# RIDA®GENE Parainfluenza

real-time RT-PCR

Art. No.: PG5805

100 Reactions

**For *in vitro* diagnostic use.**

-20 °C

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## 1. Intended use

For *in vitro* diagnostic use. RIDA®GENE Parainfluenza is a multiplex real-time RT-PCR for the direct, qualitative detection and differentiation of human parainfluenza viruses (parainfluenza virus 1, parainfluenza virus 3 and parainfluenza virus 2/4) from human nasal and throat swabs and nasopharyngeal swabs.

RIDA®GENE Parainfluenza real-time RT-PCR is intended for use as an aid in the diagnosis of parainfluenza infection.

## 2. Summary and Explanation of the test

Human Parainfluenza viruses (HPIV) are enveloped, single-stranded RNA (ss-RNA) viruses which can cause both upper respiratory infections and lower respiratory infections. Parainfluenza viruses belong to the family of Paramyxiviridae and were identified in the late 1950's in children with inflammation of the lower respiratory tract. In contrast to influenza viruses, which belong to the family of Myxoviridae, parainfluenza viruses do grow poorly in embryonated eggs and they share little antigenetic sites with influenza viruses.[1]
Parainfluenza viruses differ genetically and antigenetically and can be divided in four serogroups. Parainfluenza virus 1 and 3 which belong to the respirovirus genre, and parainfluenza virus 2 and 4 belonging to the rubilavirus genre. Mainly parainfluenza virus 1,2 and 3, but also parainfluenza virus 4 account for the major community-acquired respiratory pathogens worldwide. Although parainfluenza virus 1 and 3 most often occur in infants, small children, immunosuppressed and chronically ill people, also parainfluenza virus 2 and 4 may lead to respiratory infections. Even though all four parainfluenza virus serogroups do not differ a lot in structure or biology, there is a clear relationship between parainfluneza virus serogroup and presentation of respiratory disease, as well as the seasonal appearance of each of the four parainfluenza viruses. Epidemics of parainfluenza 1 and 2 mostly occur biannually in fall whereas parainfluenza 3 epidemics in North America and Europe occur yearly in spring and summer.[2] Clinical symptoms are, dependent on the different serogroup, croup, bronchiolitis, pneumonia and tracheobronchitis.[1] Croup (also called acute laryngotracheitis) is mostly diagnosed in small children at the age of one and two and is of parainfluenza origin in 56 – 74 % of the cases. Here, an infection of parainfluenza virus 1 is most common. Bronchiolitis is present in children in their first year of age and is in 90 % of the cases leads due to viral infection. Bronchiolitis can occur through infection with all four parainfluenza virus serogroups but parainfluenza virus 1 and 3 are most common. Parainfluenza virus dependent pneumonia is most common in children between the age of two and three. Also pneumonia can be caused by all four parainfluenza serogroups. Patients

without any of the above mentioned symptoms most often are diagnosed with tracheobronchitis. 20 – 30 % of children with lower respiratory tract infections have tracheobronchitis and 25 % are due to parainfluenza virus 3, 1 and 2.
In general, all parainfluenza virus serogroups can cause one or more respiratory tract infections and in 5 -20 % of all lower respiratory tract infections more than one parainfluenza virus serogroup is detected.[1] Infections by pathogens of the paramoxyviridae family do have the biggest economic consequences. In the US, hospitalization due to parainfluenza virus 1 and 2 leads to estimated costs of around 186 mio $ and every croup epidemic caused by parainfluenza virus 1 costs 30 mio. $.[1]

## 3. Test principle

The RIDA®GENE Parainfluenza assay is a multiplex real-time RT-PCR for the direct, qualitative detection and differentiation of parainfluenza viruses Parainfluenza 1, Parainfluenza 3, Parainfluenza 2/4). The detection is done in a one step real-time RT-PCR format where the reverse transcription is followed by the PCR in the same reaction tube. The isolated RNA is transcribed into cDNA by a reverse transcriptase. Gene fragments specific for parainfluenza virus (parainfluenza 1, parainfluenza 3, parainfluenza 2/4) are subsequently amplified by real-time PCR. The amplified targets (HN-Gene) are detected with hydrolysis probes, which are labeled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the presence of a target the probes hybridize to the amplicons. During the extension step the Taq-polymerase breaks the reporter-quencher proximity. The reporter emits a fluorescent signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the amount of formed amplicons. The RIDA®GENE Parainfluenza assay contains an Internal Control RNA (ICR) as an internal control of sample preparation procedure and to determine possible PCR-inhibition.

