RIDA®GENE Norovirus

REF PG1405

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Norovirus is a real-time RT-PCR for the direct, qualitative detection of norovirus (genogroup I and II) from human stool samples.[1,2]

RIDA®GENE Norovirus real-time RT-PCR is intended for use as an aid in diagnosis of gastroenteritis caused by noroviruses.

2. Summary and Explanation of the test

Noroviruses cause by far the most cases of non-bacterial gastroenteritis outbreaks.[3,4,5] A gastroenteritis caused by norovirus is manifested by severe nausea, vomiting and diarrhea. Noroviruses are egested by stool and with the vomit. An airborne transmission through aerosols containing the virus is often the cause of a very rapid spreading in shared facilities.[6,7,8] The CDC estimates that more than 21 million cases of acute gastroenteritis, 70.000 hospitalisations and 800 deaths are caused by norovirus infections each year in the United States.[9]
Noroviruses belong to the family of *Caliciviridae*. Because of their morphology (small round structured virus (SRSV)), they can be distinguished from the traditional calicivirus. The SRSVs were named after the place of their isolation. Thus, the name Norwalk-like stood for all viruses which have been isolated during outbreaks of gastroenteritis. The name originated from the first SRSV isolation in the city of Norwalk, Ohio, in the US in 1972. Later, other isolates like Snow Mountain agent, Hawaii agent and Montgomery County agent were named in a similar way.
Noroviruses are small, non-enveloped viruses with a single-stranded RNA (ssRNA). They can be grouped in 7 genogroups with currently over 30 genotypes and a multiplicity of clades. So far, human pathogens have only been described from genogroup I (GI) with 9 genotypes, from genogroup II (GII) with 22 genotypes and from genogroup IV (GIV) with two genotypes.[10,11]

3. Test principle

The RIDA®GENE Norovirus multiplex real-time RT-PCR is a molecular diagnostic test for the direct, qualitative detection of norovirus RNA (genogroup GI and GII) from human stool samples. The detection is done in a one step real-time RT-PCR format where the reverse transcription is followed by the PCR in the same reaction tube. The isolated RNA is transcribed into cDNA by a reverse transcriptase. Gene fragments specific for norovirus GI and GII are subsequently amplified by real-time PCR. The amplified targets (ORF1/ORF2 junction region) are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-Polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Norovirus assay contains an Internal Control RNA (ICR) as an internal control of sample preparation procedure and/or to determine possible PCR-inhibition.

4. Reagents provided

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

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 µl	yellow
2	Enzyme Mix	1x	80 µl	red
R	Internal Control RNA	2x	1700 µl	brown
N	No Template Control	1x	450 µl	white
P	Positive Control	1x	200 µl	blue

5. Storage instructions

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

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Noroviurs real-time RT-PCR Assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2: Necessary equipment

Extraction platform	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
bioMérieux	NucliSENS easy®MAG™
Qiagen	QIAsymphony SP/AS
Real-time PCR instruments	
Roche	LightCycler® 2.0, LightCycler® 480II
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).

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

- RIDA®GENE Color Compensation Kit IV (PG0004) for use with the LightCycler® 480II
- RIDA®GENE Color Compensation Kit II (PG0002) for use with the LightCycler® 2.0
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves
- PCR water (BioScience grade, nuclease-free, DEPC treated water)

7. Precautions for users

For *in-vitro* diagnostic use.
- This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed.
- The instruction manual for the test procedure has to be followed.
- Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes.
- During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure.
- Do not smoke, eat or drink in areas where samples or reagents are being used.
- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.
- All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Safety Data Sheets (SDS) at www.r-biopharm.com.

8. Collection and Storage of samples

8.1 Sample Preparation from stool samples

For RNA isolation of human stool samples, use a commercially available RNA extraction kit (e.g. RIDA® Xtract (R-Biopharm)) or RNA extraction system (e.g. Maxwell® RSC (Promega)). Extract viral RNA according to the manufacturer's instructions.
We recommend to dilute the stool sample before extraction 1:10 with water. Vortex intensely and centrifuge at 13,000 x g for 1 min. Use from the supernatant an appropriate volume according to the manufacturer's instruction.

The RIDA®GENE Norovirus assay contains an Internal Control RNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control RNA can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as PCR inhibition control.

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

If the Internal Control RNA is used as a extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control RNA has to be added during extraction procedure. The Internal Control RNA should always be

added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the Internal Control RNA to the negative control and positive control PCR Mix.

9. Test procedure

9.1 Master-Mix preparation

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

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

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

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

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

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

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

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

9.2 Preparation of the RT-PCR-Mix

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

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

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

Samples: Add 5 µl RNA-Extract to the pre-pipetted Master-Mix.

