RIDA®GENE RSV & hMPV

REF PG5905

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1. Intended use

For *in vitro* diagnostic use. The RIDA®GENE RSV & hMPV test, performed on Roche LightCycler® 480 II, is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of respiratory syncytial virus (A/B) and human metapneumovirus (A/B) RNA in untreated human nasal/throat swabs and BAL from persons with signs and symptoms of acute respiratory infection.

The RIDA®GENE RSV & hMPV test is intended to support the diagnosis of viral (RSV & hMPV) infections in patients with symptoms of respiratory infection in connection with other clinical and laboratory findings.
Negative results do not rule out infection with RSV or hMPV 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

The human respiratory syncytial virus (RSV) is considered one of the most potent pathogens for acute infections of the lower respiratory tract such as lung inflammation and bronchiolitis[1,2,3]. A high incidence of RSV infections has been found especially in children less than 6 months old[4,5]. RSV is associated with elevated morbidity and mortality in not just children but also the elderly, patients with prior illnesses, and immunocompromised adults[2,6]. RSV is a nonsegmented, enveloped RNA virus that belongs to the Pneumoviridae family[2,3]. The viral genome, a single-stranded RNA (ssRNA) molecule, codes for 11 proteins[6]. Given the antigenicity of its G protein, the strain is divided into two antigen groups: RSV A and B. Currently, 14 genotypes of RSV A and 25 genotypes of RSV B are known[2,6]. Several studies have come unanimously to the conclusion that RSV is the most commonly detected respiratory virus[2,6,7]. In particular, RSV outbreaks occur during winter from November to March. The height of infections is mainly in January[1,3,8]. RSV generally manifests together with other respiratory viruses[2,3].

The human metapneumovirus (hMPV) was isolated for the first time in 2001 from respiratory tract samples of children under 5 years of age[9]. The human metapneumovirus is an enveloped virus-like particle that belongs to the Pneumoviridae family like RSV. The viral genome is a single-stranded RNA (ssRNA) which consists of 8 genes. The hMPV strain was divided into two different genotypes, A and B. Each group is also further divided into two subgenotypes 1 and 2 (A1, A2, B1, B2) that are based on the genetic differences in the surface proteins F and G. Both genotypes can coexist and circulate simultaneously during the same period, whereas group A is generally the dominant genotype. However, the distribution depends on the seasonality and region[3,5,9,10]. Infections with hMPV can occur throughout the year, but infection usually peaks at the end of the winter season. Clear seasonality is evident, especially with high infection rates in January[9,11].

Coinfections with other pathogens of the respiratory tract, such as the respiratory syncytial virus (RSV), have been described several times[9].

According to studies, RSV and hMPV, in addition to influenza, are the predominant viral pathogens during the winter season and, therefore, show consistent seasonality[12]. The most common symptoms of acute respiratory tract infections are fever, cough, sore throat, headaches, tachypnea, rales, stridor, wheezing, or symptoms of the upper respiratory tract (rhinorrhea and sneezing), and fatigue [9,11].

3. Test principle

The RIDA®GENE RSV & hMPV test is a multiplex real-time RT-PCR test for direct qualitative detection and differentiation of respiratory syncytial virus (A/B) and human metapneumovirus (A/B) RNA from human nasal/throat swabs and BAL.
After DNA isolation, the specific gene fragments of RSV (F gene) and hMPV (F glycoprotein) (if present) 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 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 RSV & hMPV test contains an Internal Control RNA (ICR) to be able to control the 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

REF	Kit code	Reagent	Amount		Lid color
PGZ5905RM	**1**	Reaction Mix	2 ×	1050 µl	yellow, ready for use
PGZ5905EM	**2**	Enzyme Mix	1 ×	80 µl	red, ready for use
PGZ5905IC	**R**	Internal Control RNA	2 ×	1700 µl	orange, ready for use
PGZ5905NC	**N**	No Template Control	1 ×	450 µl	white, ready for use
PGZ5905PC	**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 thaw-freeze cycles

6. Reagents required but not provided

6.1 Reagents provided

The following reagents are needed to perform the RIDA®GENE RSV & hMPV test:

Reagents
PCR water (nuclease-free)

6.2 Laboratory equipment

The following equipment is needed to perform the RIDA®GENE RSV & hMPV 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, 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 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 combine the components (Reaction Mix, Enzyme Mix, Internal Control RNA, Positive Control, No Template Control) of a lot from one kit 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.

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

For users in the European Union: Report all serious adverse events associated with the product to R-Biopharm AG and the appropriate national authorities.

