RIDA®GENE Coronavirus

REF PG6805

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1. Intended use

For *in vitro* diagnostic use. The RIDA®GENE Coronavirus 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 coronaviruses (HKU1, NL63, 229E, OC43) and MERS-CoV from untreated human nasal/throat swabs from people with signs and symptoms of respiratory infection.

The RIDA®GENE Coronavirus Test is intended to support the differential diagnosis of coronavirus (HKU1, NL63, 229E, OC43) and MERS CoV infections in patients with symptoms of respiratory infection in conjunction with other clinical findings and laboratory findings.

Negative results do not rule out infection with coronaviruses (HKU1, NL63, 229E, OC43) and MERS, 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

Coronaviruses belong to the family of Coronaviridae and are single-stranded (ss) RNA viruses. Due to their high genetic variability, individual virus species can overcome the species barrier and infect different host species. Examples of these cross-species transmissions include infections with SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus), which were responsible for the SARS pandemic in 2002/2003[(1)], and the MERS-CoV (Middle-East Respiratory Syndrome Coronavirus), which appeared in 2012[(2)].
SARS-CoV cause symptoms of atypical pneumonia and claimed over 1,000 deaths during the 2002/2003 pandemic. It is believed that the original reservoir hosts are civets and bats. Even if the various routes of transmission are not fully clarified, transmission takes place primarily via droplet infection. Transmission by smear infection or via the fecal-oral route cannot be ruled out.
By 2015, more than 1,100 MERS-CoV infections were confirmed worldwide and more than 420 MERS-CoV-associated deaths were registered[(3.4)]. The majority of these cases have been identified in the Arabian Peninsula. The original host has not yet been clearly identified for MERS either, but dromedaries are suspected to be the primary source of transmission[(5)]. After an incubation period of 1 to 2 weeks, flu-like symptoms appear; severe cases can lead to pneumonia and acute respiratory distress.
In addition to SARS-CoV and MERS-CoV, the various human pathogenic coronaviruses HKU1, NL63, 229E, OC43 are the triggers of mild respiratory infections up to severe acute respiratory syndromes[(6)]. Although all four coronaviruses occur globally, they are detected in different regions around the world at different times of the year.
The human coronavirus 229E (HCoV-229E) belongs to the genus Alphacoronavirus and, together with the human coronavirus OC43 (HCoV-OC43, genus:

Betacoronavirus), which is often responsible for colds[2]. Other Beta coronaviruses are SARS-CoV, MERS-CoV and Coronavirus HKU1. The last was diagnosed in 2005 in a hospitalized patient with acute respiratory syndrome and pneumonia in China[7]. Infections with the human coronavirus NL63 (HCoV-NL63) are similar to those of parainfluenza infections. The virus was first discovered in a child with bronchiolitis in the Netherlands in 2003 and has since been detected worldwide in young children and immunosuppressed patients with acute respiratory syndrome[8].

3. Test principle

RIDA®GENE Coronavirus is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of coronavirus (HKU1, NL63, 229E, OC43) and MERS-CoV RNA. After the RNA has been isolated, the specific gene fragments of coronaviruses and MERS-CoV (ORF1) are amplified (if present). 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 the extension, the 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 Coronavirus Test contains an Internal Control RNA (ICR) in order to be able to control the sample preparation and/or a potential PCR inhibition.

4. Reagents provided

Table 1: Reagents provided (The reagents provided in the kit are sufficient for 100 determinations.)

Kit code	Reagent	Amount		Lid color
1	Reaction Mix	2 ×	700 µl	Yellow, ready for use
2	PP Mix	1 ×	770 µl	Light green, ready for use
3	Enzyme Mix	1 ×	80 µl	Red, ready for use
R	Internal Control RNA	2 ×	1800 µl	Brown, ready for use
N	PCR Water	1 ×	500 µl	White, ready for use
P	Positive Control	1 ×	100 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.
- The reagents should be gently thawed before use (e.g. in the refrigerator at +2 to +8°C).
- Repeated freezing/thawing of up to 15 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 to +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	15 thaw-freeze cycles

6. Reagents required but not provided

6.1 Reagents provided

The following reagents are required to carry out the RIDA®GENE Coronavirus Tests:

Reagents
PCR water (nuclease-free)

6.2 Laboratory equipment

The following equipment is needed to carry out the RIDA®GENE Coronavirus Tests:

Equipment
Extraction platform: Maxwell® RSC (Promega)
Real-time PCR instrument: LightCycler® 480 II (Roche)
RIDA®GENE Color Compensation Kit IV (PG0004)
Real-time PCR consumables (plates (low profile, white wells, clear frame), reaction vessels, foils)
Centrifuge with rotor for plates
Vortexer
Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1,000 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 automation@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.
Exchanging and mixing the components (Reaction Mix, PP Mix Enzyme Mix, Internal Control RNA, Positive Control, PCR Water) of a batch from one kit with the components of another batch is prohibited.
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.

