RIDA®UNITY Norovirus I & II

REF UN1415

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1. Intended use

For *in vitro* diagnostic use. The RIDA®UNITY Norovirus I & II test, performed on the RIDA®UNITY platform, is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of norovirus RNA of genogroups I (GI) and II (GII) in untreated human stool samples from people with signs and symptoms of acute gastroenteritis.

The RIDA®UNITY Norovirus I & II test is intended to support the differential diagnosis of norovirus genogroup I (GI) and II (GII) infections in patients with symptoms of gastroenteritis in connection with other clinical and laboratory findings.

Negative results do not rule out norovirus infection 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

Noroviruses are one of the world's most common causes of nonbacterial gastroenteritis in people of all age groups and result in about 70,000 to 200,000 deaths each year.(1) They are responsible for about 20 % of all cases of acute gastroenteritis worldwide. Human noroviruses, formerly called Norwalk viruses, were first identified in 1972 in stool samples collected during an outbreak of gastroenteritis in Norwalk, Ohio, USA, making them the first viral pathogens proven to cause gastroenteritis.(2)
Noroviruses belong to the family *Caliciviridae* and are small, nonenveloped viruses with single-stranded RNA (ssRNA). Based on the genetic sequences of viral RNA-dependent RNA polymerase and the capsid protein, noroviruses are divided into 10 genogroups (GI to GX) with currently more than 49 different genotypes (9 × GI, 27 × GII, 3 × GIII, 2 × GIV, 2 × GV, 2 × GVI, and 1 genotype each for GVII, GVIII, GIX [formerly GII.15], and GX) and a variety of strains. Genogroups I, II, and sometimes IV are the most important in terms of human pathogenicity.(1) In the United States, more than 99 % of all norovirus outbreaks are caused by GI and GII viruses.(3) Gastroenteritis due to noroviruses is often caused by the presence of virions in feces and vomit, whereas it takes only 10 infectious particles to cause disease. The high environmental stability and low infection dose make for high transmissibility of the norovirus.(4) Typical symptoms of norovirus infections are diarrhea, vomiting, and nausea.(3)

3. Test principle

The RIDA®UNITY Norovirus I & II multiplex real-time RT-PCR is a molecular diagnostic PCR for the direct qualitative detection and differentiation of norovirus genogroup I (GI) RNA and norovirus genogroup II (GII) and GIV RNA 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 in a one-step real-time RT-PCR format, that is, reverse transcription (RT) and subsequent PCR are performed in one reaction vial. In the process, the isolated RNA is transcribed into cDNA with the help of reverse transcriptase. The gene fragments specific to the GI and GII/GIV norovirus are then amplified using real-time PCR.
The amplified target sequences (ORF1/ORF2 junction region) are detected using the hydrolysis probes that are labeled with the quencher at one end and a fluorescent reporter dye (fluorophore) at the other end. 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
UNZ1415RM	Reaction Mix	1 ×	1935 µL	yellow, ready for use
UNZ1415EM	Enzyme Mix	1 ×	350 µL	red, ready for use
UNZ1415PC	Positive Control	1 ×	200 µL	blue, ready for use
UNZ1415NC	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 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 - 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 to -28 °C	Can be used until the printed expiration date
opened	-16 to -28 °C	8 freeze-thaw cycles

6. Reagents required but not provided

The RIDA®UNITY Norovirus I & 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 Norovirus I & 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 Norovirus I & 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 Norovirus I & II kit can be used in combination 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 Norovirus I & 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 6.

Tab. 3: Sample storage - Detection of norovirus GI

Native samples - stool		
20 - 25 °C	2 - 8 °C	-20 / -80 °C
≤ 7 days	≤ 9 days	≤ 6 months

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

At a storage temperature of -20 / -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 norovirus GII

Native samples - stool		
20 - 25 °C	2 - 8 °C	-20 / -80 °C
≤ 7 days	≤ 9 days	≤ 6 months

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

At a storage temperature of -20 / -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.

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).

9. Test procedure

Both the samples and the reagents of the RIDA®UNITY Norovirus I & II are placed 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 Norovirus I & 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 Norovirus I & II assay was verified exclusively 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. 7: 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. 8: 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. 9: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Note
R-Biopharm RIDA®UNITY	Norovirus GII	FAM	SEEK channel GGII
	Internal Control	HEX	SEEK channel ICD
	Norovirus GI	Cy5	SEEK channel GGI
Bio-Rad CFX96™ Dx	Norovirus GII	FAM	SEEK channel GGII
	Internal Control	VIC	SEEK channel ICD
	Norovirus GI	Cy5	SEEK channel GGI

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. 10).

Tab.10: 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, synthetic RNA 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.11: Result interpretation*

Detection of			
Norovirus GII	**Norovirus GI**	**IC**	**Result**
+	**-**	**+/-**	**Norovirus GII[1] detectable**
-	**+**	**+/-**	**Norovirus GI detectable**
+	**+**	**+/-**	**Norovirus GII[1] and norovirus GI detectable**
-	**-**	**+**	**Target genes are not detectable**
-	**-**	**-**	**Invalid**

*+ = positive
- = negative
[1] See Limitations of the method (Section 10).