## 4. Reagents provided

Tab.1: Reagents provided (Reagents provided in the kit are sufficient for 100 determinations)

| Kit Code | Reagent | Amount | Lid Color |
|---|---|---|---|
| 1 | Reaction Mix | 2x 700 µl | yellow |
| 2 | PP-Mix | 1x 770 µl | green |
| 3 | Enzyme Mix | 1x 80 µl | red |
| R | Internal Control RNA | 2x 1800 µl | brown |
| N | PCR Water | 1x 500 µl | white |
| P | Positive Control | 1x 100 µl | blue |

## 5. Storage instructions

- Protect all reagents from light and store at -20 °C. All reagents can be used until the expiration date. After expiry the quality guarantee is no longer valid.
- Carefully thaw reagents before using (e.g. in a refrigerator at 2 - 8 °C).
- Reagents can sustain up to 5 freeze/thaw cycles without influencing the assay performance (e.g. after the first thawing separate it in aliquots and freeze immediately).
- During PCR preparation all the reagents should be stored cold in an appropriate way (2 - 8 °C).

## 6. Additional necessary reagents and necessary equipment

- Sterile, media-free Rayon or Nylon flocked swabs (e.g. Copan Diagnostic Inc., catalogue no. 155C or 552C)
- The RIDA®GENE Parainfluenza real-time RT-PCR Assay is suitable for use with following extraction platforms and real-time PCR instruments:
- Extraction platforms:
  - RIDA® Xtract (R-Biopharm)
  - Maxwell®16 MDX (Promega)
  - QIAsymphony SP/AS, QIAcube (Qiagen)
  - NucliSENS® easyMag™ (bioMérieux)
  - Magna Pure 96, Magna Pure LC2.0 (Roche)
  - m2000sp, m24sp (Abbott)
- Real-time PCR instrument:

| | |
|---|---|
| Roche: | LightCycler® 480II |
| Agilent Technologies: | Mx3005P |
| Applied Biosystems: | ABI 7500 |
| Abbott: | m2000rt |
| Bio-Rad: | CFX96™ |
| Cepheid: | SmartCycler® |
| QIAGEN: | Rotor-Gene Q |

**Note: Only use 0.1 ml tubes on the Rotor-Gene Q (QIAGEN).**

If you want to use otherextraction platforms or real-time PCR instruments please contact R-Biopharm at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit I (PG0001) for use with the LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortexer
- Pipettes (0.5 – 20 µl, 20 – 200 µl, 100 – 1000 µl)
- Filter tips
- Powder-free disposal gloves

## 7. Precautions for users

For *in-vitro* diagnostic use.
This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories have to be followed. The instruction manual for the test procedure has to be followed. Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes. During handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash your hands after finishing the test procedure. Do not smoke, eat or drink in areas where samples or reagents are being used.

- Extraction, PCR preparation and the PCR run should be separated in different rooms to avoid cross-contaminations.
- Samples must be treated as potentially infectious as well as all reagents and materials being exposed to the samples and have to be handled according to the national safety regulations.
- Do not use the kit after the expiration date.

All reagents and materials used have to be disposed properly after use. Please refer to the relevant national regulations for disposal.

For more details see Material Safety Data Sheets (MSDS) at www.r-biopharm.com

## 8. Sample collection and Storage

### 8.1 Specimen collection

Moisten the swab with sterile saline or use dry swabs. Nasal / throat specimen should be collected with the recommended swab (refer to section 6. Additional necessary reagents and necessary equipment) according to manufacturer's instructions.

***NOTE:*** *Calcium alginate swabs and swabs with wooden or aluminum shafts and/or cotton tips may inhibit PCR and can lead to false negative results. Use the recommended swabs for specimen collection only.*

### 8.2 RNA Preparation

For RNA isolation of nasal or throat swabs or nasopharyngeal swabs the following procedure is recommended: Add 200 µl water (RNase-free) into a preparation tube. Insert the swab into the water and cut or break the swab stem. Cap the preparation tube tightly, vortex shortly and continue according to manufacturer's instruction of the RNA-extraction kit or RNA-extraction system.

The RIDA®GENE Parainfluenza assay contains an Internal Control RNA (ICR), which can either be used as PCR inhibition control or as extraction control for the sample preparation procedure and as a PCR inhibition control.

If the ICR is used only as a PCR inhibition control, 1 µl of the ICR should be added to the Master- Mix (see Tab. 3).

If the ICR is used as a extraction control for the sample preparation procedure and as PCR inhibition control, 20 µl of the ICR has to be added during extraction procedure. The ICR should always be added to the specimen-lysis buffer mixture and must **not** be added directly to the specimen. We also recommend to add 1 µl of the ICR to the negative and positive control RT-PCR Mix.