Hazardous materials are indicated according to labeling obligations. For more details, refer to the Safety Data Sheets (SDS).

8. Collection and storage of samples

8.1 RNA preparation from nasal and throat swabs

A commercially available nucleic acid extraction kit (e.g. RIDA® Xtract (R-Biopharm)) or nucleic acid extraction system (e.g., Maxwell® RSC (Promega)) is recommended for preparing RNA from smears. Pay attention to the information provided by the manufacturer.

The RIDA®GENE Coronavirus Test contains an Internal Control RNA, which indicates possible PCR inhibition, checks the integrity of the reagents and confirms a 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 to be used only as an inhibition control for amplification, 1 µl of the Internal Control RNA must be added to the Master Mix (Table 3).

When the Internal Control RNA is to be used as an extraction control for sample preparation and as inhibition control for amplification, then 20 µl of the Internal Control RNA must be used during extraction. We recommend that Internal Control RNA be added to the sample lysis buffer mix whenever possible, not directly to the sample material. We recommend that 1 µl per reaction of the Internal Control RNA be pipetted to the PCR mix of the negative control and 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 pipette loss (Table 3, Table 4). Thaw, vortex (except for Enzyme Mix) and centrifuge briefly before using the Reaction Mix, the Enzyme-Mix, the Positive Control, the PCR Water and the Internal Control RNA. Reagents must always be appropriately cooled during the work steps (+2 to +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	12.5 µl	137.5 µl
2	PP Mix	6.9 µl	75.9 µl
3	Enzyme Mix	0.7 µl	7.7 µl
	Total	**20.1 µl**	**221.1 µl**

Mix the Master Mix and then centrifuge for short time.

Table 4: Example of the calculation and production 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	12.5 µl	137.5 µl
2	PP-Mix	6.9 µl	75.9 µl
3	Enzyme-Mix	0.7 µl	7.7 µl
R	Internal Control RNA	1.0 µl	11 µl
	Total	**21.1 µl**	**232.0 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 (vials/plates).

Negative control: 5 µl PCR Water pipetted into the respective Master Mix.

Note: When using the Internal Control RNA as an extraction control for sample preparation and as an inhibition control, we recommend that 1 µl of the Internal Control RNA be pipetted to each PCR Mix of the negative control.

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

Positive control: 5 µl Positive Control pipetted to the respective Master Mix.

Note: When using the Internal Control RNA as an extraction control for sample preparation and as an inhibition control, we recommend that 1 µl of the Internal Control RNA be pipetted to each PCR Mix of the positive control.

Seal the reaction vials or plates, briefly centrifuge at slow speed, and transfer into the real-time PCR instrument. Start PCR according to PCR instrument set-up (Table 5, Table 6).

9.3 PCR instrument set-up

9.3.1 Universal real-time RT-PCR profile

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

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 take place in the same step.

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

9.4 Detection channel setting

Table 6: Selection of appropriate detection channels

Real-time PCR instrument	Detection	Detection channel	Comment
Roche LightCycler® 480 II	Coronaviruses (HKU1, NL63, 229E, OC43)	465/510	**RIDA®GENE Color Compensation Kit I (PG0001)**
	ICR	533/580	
	MERS-CoV	618/660	

10. Quality control

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 available 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 Quality Assurance Certificate
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 in 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			
Coronaviruses (HKU1, NL63, 229E, OC43)	**MERS-CoV**	**ICR**	**Result**
positive	negative	**positive/negative**	**Coronaviruses (HKU1, NL63, 229E, OC43) detectable**
negative	**positive**	**positive/negative**	**MERS-CoV detectable**
positive	**positive**	**positive/negative**	**Coronaviruses (HKU1, NL63, 229E, OC43) and MERS-CoV detectable**
pegative	negative	**positive**	**Negative (target genes are not detectable)**
negative	negative	**negative**	**Invalid**

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

A sample is also rated positive if the sample RNA shows amplification, but the Internal Control RNA does not show amplification in the detection system. Detection of Internal Control RNA is not necessary in this case, since high concentrations of the amplicon can lead to a weak or absent signal of the Internal Control RNA.