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

A sample is also positive if the sample 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 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 RNA 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 Norovirus I & II test detects norovirus GI and norovirus GII RNA 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 and validated only for stool samples.
5. Improper specimen 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 Norovirus I & II.
9. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target genes (norovirus GI and GII; ORF1/ORF2 junction region) are present.
10. Noroviruses of genogroup IV, which, in very rare cases, can also infect humans, are likewise detected using RIDA®UNITY Norovirus I & II.
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 Norovirus I & II multiplex real-time PCR was compared in an external laboratory with a CE-marked reference test based on 237 stool samples from patients with symptoms of gastrointestinal infection.

The results show high sensitivity and specificity for the detection of norovirus GI or GII in human stool samples.

Tab. 12: Detection of norovirus GI

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Norovirus I & II - Norovirus GI	Positive	33	0	33
	Negative	3	201	204
	Total	36	201	237

Relative sensitivity (95 % CI)	91.7 % (77.5 % - 98.2 %)
Relative specificity (95 % CI)	100 % (98.2 % - 100 %)

Tab. 13: Detection of norovirus GII

		Reference PCR		
		Positive	Negative	Total
RIDA®UNITY Norovirus I & II - Norovirus GII	Positive	126	0	126
	Negative	4	107	111
	Total	130	107	237

Relative sensitivity (95 % CI)	96.9 % (92.3 % - 99.2 %)
Relative specificity (95 % CI)	100 % (96.6 % - 100 %)

13.2 Analytical performance characteristics

13.2.1 Detection limit (LoD 95%)

A positive sample (negative stool pool, spiked with positive clinical stool samples) 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. This was followed by a probit analysis. Next, the calculated LoD was confirmed with 20 replicates per target for the calculated dilution step/concentration.

For the detection of norovirus GI and norovirus GII RNA using the RIDA®UNITY Norovirus I & II assay on the UNITY System, the following limits of detection (LoD) were identified.
The results of these measurements are shown in Table 15.

Tab. 15: Results of the limit of detection of the RIDA®UNITY Norovirus I & II test for the parameters norovirus GI and norovirus GII in stool samples

	Norovirus GI	Norovirus GII
LoD	4.85E-04 [dilution factor]**	5.98E-05 [dilution factor]*

(*) Relative dilution of the stock concentration. Positive clinical sample with Ct range of 26 - 27
(**) Relative dilution of the stock concentration. Positive clinical sample with Ct range of 30 - 31

The LoD for the parameter norovirus GI in stool samples was determined to be 4.85E-04 [dilution factor].

The LoD for the parameter norovirus GII in stool samples was determined to be 5.98E-05 [dilution factor].

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 16.

Tab. 16: Potentially interfering substances

Potentially interfering substance	Concentration
Ciprofloxacin-ratiopharm® 500 mg film-coated tablets (ciprofloxacin)	0.375 % [w/v]
Ethanol	5 % based on the eluate
Guanidinium hydrochloride	5 % based on the eluate
Human blood	5 % [v/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 either they naturally occur in stool samples, or they cause corresponding symptoms as gastrointestinal pathogens. Bacterial cultures (between 10^7 and 10^9 CFU/mL) and fungal cultures or viral isolates (from positive stool samples) of the respective organisms were used for the analyses.

The RIDA®UNITY Norovirus I & II multiplex real-time PCR is specific to norovirus GI and norovirus GII. No cross-reactivities with the following species were detected (see Tab. 17):

Tab. 17: Potentially cross-reactive organisms.

Organism	Test result*	
	Norovirus GI	Norovirus GII
Adenovirus	-	-
Aeromonas hydrophila	-	-
Arcobacter butzleri	-	-
Astrovirus	-	-
Bacillus cereus	-	-
Bacteroides fragilis	-	-
Campylobacter fetus	-	-
Campylobacter lari	-	-
Campylobacter upsaliensis	-	-
Campylobacter coli	-	-
Campylobacter jejuni	-	-
Candida albicans	-	-
Citrobacter freundii	-	-
Clostridium difficile	-	-
Clostridium perfringens	-	-
Clostridium sordellii	-	-
E. coli (O157:H7)	-	-
E. coli (O26:H-)	-	-
E. coli (O6)	-	-
Enterobacter cloacae	-	-
Enterococcus faecalis	-	-
Klebsiella oxytoca	-	-
Proteus vulgaris	-	-
Pseudomonas aeruginosa	-	-

Rotavirus	-	-
Salmonella enterica (serovar Enteritidis)	-	-
Salmonella enterica (serovar Typhimurium)	-	-
Serratia liquefaciens	-	-
Shigella flexneri	-	-
Staphylococcus aureus	-	-
Staphylococcus epidermidis	-	-
Vibrio parahaemolyticus	-	-
Yersinia enterocolitica	-	-

13.2.3 Precision

The precision of the RIDA®UNITY Norovirus I & 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 on different instruments under reproducible conditions.