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

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

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

9.3 PCR instrument set-up

9.3.1 Universal real-time RT-PCR profile

Tab. 5: Universal real-time RT-PCR profile for LightCycler® series

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

Note: Annealing and Extension occur in the same step.

Tab. 6: Universal real-time RT-PCR profile for Mx3005P, ABI7500, Rotor-Gene Q and CFX96™

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

Note: Annealing and Extension occur in the same step

Note: The universal real-time PCR profile can also be used for DNA assays if RIDA®GENE DNA and RIDA®GENE RNA real-time PCR assays are combined in one run.

9.4 Detection channel set-up

Tab. 7: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection Channel	Note
Roche LightCycler® 2.0	Norovirus	530	**RIDA®GENE Color Compensation Kit II (PG0002) is required**
	ICR	560	
Roche LightCycler® 480II	Norovirus	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICR	533/580	
ABI 7500	Norovirus	FAM	**Check that passive reference option ROX is none**
	ICR	VIC	
Agilent Techn. Mx3005P	Norovirus	FAM	**Check that reference dye is none**
	ICR	HEX	
Qiagen Rotor-Gene Q	Norovirus	Green	**The gain settings have to be set to 5**
	ICR	Yellow	
Bio-Rad CFX 96™	Norovirus	FAM	
	ICR	VIC	

Note: Upon usage with the LightCycler® 2.0, the "Seek Temperature" has to be increased from "30" to "58".

10. Quality Control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instructions. Negative control and positive control have to show correct results (see Table 8, Fig. 1) in order to determine a valid run.

The Positive Control has a concentration of 10^3 copies/µl. In each PCR run it is used in a total amount of 5 x 10^3 copies.

Tab. 8: For a valid run, the following conditions must be met:

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

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

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

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

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

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

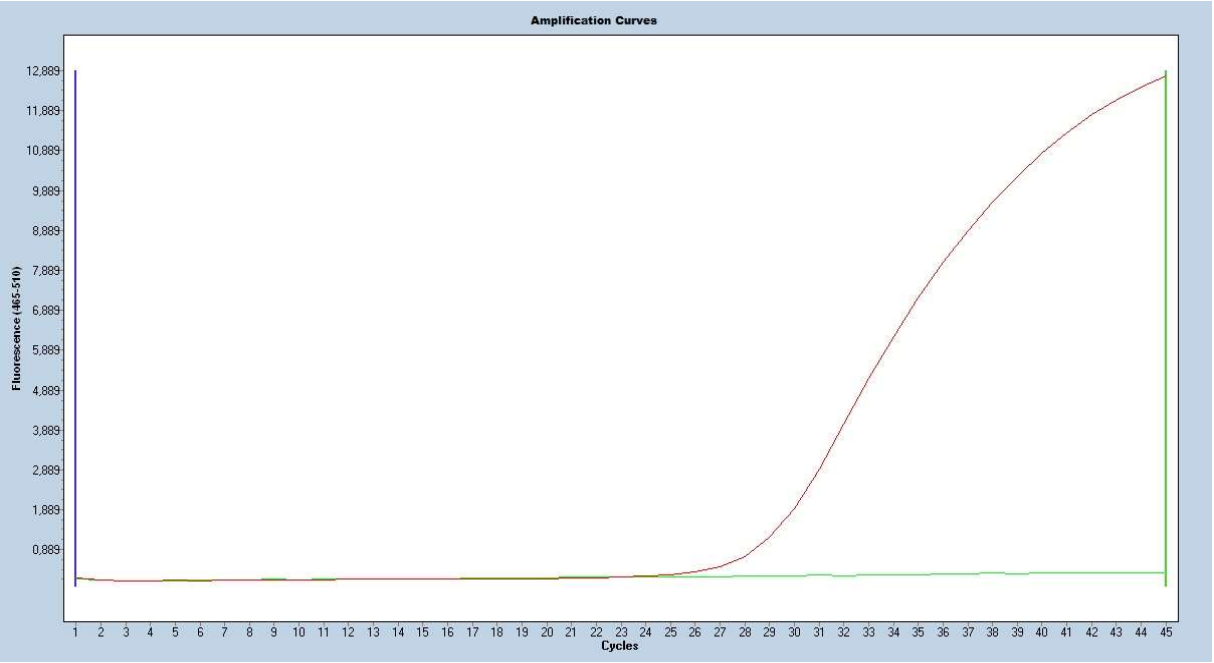

Fig. 1: Correct run of the positive control and negative control (Norovirus) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 9.

Tab.9: Sample interpretation

Norovirus	ICR	Result
positive	**positive/negative**	**Norovirus detected**
negative	**positive**	**Norovirus not detected**
negative	negative	**Invalid**

Norovirus is detected, if the sample RNA and the Internal Control RNA show an amplification signal in the detection system.

Norovirus is also detected, if the sample RNA shows an amplification signal but none for the Internal Control RNA in the detection system. The detection of the Internal Control RNA is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control RNA.

