RIDA®GENE Parainfluenza

REF PG5805

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1. Intended use

For *in vitro* diagnostic use. The RIDA®GENE Parainfluenza test, performed on Roche LightCycler® 480 II, is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of parainfluenza 1, parainfluenza 3, and parainfluenza 2/4 RNA in untreated human nasal/throat swabs from persons with signs and symptoms of acute respiratory infection.

The RIDA®GENE Parainfluenza test is intended to support the diagnosis of parainfluenza (parainfluenza 1, parainfluenza 3, and parainfluenza 2/4) infections in patients with symptoms of respiratory infection in connection with other clinical and laboratory findings.
Negative results do not rule out infection with parainfluenza virus (parainfluenza 1, parainfluenza 3, and parainfluenza 2/4) 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

Human parainfluenza viruses (HPIVs) are enveloped, single-stranded RNA viruses from the family *Paramyxoviridae*[1-3]. They can cause a number of respiratory illnesses, by initially infecting the pseudostratified mucociliary airway epithelium of the nose and oropharynx before spreading to the large and small airways[1]. HPIVs are the second leading cause of hospitalizations in children under age 5[2] and are responsible for up to 17 % of hospitalizations[3].
HPIVs can be divided genetically and antigenically into four serotypes that can infect humans: HPIV-1, HPIV-2, HPIV-3, and HPIV-4[1, 2]. HPIV-1 and -3 belong to the genus *Respirovirus*, while HPIV-2 and -4 are classified in the genus *Rubulavirus*[1, 3]. The clinical presentation varies from type to type: HPIV-1 and -2 often cause croup and flu-like symptoms, while HPIV-3 is commonly associated with pneumonia and bronchiolitis. So far, HPIV-4 is not as well characterized, but it is suspected to have a clinical presentation similar to that of HPIV-3[2]. Even the seasonal pattern is different among the four serotypes: HPIV-1 usually occurs in the fall of odd-numbered years, while HPIV-2 occurs in the fall of even-numbered years. HPIV-3 infections break out annually from April to June[2] and are, therefore, the main cause of clinically significant infections among parainfluenza viruses[1]. For instance, 60 % of children aged 2 years are infected with HPIV-3, while the number increases to 80 % among children up to 4 years old[3]. Generally, infants, toddlers, older people, and immunocompromised people have a higher risk of a severe HPIV infection, while the HPIV infection in healthy adults is usually limited to mild symptoms of the upper airways[2, 3]. HPIV is normally transmitted from human to human through the air from coughing and sneezing, by close human contact, or by touching of hands or objects containing HPIV and then touching the mouth, eyes, or nose[4].

3. Test principle

The RIDA®GENE Parainfluenza test is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of parainfluenza 1, parainfluenza 3, and parainfluenza 2/4 in human nasal/throat swabs.
After RNA isolation, the specific gene fragments (if present) of parainfluenza 1, parainfluenza 3, and parainfluenza 2/4 (HN gene) 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 Parainfluenza 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

REF	Kit code	Reagent	Amount		Lid color
PGZ5805RM	**1**	Reaction Mix	2 ×	1050 µL	yellow, ready for use
PGZ5805EM	**2**	Enzyme Mix	1 ×	80 µL	red, ready for use
PGZ5805IC	**R**	Internal Control RNA	2 ×	1700 µL	brown, ready for use
PGZ5805NC	**N**	No Template Control	1 ×	450 µL	white, ready for use
PGZ5805PC	**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 Parainfluenza test:

Reagents
PCR water (nuclease-free)

6.2 Laboratory equipment

The following equipment is needed to perform the RIDA®GENE Parainfluenza 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

For 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 Parainfluenza 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 Parainfluenza 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	Parainfluenza 1	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	ICR	533/580	
	Parainfluenza 3	533/610	
	Parainfluenza 2/4	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	+	N/A *1	See Certificate of Analysis
Negative control	-	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 13.2.1), the threshold was set as follows:

Detection	High threshold (% total fluorescence)
Parainfluenza 1	2.39 %
Parainfluenza 2	20.21 %
Parainfluenza 3	9.09 %
Parainfluenza 4A	6.62 %

The result interpretation is done according to Table 8.

Table 8: Result interpretation*

Detection of				
Parainfluenza 1	**Parainfluenza 3**	**Parainfluenza 2/4**	**ICD**	**Result**
+	-	-	**+/-**	**Parainfluenza 1 detectable**
-	**+**	-	**+/-**	**Parainfluenza 3 detectable**
-	-	**+**	**+/-**	**Parainfluenza 2/4 detectable**
+	**+**	-	**+/-**	**Parainfluenza 1 and parainfluenza 3 detectable**
+	-	**+**	**+/-**	**Parainfluenza 1 and parainfluenza 2/4 detectable**
-	**+**	**+**	**+/-**	**Parainfluenza 3 and parainfluenza 2/4 detectable**
+	**+**	**+**	**+/-**	**Parainfluenza 1, parainfluenza 3, and parainfluenza 2/4 detectable**
-	-	-	**+**	**Target genes not detectable**
-	-	-	**-**	**Invalid**