A sample is rated negative if the sample RNA shows no amplification, while the Internal Control RNA shows amplification in the detection system. Detection of the Internal Control RNA can rule out inhibition of the PCR reaction.

A sample is rated invalid when the sample RNA and the Internal Control RNA do not show amplification in the detection system. PCR inhibitors are present in the sample or an error occurred during the extraction process. The extracted sample should be diluted 1:10 with PCR Water and re-amplified, or the isolation and purification of the sample should be improved.

12. Limitations of the method

1. The RIDA®GENE Coronavirus Test detects coronavirus (HKU1, NL63, 229E, OC43) RNA and MERS-CoV RNA from 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 only verified for untreated human 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 regions can interfere with the detection of new or unknown variants, and can lead to false negative results with the RIDA®GENE coronavirus.
8. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the corresponding target genes (ORF1) are present.
9. This assay should be performed in accordance with the EU Regulation on good laboratory practice (GLP). Users must follow the manufacturer's instructions precisely when performing the test.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE Coronavirus multiplex real-time RT-PCR has a detection limit of ≥ 50 RNA copies/reaction for coronaviruses (HKU1, NL63, 229E, OC43) and MERS-CoV (Fig. 1, Fig. 2).

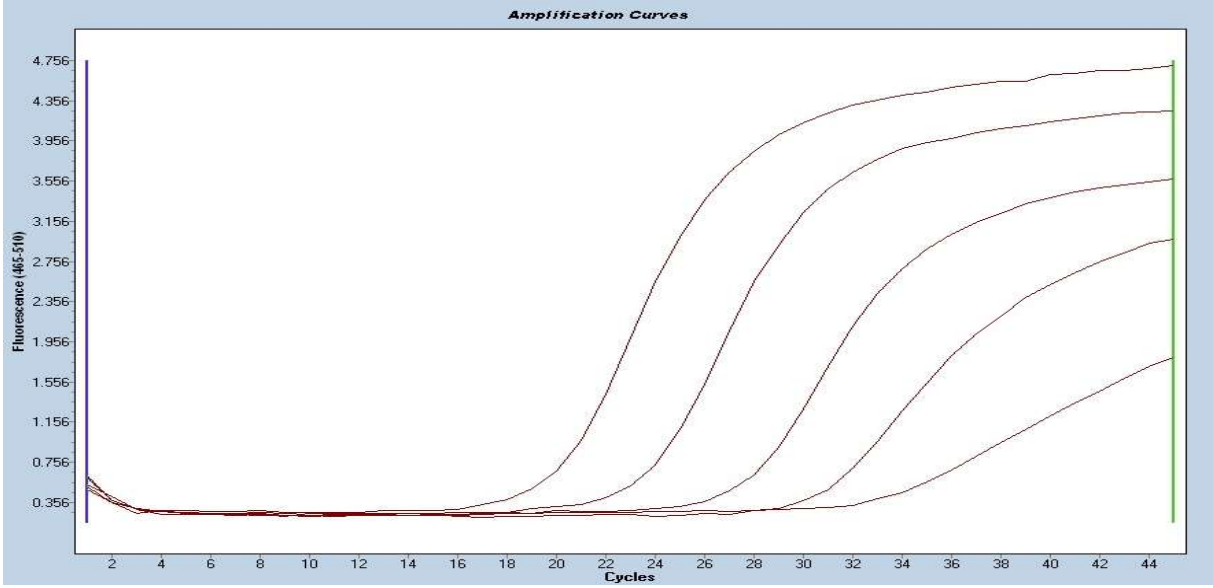

Figure 1: Coronavirus dilution series (HKU1, NL63, 229E, OC43) (10^5 – 10^1 RNA copies/µl) on the LightCycler® 480 II