Norovirus is not detected, if the sample RNA shows no amplification signal, but an amplification signal for the Internal Control RNA in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control RNA.

A sample is invalid, if the sample RNA and Internal Control RNA show no amplification signal in the detection system. The sample contains a PCR inhibitor.

The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This assay is only validated for stool samples.
3. Inappropriate specimen collection, transport, storage and processing or a viral load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new norovirus genotypes resulting in a false negative result with the RIDA®GENE Norovirus assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target gene (ORF1/ORF2 junction region).
8. Norovirus genogroup IV, which very rarely infect humans (see Tab. 11), will be also detected by the RIDA®GENE Norovirus assay.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE Norovirus multiplex real-time RT-PCR has a limit of detection of ≥ 50 RNA copies per reaction (see Fig. 2).

The following figures 2 and 3 show a dilution series of Norovirus GI and GII (5×10^5 – 5×10^1 RNA copies per µl) on the LightCycler® 480II.

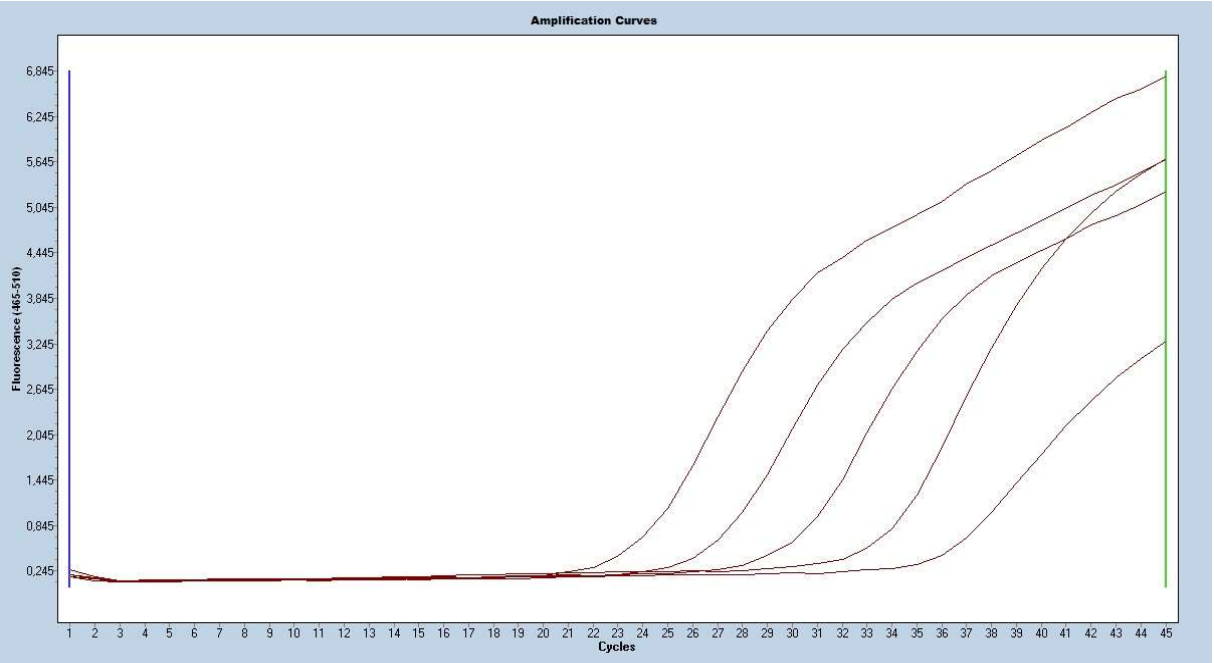

Fig. 2: Dilution series Norovirus GI (5×10^5 – 5×10^1 RNA copies per µl) on the LightCycler® 480II

