1.48%	0.67%	0.59%	4.84%
4	Ct	28.9	26.3	26.2	29.2	26.7	26.6	27.5
	CV (%)	0.68%	1.07%	0.95%	1.63%	1.05%	0.88%	5.49%
5	Ct	30.3	27.6	27.4	30.1	27.7	27.6	28.5
	CV (%)	0.90%	0.85%	0.67%	1.52%	0.93%	0.84%	5.12%

Tab. 18: Results of the precision of the RIDA®UNITY Viral Stool Panel II test for rotavirus 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	32.7	30.4	30.4	32.2	30.1	29.8	30.7
	CV (%)	0.79%	0.67%	0.50%	1.37%	1.45%	1.06%	4.46%
3	Ct	32.6	29.9	29.7	32.0	29.7	29.4	30.4
	CV (%)	0.99%	1.09%	1.02%	1.73%	1.56%	1.36%	4.85%
4	Ct	30.8	28.4	28.1	30.3	28.0	27.6	28.6
	CV (%)	1.53%	1.44%	1.47%	1.64%	1.84%	1.46%	5.44%
5	Ct	31.4	28.6	28.4	31.1	28.8	28.6	29.5
	CV (%)	0.55%	0.81%	0.89%	1.94%	1.67%	1.62%	5.01%

Tab. 19: Results of the precision of the RIDA®UNITY Viral Stool Panel II test for astrovirus 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	23.1	22.8	22.7	22.5	22.2	22.3	22.3
	CV (%)	1.41%	1.11%	1.24%	1.42%	1.47%	1.52%	1.56%
2	Ct	25.9	25.2	25.1	25.9	25.4	25.5	25.6
	CV (%)	1.12%	1.31%	1.10%	0.79%	1.14%	1.02%	1.44%
3	Ct	29.3	28.4	28.6	29.1	28.6	28.7	28.8
	CV (%)	0.80%	0.88%	0.85%	0.81%	1.14%	1.12%	1.44%
4	Ct	32.4	31.5	31.8	32.3	31.8	31.8	32.0
	CV (%)	0.99%	1.01%	0.93%	1.05%	0.99%	0.90%	1.34%
5	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Tab. 20: Results of the precision of the RIDA®UNITY Viral Stool Panel II test for astrovirus 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	24.4	24.1	23.9	23.5	23.4	23.4	23.5
	CV (%)	1.07%	0.94%	1.26%	2.12%	2.26%	1.65%	2.03%
2	Ct	27.3	26.8	27.1	27.7	27.3	27.5	27.5
	CV (%)	0.86%	1.10%	0.64%	1.55%	2.31%	1.52%	1.87%
3	Ct	31.0	30.1	30.5	30.8	30.4	30.6	30.6
	CV (%)	0.73%	0.77%	1.07%	1.37%	2.08%	1.39%	1.76%
4	Ct	33.4	32.7	33.1	34.1	33.7	33.6	33.8
	CV (%)	0.90%	1.06%	1.32%	1.50%	1.86%	1.11%	1.64%
5	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Tab. 21: Results of the precision of the RIDA®UNITY Viral Stool Panel II test for adenovirus 40/41 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	28.0	28.3	28.1	26.4	26.3	26.3	26.3
	CV (%)	0.82%	1.03%	1.27%	1.52%	1.53%	1.48%	1.51%
2	Ct	28.0	27.9	27.9	28.1	27.9	27.8	28.0
	CV (%)	1.18%	1.22%	1.18%	1.02%	1.01%	1.01%	1.14%
3	Ct	31.5	31.5	31.6	31.5	31.2	31.2	31.3
	CV (%)	1.33%	1.60%	2.22%	1.32%	1.24%	1.51%	1.42%
4	Ct	34.6	34.5	34.5	34.7	34.4	34.3	34.5
	CV (%)	1.06%	1.02%	2.14%	1.30%	1.28%	1.33%	1.42%
5	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Tab. 22: Results of the precision of the RIDA®UNITY Viral Stool Panel II test for adenovirus 40/41 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	28.6	29.1	29.1	26.1	26.2	26.1	26.1
	CV (%)	1.66%	1.52%	1.42%	1.55%	1.61%	1.72%	1.63%
2	Ct	28.4	28.1	28.1	28.4	28.2	28.2	28.3
	CV (%)	0.91%	1.00%	1.11%	1.06%	1.04%	1.06%	1.07%
3	Ct	31.7	31.5	31.6	31.6	31.4	31.6	31.5
	CV (%)	1.11%	0.80%	1.04%	1.38%	1.09%	1.34%	1.31%
4	Ct	33.9	33.6	33.7	34.8	34.6	34.5	34.6
	CV (%)	0.73%	1.95%	1.07%	1.24%	1.15%	1.21%	1.26%
5	Ct	-	-	-	-	-	-	-
	CV (%)	N/A	N/A	N/A	N/A	N/A	N/A	N/A

13.2.4 Analytical reactivity

The reactivity of the RIDA®UNITY Viral Stool Panel II multiplex real-time PCR test was examined on a defined panel of rotavirus, astrovirus and adenovirus serotypes (see Table 23).

Tab. 23: Analytical reactivity testing

Strain	Result*		
	Rotavirus	Astrovirus	Adenovirus 40/41
Adenovirus Type 41 stock strain: TAK	-	-	**+**
Adenovirus Type 40	-	-	**+**
Astrovirus Type 2	-	**+**	-
Astrovirus Type 8	-	**+**	-
Rotavirus G1 10-G1048.01	**+**	-	-
Rotavirus G2 10-G1098.01	**+**	-	-
Rotavirus G3 10-G1047.01	**+**	-	-
Rotavirus G4 10-G1120.01	**+**	-	-
Rotavirus G4 10-G1557.01	**+**	-	-
Rotavirus G9 10-G0164.01	**+**	-	-
Rotavirus G12 10-G0523.01	**+**	-	-
Rotavirus G12 10-G0817.01	**+**	-	-

*+ = positive (at least 2 of 3 replicates positive)
- = negative

14. Version history

Version number	Section and designation
2022-09-08	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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