The summary of safety and performance (SSP) for this product will be available at https://ec.europa.eu/tools/eudamed once the European Database on Medical Devices (EUDAMED) gets underway. In the database, search for the device using the UDI-DI located on the outer packaging of the device.

8. Collection and storage of samples

8.1 RNA preparation from nasal and throat swabs & BAL

For RNA preparation from nasal and throat swabs and BAL, 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 RSV & hMPV 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 to be 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 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 adding the Internal Control RNA to the sample cartridge before adding the sample, if possible. We recommend pipetting 1 µl of the Internal Control RNA per reaction 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 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 Enzyme Mix), and centrifuge briefly. 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: Pipette 5 µl of the No Template Control into the pre-pipetted master mix.

Note: **When the Internal Control RNA is used as an extraction control for sample preparation and as an inhibition control, we recommend adding 1 µl of the Internal Control RNA to each PCR mix of the negative control.**

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

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

Note: When the Internal Control RNA is used as an extraction control for sample preparation and as an inhibition control, we recommend adding 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 set-up (Table 5, Table 6).

9.3 PCR instrument set-up

9.3.1 Universal real-time PCR profile

To harmonize the RIDA®GENE assays, the RIDA®GENE RSV & hMPV assay was verified in the universal profile. This makes it possible to combine DNA and RNA assays with each other. Reverse transcription therefore comes first in the universal profile.

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	RSV	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	ICR	533/580	
	hMPV	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 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

At this time, there is no internationally recognized reference method or reference material for standardization. The control materials can be metrologically traced to internal R-Biopharm AG standards based on specific RNA amplificates.

For further information on metrological traceability, please contact R-Biopharm AG.

The adjusted values, fluctuations, and further details can be found in the enclosed certificate of analysis (CoA).

The data are evaluated in LightCycler® 480 II using the fit point method. Signals greater than the threshold are considered a positive result.

To determine the limit of detection (LoD 95 %) (Section 13.1.1), the threshold was set as follows:

Detection	Matrix	High threshold (% total fluorescence)
RSV A	BAL	4.9 %
	Nasal/throat swab	6.5 %
RSV B	BAL	3.5 %
	Nasal/throat swab	6.0 %
hMPV A1	BAL	7.9 %
	Nasal/throat swab	7.0 %
hMPV A2	BAL	9.9 %
	Nasal/throat swab	10.9 %

The result interpretation is done according to Table 8.

Table 8: Result interpretation

Detection of			
RSV	**hMPV**	**ICR**	**Result**
positive	negative	**positive/ negative**	**RSV detectable**
negative	**positive**	**positive/ negative**	**hMPV detectable**
positive	**positive**	**positive/ negative**	**RSV and hMPV detectable**
negative	negative	**positive**	**Target gene 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 the 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, but 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.

12. Limitations of the method

1. The RIDA®GENE RSV & hMPV test detects respiratory syncytial virus (A/B) and human metapneumovirus (A/B) RNA from untreated human nasal/throat swabs and BAL. 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 and BAL.
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 RIDA®GENE RSV & hMPV.
8. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target genes (RSV (F gene) and hMPV (F glycoprotein)) are present.
9. Even in small amounts, the substances dihydrocodeine and azithromycin can have interfering properties. Paracodine interferes starting at a concentration of 3.0 % [v/v]; azithromycin interferes starting at a concentration of 25.2 mg/ml.
10. This assay should be performed in compliance with the regulation on good laboratory (GLP). Users must follow the manufacturer's instructions precisely when performing the test.

13. Performance characteristics

13.1 Analytical performance characteristics

13.1.1 Detection limit (LoD 95 %)

A positive control specimen (negative BAL or nasal/throat swab, spiked) was measured in five dilution steps (in 0.25-log steps) for each target and matrix with 20 replicates per step in one lot to determine the LoD. This was followed by a probit analysis. Next, the calculated LoD was confirmed with 20 replicates per target and matrix for the calculated dilution step/concentration.
The following strains were used for testing:

RSV A 2006 isolate, ZeptoMetrix, #0810040ACF
RSV B strain: CH93(18)-18, ZeptoMetrix, #0810040CF
hMPV-16 type A1, strain: IA10-2003, ZeptoMetrix, #0810161CF
hMPV-8 type B2, strain: Peru6-2003, ZeptoMetrix, #0810159CF

For the detection of RSV and hMPV RNA with the assistance of the RIDA®GENE RSV & hMPV assay, the MagNA Pure 96 instrument, and LightCycler® 480 II, the limits of detection (LoD) shown in Table 9 were determined.

