

RIDA® GENE Viral Stool Panel I

REF PG1315



1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Viral Stool Panel I is a multiplex real-time RT-PCR for the direct, qualitative detection and differentiation of Norovirus, Rotavirus, Adenovirus 40/41 and Astrovirus in human stool samples.^{1,2,3,4} The RIDA®GENE Viral Stool Panel I multiplex real-time RT-PCR is intended for use as an aid in diagnosis of gastroenteritis caused by Norovirus, Rotavirus, Adenovirus 40/41 and Astrovirus, respectively.

2. Summary and explanation of the test

Acute Gastroenteritis is one of the main causes of morbidity and mortality worldwide. Especially in children, enteric viruses are the primary cause of gastroenteritis. In the US, viral infections cause approximately 30.8 million cases of gastroenteritis, yearly.⁵ The most important pathogens causing diarrhea are Norovirus, Rotavirus, Adenovirus and Astrovirus.

Noroviruses belong to the family of *Caliciviridae* and are single-stranded RNA (ssRNA) viruses. Gastroenteritis caused by Norovirus is manifested by severe nausea, vomiting and diarrhoea. Noroviruses are egested by stool and with the vomit. 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.^{7,8} In the US, it is estimated, that more than 21 million cases of acute gastroenteritis, 70,000 hospitalisations and 800 deaths are caused by Norovirus infections each year.⁶

Rotaviruses belong to the *Reoviridae* family of non-enveloped icosahedral double-stranded RNA (dsRNA) viruses. Symptoms of Rotavirus infection are usually vomiting, watery diarrhoea and abdominal pain. The virus is transmitted by the fecal-oral route through contaminated hands and objects. Rotavirus is the main cause of diarrhoea in children aged under five and is responsible for the death of an estimated 611,000 children worldwide each year.⁹ Rotaviruses are classified in seven serogroups A – G, whereby the viruses of serogroup A are of major epidemiologic importance.¹⁰

Adenoviruses belong to the *Adenoviridae* family of non-enveloped icosahedral double-stranded (dsDNA) viruses. One differentiates 56 serotypes of human adenoviruses and they are classified into seven groups (A - G). Adenoviruses mainly cause respiratory diseases, whereas Gastroenteritis is primarily caused by serotype 40 and 41.^{11,12}

Astroviruses are single-stranded RNA (ssRNA) viruses and belong to the family of *Astroviridae*. An astroviral-dependent Gastroenteritis is primarily manifested by diarrhoea, but can also be accompanied by vomiting and fever. In developed countries, the Astrovirus incidence is between 2 – 9%, where the disease mainly affects children under the age of two.¹³ Today, there are 8 serotypes described, with

serotypes 1-5 being most relevant. The infection is transmitted by contaminated foods, by water and by the fecal-oral route.¹⁵

3. Test principle

The RIDA®GENE Viral Stool Panel I assay is a multiplex real-time PCR for the direct, qualitative detection and differentiation Norovirus RNA, Rotavirus RNA, Adenovirus 40/41 DNA and Astrovirus RNA in human stool samples. Detection takes place in one-step real-time RT-PCR format, i.e. the reverse transcription (RT) and the subsequent PCR takes place in the same reaction tube. The isolated RNA is transcribed into cDNA using a reverse transcriptase. Subsequently amplification of the gene fragments specific for Norovirus (ORF1/ORF2 junction Region), Rotavirus (NSP3), Adenovirus 40/41 (Hexon), and Astrovirus (CAP; capsid protein) occurs. The amplified targets 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 Viral Stool Panel I assay contains an **Internal Control RNA** (ICR) as an internal control of sample preparation procedure and 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 Viral Stool Panel I multiplex 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
Real-time PCR instrument	
Roche	LightCycler® 480II
Agilent Technologies	Mx3005P

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) to run the LightCycler® 480II
- 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)

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 DNA/RNA isolation of human stool samples, use a commercially available nucleic acid extraction kit (e.g. RIDA[®] Xtract (R-Biopharm)) or nucleic acid extraction system (e.g. Maxwell[®] RSC (Promega)). Extract viral nucleic acid 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 Viral Stool Panel I 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 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.

Sample: 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® 480II

<u>Reverse Transcription</u>	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
<u>PCR</u> Denaturation	10 sec, 95 °C
Annealing/Extension	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

<u>Reverse Transcription</u>	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
<u>PCR</u> Denaturation	15 sec, 95 °C
Annealing/Extension	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® 480II	Adenovirus	440/488	RIDA® GENE Color Compensation Kit IV (PG0004) is required
	Norovirus	465/510	
	ICR	533/580	
	Rotavirus	533/610	
	Astrovirus	618/660	
Agilent Techn. Mx3005P	Adenovirus	ATTO	Check that reference dye is none NOTE: The “Filter Set Gain Settings” for ATTO have to be set to 8
	Norovirus	FAM	
	ICR	HEX	
	Rotavirus	ROX	
	Astrovirus	Cy5	

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, Fig. 2, Fig. 3) in order to determine a valid run.

