RIDA®GENE Adenovirus

REF PG1005

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Adenovirus is a multiplex real-time PCR for the direct, qualitative detection of Adenovirus in human stool samples, throat rinsing fluid, sputum and bronchoalveolar lavage (BAL).

The RIDA®GENE Adenovirus real-time PCR is intended for use as an aid in diagnosis of respiratory infections caused by adenoviruses.

2. Summary and explanation of the test

Adenoviruses are non-enveloped ikosaedric double-stranded DNA (dsDNA) viruses and belong to the family of *Adenoviridae.* They were isolated from human pharyngeal tonsils (Adenoides), where their name originated from. [1] One differentiates 56 serotypes of human adenoviruses and they are classified into seven groups (A - G).[4,5] Adenoviruses cause a variety of different clinical pictures. Besides ocular and gastrointestinal infections, adenoviruses mostly cause respiratory disease. The latter one is primarily observed in small children under the age of four since they lack humoral immunity. However, 1 – 7 % of adult respiratory infections are caused by adenoviruses.[1] The symptoms of an adenovirus infection reach from cold, acute bronchitis to pneumonia and in immunocompromised patients, also acute respiratory distress syndrome (ARDS) is observed. Acute respiratory infections are mainly caused by serotypes 1, 2, 3, 4, 6, 7, 14 and 21, whereas serotypes 1, 2, 3, 4 and 7 are the major causes of pneumonia. Many adenoviruses are endemic with adenovirus outbreaks being often described on military bases.[2] In 2006/2007, a new adenovirus variant of serotype 14 lead to a major respiratory disease outbreak with a mortality rate of 5%.[3] The clinical picture of an adenovirus infection is also dependent on its viral entry to the host. Inhalation with adenovirus 7 leads to major infection of the lower respiratory tract but oral uptake of adenovirus 7, if at all, may only lead to mild infection. The RIDA®GENE Adenovirus assay was developed as an aid in diagnosis of respiratory infections, although Adenovirus serotypes, which cause primarily gastrointestinal infections (serotype 40 and 41), can be detected from stool samples.

3. Test principle

The RIDA®GENE Adenovirus is a multiplex real-time PCR for the direct, qualitative detection of Adenovirus in human stool samples, throat rinsing fluid, sputum and bronchoalveolar lavage (BAL).
After DNA isolation, amplification of gene fragments (Hexon, if present) specific for Adenovirus occurs. The amplified target for Adenovirus is 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 Adenovirus assay contains an Internal Control DNA (ICD) 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	Taq-Polymerase	1x	80 µl	red
D	Internal Control DNA	2x	1700 µl	orange
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 and fully defrost 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 reagents should be stored cold in an appropriate way (2 - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE Adenovirus multiplex real-time PCR assay is suitable for use with following extraction platforms and real-time PCR instruments:

Tab. 2 Necessary equipment

Extraction platforms	
R-Biopharm	RIDA® Xtract
Promega	Maxwell® RSC
Roche	MagNA Pure 96

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 II (PG0002) for use with the LightCycler® 2.0
- RIDA®GENE Color Compensation Kit IV (PG0004) for use with 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 DNA preparation from stool samples

For DNA isolation of human stool samples, use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.
We recommend to dilute the stool samples before extraction 1:3 with water (or with S.T.A.R. Buffer if the MagNA Pure 96 is used). Vortex the diluted stool sample intensely and centrifuge at 1000 x g for 30 sec. Use from the supernatant the appropriate volume according to the manufacturer's instruction.

The RIDA®GENE Adenovirus assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA 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 DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA 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 DNA to the negative control and positive control PCR Mix.

8.2 DNA preparation from throat rinsing fluid, sputum and bronchoalveolar lavage (BAL)

For DNA isolation of throat rinsing fluid, sputum and bronchoalveolar lavage (BAL), use a commercially available DNA isolation kit (e.g. RIDA® Xtract (R-Biopharm)) or DNA extraction system (e.g. Maxwell® RSC (Promega)). Extract DNA according to the manufacturer's instructions.

The RIDA®GENE Adenovirus assay contains an Internal Control DNA that detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient. The Internal Control DNA 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 DNA is used only as a PCR inhibition control, 1 µl of the Internal Control DNA should be added to the Master-Mix (see Tab.4).

If the Internal Control DNA is used as an extraction control for the sample preparation procedure **and** as PCR inhibition control, 20 µl of the Internal Control DNA has to be added during extraction procedure. The Internal Control DNA 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 DNA 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 Taq-Polymerase, the Positive Control, the No Template Control and the Internal Control DNA 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 (ICD 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	Taq-Polymerase	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 (ICD 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	Taq-Polymerase	0.7 µl	7.7 µl
D	Internal Control DNA	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 DNA 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 DNA to the PCR-Mix of the negative control.