Table 9: Limits of detection results of the RIDA®GENE RSV & hMPV test for the parameters RSV and hMPV.

	RSV A	RSV B	hMPV A1	hMPV B2
LoD BAL	190.11 $TCID_{50}$*/ml	4.15 $TCID_{50}$*/ml	1.01 $TCID_{50}$*/ml	0.63 $TCID_{50}$*/ml
LoD nasal/throat swab	193.20 $TCID_{50}$*/ml	6.89 $TCID_{50}$*/ml	5.70 $TCID_{50}$*/ml	5.22 $TCID_{50}$*/ml

*TCID: Tissue culture infectious dose

The LoD for the parameter RSV A in BAL was determined at 190.11 $TCID_{50}$*/ml.

The LoD for the parameter RSV A in the nasal/throat swab was determined at 193.20 $TCID_{50}$*/ml.

The LoD for the parameter RSV B in BAL was determined at 4.15 $TCID_{50}$*/ml.

The LoD for the parameter RSV B in the nasal/throat swab was determined at 6.89 $TCID_{50}$*/ml.

The LoD for the parameter hMPV A1 in BAL was determined at 1.01 $TCID_{50}$*/ml.

The LoD for the parameter hMPV A1 in the nasal/throat swab was determined at 5.70 $TCID_{50}$*/ml.

The LoD for the parameter hMPV B2 in BAL was determined at 0.63 $TCID_{50}$*/ml.

The LoD for the parameter hMPV A1 in the nasal/throat swab was determined at 5.22 $TCID_{50}$*/ml.

13.1.2 Device detection limit

For determining the device detection limit, 20 replicates of a control sample (50 copies/reaction) 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 RT-PCR inhibitors and interfering substances can lead to false-negative or invalid results. Therefore, the effects of various substances that may exist given their widespread use for respiratory infections or widespread occurrence in the corresponding specimens were investigated.
Substances that could possibly significantly influence the test results were first examined in an interference screen. Various substances 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) were initially examined in high concentrations (three times the daily dose or simulation of the “worst case”). 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
Mucosolvan (ambroxol hydrochloride)*	10 % [v/v]
Amoxicillin	1 mg/ml
Human blood	2 % [v/v]
Mucins	60 µg/ml
Nasivin 0.05 % (oxymetazoline)**	10 % [v/v]
Sodium chloride	10 % [v/v]
Oseltamivir phosphate	25 mg/ml
ratioAllerg 50 µg (beclomethasone dipropionate)**	10 % [v/v]
Tobramycin	4 µg/ml

*Substance only tested for the matrix BAL
**Substances only tested for the matrix nasal/throat swab

Inhibitory effects were observed for the substances paracodine (3 %) and Azithromycin-ratiopharm (25.2 mg/ml) (see the limitations of the method).

Cross-reactivity

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

The RIDA®GENE RSV & hMPV multiplex real-time RT-PCR is specific to RSV and hMPV. No cross-reactivities with the following species were detected (Table 11):

Table 11: Potentially cross-reactive organisms

Organism	Test result	
	RSV	hMPV
Acinetobacter baumannii strain 5377	negative	negative
Adenovirus 1, Human, Adenoid 71 strain	negative	negative
Adenovirus 4	negative	negative
Adenovirus 7, Human, strain Gomen	negative	negative
Adenovirus 31	negative	negative
Adenovirus 34	negative	negative
Adenovirus 37	negative	negative
Aspergillus terreus	negative	negative
Bordetella parapertussis strain 12822	negative	negative
Bordetella pertussis Tohama 1	negative	negative
Candida albicans	negative	negative
Chlamydophila pneumoniae	negative	negative
Chlamydia psittaci	negative	negative
Clostridium perfringens	negative	negative
Corynebacterium diphtheriae	negative	negative
Echovirus 11	negative	negative
Epstein-Barr virus strain B95-8	negative	negative
Escherichia coli (O26:H-)	negative	negative
Escherichia coli (O6)	negative	negative
Escherichia coli (O157:H7)	negative	negative
Enterobacter cloacae	negative	negative
Enterovirus type 71, strain 2003 isolate	negative	negative
Haemophilus influenzae	negative	negative
Herpes simplex virus 1 strain McIntyre	negative	negative
Herpes simplex virus 2 strain MS	negative	negative
Human coronavirus 229E	negative	negative
Human coronavirus OC43	negative	negative
Human coxsackievirus A2, strain Fleetwood	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 serotype 3	negative	negative
Human parainfluenza virus 4a strain M-25	negative	negative

