RIDA®GENE Pediatric Viral Panel

REF PG4725

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1. Intended use

For *in vitro* diagnostic use. The RIDA®GENE Pediatric Viral Panel test, performed on the LightCycler® 480 II real-time PCR instrument, is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of rhinovirus/ enterovirus/ parechovirus RNA, bocavirus DNA, and adenovirus DNA in untreated human nasal/throat swabs from people with signs and symptoms of a respiratory infection.

The RIDA®GENE Pediatric Viral Panel test is intended to support the differential diagnosis of rhinovirus/enterovirus/parechovirus, bocavirus, and adenovirus infections in patients with symptoms of a respiratory infection in connection with other clinical and laboratory findings.
Negative results do not rule out infection with rhinovirus/enterovirus/parechovirus, bocavirus, or adenovirus 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

Viral respiratory tract infections are one of the primary causes of hospitalizations and clinic visits in children and older people.[1-6] In addition to the known respiratory viruses like influenza and SARS-CoV-2, adenoviruses, bocaviruses, enteroviruses, parechoviruses, and rhinoviruses can cause respiratory tract infections.

Human adenoviruses (HAdVs) belong to the family Adenoviridae and the genus Mastadenovirus. They are icosahedron-shaped, nonenveloped, double-stranded DNA viruses with a diameter of 65 to 110 nm. They are divided into seven species, (A to G), which comprise about 67 serotypes.[7,8]

Human bocaviruses (HBoVs) are icosahedral, small, nonenveloped parvoviruses.[9-11] They are about 18 nm to 26 nm in diameter and have single-stranded DNA with a genome size of 4 kb to 6 kb (plus terminal sequences of 32 to 52 nucleotides). The role of HBoVs as harmless passengers is still contentiously debated because they are often isolated in combination with other pathogens. HBoVs comprise four subtypes: HBoV1, HBoV2, HBoV3, and HBoV4.[9,10]

Enteroviruses and rhinoviruses are single-stranded, icosahedral, nonenveloped RNA viruses that have a genome size of about 7.2 kb.[12-15] Before molecular tools were introduced to classify pathogens, rhinoviruses and enteroviruses were considered to be two very different pathogens.[15] Today, we know that they are genetically related to one another. They both belong to the genus Enterovirus within the family Picornaviridae.[13-17] Human enteroviruses (HEVs) comprise four species, HEV-A, HEV-B, HEV-C, and HEV-D, with more than 200 different serotypes. Rhinoviruses are divided into three species, rhinovirus A, rhinovirus B, and rhinovirus C, which comprise about 150 different types.[12-18] Like enteroviruses and rhinoviruses, human parechoviruses (HPeVs) belong to the family Picornaviridae, but they are classified under the genus Parechovirus.[16,17,19-22] HPeVs are single-stranded, nonenveloped

RNA viruses. HPeVs comprise 19 genotypes (HPeV1 to HPeV19), whereas HPeV1 and HPeV3 are the most common.[19,20]

HAdV infections that affect the respiratory tracts are reported with common cold-like symptoms including fever, sore throat, vomiting, and diarrhea, and severe infections are reported with conjunctivitis and lung inflammation.[3,7,8] HBoV infections induce wheezing and cough as common symptoms. Both viruses are distributed worldwide and occur throughout the year, whereas HBoV reaches a peak in the winter and spring months.[8,9]

Reported symptoms of HEV respiratory tract infections include cough, shortness of breath, wheezing, and fever.[14] HEV infections are more common in the summer and fall in temperate climates and occur throughout the year in tropical regions.[13] Rhinoviruses are distributed worldwide, with higher prevalence in spring and fall.[12,15] They occur commonly in patients with pneumonia, bronchiolitis, or an infection of the middle ear and can occur with or without copathogens. Rhinoviruses cause symptoms like rhinorrhea, sore throat, cough, headache, nasal congestion, and malaise.[15] Common symptoms of an HPeV infection are common cold symptoms such as stuffy nose and fever as well as cough.[20,21,23]

Usually viral respiratory infections with these viruses are self limiting or asymptomatic, but especially children, older people, and patients with immunodeficiency can be affected by a severe disease.[7-9,14-16,19,22]

3. Test principle

The RIDA®GENE Pediatric Viral Panel test is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of rhinovirus/enterovirus/parechovirus RNA, bocavirus DNA, and adenovirus DNA in human nasal/throat swabs.
After DNA isolation, (if present) the specific gene fragments of rhinovirus/enterovirus/parechovirus (5'UTR), bocavirus (5'NTR), and adenovirus (hexon) are amplified. The amplified target sequences are detected using hydrolysis probes that are labeled with a quencher at one end and a fluorescent reporter dye (fluorophore) at the other. The probes hybridize to the amplicon in the presence of a target sequence. During extension, Taq-Polymerase separates the reporter from the quencher. The reporter emits a fluorescent signal that is detected by the optical unit of a real-time PCR instrument. The fluorescent signal increases with the quantity of formed amplicons. The RIDA®GENE Pediatric Viral Panel test contains an Internal Control RNA (ICR) to be able to control sample preparation and/or any potential PCR inhibition.