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

The obtained coefficients of variation of the respective measurements taken using the RIDA®UNITY Norovirus I & II real-time PCR test were no more than 4.82 % on the RIDA®UNITY and no more than 3.25 % on CFX96™ Dx.

Tab. 18: Results of the precision of the RIDA®UNITY Norovirus I & II test for norovirus GI (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	25.6	24.1	24.3	30.5	29.6	29.4	29.8
	CV (%)	1.27	0.58	0.97	2.97	1.51	2.16	3.04
2	Ct	29.5	29.2	29.3	29.1	28.4	28.3	28.6
	CV (%)	0.42	1.06	0.99	3.11	2.40	2.69	3.10
3	Ct	34.8	33.3	34.1	26.4	25.5	25.4	25.8
	CV (%)	1.11	1.00	1.44	3.01	1.77	2.40	3.14
4	Ct	33.0	33.5	31.9	29.7	28.8	28.7	29.1
	CV (%)	1.50	1.30	0.93	3.17	2.34	2.75	3.35
5	Ct	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	CV (%)	/	/	/	/	/	/	/

Tab. 19: Results of the precision of the RIDA®UNITY Norovirus I & II test for norovirus GI (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	26.7	26.1	25.4	29.6	29.0	28.7	29.1
	CV (%)	0.97	0.94	0.93	2.49	1.62	1.50	2.44
2	Ct	30.3	30.0	29.6	28.2	27.4	27.2	27.6
	CV (%)	0.72	0.90	0.51	2.20	1.75	1.66	2.66
3	Ct	33.1	32.6	32.5	25.7	25.2	25.0	25.3
	CV (%)	0.81	0.74	0.82	1.91	1.40	1.48	2.13
4	Ct	32.4	31.9	31.7	28.9	28.3	28.0	28.4
	CV (%)	1.16	0.74	0.64	2.83	1.95	1.98	2.72
5	Ct	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	CV (%)	/	/	/	/	/	/	/

Tab. 20: Results of the precision of the RIDA®UNITY Norovirus I & II test for norovirus GII (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	23.4	21.7	21.7	28.4	26.9	26.8	27.4
	CV (%)	1.12	0.39	1.00	4.02	1.98	2.64	4.46
2	Ct	27.9	26.8	26.8	30.6	29.4	29.2	29.7
	CV (%)	0.76	0.77	0.75	3.42	2.33	2.64	3.69
3	Ct	33.6	31.6	32.3	23.7	22.6	22.4	22.9
	CV (%)	1.43	0.85	1.43	3.93	2.16	2.63	4.31
4	Ct	31.7	31.4	30.1	27.3	25.8	25.7	26.3
	CV (%)	1.45	1.31	0.96	4.30	2.86	3.34	4.82
5	Ct	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	CV (%)	/	/	/	/	/	/	/

Tab. 21: Results of the precision of the RIDA®UNITY Norovirus I & II test for norovirus GII (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	24.2	23.4	22.7	27.7	27.2	26.8	27.2
	CV (%)	1.07	1.02	1.23	3.19	2.06	1.91	2.94
2	Ct	28.6	28.3	27.8	29.1	28.3	28.1	28.5
	CV (%)	0.93	0.65	0.83	2.23	1.65	1.93	2.70
3	Ct	32.0	31.4	31.2	22.9	22.4	22.2	22.5
	CV (%)	0.85	0.90	0.61	2.28	1.42	1.57	2.45
4	Ct	30.8	30.2	30.0	26.6	26.1	25.8	26.2
	CV (%)	1.11	0.69	0.85	3.25	2.27	2.23	3.03
5	Ct	Negative	Negative	Negative	Negative	Negative	Negative	Negative
	CV (%)	/	/	/	/	/	/	/

13.2.4 Analytical reactivity

The reactivity of the RIDA®UNITY Norovirus I & II multiplex real-time RT-PCR test was examined on a defined panel of various norovirus genotypes of genogroups I and II (see Tab. 22).

Tab. 22: Analytical reactivity testing

Strain	Result*	
	Norovirus GII	Norovirus GI
Norovirus P31-GII4 Sydney	+	-
Norovirus GII.P4 New Orleans/GII.4 Sydney	+	-
Norovirus GII.P16-GII.4 Sydney	+	-
Norovirus GII.P16-GII.2	+	-
Norovirus GII.P31-GII.4 Sydney	+	-
Norovirus GII.7	+	-
Norovirus GI.3	-	+

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

14. Version history

Version number	Section and designation
2022-08-09	Release version

15. Explanation of symbols

General symbols

IVD	For *in vitro* diagnostic use
	Follow instructions for use
LOT	Batch number
	Use before
	Storage temperature
REF	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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