Sample: Add 5 µl DNA 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 DNA 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 DNA to the PCR-Mix of the positive control.

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The PCR reaction should be started according to the PCR instrument set-up see (Tab. 5, Tab. 6, Tab.7, Tab. 8).

9.3 PCR Instrument set-up

9.3.1 DNA real-time PCR profile

Tab. 5: DNA real-time PCR profile for LightCycler® series and Rotor-Gene Q

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 occur in the same step.

Tab. 6: DNA real-time PCR profile for Mx3005P, ABI7500, CFX96™

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 occur in the same step.

9.3.2 Universal real-time PCR profile

Note: The universal real-time PCR profile should only be used for DNA assays when combining RIDA®GENE DNA and RNA real-time PCR assays in one run.

Tab. 7: Universal real-time PCR profile for LightCycler® series

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 occur in the same step.

Tab. 8: Universal real-time PCR profile for Mx3005P, ABI7500, CFX96™ and Rotor-Gene Q

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 occur in the same step.

9.4 Detection channel set-up

Tab. 9: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Note
Roche LightCycler® 2.0	Adenovirus	530	**RIDA®GENE Color Compensation Kit II (PG0002) is required**
	ICD	560	
Roche LightCycler® 480II	Adenovirus	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required**
	ICD	533/580	
Agilent Techn. Mx3005P	Adenovirus	FAM	**Check that reference dye is none**
	ICD	HEX	
ABI 7500	Adenovirus	FAM	**Check that passive reference option ROX is none**
	ICD	VIC	
Bio-Rad CFX96™	Adenovirus	FAM	**-**
	ICD	VIC	
Qiagen Rotor-Gene Q	Adenovirus	Green	**The gain settings have to be set to 5, according to the default settings**
	ICD	Yellow	

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. Positive control and negative control have to show correct results (see Table 10, 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. 10: For a valid run, the following conditions must be met:

Sample	Assay result	ICD 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 ICD 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

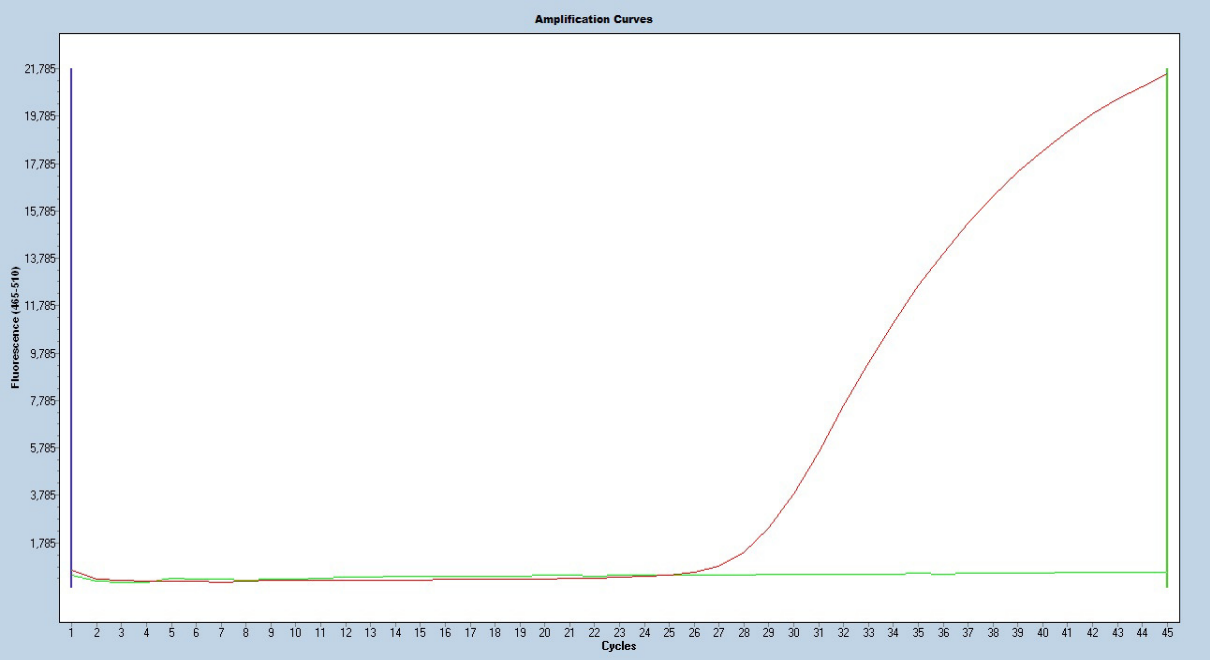

Fig. 1: Correct run of the positive control and negative control (Adenovirus) on the LightCycler® 480II

11. Result interpretation

The result interpretation is done according to Table 11.