4. Reagents provided

The reagents in the kit are sufficient for 100 determinations.

Table 1: Reagents provided

Kit code	Reagent	Amount		Lid color
1	Reaction Mix	2 ×	1050 µL	yellow, ready for use
2	Enzyme Mix	1 ×	80 µL	red, ready for use
R	Internal Control RNA	2 ×	1700 µL	brown, ready for use
N	No Template Control	1 ×	450 µL	white, ready for use
P	Positive Control	1 ×	200 µL	blue, ready for use

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 -20 °C and, if unopened, can be used until the expiration date printed on the label. After the expiration date, the quality guarantee is no longer valid.
- All reagents should be carefully thawed prior to use (e.g., in a refrigerator at 2 °C - 8 °C).
- Repeated freezing/thawing of up to 20 times does not have an impact on the test property (if necessary, create aliquots after the first thaw and refreeze reagents immediately).
- Cool all reagents appropriately during PCR preparation (2 °C - 8 °C).

Table 2: Storage conditions and information

	Storage temperature	Maximum storage time
unopened	-20 °C	Can be used until the printed expiration date
opened	-20 °C	20 freeze-thaw cycles

6. Reagents required but not provided

6.1 Reagents

None.

6.2 Laboratory equipment

The following equipment is needed for performing the RIDA®GENE Pediatric Viral Panel test:

Equipment
Extraction platform: MagNA Pure 96 instrument (Roche)
Real-time PCR instrument: LightCycler® 480 II (Roche)
RIDA®GENE Color Compensation Kit IV (PG0004) (R-Biopharm)
Real-time PCR consumables (plates (low profile, white wells, clear frame), reaction vials, films)
Centrifuge with rotor for plates
Vortexer
Pipettes (0.5 - 20 µL, 20 - 200 µL, 100 - 1000 µL)
Pipette tips with filters
Powder-free disposable gloves

For questions on the use of equipment for automated processing, please contact R-Biopharm AG at pcr@r-biopharm.de.

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.
This test must be carried out only by qualified laboratory personnel. The guidelines for working in medical laboratories must be followed.
Always adhere strictly to the operating manual 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.
Separate rooms, special clothing, and instruments for extraction, PCR preparation, and PCR must be used to prevent cross-contamination and false-positive results.
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,
Internal Control RNA, Positive Control, No Template Control) from one kit lot with the components of another lot.
Do not use the kit after the expiration date. 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

8.1 DNA/RNA preparation from nasal and throat swabs

For DNA/RNA preparation from nasal and throat swabs, the MagNA Pure 96 DNA/Viral NA SV Kit on the MagNA Pure 96 instrument (Roche) is recommended. For this, use the Pathogen Universal 200 protocol and elute in 50 µL. The manufacturer's instructions must be observed.

The RIDA®GENE Pediatric Viral Panel test contains an Internal Control RNA that indicates potential PCR inhibition, checks the integrity of the reagents, and confirms successful nucleic acid extraction. The Internal Control RNA can be used either solely as an inhibition control or as a process control (extraction and inhibition control).

When the Internal Control RNA is used only as an inhibition control for amplification, 1 µL of the Internal Control RNA must be added to the Master Mix (see Table 4).

When the Internal Control RNA is used as an extraction control for sample preparation **and** as an inhibition control for amplification, then 20 µL of the Internal Control RNA must be used during extraction. It is recommended, if possible, to add the Internal Control RNA to the sample cartridge before adding the sample. It is recommended to pipette 1 µL of the Internal Control RNA per reaction into the PCR mix of the negative control and of the positive control.

9. Test procedure

9.1 Preparation of the Master Mix

The total number of the reactions needed for PCR (samples and control reactions) must be calculated. One positive control and one negative control must be included in each test run.