Human rhinovirus genogroup A	negative	negative
Influenza A H1N1 Brisbane/59/07	negative	negative
Influenza A H3N2 Texas/50/12	negative	negative
Influenza B/Washington/02/2019	negative	negative
Influenza B/Colorado/6/2017	negative	negative
Klebsiella pneumoniae strain MGH 78578	negative	negative
Lactobacillus plantarum	negative	negative
Legionella pneumophila subsp. *Pneumophila*	negative	negative
Moraxella catarrhalis	negative	negative
Mycoplasma pneumoniae strain FH of Eaton Agent	negative	negative
Neisseria meningitidis strain FAM18	negative	negative
Pneumocystis jirovecii	negative	negative
Proteus vulgaris	negative	negative
Pseudomonas aeruginosa	negative	negative
Serratia marcescens	negative	negative
Streptococcus pneumoniae strain NCTC 7465	negative	negative
Streptococcus pyogenes	negative	negative
Streptococcus salivarius	negative	negative
Staphylococcus aureus	negative	negative
Staphylococcus epidermidis	negative	negative

*CFU: Colony-forming units

13.1.4 Precision

The precision of the RIDA®GENE RSV & hMPV 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.

Inter-lot precision: 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 RSV & hMPV real-time RT-PCR test on LightCycler® 480 II was 1.93 %.

Table 12: Results of the precision of the RIDA®GENE RSV & hMPV test for RSV.

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	34.3	34.6	34.6	33.6	33.6	33.7	33.7
	CV (%)	1.72 %	1.31 %	1.27 %	1.47 %	1.63 %	1.88 %	1.66 %
3	Ct	32.1	32.6	32.6	31.2	31.1	31.6	31.3
	CV (%)	0.94 %	1.06 %	0.93 %	1.72 %	1.62 %	1.86 %	1.85 %
4	Ct	25.9	25.9	26.1	24.6	24.5	25.0	24.7
	CV (%)	0.87 %	0.67 %	0.68 %	1.35 %	1.50 %	1.84 %	1.93 %
5	Ct	22.4	22.7	22.8	21.3	21.3	21.7	21.4
	CV (%)	0.84 %	0.73 %	0.66 %	1.25 %	1.84 %	1.62 %	1.85 %

Table 13: Results of the precision of the RIDA®GENE RSV & hMPV test for hMPV.

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	33.6	33.5	33.7	32.9	33.0	32.8	32.9
	CV (%)	0.80 %	1.17 %	0.83 %	1.44 %	1.43 %	1.64 %	1.54 %
3	Ct	31.6	31.8	31.7	30.6	30.6	30.8	30.7
	CV (%)	0.80 %	0.50 %	0.80 %	1.40 %	1.11 %	1.27 %	1.29 %
4	Ct	25.1	25.4	25.4	24.1	24.1	24.4	24.2
	CV (%)	0.97 %	0.72 %	0.69 %	1.92 %	1.80 %	1.80 %	1.91 %
5	Ct	21.8	21.9	21.8	20.9	20.8	20.9	20.9
	CV (%)	0.83 %	0.94 %	0.79 %	1.27 %	1.65 %	1.63 %	1.53 %

13.1.5 Analytical reactivity

The reactivity of the RIDA®GENE RSV & hMPV real-time RT-PCR test was investigated using RSV strains and hMPV subtypes (Table 14). The tested RSV strains and hMPV subtypes were detected using the RIDA®GENE RSV & hMPV real-time RT-PCR test.

Table 14: Analytical reactivity testing

Strain	Concentration	Result*	
		RSV	hMPV
hMPV 5 type B1	$10^{-1.70}$ U/ml	-	+
hMPV 8 type B2	$10^{2.10}$ U/ml	-	+
hMPV 16 type A1	$10^{0.82}$ U/ml	-	+
hMPV 20 type A2	$10^{0.34}$ U/ml	-	+
RSV A (Isolate: 2006 Isolate)	$10^{2.53}$ U/ml	+	-
RSV A strain Long	3.74 x 10^{1} PFU**/ml	+	-
RSV B (strain:CH93(18)-18)	$10^{0.82}$ U/ml	+	-
RSV B strain 9320	8.9 x 10^{0} $TCID_{50}$/ml	+	-

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

**Plaque-forming units

14. Version history

Version number	Section and designation
2015-11-16	Release version
2021-08-24	General revision: 1. Intended use 2. Summary and explanation of the test 3. Test principle 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 11. Result interpretation 12. Limitations of the method 13. Performance characteristics

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
	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

16. References

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