Tab. 11: Sample interpretation

Target genes		
Adenovirus	**ICD**	**Result**
positive	**positive/negative**	**Adenovirus detected**
negative	**positive**	**Target genes not detected**
negative	**negative**	**Invalid**

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

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

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

A sample is invalid, if the sample DNA and Internal Control DNA show no amplification signal in the detection system. The sample contains a PCR inhibitor or a failure occurred in the extraction procedure. 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 suitable for human stool samples, throat rinsing fluid, sputum and BAL samples.
3. Inappropriate specimen collection, transport, storage and processing or a pathogen 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 variants resulting in a false negative result with the RIDA®GENE Adenovirus 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 (Hexon).

13. Performance characteristics

13.1 Clinical Performance

In a retrospective clinical validation study we analyzed 118 extracted respiratory specimens with the RIDA®GENE Adenovirus assay and an in-house real-time PCR assay in an institute in Germany.

Tab. 12: Correlation of the Adenovirus results with the RIDA®GENE Adenovirus real-time PCR and reference in-house real-time PCR

		In-house real-time PCR			
		Positive	Negative	Total	
RIDA®GENE Adenovirus	Positive	16	0	16	PPV: 100 %
	Negative	0	102	102	NPV: 100%
	Total	16	102	118	

13.2 Analytical sensitivity

The RIDA®GENE Adenovirus multiplex real-time PCR has a detection limit of ≥ 10 DNA copies per reaction for Adenovirus.

The following figure 2 shows a dilution series Adenovirus (10^5 - 10^1 DNA copies per µl) on the LightCycler® 480II.

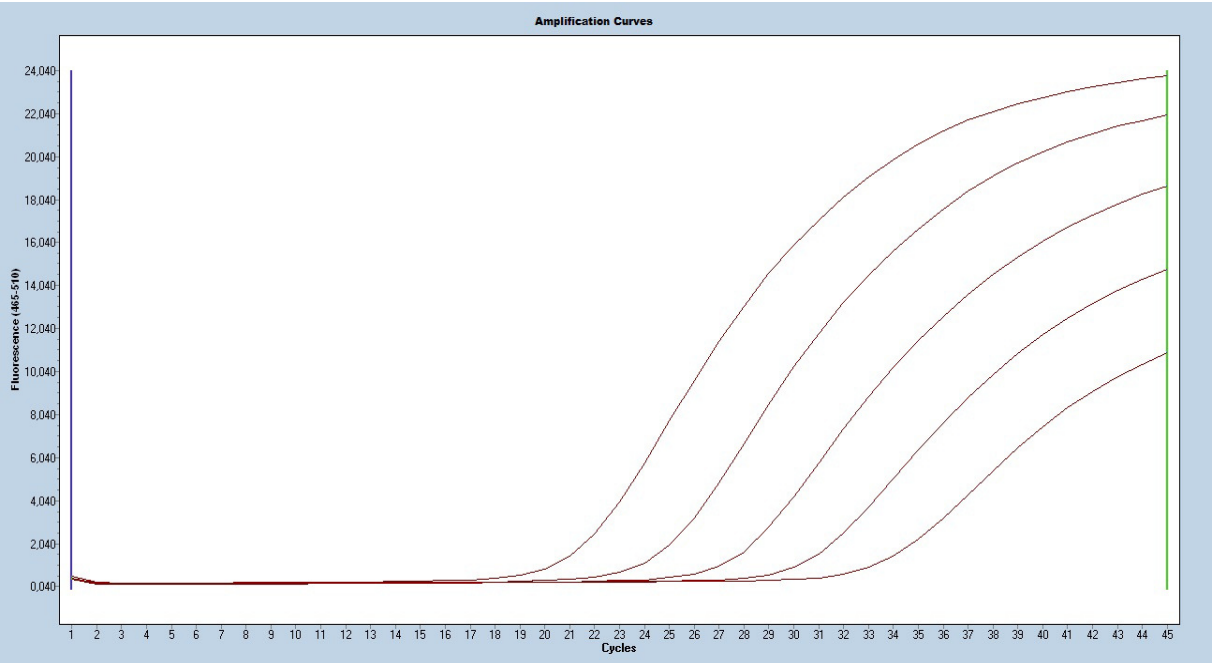

Fig. 2: Dilution series Adenovirus (10^5 – 10^1 DNA copies per µl) on the LightCycler® 480II

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

13.3 Analytical specificity

The analytical specificity of the RIDA®GENE Adenovirus multiplex real-time PCR is specific for Adenovirus. No cross-reaction could be detected for the following species (see Tab. 13):