## 9. Test procedure

### 9.1 Master-Mix preparation

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and negative control must be included in each assay run.

We recommend to calculate an additional volume of 10 % to compensate imprecise pipetting (see Tab. 2, Tab. 3). Thaw, mix gently and centrifuge briefly the Reaction Mix, the PP-Mix, the Enzyme Mix, the Positive Control, the PCR Water and the ICR before using. Keep reagents appropriately cold during working step (2 - 8 °C).

Tab. 2: Calculation and pipetting example for 10 reactions of the Master Mix (ICR as extraction and PCR inhibition control)

| Kit code | Master-Mix components | Volume per reaction | 10 reactions (10 % extra) |
|---|---|---|---|
| 1 | Reaction Mix | 12.5 µl | 137.5 µl |
| 2 | PP-Mix (Primer-Probe-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 components of the Master Mix gently and briefly spin down.

Tab. 3: Calculation and pipetting example for 10 reactions of the Master Mix (ICR only as PCR inhibition control)

| Kit Code | Master-Mix components | Volume per reaction | 10 reactions (10 % extra) |
|---|---|---|---|
| 1 | Reaction Mix | 12.5 µl | 137.5 µl |
| 2 | PP-Mix (Primer-Probe-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** | **232.1 µl** |

Mix the components of the Master Mix gently and briefly spin down.

## 9.2 Preparation of the RT-PCR Mix

Pipette 20 µl of the Master-Mix in each reaction vial (tube or plate).

**Negative control:** Add 5 µl PCR Water as negative control to the pre-pipetted Master Mix.

***Note:*** *If the ICR is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the ICR to the negative control RT-PCR Mix.*

**Sample:** Add 5 µl RNA-Extract to the pre-pipetted Master Mix.

**Positive control:** Add 5 µl Positive Control to the pre-pipetted Master Mix.

***Note:*** *If the ICR is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the ICR to the positive control RT-PCR Mix.*

Cover tubes or plate. Spin down and place in the real-time PCR instrument. The RT-PCR reaction should be started according to the PCR instrument Set-up (see Tab. 4).

## 9.3 PCR Instrument Set-up

Tab. 4: Real-time PCR profile

| | |
|---|---|
| Reverse Transcription<br>Initiale Denaturation<br><br>Cycles<br>PCR Denaturation<br>Annealing/Extension | 10 min, 58 °C<br>1 min, 95 °C<br><br>45 Cycles<br>15 sec, 95 °C<br>30 sec, 55 °C |
| Temperature Transition Rate / Ramp Rate | Maximum |

***Note:*** *Annealing and Extension occur in the same step.*

***Note:*** ***Check that the "Manual Thres. Fluor Units" for Channel 1 is set to 30.0 and for Channel 2 to 4 is set to 5.0 on the SmartCycler® (Cepheid). Due to variations between different cyclers, it may be required to individually adapt the "Manual Thres. Fluor Units" for channel 1.***

## 9.4 Detection Channel Set-up

Tab. 5: Selection of appropriate detection channels

| Real-time PCR instrument | Detection | Detection Channel | Note |
|---|---|---|---|
| Roche LightCycler® 480II | Parainfluenza 1 | 465/510 | RIDA®GENE Color Compensation Kit I (PG0001) is required |
| | ICR | 533/580 | |
| | Parainfluenza 3 | 533/610 | |
| | Parainfluenza 2/4 | 618/660 | |
| Cepheid SmartCycler® | Parainfluenza 1 | Kanal 1 | Check that the "Manual Thres. Fluor Units" for Channel 1 is set to 30.0 and for Channel 2 - 4 is set to 5.0* |
| | ICR | Kanal 2 | |
| | Parainfluenza 3 | Kanal 3 | |
| | Parainfluenza 2/4 | Kanal 4 | |
| ABI 7500 | Parainfluenza 1 | FAM | Check that passive reference option ROX is none |
| | ICR | VIC | |
| | Parainfluenza 3 | ROX | |
| | Parainfluenza 2/4 | Cy5 | |
| Abbott m2000rt | Parainfluenza 1 | FAM | - |
| | ICR | VIC | |
| | Parainfluenza 3 | ROX | |
| | Parainfluenza 2/4 | Cy5 | |
| Stratagene Mx3005P | Parainfluenza 1 | FAM | Check that passive reference option ROX is none |
| | ICR | HEX | |
| | Parainfluenza 3 | ROX | |
| | Parainfluenza 2/4 | Cy5 | |
| Qiagen Rotor-Gene Q | Parainfluenza 1 | Green | The gain settings have to be set to 5 |
| | ICR | Yellow | |
| | Parainfluenza 3 | Orange | |
| | Parainfluenza 2/4 | Red | |
| Bio-Rad CFX96™ | Parainfluenza 1 | FAM | - |
| | ICR | VIC | |
| | Parainfluenza 3 | ROX | |
| | Parainfluenza 2/4 | Cy5 | |

*** Due to variations between different cyclers, it may be required to individually adapt the "Manual Thres. Fluor Units" for channel 1.**

## 10. Quality Control

The analysis of the samples is done by the software of the used real-time PCR instrument according to the manufacturer` s instructions. Positive and negative controls have to show correct results (see Table 6, Fig. 1, Fig. 2, Fig. 3) in order to determine a VALID run.