Adding an additional 10 % volume to the Master Mix is recommended in order to compensate for any pipetting loss (see Table 3, Table 4). Prior to use, thaw the Reaction Mix, the Enzyme Mix, the Positive Control, the No Template Control, and the Internal Control RNA, vortex (except for the enzyme mix), and centrifuge for a short time. Reagents must always be cooled appropriately during the work steps (2 °C - 8 °C).

Table 3: Example of the calculation and preparation of the Master Mix for 10 reactions (ICR as extraction and inhibition control)

Kit code	Components of the Master Mix	Quantity per reaction	10 reactions (plus 10 %)
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 Master Mix and then centrifuge for short time.

Table 4: Example of the calculation and preparation of the Master Mix for 10 reactions (ICR only as inhibition control)

Kit code	Components of the Master Mix	Quantity per reaction	10 reactions (plus 10 %)
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 Master Mix and then centrifuge for short time.

9.2 Preparation of the PCR mix

Pipette 20 µL of the Master Mix into each reaction vial (plates).

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

Note: When the Internal Control RNA is used as an extraction control for sample preparation and as an inhibition control, it is recommended to add 1 µL of the Internal Control RNA to each PCR mix of the negative control.

Samples: Add 5 µL eluate to each pre-pipetted Master Mix.

Positive control: Add 5 µL of the Positive Control to each pre-pipetted Master Mix.

Note: When the Internal Control RNA is used as an extraction control for sample preparation and as an inhibition control, it is recommended to add 1 µL of the Internal Control RNA to each PCR mix of the positive control.

Seal the plates, briefly centrifuge at slow speed, and transfer to the real-time PCR instrument. Start PCR according to PCR instrument setup (see Table 5, Table 6).

9.3 PCR instrument setup

9.3.1 Universal real-time PCR profile

To harmonize the RIDA®GENE assays, the RIDA®GENE Pediatric Viral Panel assay was verified in the universal profile. This makes it possible to combine DNA and RNA assays with each other.

Table 5: Universal real-time PCR profile for LightCycler® 480 II

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.

9.4 Detection channel setting

Table 6: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Comment
Roche LightCycler® 480 II	Rhinovirus/ enterovirus/ parechovirus	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	ICR	533/580	
	Bocavirus	533/610	
	Adenovirus	618/660	

10. Quality control - indication of instability or expiration of reagents

Samples are evaluated using the analysis software of the real-time PCR instrument according to the manufacturer's instructions. Negative and positive controls must show the correct results (see Table 7).

The Positive Control is present in a concentration of 10^3 copies/µL. It is used in a total quantity of 5 x 10^3 copies in every PCR run.

Table 7: A valid PCR run must meet the following conditions:

Sample	Result	ICR Ct	Target gene Ct
Positive control	Positive	N/A *[1]	See Certificate of Analysis
Negative control	Negative	Ct > 20	0

[1] A Ct value for the ICR is not needed to obtain a positive result of the positive control.

If the positive control is not within the specified Ct range but the negative control is valid, all reactions need to be reanalyzed, including the controls.

If the negative control is not negative, but the positive control is valid, all reactions need to be reanalyzed, including the controls.

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. Result interpretation

Table 8: Result interpretation*

Detection of				
Rhinovirus/ enterovirus/ parechovirus	**Bocavirus**	**Adenovirus**	**ICR**	**Result**
+	-	-	+/-	**Rhinovirus/enterovirus/ parechovirus detectable**
-	+	-	+/-	**Bocavirus detectable**
-	-	+	+/-	**Adenovirus detectable**
+	+	-	+/-	**Rhinovirus/enterovirus/ parechovirus & bocavirus detectable**
+	-	+	+/-	**Rhinovirus/enterovirus/ parechovirus & adenovirus detectable**
-	+	+	+/-	**Bocavirus & adenovirus detectable**
+	+	+	+/-	**Rhinovirus/enterovirus/ parechovirus; bocavirus & adenovirus detectable**
-	-	-	+	**Target genes not detectable**
-	-	-	-	**Invalid**

* + = positive
- = negative

A sample is positive if the sample RNA and the Internal Control RNA 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 RNA in the detection system. Detecting the Internal Control RNA is not necessary in this case because high amplicon concentrations can result in a weak or absent signal of the Internal Control RNA.

A sample is negative if the sample RNA does not show an amplification signal, but an amplification signal is visible for the Internal Control RNA in the detection system. Inhibition of the PCR reaction can be ruled out by the detection of the Internal Control RNA.

A sample is invalid if the sample RNA and the Internal Control RNA do not show an amplification signal in the detection system. PCR inhibitors are present in the sample or an error occurred during the extraction process.