Tab. 13: Cross-reactivity testing

Acinetobacter baumannii	-	Coronavirus 229E, human	-	Norovirus GGII	-
Arcobacter butzleri	-	Coxsackie virus B4, human	-	Parainfluenza virus 1 strain C35, human	-
Aeromonas hydrophila	-	Cytomegalovirus, human	-	Parainfluenza virus 2 strain Greer, human	-
Bacillus cereus	-	*E. coli* (O157:H7)	-	Parainfluenza virus, serotype 3	-
Bacteroides fragilis	-	*E. coli* (O26:H-)	-	Parainfluenza virus 4b strain CH19503, human	-
Bordetella parapertussis	-	*E. coli* (O6)	-	*Proteus vulgaris*	-
Bordetella pertussis	-	*Entamoeba histolytica*	-	*Pseudomonas aeruginosa*	-
Campylobacter coli	-	*Enterobacter cloacae*	-	Respiratory syncitial virus, human, strain Long	-
Campylobacter fetus subsp. *Fetus*	-	*Enterococcus faecalis*	-	Respiratory syncitial virus, human, strain 9320	-
Campylobacter lari subsp. *Lari*	-	Epstein-Barr-Virus B95-8 strain	-	Rhinovirus, human, Genogruppe A	-
Campylobacter jejuni	-	*Giardia intestinalis* Portland 1	-	Rotavirus	-
Campylobacter upsaliensis	-	*Giardia intestinalis WB* Clone C6	-	*Salmonella enteritidis*	-
Candida albicans	-	*Haemophilus influenzae* Rd	-	*Salmonella typhimurium*	-
Citrobacter freundii	-	Herpes simplex virus 1 strain McIntyre	-	*Serratia liquefaciens*	-
Clostridium bifermentas	-	Herpes simplex virus 2 strain MS	-	*Shigella flexneri*	-
Clostridium difficile	-	Influenza virus A/PR/8/34	-	*Staphylococcus aureus*	-
Clostridium novyi	-	*Klebsiella pneumoniae*	-	*Staphylococcus epidermidis*	-
Clostridium perfringens	-	*Klebsiella oxytoca*	-	*Staphylococcus haemolyticus*	-
Clostridium septicum	-	*Legionella pneumophila* subsp. *pneumophila*	-	*Staphylococcus hominis* subsp. *novobiosepticus R22*	-
Clostridium sordellii	-	*Mycoplasma pneumoniae*	-	*Streptococcus pneumoniae*	-
Clostridium sporogenes	-	Metapneumovirus, human	-	Varicella Zoster Virus (Type B)	-
Cryptosporidium muris	-	*Neisseria meningitides*	-	*Vibrio parahaemolyticus*	-
Cryptosporidium parvum	-	Norovirus GGI	-	*Yersinia enterocolitica*	-

13.4 Analytical reactivity

The reactivity of the RIDA®GENE Adenovirus multiplex real-time PCR was evaluated against one serotype exemplary for each Adenovirus serogroup (see Tab. 14). All tested adenoviruses were detected by the RIDA®GENE Adenovirus multiplex real-time PCR.

Tab. 14: Analytical reactivity testing

Adenovirus					
Serogroup A					
Serotype 31	+				
Serogroup B					
Serotype 7A	+	Serotype 11	+		
Serogroup C					
Serotype 1	+	Serotype 5	+		
Serogroup D					
Serotype 37	+				
Serogroup E					
Serotype 4	+				
Serogroup F					
Serotype 40	+	Serotype 41	+		

14. Version history

Version number	Chapter and designation
2014-08-14	Release version
2018-10-30	General revision 2. Summary and explanation of the test 4. Reagents provided 6. Additional necessary reagents and necessary equipment 8. Collection and storage of samples 9. Test procedure 10. Quality Control 13. Performance characteristics 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

Not applicable

16. Literature

1. Cesario T. Viruses associated with pneumonia in adults. Clin Infect Diseases 2012, 55:107–113.
2. Sanchez J, *et al.* Epidemic of adenovirus-induced respiratory illness among US military recruits-epidemiologic and immunologic risk factors in healthy young adults. J Med Virol 2001, 65:710–718.
3. Tate J, *et al.* Outbreak of severe disease associated with emergent human adenovirus serotype 14 at a US Air Force training facility. J Infect Dis 2009, 199:1419–1426.
4. Robert Koch Institut. Keratoconjunctivitis epidemica und andere Konjunktivitidendurch Adenoviren. RKI-Ratgeber Infektionskrankheiten – Merkblätter für Ärzte 2010.
5. Robinson CM, *et al.* Molecular evolution of human species D adenoviruses. Infection, Genetics and Evolution 2011, 11: 1208-1217.