The positive control has a concentration of $10^3$ copies/µl. In each PCR run it is used in a total amount of 5 x $10^3$ copies.

Tab. 6: For a VALID run, the following conditions must be met:

| Sample | Assay result | ICR Ct | Target Ct |
|---|---|---|---|
| PTC | Positive | NA *[1] | See QAC |
| NTC | Negative | Ct > 20 | 0 |

*[1] *No Ct value is required for the ICR to make a positive call for the positive control.*

If the Positive Control (PTC) is not positive within the specified Ct range but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and a new Positive Control.

If the Negative Control (NTC) is not negative but the Positive control is valid prepare all new reactions using remaining purified nucleic acids and a new Negative Control.

If the required criteria are not met, following items have to be checked before repeating the test:

- Expiry of the used reagents
- Functionality of the used instrumentation
- Correct performance of the test procedure

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

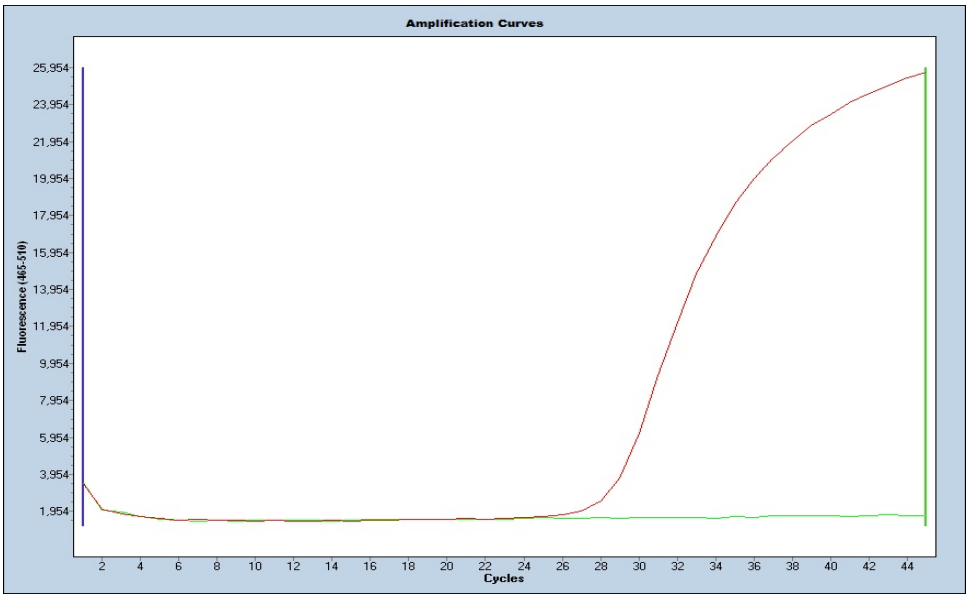


Fig. 2: Correct run of the positive and negative control (parainfluenza 3) on the LightCycler® 480II