* + = positive
- = negative

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 Parainfluenza test detects parainfluenza 1, parainfluenza 3, and parainfluenza 2/4 RNA 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 with RIDA®GENE Parainfluenza.
8. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target genes (parainfluenza 1, parainfluenza 3, and parainfluenza 2/4 (HN gene)) are present.
9. Even in small amounts, the substances human blood and Paracodin (dihydrocodeine) can have interfering properties. Human blood interferes starting at a concentration of 2.4 % [v/v]. Paracodin (dihydrocodeine) interferes starting at a concentration of 3 % [v/v].
10. This assay should be performed in compliance with the regulation on good laboratory practice (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 %)

The LoD was determined by measuring a positive control sample (negative nasal/throat swabs, spiked) in five dilution steps (in 0.25-log steps) for each target and matrix with 20 replicates per step in one lot. 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:

Parainfluenza 1: ZeptoMetrix (#0810014CF)
Parainfluenza 2: ZeptoMetrix (#0810015CF)
Parainfluenza 3: ZeptoMetrix (#0810014CF)
Parainfluenza 4a: ZeptoMetrix (#0810060CF)

For the detection of parainfluenza 1, parainfluenza 3, and parainfluenza 2/4 RNA using the RIDA®GENE Parainfluenza assay, the MagNA Pure 96 Instrument, and LightCycler® 480 II, the following limits of detection (LoD) were determined.
The results of these measurements are shown in Table 9.

Table 9: Limit of detection results for the RIDA®GENE Parainfluenza test for the parameters Parainfluenza 1, Parainfluenza 3, and Parainfluenza 2/4

	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Parainfluenza 4
LoD	0.05 [$TCID_{50}$/mL]	354.00 [$TCID_{50}$/mL]	3.69 [$TCID_{50}$/mL]	67.61 [$TCID_{50}$/mL]

The LoD for the parameter Parainfluenza 1 in nasal-/throat swabs was determined at 0.05 [$TCID_{50}$/mL].

The LoD for the parameter Parainfluenza 2 in nasal-/throat swabs was determined at 354.00 [$TCID_{50}$/mL].

The LoD for the parameter Parainfluenza 3 in nasal-/throat swabs was determined at 3.69 [$TCID_{50}$/mL].

The LoD for the parameter Parainfluenza 4 in nasal/throat swabs was determined at 67.61 [$TCID_{50}$/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
Amoxicillin	1 mg/mL
Ethanol*	5 % [v/v]
Guanidine hydrochloride*	5 % [w/v]
Mucin	60 µg/mL
Mucosolvan	10 % [v/v]
Nasivin/oxymetazoline	10 % [v/v]
Sodium chloride	10 % [v/v]
Oseltamivir	25 mg/mL
Paracetamol	10 mg/mL

* based on the eluate volume

Inhibitory effects were observed for the substances human blood (2.4 %) and Paracodin (dihydrocodeine) (3 %) (see Limitations of the method).

Cross-reactivity

Various organisms commonly found in nasal/throat swabs (bacteria, parasites, fungi, and viruses) were examined. The microorganisms to be investigated for this assay were chosen because either they naturally occur in nasal/throat swabs, or they cause corresponding symptoms as respiratory pathogens. Bacterial, fungal, or viral cultures; supernatants of bacterial, fungal, or viral cultures; and LGC and NIBSC standards of the respective organisms were used for the analyses.

The RIDA®GENE Parainfluenza multiplex real-time PCR is specific for parainfluenza 1, parainfluenza 3, and parainfluenza 2/4. No cross-reactivities with the following species were detected (see Table 11):

Table 11: Potentially cross-reactive organisms

Organism	Test result*		
	Parainfluenza 1	***Parainfluenza 3***	***Parainfluenza 2/4***
Adenovirus 7, Human, strain Gomen	-	-	-
Adenovirus 1, Human, Adenoid 71 strain	-	-	-
Bordetella pertussis Tohama 1	-	-	-
Candida albicans	-	-	-
Chlamydia pneumoniae	-	-	-
Enterovirus type 71	-	-	-
Epstein-Barr virus B95-8	-	-	-
Haemophilus influenzae	-	-	-
Human coronavirus 229E	-	-	-
Human coronavirus OC43	-	-	-
Human coronavirus NL63	-	-	-
Human metapneumovirus	-	-	-
Influenza A H1N1 Brisbane/59/07	-	-	-
Influenza A H3N2 Texas/50/12	-	-	-
Influenza B/Washington/02/2019	-	-	-
Influenza virus B/Colorado/6/2017	-	-	-
Lactobacillus acidophilus	-	-	-
Lactobacillus crispatus strain VPI3199	-	-	-
Legionella pneumophila	-	-	-
MERS-CoV	-	-	-
Moraxella catarrhalis	-	-	-
Mycoplasma pneumoniae strain (FH of Eaton Agent)	-	-	-
Pneumocystis jirovecii (NATtrol Recombinant External Run Control)	-	-	-
Pseudomonas aeruginosa	-	-	-
Rhinovirus 1A strain MRC-5	-	-	-
RSV (strain Long)	-	-	-
RSV (strain 9320)	-	-	-
SARS	-	-	-