12. Limitations of the method

1. The RIDA®GENE Pediatric Viral Panel test detects rhinovirus/ enterovirus/ parechovirus RNA, bocavirus DNA, and adenovirus DNA in untreated human nasal/throat swabs. 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 validated only for nasal/throat swabs.
4. Improper sampling, transport, storage, and handling, or a pathogen load below the test's analytical sensitivity can lead to false-negative results.
5. The presence of PCR inhibitors can lead to false-negative or invalid results.
6. 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.
7. 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 the RIDA®GENE Pediatric Viral Panel.
8. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target genes (rhinovirus/ enterovirus/ parechovirus (5'UTR), bocavirus (5'NTR), and adenovirus (hexon)) are present.
9. The substances beclomethasone dipropionate and dihydrocodeine can have interfering properties even in low amounts. Starting at a concentration of 5 % [v/v], ratioAllerg® 50 µg (beclomethasone dipropionate) has an interfering effect on the detection of adenovirus. Paracodin® N drops (dihydrocodeine) have an interfering effect on the detection of enterovirus/rhinovirus/parechovirus and the detection of the IC starting at the lowest tested concentration of 3.0 % [v/v].
10. This assay should be performed in compliance with the regulation on good laboratory practice (GLP). Users must precisely follow the manufacturer's instructions when performing the test.

13. Performance characteristics

13.1 Analytical performance characteristics

13.1.1 Device detection limit

For determining the device detection limit, 20 replicates of a control sample (50 copies/reaction (rhinovirus/ enterovirus/ parechovirus, bocavirus, adenovirus)) were measured on LightCycler® 480 II. All replicates were positive.

The device detection limit is therefore 50 copies/reaction.

13.1.3 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 could be present given their widespread use in respiratory infections or their widespread occurrence in the corresponding samples (nasal/throat swabs) 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. If a potential interference was found in this interference screen for an examined substance, a dose-effect relationship was established between the concentration of the substance in question and the interference.
No interference was found for the substances listed in Table 10.

Table 10: Potentially interfering substances

Potentially interfering substance	Concentration
Amoxicillin-ratiopharm® 1000 mg film-coated tablets (amoxicillin)	1 mg/mL
Azithromycin-ratiopharm® 500 mg film-coated tablets (azithromycin)	84 mg/mL
Human blood	2 % [v/v]
Mucins	60 µg/mL
Nasenspray-ratiopharm® nasal spray (xylometazoline)	10 % [v/v]
Sodium chloride	10 % [v/v]
Neo-angin® spray (benzydamine hydrochloride)	10 % [v/v]
Oseltamivir phosphate	25 mg/mL
Paracetamol-ratiopharm® 500 mg tablets (paracetamol)	10 mg/mL

Inhibitory effects were observed for the substances ratioAllerg® 50 µg (beclomethasone dipropionate) (5 % [v/v]) and Paracodin® N drops (dihydrocodeine) (3.0 % [v/v]) (see Limitations of the method).

Cross-reactivity

Various organisms (bacteria, viruses, fungi) that are commonly found in the matrix of nasal/throat swabs were investigated. The microorganisms to be investigated for this assay were chosen because either they naturally occur in the matrix or they cause corresponding symptoms as respiratory pathogens. Bacterial cultures (between 10^6 and 10^9 CFU*/mL); fungal or viral cultures; supernatants of bacterial fungal, or viral cultures; and LGC and NIBSC standards of the particular organisms were used for the analyses.

The RIDA®GENE Pediatric Viral Panel multiplex real-time RT-PCR is specific for rhinovirus/enterovirus/parechovirus, bocavirus, and adenovirus. No cross-reactivities with the following species were detected (see Table 11):

Table 11: Potentially cross-reactive organisms

Organism	Test result*		
	Rhinovirus/ enterovirus/ parechovirus	Bocavirus	Adenovirus
Acinetobacter baumannii strain 5377	-	-	-
Aspergillus terreus	-	-	-
Bordetella parapertussis strain 12822	-	-	-
Bordetella pertussis Tohama 1	-	-	-
Candida albicans	-	-	-
Chlamydophila pneumoniae	-	-	-
Chlamydia psittaci	-	-	-
Clostridium perfringens	-	-	-
Corynebacterium diphtheriae	-	-	-
Epstein-Barr virus strain B95-8	-	-	-
Escherichia coli (O157:H7)	-	-	-
Enterobacter cloacae	-	-	-
Haemophilus influenzae	-	-	-
Herpes simplex virus 1 strain McIntyre	-	-	-
Herpes simplex virus 2 strain MS	-	-	-
Human coronavirus 229E	-	-	-
Human coronavirus OC43	-	-	-