The Positive Control for Adenovirus 40/41, Norovirus, Rotavirus and Astrovirus has a concentration of 10^3 copies/ μ l. In each PCR run it is used in a total amount of 5×10^3 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

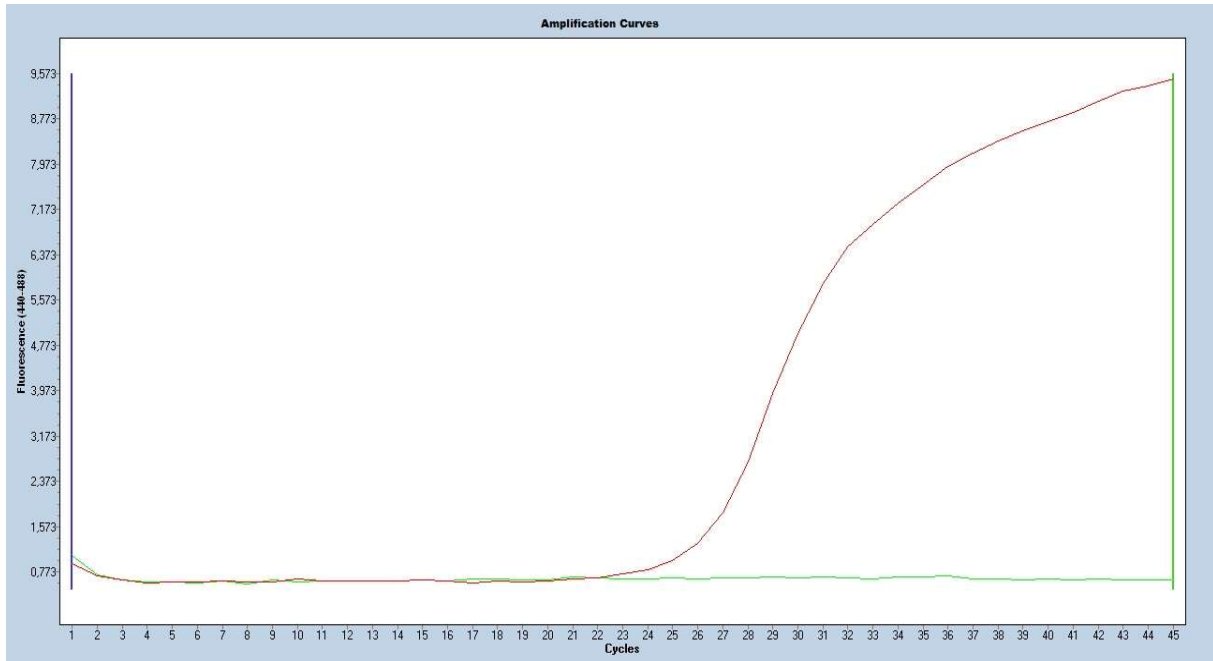


Fig. 1: Correct run of the positive control and negative control (Adenovirus) on the LightCycler® 480II