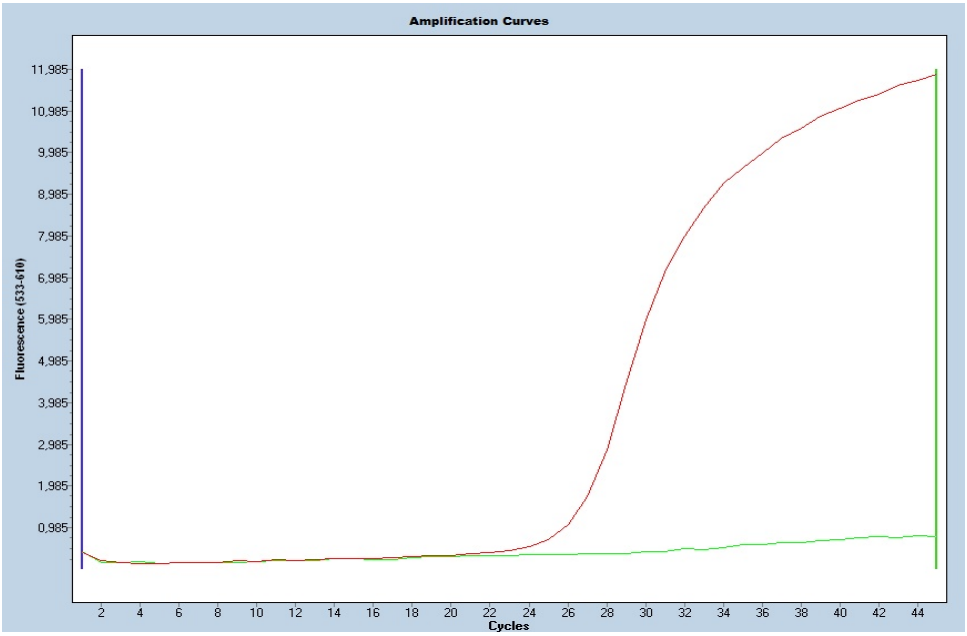
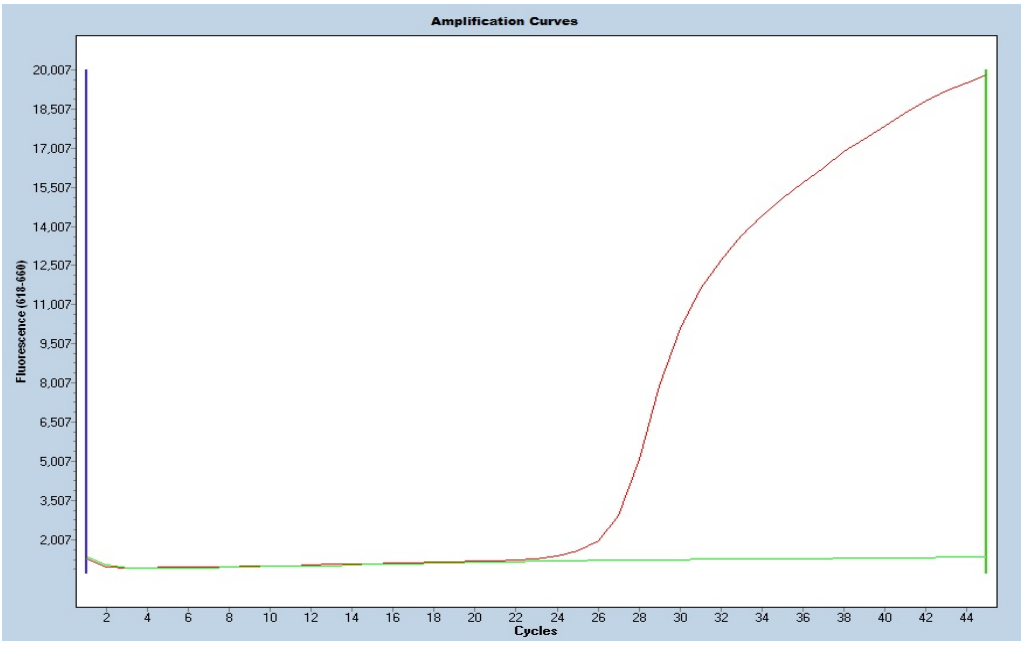



Fig. 3: Correct run of the positive and negative control (parainfluenza 2/4) on the LightCycler® 480II



## 11. Evaluation and interpretation

The result interpretation is done according to Table 7.

Tab. 7: Sample interpretation

| Target genes | | | | |
|---|---|---|---|---|
| **HN-Gene (specific for Parainfluenza 1)** | **HN-Gene (specific for Parainfluenza 3)** | **HN-Gene (specific for Parainfluenza 2/4)** | **Internal Control RNA (ICR)** | **Result** |
| **positive** | negative | negative | **positive/negative** | **Parainfluenza 1** |
| negative | **positive** | negative | **positive/negative** | **Parainfluenza 3** |
| negative | negative | **positive** | **positive/negative** | **Parainfluenza 2/4** |
| negative | negative | negative | **positive** | **Negative (Target genes are not detectable)** |
| negative | negative | negative | negative | **Not evaluable** |

A sample is evaluated negative, if the sample shows no amplification signal in the detection system, but the Internal Control RNA (ICR) is positive. An inhibition of the PCR reaction or a failure in the extraction procedure can be excluded by the detection of the Internal Control RNA (ICR).

A sample is evaluated positive, if both, the sample and the Internal Control RNA, (ICR) show an amplification signal in the detection system.

A sample is evaluated positive, if the sample RNA shows an amplification signal in the detection system, but the Internal Control RNA (ICR) is negative. The detection of the internal amplification control is not necessary, because high concentrations of the amplicon can cause a weak or absent signal of the internal amplification control.