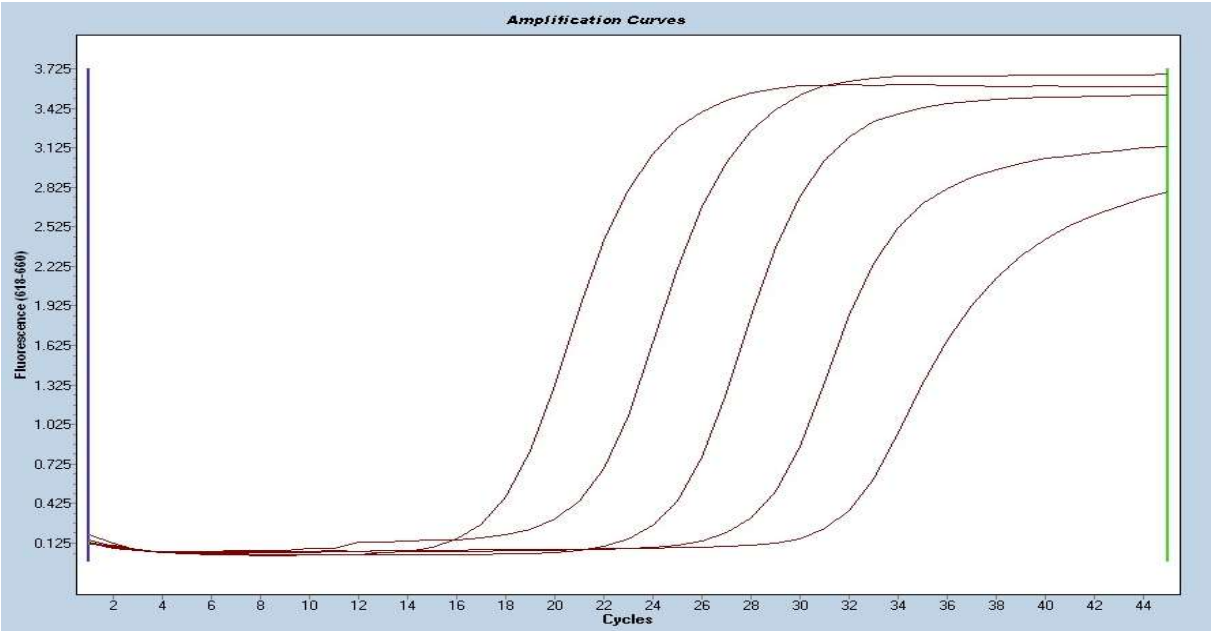

Figure 2: MERS dilution series (10^5 – 10^1 RNA copies/µl) the LightCycler® 480 II

The detection limit of the overall method depends on the sample matrix, RNA extraction and the RNA content.

13.2 Analytical specificity

The RIDA®GENE Coronavirus real-time RT-PCR is specific for coronaviruses. No cross-reactivities with the following species were detected (Table 9):

Table 9: Potentially cross-reactive organisms

Organism	Test result	
	Coronaviruses (HKU1, NL63, 229E, OC43)	MERS-CoV
Acinetobacter baumannii strain 5377	negative	negative
Adenovirus	negative	negative
Adenovirus 1, Human, Adenoid 71 strain	negative	negative
Adenovirus 7, Human, strain Gomen	negative	negative
Adenovirus 40, human, strain Dugan	negative	negative
Adenovirus 41, human, strain Tak	negative	negative
Aeromonas hydrophila	negative	negative
Arcobacter butzleri	negative	negative
Astrovirus	negative	negative
Bacillus cereus	negative	negative
Bacteroides fragilis	negative	negative
Bordetella parapertussis strain 12822	negative	negative
Bordetella pertussis Tohama 1	negative	negative
Campylobacter coli	negative	negative
Campylobacter jejuni	negative	negative
Campylobacter fetus subsp. *fetus*	negative	negative
Campylobacter lari subsp. *lari*	negative	negative
Campylobacter upsaliensis	negative	negative
Candida albicans	negative	negative
Citrobacter freundii NCTC 9750	negative	negative
Clostridium bifermentans	negative	negative
Clostridium difficile	negative	negative
Clostridium perfringens	negative	negative
Clostridium sporogenes	negative	negative
Clostridium septicum	negative	negative
Clostridium novyi	negative	negative
Clostridium sordellii	negative	negative
E. coli (O26:H-)	negative	negative
E. coli (O6)	negative	negative