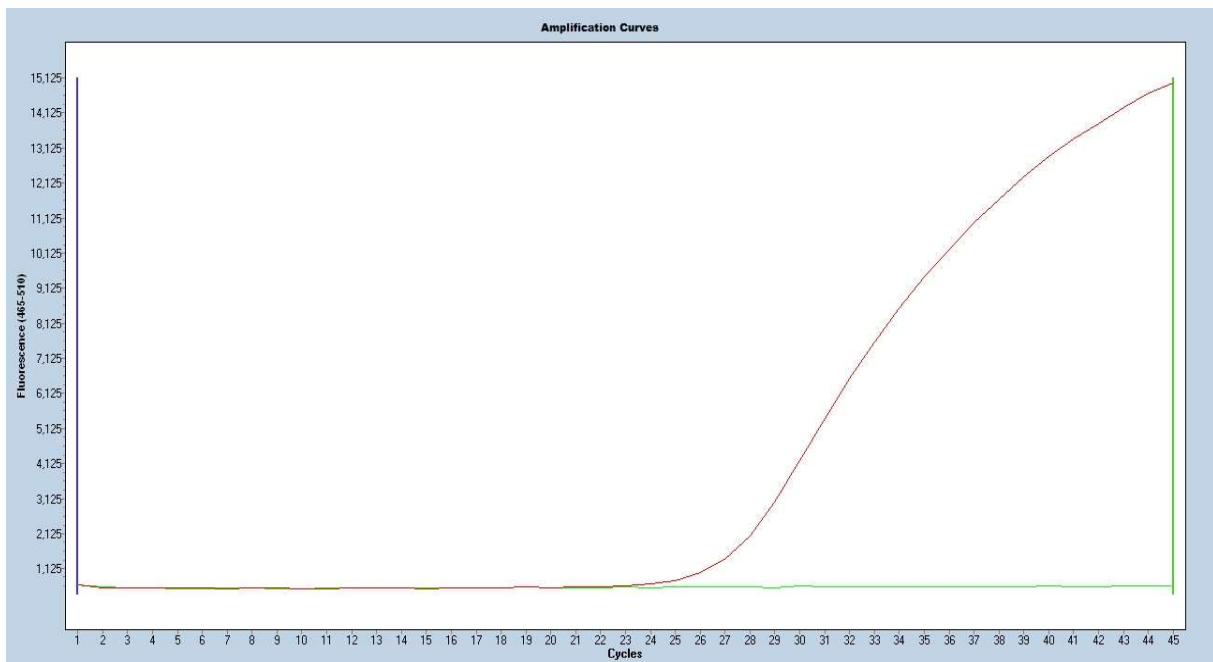


Fig. 2: Correct run of the positive control and negative control (Norovirus) on the LightCycler® 480II

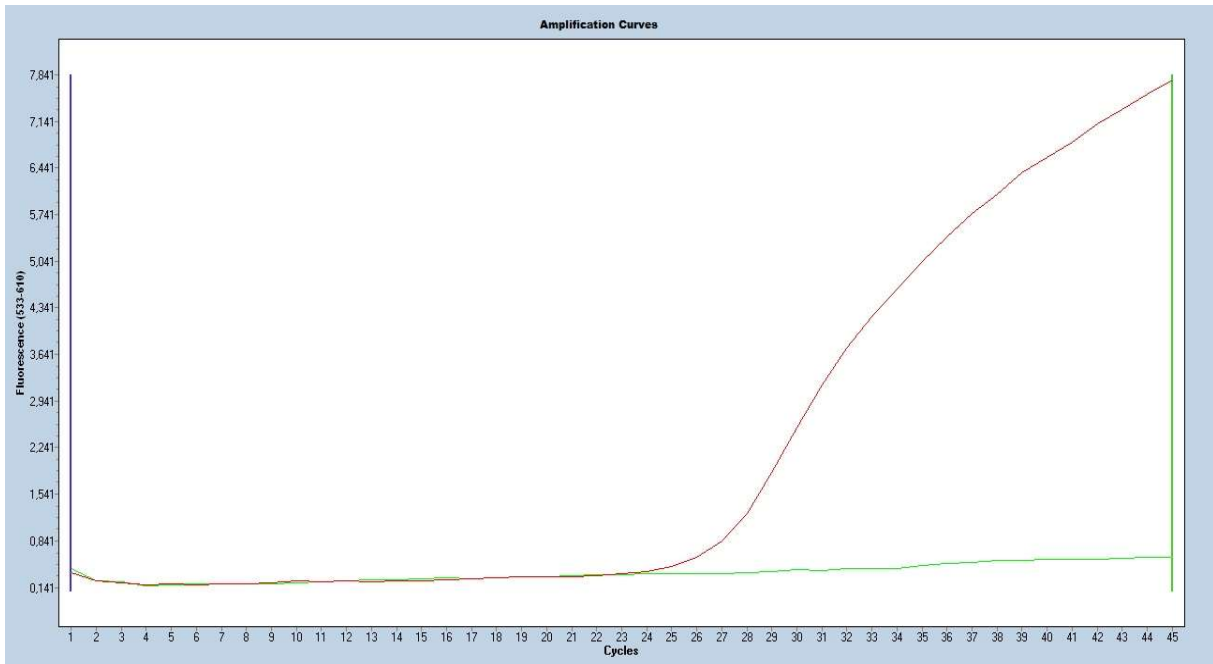


Fig. 3: Correct run of the positive control and negative control (Rotavirus) on the LightCycler® 480II