Staphylococcus aureus	-	-	-
Staphylococcus epidermidis	-	-	-
Streptococcus salivarius	-	-	-
Streptococcus pneumoniae (NCTC 7465)	-	-	-
Streptococcus pyogenes	-	-	-

* - = negative

13.1.4 Precision

The precision of the RIDA®GENE Parainfluenza real-time 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 different operators 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 obtained coefficients of variation of each measurement using the RIDA®GENE Parainfluenza real-time PCR test on LightCycler® 480 II were less than 3.06 %.

Table 12: Precision results for the RIDA®GENE Parainfluenza test for parainfluenza 1.

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	-	-	-	-	-	-	-
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	30.9	30.7	30.8	30.8	30.6	30.5	30.6
	CV (%)	0.68 %	0.78 %	0.82 %	1.06 %	1.07 %	1.01 %	1.11 %
3	Ct	31.1	30.7	30.9	30.6	30.5	30.5	30.6
	CV (%)	0.91 %	1.06 %	1.13 %	1.19 %	0.91 %	0.90 %	0.99 %
4	Ct	27.3	27.0	27.1	26.9	26.9	27.0	26.9
	CV (%)	0.57 %	1.09 %	0.70 %	1.28 %	1.39 %	1.30 %	1.32 %
5	Ct	23.4	23.7	23.8	23.7	23.6	23.7	23.6
	CV (%)	0.87 %	1.19 %	0.65 %	1.39 %	1.51 %	1.25 %	1.39 %

Table 13: Precision results for the RIDA®GENE Parainfluenza test for parainfluenza 3.

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	-	-	-	-	-	-	-
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	31.3	31.1	30.8	30.8	30.8	30.5	30.7
	CV (%)	0.74 %	1.03 %	1.30 %	1.55 %	1.37 %	1.16 %	1.45 %
3	Ct	32.2	31.9	31.7	31.3	31.6	31.5	31.5
	CV (%)	0.92 %	1.33 %	1.03 %	2.04 %	1.39 %	1.44 %	1.69 %
4	Ct	26.0	25.7	25.5	25.0	25.4	25.2	25.2
	CV (%)	1.06 %	1.57 %	1.17 %	2.78 %	2.61 %	1.85 %	2.51 %
5	Ct	22.5	22.7	21.9	21.8	22.0	21.9	21.9
	CV (%)	1.12 %	1.48 %	1.39 %	2.63 %	2.32 %	1.98 %	2.35 %

Table 14: Precision results for the RIDA®GENE Parainfluenza test for parainfluenza 2/4.

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	-	-	-	-	-	-	-
	CV (%)	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Ct	31.6	32.0	31.6	32.1	31.5	31.6	31.7
	CV (%)	1.29 %	1.44 %	2.46 %	1.55 %	2.02 %	1.90 %	2.05 %
3	Ct	31.9	32.2	32.2	31.8	31.9	31.8	31.8
	CV (%)	1.88 %	3.06 %	2.60 %	2.11 %	2.12 %	2.30 %	2.14 %
4	Ct	27.0	26.9	26.8	26.5	26.6	26.9	26.7
	CV (%)	1.48 %	2.66 %	2.42 %	2.46 %	2.92 %	2.13 %	2.56 %
5	Ct	22.7	23.6	23.3	23.2	23.1	23.1	23.1
	CV (%)	1.30 %	2.05 %	2.03 %	1.90 %	2.86 %	2.28 %	2.38 %

13.1.5 Analytical reactivity

The reactivity of the RIDA®GENE Parainfluenza multiplex real-time PCR test was investigated using various parainfluenza strains (see Table 15).

Table 15: Analytical reactivity testing

Strain	Concentration	Result*		
		Parainfluenza 1	Parainfluenza 3	Parainfluenza 2/4
Parainfluenza 1 C35	0.417 ng	**+**	-	-
Parainfluenza 2 Greer	0.01005 ng	-	-	**+**
Parainfluenza 3 C243	0.0201 ng	-	**+**	-
Parainfluenza 4a M-25	$10^{3.25}$ TCID50/0.2 mL	-	-	**+**
Parainfluenza 4b CH 19503	1.524 ng	-	-	**+**

* + = positive
- = negative

14. Version history

Version number	Section and designation
2021-10-27	Previous version
2022-01-20	General revision: 4. Reagents provided

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
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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4. Centers for Disease Control and Prevention. Human Parainfluenza Viruses (HPIVs) - Transmission 2019 [Available from: https://www.cdc.gov/parainfluenza/about/transmission.html.] 15.10.2021