Human coronavirus SARS-CoV-2 (isolate: Italy-INMI1)	-	-	-
MERS-CoV strain: Florida/USA-2 Saudi Arabia 2014	-	-	-
Human cytomegalovirus	-	-	-
Human metapneumovirus	-	-	-
Human parainfluenza virus 1 strain C35	-	-	-
Human parainfluenza virus 2 strain Greer	-	-	-
Parainfluenza virus serotype 3	-	-	-
Parainfluenza virus 4a strain M-25	-	-	-
Human respiratory syncytial virus strain long	-	-	-
Human respiratory syncytial virus strain 9320	-	-	-
Influenza A/Hong Kong/2671/2019 (H3N2)	-	-	-
Influenza A/Victoria/2570/2019 (H1N1)	-	-	-
Influenza B/Washington/02/2019	-	-	-
Influenza B/Phuket/3073/2013 (Yamagata lineage)	-	-	-
Klebsiella pneumoniae strain MGH 78578	-	-	-
Legionella pneumophila subsp. *pneumophila*	-	-	-
Moraxella catarrhalis	-	-	-
Mycoplasma pneumoniae strain FH of Eaton Agent	-	-	-
Neisseria meningitidis strain FAM18	-	-	-
Proteus vulgaris	-	-	-
Pseudomonas aeruginosa	-	-	-
Serratia marcescens	-	-	-
Staphylococcus aureus	-	-	-
Staphylococcus epidermidis	-	-	-
Streptococcus pneumoniae strain NCTC 7465	-	-	-
Streptococcus pyogenes	-	-	-
Streptococcus salivarius	-	-	-

- = negative

13.1.4 Precision

The precision of the RIDA®GENE Pediatric Viral Panel real-time RT-PCR test was determined for the following levels of consideration.

Intra-assay precision: Determination of 5 control samples using 20 replicates each on LightCycler® 480 II under identical conditions.

Inter-assay precision: Determination of 5 control samples in 20 runs in duplicate on 10 work days (2 runs per day) performed by two different technicians under reproducible conditions.

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

The precision data were obtained using five control samples, as well as the PTC and NTC belonging to the assay.

The maximum obtained coefficient of variation of the measurements with the RIDA®GENE Pediatric Viral Panel real-time RT-PCR test on the LightCycler® 480 II was 3.16 %.

Table 12: Results of the precision of the RIDA®GENE Pediatric Viral Panel test for rhinovirus/enterovirus/parechovirus.

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	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	31.0	30.6	30.9	31.9	31.9	31.6	31.8
	CV (%)	1.11 %	1.49 %	0.75 %	2.09 %	2.43 %	2.06 %	2.23 %
3	Ct	32.0	31.9	31.8	33.3	32.8	33.0	33.0
	CV (%)	1.22 %	1.29 %	1.19 %	2.04 %	2.50 %	1.91 %	2.26 %
4	Ct	22.8	22.8	22.5	23.9	23.7	23.7	23.8
	CV (%)	1.12 %	0.96 %	0.91 %	2.87 %	3.16 %	2.45 %	2.84 %
5	Ct	26.1	25.9	26.2	27.2	26.9	27.0	27.1
	CV (%)	0.83 %	0.83 %	0.78 %	2.12 %	2.00 %	2.19 %	2.13 %

Table 13: Results of the precision of the RIDA®GENE Pediatric Viral Panel test for bocavirus.

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	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	30.5	30.5	30.5	30.9	30.9	30.8	30.9
	CV (%)	0.91 %	0.67 %	0.70 %	1.25 %	1.03 %	1.16 %	1.16 %
3	Ct	31.4	31.5	31.6	32.2	32.1	32.2	32.2
	CV (%)	0.91 %	0.77 %	1.15 %	1.24 %	1.43 %	1.83 %	1.52 %
4	Ct	22.5	23.0	22.6	23.5	23.4	23.4	23.4
	CV (%)	1.03 %	0.76 %	0.53 %	1.26 %	1.66 %	1.56 %	1.50 %
5	Ct	25.7	25.7	26.2	26.5	26.5	26.6	26.5
	CV (%)	0.69 %	0.49 %	0.74 %	1.17 %	1.15 %	0.96 %	1.10 %

Table 14: Results of the precision of the RIDA®GENE Pediatric Viral Panel test for adenovirus.