E. coli (O157:H7)	negative	negative
Enterobacter cloacae	negative	negative
Enterococcus faecalis	negative	negative
Epstein-Barr virus strain B95-8	negative	negative
Haemophilus influenzae Rd	negative	negative
Herpes simplex virus 1 strain McIntyre	negative	negative
Herpes simplex virus 2 strain MS	negative	negative
Human metapneumovirus	negative	negative
Human coxsackievirus B4	negative	negative
Human cytomegalovirus	negative	negative
Human parainfluenza virus 1 strain C35	negative	negative
Human parainfluenza virus 2 strain Greer	negative	negative
Human parainfluenza virus 4b strain CH19503	negative	negative
Human parainfluenza virus serotype 3	negative	negative
Human respiratory syncytial virus strain long	negative	negative
Human respiratory syncytial virus strain 9320	negative	negative
Human rhinovirus genogroup A	negative	negative
Influenza virus infectious A/PR/8/34	negative	negative
Klebsiella pneumoniae strain MGH 78578	negative	negative
Legionella pneumophila subsp. *pneumophila*	negative	negative
Klebsiella oxytoca	negative	negative
Mycoplasma pneumoniae strain FH of Eaton Agent	negative	negative
Neisseria meningitidis strain FAM18	negative	negative
Proteus vulgaris	negative	negative
Pseudomonas aeruginosa	negative	negative
Rotavirus	negative	negative
Salmonella enteritidis	negative	negative
Salmonella typhimurium	negative	negative
Serratia liquefaciens	negative	negative
Shigella flexneri	negative	negative
Staphylococcus aureus	negative	negative
Staphylococcus epidermidis	negative	negative

Staphylococcus haemolyticus SM131	negative	negative
Staphylococcus hominis subsp. *novobiosepticus* R22	negative	negative
Streptococcus pneumoniae strain NCTC 7465	negative	negative
Varicella Zoster Virus (Type B)	negative	negative
Vibrio parahaemolyticus	negative	negative
Yersinia enterocolitica	negative	negative

Interfering substances

The presence of RT-PCR inhibitors and interfering substances can lead to false negative or invalid results. Correspondingly, the effects were investigated of various substances that may exist given their widespread use for respiratory infections, or widespread existence in the corresponding specimens.
Substances that could possibly significantly influence the test results were first examined in an interference screen. Various substances were identified that could be present either as residue from the extraction, due to widespread use in respiratory infections (various pharmacy or prescription drugs), or due to widespread occurrence in the corresponding control samples (e.g. mucins on the surface of mucous membranes or blood), and initially checked in high concentrations (three times the daily dose or simulation of the "worst case"). If a possible 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
Ciprofloxacin 500 mg (Ciprofloxacin)	25 mg/ml
Ampicillin	25 mg/ml
ratioAllerg 50 µg (beclomethasone dipropionate)	10 % [v/v]
Human blood	2 % [v/v]
Erythromycin	10 % [v/v]
Flutide Nasal (Fluticasone Propionate)	25 mg/ml
Robitussin (guaifenesin/dextromethorphan)	10 % [v/v]
Chloraseptic® Sore Throat Spray (Phenol)	10 % [v/v]
Mucins	60 µg/ml
Sodium chloride	10 % [v/v]
Nasivin 0.05 % (oxymetazoline)	10 % [v/v]
Tobramycin	4 µg/ml
Oseltamivir phosphate	25 mg/ml

13.3 Analytical reactivity

The reactivity of the RIDA®GENE Coronavirus multiplex real-time RT-PCR was examined with various subtypes of the coronavirus 11). Coronavirus subtypes of the sample panel were detected with the RIDA®GENE Coronavirus multiplex real-time RT-PCR. The reactivity of the subtype HKU1 was analyzed by sequence matching.

Table 11: Analytical reactivity testing

Strain – Subtype	Concentration	Result	
		Coronavirus	MERS-CoV
Coronavirus – HKU1	*	**positive**	negative
Coronavirus – OC43	$1.5x10^{-1}$ U/ml	**positive**	negative
Coronavirus – NL63	$1.2x10^{-1}$ U/ml	**positive**	negative
Coronavirus – 229E	$1.2x10^{0}$ U/ml	**positive**	negative
Coronavirus – MERS	$1.0x10^{-5}$ U/ml	negative	**positive**

*Analytical reactivity to coronavirus HKU1 was detected with a BLAST analysis.

14. Version history

Version number	Section and designation
2017-09-20	Previous version
2021-08-13	General revision: 1. Intended use 4. Reagents provided 5. Storage instructions 6. Reagents required but not provided 7. Warnings and precautions for the users 8. Collection and storage of samples 9. Test procedure 10. Quality control 12. Limitations of the method 13. Performance characteristics
2022-04-19	6. Reagents required but not provided (RIDA®GENE Color Compensation Kit IV (PG0004))

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Comply with operating manual
LOT	Batch number
	Use before
	Storage temperature
REF	Item number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Reaction Mix	Reaction Mix
PP Mix	Primer/sample mix
Enzyme Mix	Enzyme Mix
Internal Control RNA	Extraction/inhibition control
PCR Water	Negative Control
Positive Control	Positive Control

16. References

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