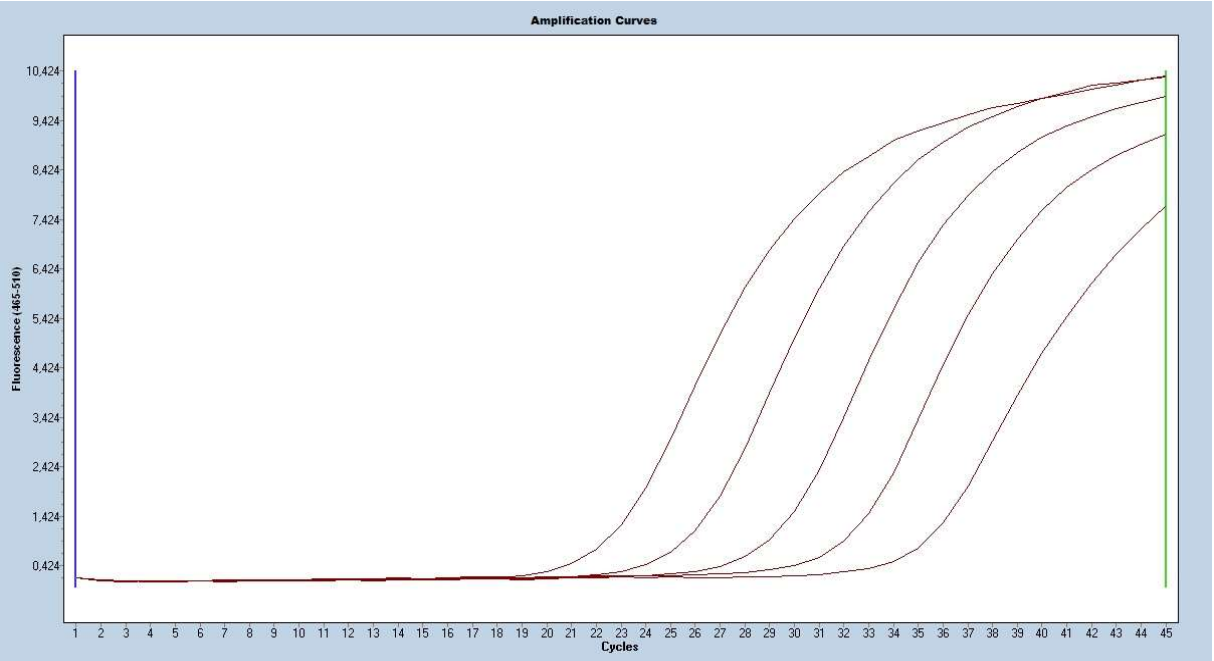

Fig. 3: Dilution series Norovirus GII (5×10^5 – 5×10^1 RNA copies per µl) on the LightCycler® 480II

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

13.2 Analytical specificity

The RIDA®GENE Norovirus multiplex real-time RT-PCR is specific for Norovirus of the genogroups I and II from human stool samples. No cross-reaction could be detected for the following species (see Tab. 10):

Tab. 10: Cross-reactivity testing

Adenovirus	-	*Candida albicans*	-	*Proteus vulgaris*	-
Aeromonas hydrophila	-	*Citrobacter freundii*	-	*Pseudomonas aeruginosa*	-
Arcobacter butzleri	-	*Clostridium difficile*	-	Rotavirus	-
Astrovirus	-	*Clostridium perfringens*	-	*Salmonella enteritidis*	-
Bacillus cereus	-	*Clostridium sordellii*	-	*Salmonella typhimurium*	-
Bacteroides fragilis	-	*E. coli* (O6)	-	*Serratia liquefaciens*	-
Campylobacter coli	-	*E. coli* (O26:H-)	-	*Shigella flexneri*	-
Campylobacter fetus subsp. *fetus*	-	*E. coli* (O157:H7)	-	*Staphylococcus aureus*	-
Campylobacter jejuni	-	*Enterobacter cloacae*	-	*Staphylococcus epidermidis*	-
Campylobacter lari subsp. *lari*	-	*Enterococcus faecalis*	-	*Vibrio parahaemolyticus*	-
Campylobacter upsaliensis	-	*Klebsiella oxytoca*	-	*Yersinia enterocolitica*	-

13.3 Analytical reactivity

The reactivity of the RIDA®GENE Norovirus multiplex real-time RT-PCR was evaluated against multiple genotypes of the Norovirus genogroup I, II and IV (see Tab. 11). All Norovirus genotypes of the panel were detected by the RIDA®GENE Norovirus real-time RT-PCR.

Tab. 11: Analytical reactivity testing

Norovirus genogroup I					
GGI.1 – Norwalk	+	GGI.3 – Desert Shield, Birmingham	+	GGI.6 – Hesse	+
GGI.2 – Southampton, Whiterose	+	GGI.4 – Chiba, Malta	+	GGI.7 – Winchester	+
GGI.2 – Southampton, Southampton	+	GGI.5 – Musgrove	+	GGI.8 – Boxer	+
Norovirus genogroup II					
GGII.1 – Hawaii	+	GGII.4 – Sydney 2012	+	GGII.10 – Erfurt	+
GGII.2 – Melksham	+	GGII.6 – Seacroft	+	GGII.b – Hilversum	+
GGII.3 – Toronto	+	GGII.7 – Leeds	+	GII.c – Den Haag	+
GGII.4 – Bristol, Grimsby 2004	+				
Norovirus genogroup IV					
GGIV.1 – Alphatron	+				

14. Version history

Version number	Chapter and designation
2018-05-16	Previous version
2021-01-28	General revision 10. Quality control (Spelling mistake) 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

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

Testspecific symbols

Reaction Mix

Enzyme-Mix

Internal Control RNA

No Template Control

Positive Control

16. Literature

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