A sample is evaluated invalid, if both, the sample and the Internal Control RNA (ICR) show no amplification signal in the detection system. The sample contained a RT-PCR inhibitor or a failure occurred in the extraction procedure. The extracted sample needs to be further diluted with PCR water (1:10) and re-amplified, or the isolation and purification of the sample has to be improved.

## 12. Limitations of the method

1. The result of molecular analysis should not lead to the diagnosis, but always be considered in the context of medical history and symptoms of the patient.
2. This test is only validated for nasal and throat swabs and nasopharyngeal swabs.
3. Inappropriate specimen collection, transport, storage and processing or a viral load in the specimen below the analytical sensitivity can result in false negative results.
4. The presence of PCR inhibitors may cause invalid results.
5. Mutations or polymorphisms in primer or probe binding regions may affect detection of new variants resulting in a false negative result with the RIDA®GENE Parainfluenza assay.
6. As with all PCR based *in vitro* diagnostic tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.

## 13. Performance characteristics

### 13.1 Analytical sensitivity

The RIDA®GENE Parainfluenza multiplex real-time RT-PCR has a detection limit of ≥ 50 RNA copies per reaction for parainfluenza 1, parainfluenza 3 and parainfluenza 2/4, respectively (see Fig. 4, Fig. 5, Fig. 6).

Fig. 4: Dilution series parainfluenza 1 ($10^5$ – $10^1$ RNA copies per µl) on the LightCycler® 480II

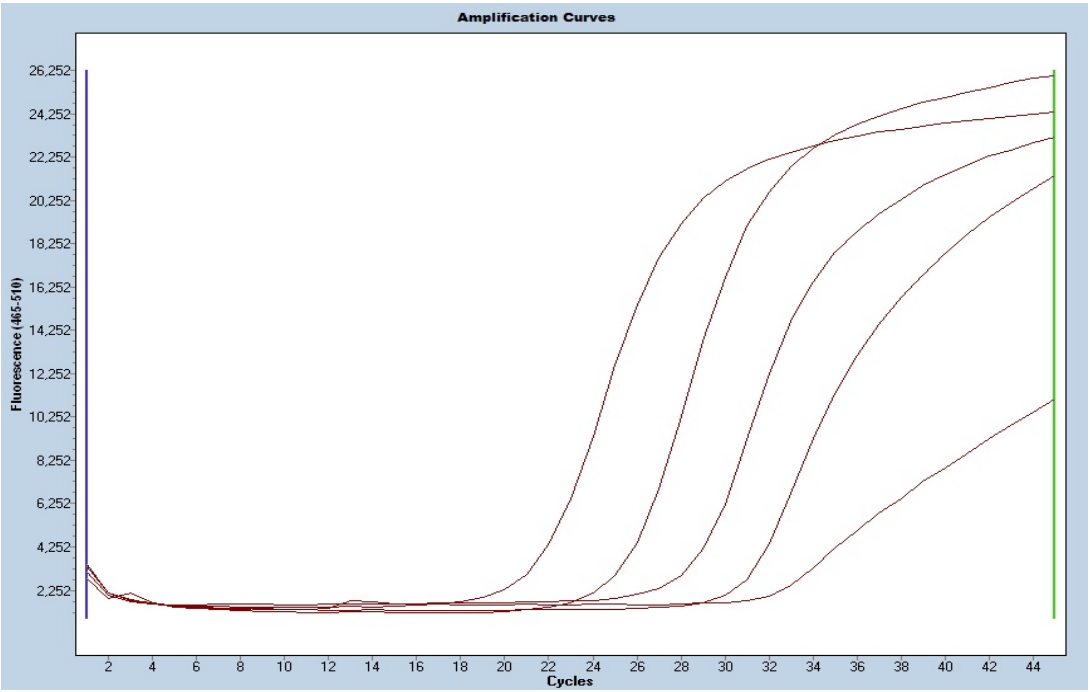


Fig. 5: Dilution series parainfluenza 3 ($10^5$ – $10^1$ RNA copies per µl) on the LightCycler® 480II