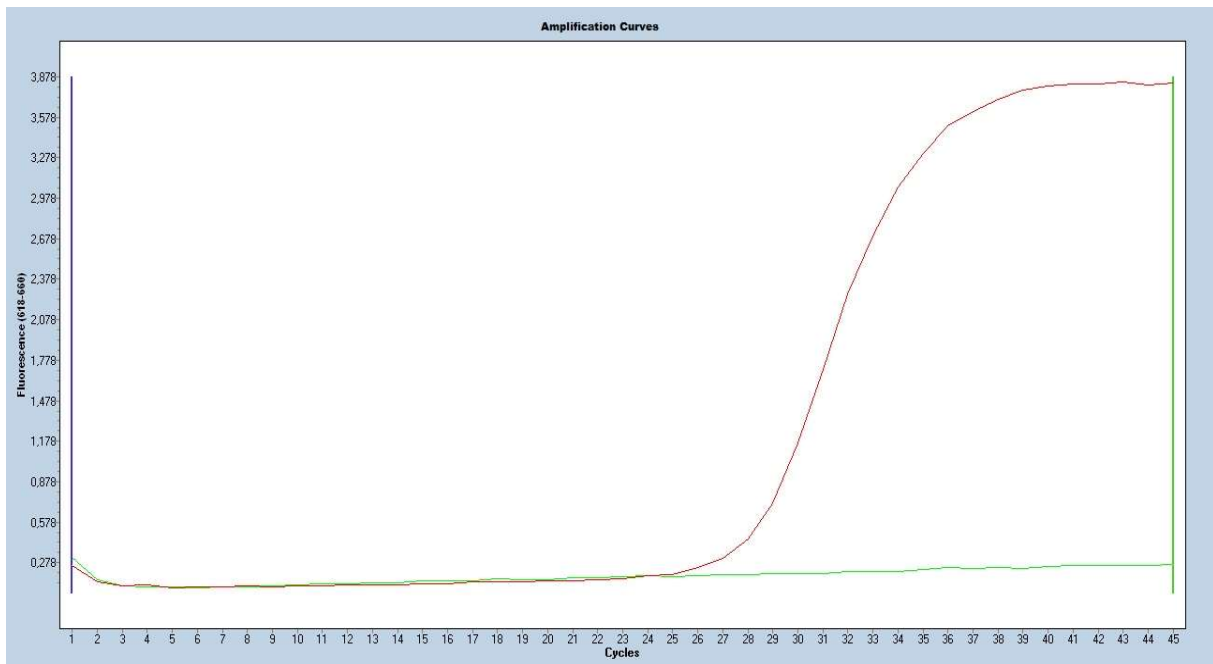


Fig. 4: Correct run of the positive control and negative control (Astrovirus) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 9.

Tab. 9: Sample interpretation

Target genes				ICR	Result
Adenovirus	Norovirus	Rotavirus	Astrovirus		
positive	negative	negative	negative	positive/negative	Adenovirus detected
negative	positive	negative	negative	positive/negative	Norovirus detected
negative	negative	positive	negative	positive/negative	Rotavirus detected
negative	negative	negative	positive	positive/negative	Astrovirus detected
positive	positive	negative	negative	positive/negative	Adenovirus and Norovirus detected
positive	negative	positive	negative	positive/negative	Adenovirus and Rotavirus detected
positive	negative	negative	positive	positive/negative	Adenovirus and Astrovirus detected
negative	positive	positive	negative	positive/negative	Norovirus and Rotavirus detected
negative	positive	negative	positive	positive/negative	Norovirus and Astrovirus detected
negative	negative	positive	positive	positive/negative	Rotavirus and Astrovirus detected
positive	positive	positive	negative	positive/negative	Adenovirus, Norovirus and Rotavirus detected
positive	positive	negative	positive	positive/negative	Adenovirus, Norovirus and Astrovirus detected
positive	negative	positive	positive	positive/negative	Adenovirus, Rotavirus and Astrovirus detected
negative	positive	positive	positive	positive/negative	Norovirus, Rotavirus and Astrovirus detected
positive	positive	positive	positive	positive/negative	Adenovirus, Norovirus, Rotavirus and Astrovirus detected
negative	negative	negative	negative	positive	Target genes not detected
negative	negative	negative	negative	negative	Invalid

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

A sample is also evaluated positive, 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**.

A sample is evaluated negative, 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 evaluated 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. The RIDA[®]GENE Viral Stool Panel I 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 new variants resulting in a false negative result with the RIDA[®]GENE Viral Stool Panel I assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target genes (Adenovirus 40/41 (Hexon), Norovirus (ORF1/ORF2 junction Region), Rotavirus (NSP3) and Astrovirus (CAP; capsid protein)).
8. Norovirus genogroup IV, which very rarely infect humans, will be also detected by the RIDA[®]GENE Viral Stool Panel I assay.
9. With RIDA[®]GENE Viral Stool Panel I the Adenovirus serotypes 40 und 41, which cause primarily Gastroenteritis, are detected only. The serotypes 1, 2, 5, 6, 12, 18, und 31 are rarely associated with acute diarrhea and therefore not detected by this real-time PCR assay.
10. Mucin can show interfering characteristics at a concentration of 2.5 % (v/v) or above.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA[®]GENE Viral Stool Panel I multiplex real-time RT-PCR has a detection limit of ≥ 50 RNA copies per reaction.