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	neg.	neg.	neg.	neg.	neg.	neg.	neg.
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	31.1	31.3	31.3	31.7	31.8	31.8	31.7
	CV (%)	1.15 %	1.08 %	1.38 %	1.50 %	1.51 %	1.09 %	1.38 %
3	Ct	32.7	32.5	32.4	33.0	32.9	33.0	33.0
	CV (%)	0.87 %	0.80 %	1.11 %	2.05 %	1.39 %	1.66 %	1.72 %
4	Ct	23.7	23.9	23.6	24.3	24.4	24.4	24.4
	CV (%)	1.05 %	0.77 %	0.65 %	1.98 %	1.46 %	1.69 %	1.72 %
5	Ct	27.1	26.8	27.4	27.3	27.4	27.5	27.4
	CV (%)	0.93 %	0.83 %	0.68 %	1.85 %	1.65 %	1.23 %	1.62 %

13.1.5 Analytical reactivity

The reactivity of the RIDA®GENE Pediatric Viral Panel real-time RT-PCR test was examined on a defined panel of virus strains/subtypes (see Table 15).

Table 15: Analytical reactivity testing

Strain	Concentration	Result*		
		Rhinovirus/ enterovirus/ parechovirus	Bocavirus	Adenovirus
Adenovirus 1, human, strain Adenoid 71 (subgroup C)	1 x 10^{-5} µg/mL	-	-	+
Adenovirus 40, human, strain Dugan (subgroup F)	1.26 U/mL	-	-	+
Adenovirus 41, human, strain Tak (subgroup F)	1.17 x 10^{1} $TCID_{50}$/mL	-	-	+
Adenovirus type 2 (subgroup C)	8.51 x 10^{3} $TCID_{50}$/mL	-	-	+
Adenovirus type 3 (subgroup B)	1 x $10^{3.23}$ U/mL	-	-	+
Adenovirus type 4 (subgroup E)	1.41 U/mL	-	-	+
Adenovirus type 11 (subgroup B)	1 x $10^{0.29}$ U/mL	-	-	+
Adenovirus type 31 (subgroup A)	1 x $10^{0.15}$ U/mL	-	-	+
Adenovirus type 37 (subgroup D)	1 x $10^{-1.98}$ U/mL	-	-	+
Adenovirus type 5 stock strain N/A (subgroup C)	1:100	-	-	+
Adenovirus type 7A (subgroup B)	1 x $10^{3.06}$ U/mL	-	-	+
Bocavirus, HBoV1	1:10^{6}	-	+	-
Enterovirus A - coxsackievirus A2, strain Fleetwood	1.51 x 10^{2} U/mL	+	-	-
Enterovirus A - enterovirus type 71,strain 2003 isolate	4.57 $TCID_{50}$/mL	+	-	-
Enterovirus B - coxsackievirus B4	1 x $10^{2.5}$ U/mL	+	-	-
Enterovirus B - echovirus 11	5.89 x 10^{1} U/mL	+	-	-
Enterovirus C - coxsackievirus A21, strain Kuykendall	1 x $10^{3.29}$ U/mL	+	-	-

Enterovirus C - coxsackievirus A24, strain DN-19	$1:10^4$	+	-	-
Enterovirus D - enterovirus type 68, strain 2007 isolate	1.26×10^2 $TCID_{50}$/mL	+	-	-
Enterovirus D - enterovirus type 70, strain J670/71	$1:10^5$	+	-	-
Parechovirus type 1, strain Harris	3.39×10^4 U/mL	+	-	-
Parechovirus type 3	4.07×10^3 $TCID_{50}$/mL	+	-	-
Rhinovirus type 1A	4.17×10^1 U/mL	+	-	-
Rhinovirus A16	1.26×10^2 U/mL	+	-	-
Rhinovirus A80	1.17×10^1 U/mL	+	-	-
Rhinovirus B14	1.05×10^1 $TCID_{50}$/mL	+	-	-
Rhinovirus B42	2.45×10^2 U/mL	+	-	-
Rhinovirus B70	1.70×10^2 U/mL	+	-	-

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

14. Version history

Version number	Section and designation
2022-03-03	Release version

15. Explanation of symbols

General symbols

	For *in vitro* diagnostic use
	Observe operating manual
	Batch number
	Use before
	Storage temperature
	Item number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Reaction Mix	Reaction Mix
Enzyme Mix	Enzyme Mix
Internal Control RNA	Extraction/inhibition control
No Template Control	Negative control
Positive Control	Positive control

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