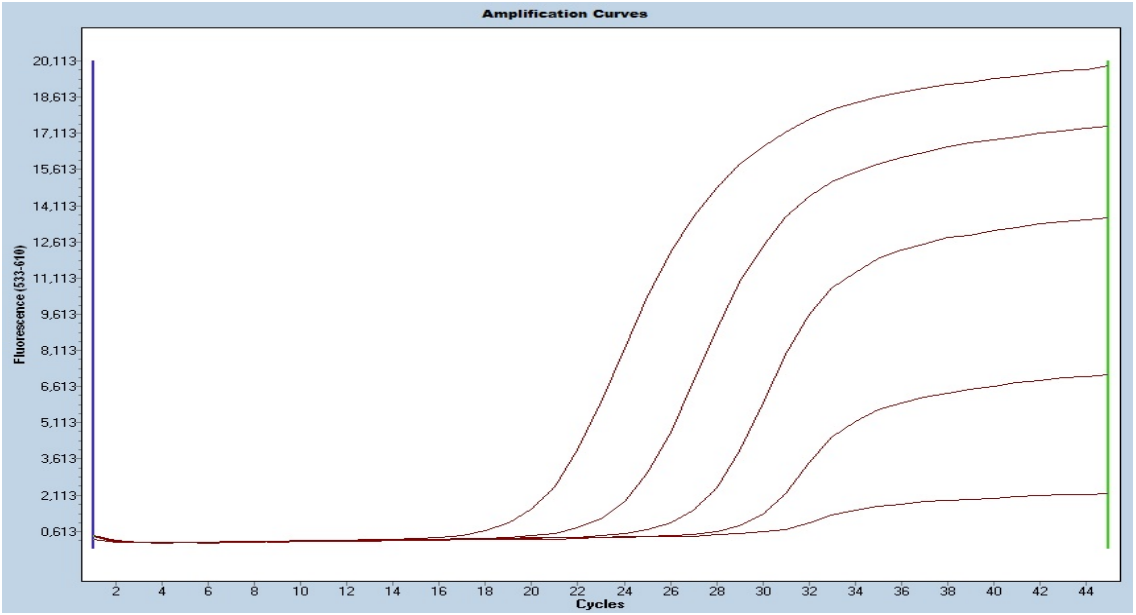
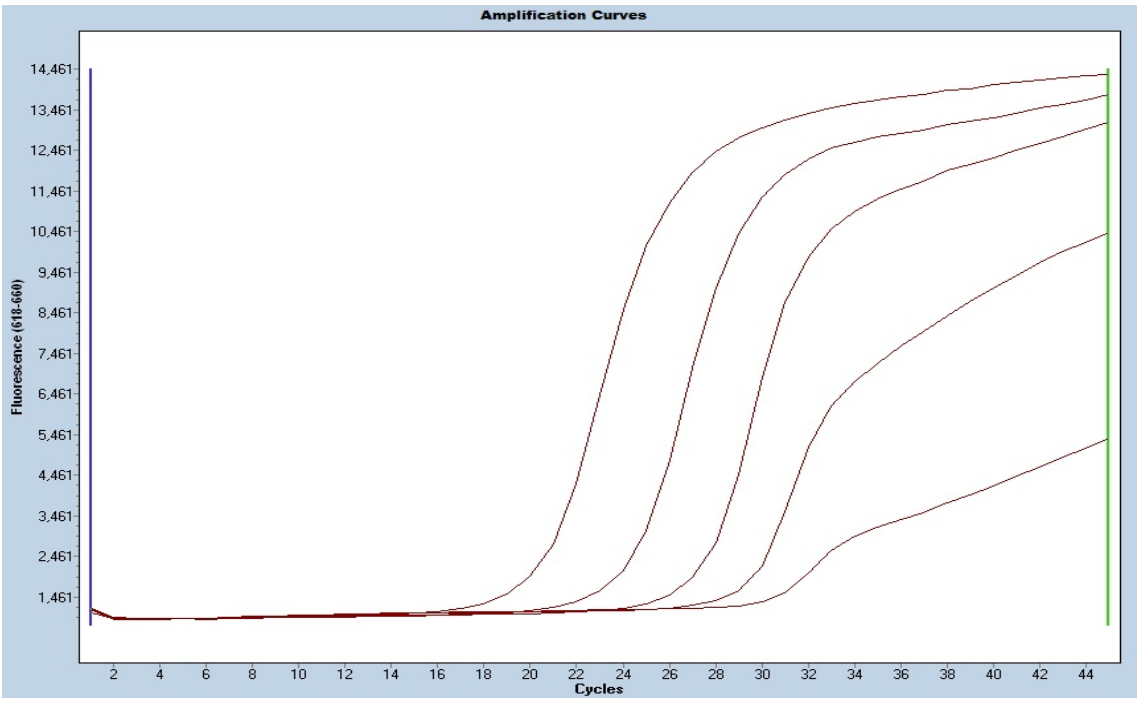



Fig. 6: Dilution series parainfluenza 2/4 ($10^5$ – $10^1$ RNA copies per µl) on the LightCycler® 480II