The following figures 5, 6, 7 and 8 show dilution series of Adenovirus ($10^5 - 10^1$ DNA copies per μl), Norovirus, Rotavirus and Astrovirus ($10^5 - 10^1$ RNA copies per μl) of on the LightCycler[®] 480II.

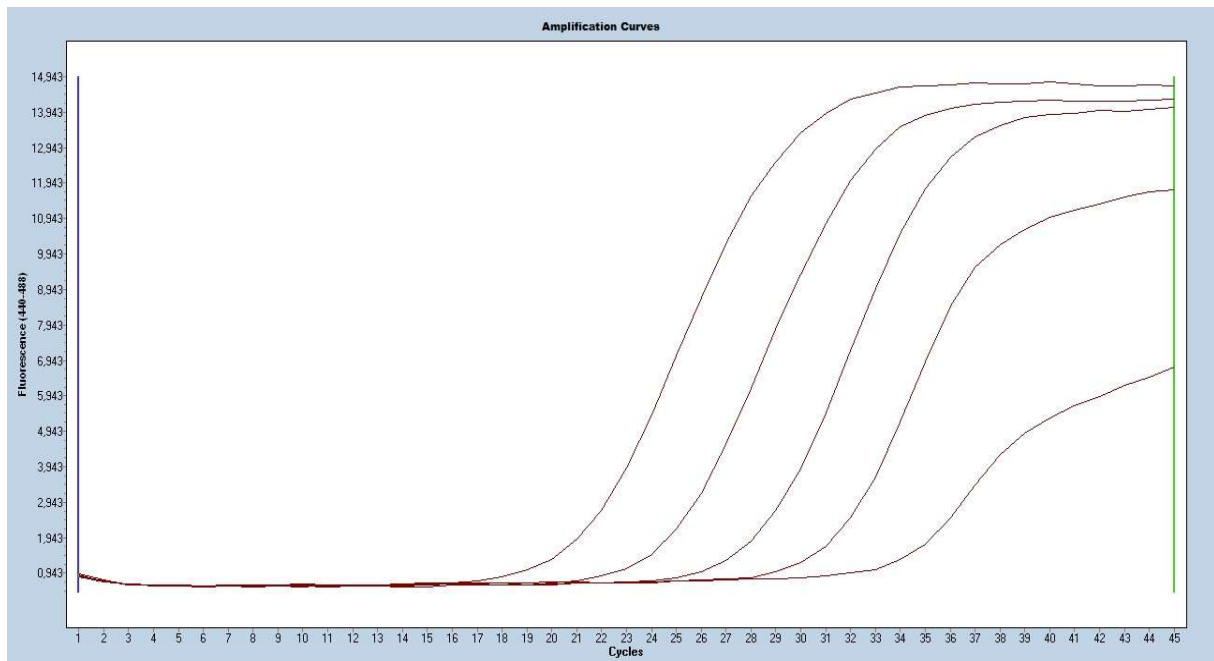


Fig. 5: Dilution series Adenovirus ($10^5 - 10^1$ DNA copies per μl) on the LightCycler[®] 480II