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

## 13.2 Analytical specificity

The RIDA®GENE Parainfluenza real-time RT-PCR is specific for parainfluenza viruses (parainfluenza 1, parainfluenza 3, parainfluenza 2/4). No cross-reaction could be detected for the following species (see Tab. 8):

Tab. 8: Cross-reactivity testing

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Acinetobacter baumannii* Strain 5377 | - | Herpes simplex virus 1 strain McIntyre | - | Human Rhinovirus Genogruppe A | - | *Staphylococcus aureus* | - |
| Adenovirus | - | Herpes simplex virus 2 strain MS | - | Influenza virus infectious A/PR/8/34 | - | *Staphylococcus haemolyticus* SM131 | - |
| Adenovirus 1, Human, strain Adenoid 71 | - | Human Coronavirus 229E | - | *Klebsiella oxytoca* | - | *Streptococcus pneumoniae* strain NCTC 7465 | - |
| Adenovirus 7, Human, Strain Gomen | - | Human Coxsackie B4 | - | *Klebsiella pneumoniae* strain MGH78578 | - | Varicella Zoster Virus (Type B) | - |
| *Bordetella parapertussis* Strain 12822 | - | Human Cytomegalovirus | - | *Legionella pneumophila* subsp. Pneumophila | - | | |
| *Bordetella pertussis* Tohama 1 | - | Human Metapneumovirus | | *Mycoplasma pneumoniae* Strain FH of Eaton Agent | - | | |
| Epstein-Barr-Virus B95-8 strain | - | Human respiratory syncitial virus strain Long | | Neisseria meningitidis Strain FAM18 | - | | |
| *Haemophilus influenzae* Rd | - | Human respiratory syncitial virus strain 9320 | - | *Serratia liquefaciens* | - | | |
| | | | | | | | |
| Adenovirus 40, Human, Strain Dugan | - | Candida albicans | - | *E. coli* (O6) | - | *Staphylococcus epidermidis* | - |
| Adenovirus 41, Human, Strain Tak | - | *Citrobacter freundii* | - | *E. coli* (O157:H7) | - | *Vibrio parahaemolyticus* | - |
| *Aeromonas hydrophila* | - | *Citrobacter freundii* NCTC 9750 | - | *Enterobacter cloacae* | - | *Yersinia enterocolitica* | - |
| *Arcobacter butzleri* | - | *Clostridium difficile* | - | *Enterococcus faecalis* | - | | |
| *Bacillus cereus* | - | *Clostridium perfringens* | - | *Proteus vulgaris* | - | | |
| *Bacteroides fragilis* | - | *Clostridium bifermentans* | - | *Pseudomonas aeruginosa* | - | | |
| *Campylobacter coli* | - | *Clostridium sporogenes* | - | Rotavirus | - | | |
| *Campylobacter jejuni* | - | *Clostridium novyi* | - | *Salmonella enteritidis* | - | | |
| *Campylobacter fetus* subsp. Fetus | - | *Clostridium septicum* | - | *Shigella flexneri* | - | | |
| *Campylobacter lari* subsp. Lari | - | *Clostridium sordelli* | - | *Salmonella typhimurium* | - | | |
| *Campylobacter upsaliensis* | - | *E. coli* (O26:H-) | - | *Staphylococcus hominis* subsp. novobiosepticus R22 | - | | |

## 13.3 Analytical reactivity

The reactivity of the RIDA®GENE Parainfluenza real-time RT-PCR was evaluated against multiple strains of parainfluenza 4 (see Tab. 9). All parainfluenza 4 strains of the panel were detected by the RIDA®GENE Parainfluenza real-time RT-PCR.

Tab. 9: Analytical reactivity testing

| Subtype | Strain | Parainfluenza 1 | Parainfluenza 3 | Parainfluenza 2/4 |
|---|---|---|---|---|
| 1 | Human Parainfluenza virus 1, Strain C35 | **positive** | negative | negative |
| 2 | Human Parainfluenza virus 1, Strain Greer | negative | negative | **positive** |
| 4a | Human Parainfluenza virus 4, Strain M25 | negative | negative | **positive** |
| 4b | Human Parainfluenza virus 4, Strain CH 19503 | negative | negative | **positive** |

## Explanation of Symbols

For *in vitro* diagnostic use


Consult instructions for use

Lot number


Expiry


Store at

Article number

Number of test


Date of manufacture


Manufacturer

## Literature

1. Henrickson KJ. Parainfluenza viruses. Clin. Microbiol. Reviews. 2003, 16(2): 242-263.
2. Berman S. Epidemiology of acute respiratory infections in children of deveioning countries. Rev. Infect. Dis. 1991, 13:454-462.