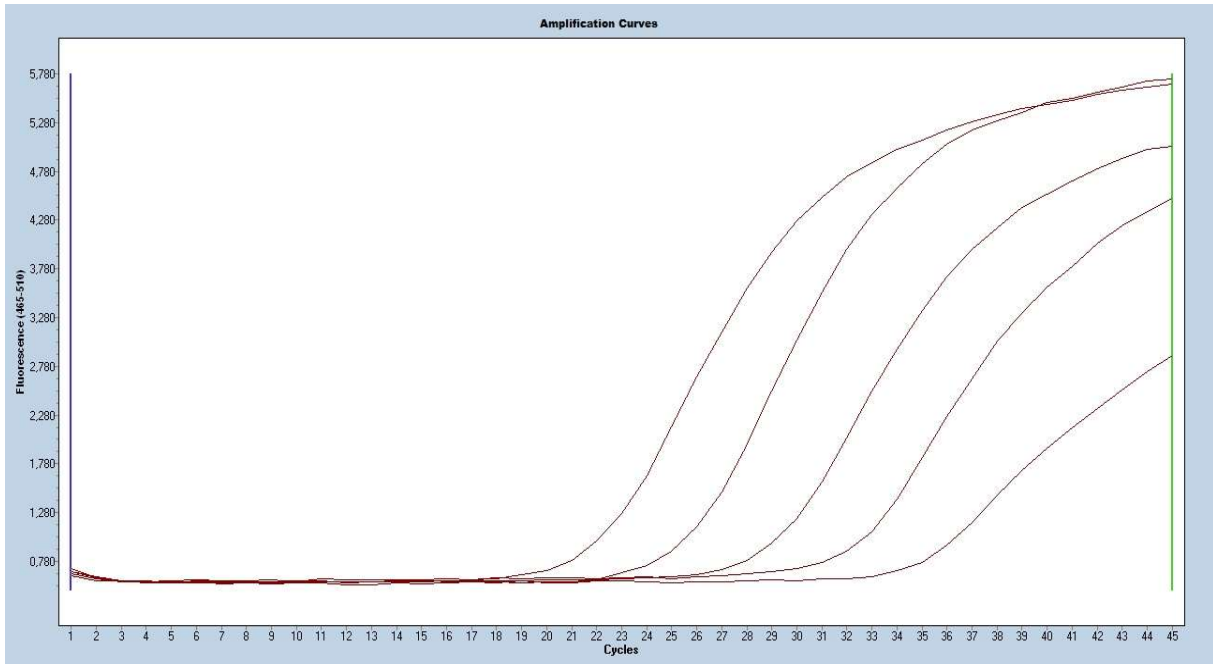


Fig. 6: Dilution series Norovirus ($10^5 - 10^1$ RNA copies per μl) on the LightCycler[®] 480II

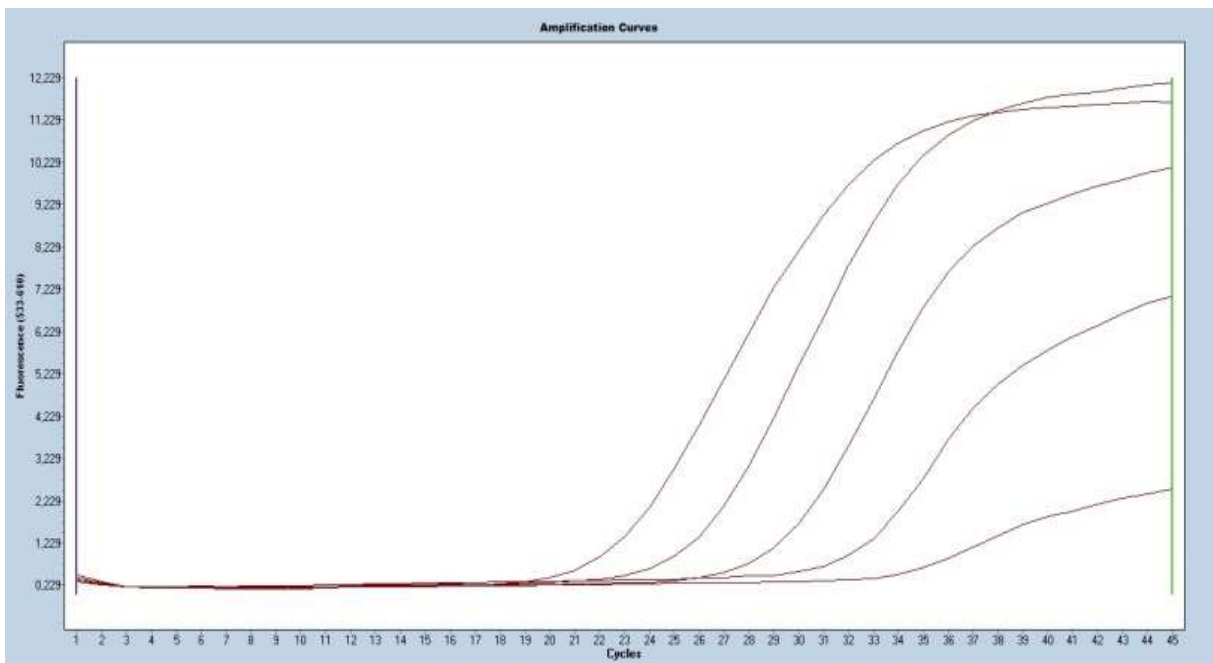


Fig.7: Dilution series Rotavirus ($10^5 - 10^1$ RNA copies per μl) on the LightCycler[®] 480II

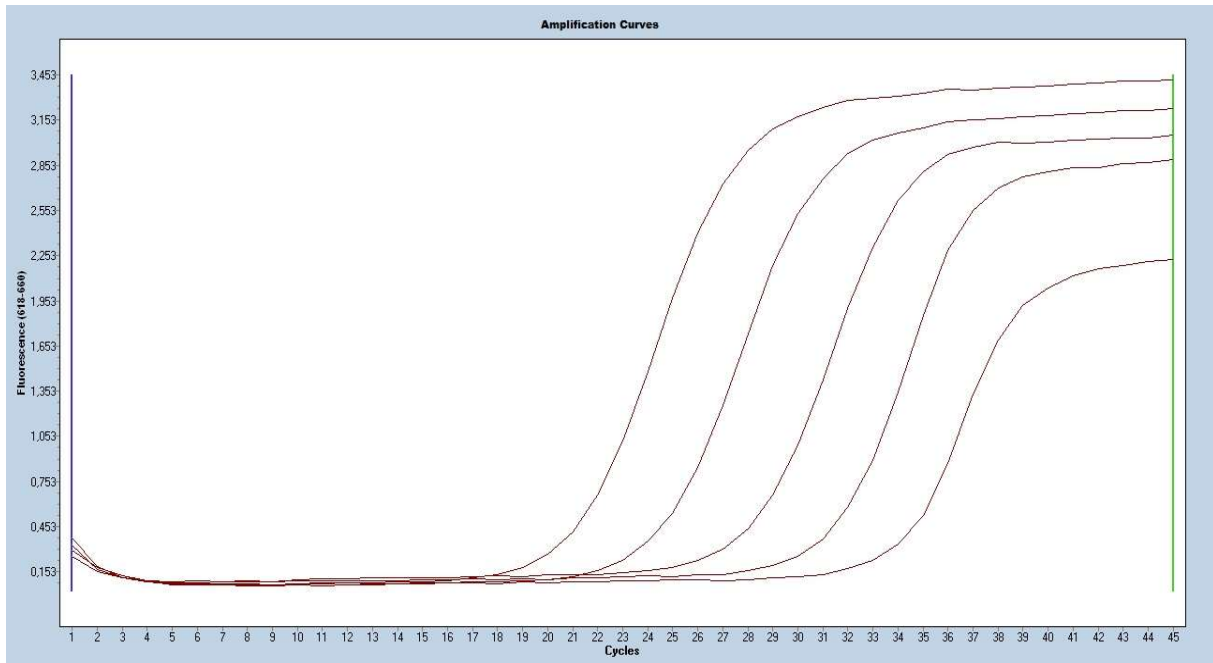


Fig. 8: Dilution series Astrovirus (10^5 – 10^1 RNA copies per μl) on the LightCycler® 480II

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

13.2 Analytical specificity

The analytical specificity of the RIDA®GENE Viral Stool Panel I multiplex real-time PCR is specific for Adenovirus, Norovirus (Genogroup I and II), Rotavirus and Astrovirus. No cross-reaction could be detected for the following species (see Tab.10):

Tab. 10: Cross-reactivity testing

Adenovirus type: 4	-	<i>Campylobacter fetus</i> subsp. <i>fetus</i>	-	<i>Cryptosporidium muris</i>	-	<i>Klebsiella oxytoca</i>	-
Adenovirus type: 5	-	<i>Campylobacter lari</i> subsp. <i>lari</i>	-	<i>Cryptosporidium parvum</i>	-	<i>Proteus vulgaris</i>	-
Adenovirus type: 7A	-	<i>Campylobacter upsaliensis</i>	-	<i>E. coli</i> (O26:H-)	-	<i>Pseudomonas aeruginosa</i>	-
Adenovirus type: 11	-	<i>Candida albicans</i>	-	<i>E. coli</i> (O6)	-	<i>Salmonella enteritidis</i>	-
Adenovirus type: 31	-	<i>Citrobacter freundii</i>	-	<i>E. coli</i> (O157:H7)	-	<i>Salmonella typhimurium</i>	-
Adenovirus type: 37	-	<i>Clostridium bifermentans</i>	-	<i>Entamoeba histolytica</i>	-	<i>Serratia liquefaciens</i>	-
<i>Aeromonas hydrophila</i>	-	<i>Clostridium difficile</i>	-	<i>Enterobacter cloacae</i>	-	<i>Shigella flexneri</i>	-
<i>Arcobacter butzleri</i>	-	<i>Clostridium novyi</i>	-	<i>Enterococcus faecalis</i>	-	<i>Staphylococcus aureus</i>	-
<i>Bacillus cereus</i>	-	<i>Clostridium perfringens</i>	-	<i>Giardia intestinalis</i> Portland 1	-	<i>Staphylococcus epidermidis</i>	-
<i>Bacteroides fragilis</i>	-	<i>Clostridium septicum</i>	-	<i>Giardia intestinalis</i> WB Clone C6	-	<i>Vibrio parahaemolyticus</i>	-
<i>Campylobacter coli</i>	-	<i>Clostridium sordellii</i>	-	<i>Giardia lamblia</i>	-	<i>Yersinia enterocolitica</i>	-
<i>Campylobacter jejuni</i>	-	<i>Clostridium sporogenes</i>	-				

13.3 Analytical reactivity

The reactivity of the RIDA®GENE Viral Stool Panel I multiplex real-time PCR was tested with Adenovirus, Norovirus, Rotavirus and Astrovirus (see Tab.11). All tested Adenovirus, Rotavirus and Astrovirus serogroups and Norovirus genogroups were detected by the RIDA®GENE Viral Stool Panel I multiplex real-time PCR assay or by sequence alignment (*).

Tab. 11: Analytical reactivity testing










Adenovirus					
Serotype 40	+	Serotype 41	+		
Norovirus					
Genogroup I					
GGI.1 – Norwalk	+	GGI.3 – Desert Shield, Birmingham	+	GGI.6 – Hesse	+
GGI.2 – Southhampton, Whiterose	+	GGI.4 – Chiba, Malta	+	GGI.7 – Winchester	+
GGI.2 – Southhampton, Southhampton	+	GGI.5 – Musgrove	+	GGI.8 – Boxer	+
Genogroup II					
GGII.1 – Hawaii	+	GGII.4 – Sydney 2012	+	GGII.17 – Kawasaki	+
GGII.2 – Melksham	+	GGII.6 – Seacroft	+	GGII.b – Hilversum	+
GGII.3 – Toronto	+	GGII.7 – Leeds	+	GGII.c – Den Haag	+
GGII.4 – Bristol, Grimsby 2004	+	GGII.10 – Erfurt	+		
Genogroup IV					
GGIV.1 – Alpatron	+				
Rotavirus					
Serogroup A					
Serotype G1	+	Serotype G2	+	Serotype G3	+
Serotype G4	+	Serotype G9	+	Serotype G12	+
Astrovirus					
Serotype 1*	+	Serotype 2	+	Serotype 3*	+
Serotype 4*	+	Serotype 5*	+	Serotype 7*	+
Serotype 8	+				

14. Version history

Version number	Chapter and designation
2018-06-14	Previous version
2021-01-27	General revision 10. Quality control (Spelling mistake) 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

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

Testspecific symbols

Reaction Mix

Enzyme-Mix

Internal Control RNA

No Template Control

Positive Control

16. Literature

1. Hoehne M, *et al.* Detection of Norovirus genogroup I and II by multiplex real-time RT-PCR using a 3'-minor groove binder-DNA probe. *BMC Infectious Diseases*. 2006; 6:69-75.
2. Pang XL, *et al.* Increased Detection of Rotavirus Using a Real Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Assay in Stool Specimens From Children With Diarrhea. *Journal of Medical Virology* 2004, 72: 496–501.
3. Heim A, *et al.* Rapid and Quantitative Detection of Human Adenovirus DNA by Real-Time PCR. *Journal of Medical Virology* 2003, 10: 228-239.
4. Kapoor A, *et al.* Multiple novel Astrovirus species in human stool. *Journal of general Virology* 2009, 90: 2965-2972.
5. Mead PS, *et al.* *EID* 1999, 5: 607-625.
6. Centers for Disease Control and Prevention. Norovirus: Overview 2012.
7. Parra GI, *et al.* Static and Evolving Norovirus Genotypes: Implications for Epidemiology and Immunity. *PLoS Pathog* 2017, 13(1): e1006136.
8. Vinjé J. Advances in laboratory methods for detection and typing of norovirus. *Journal Clinical Microbiology* 2015, 53(2):373-81.
9. Parashar UD, *et al.* Rotavirus and Severe Childhood Diarrhea. *Emerging Infectious Diseases* 2006, 12: 304-306.
10. Robert Koch Institut. Rotaviren-Gastroenteritis. RKI-Ratgeber Infektionskrankheiten. Stand 31.07.2013.
https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Rotaviren.html;jsessionid=D381EC22661EBE5C847628E9368E3401.2_cid381#doc2374564bodyText8. Aufgerufen am 09.07.2018.
11. Robert Koch Institut. Keratoconjunctivitis epidemica und andere Konjunktivitis durch Adenoviren. RKI-Ratgeber Infektionskrankheiten – Merkblätter für Ärzte 2010.
12. Robinson CM, *et al.* Molecular evolution of human species D adenoviruses. *Infection, Genetics and Evolution* 2011, 11: 1208-1217.
13. Guix S, *et al.* Human astrovirus diagnosis and typing: current and future Prospects. *Letters of Applied Microbiology* 2005, 41:103-105.
14. Wilhelmi I, *et al.* Viruses causing gastroenteritis. *Clinical Microbiology and Infection* 2003, 9: 247-262.
15. Guix S, *et al.* Human Astroviruses. *Clinical Microbiology Reviews* 2014, 27:1048-1074.