RIDA®GENE RSV & hMPV

real-time RT-PCR

Art. Nr.: PG5905

100 Reactions

For *in vitro* diagnostic use.

-20 °C

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1. Intended use

For *in vitro* diagnostic use. RIDA®GENE RSV & hMPV is a multiplex real-time RT-PCR for the direct, qualitative detection and differentiation of respiratory syncytial virus (A/B) and human metapneumovirus (1-4) from human nasal/throat swabs, nasopharyngeal swabs, throat washes and BAL.

RIDA®GENE RSV & hMPV real-time RT-PCR is intended for use as an aid in the diagnosis of RSV & hMPV infections.

2. Summary and Explanation of the test

Respiratory syncitial viruses (RSV) belong to the family of *Paramyxoviridae* and are enveloped single-stranded RNA viruses. There are two groups of RSV, group A and group B, whereas RSV A is most dominant in most years.[1]
RSV is spread worldwide and can lead to an infection of the upper and lower respiratory tract at every age. RSV is transmitted *via* smear or airborne transfection and patients show symptoms of rhinitis, cold, cough, acute bronchitis and also inflammation of the middle ear. An acute presentation may occur during the presence of a bacterial super infection and, worldwide, around 600 000 people die yearly from either direct or indirect infection with RSV.[2] Infants and small children often show an acute presentation which requires hospitalization. Here, symptoms such as fever, cold and tachypnoe occur. 50 – 70 % of infants and small children have a RSV infection in at the age of one, whereas almost 100 % had a RSV infection after the age of two.[1]
Another new representative of *Paramyxoviridae* is the human metapneumovirus (hMPV). The virus was first isolated in the Netherlands in 2001 and is genetically related to RSV. However, the course of this infection is less severe compared to RSV infections. In many cases infants are affected by this, whereas 15% of the annual cases of bronchitis are also caused by hMPV. Infants, elderly and immunosuppressed patients with severe affections of hMPV usually need hospitalization. Regarding the route of transmission little is known yet. The worldwide occurrence of hMPV is comparable with the seasonal occurrence of influenza in winter.

3. Test principle

The RIDA®GENE RSV & hMPV assay is a multiplex real-time RT-PCR for the direct, qualitative detection and differentiation of RSV & hMPV. 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 RSV (A/B) and hMPV (1-4) are subsequently amplified by real-time PCR. The amplified targets (F Gene and F-Glycoprotein) 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 RSV & hMPV 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 RSV & hMPV 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 (Promega)
  - Magna Pure 96 (Roche)
- 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 other extraction 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. Collection and Storage of Samples

8.1 Specimen collection

Moisten the swab with sterile saline or use dry swabs. Nasal/throat or nasopharyngeal specimen should be collected with the recommended swab (refer to section 6. Additional equipment and materials required) 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 Sample Preparation

For RNA isolation of nasal/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 instructions of the RNA extraction kit or RNA extraction system.

To isolate RNA from bronchoalveolar lavage, we recommend using a commercially available RNA extraction system (e.g. Maxwell®16 (Promega)). Isolate RNA according to manufacturer's instructions.

The RIDA®GENE RSV & hMPV 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 Internal Control RNA is used as a extraction control for the sample preparation procedure and as PCR inhibition control, 20 µl of the Internal Control RNA has to be added during extraction procedure. The Internal Control RNA 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 Internal Control RNA 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 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 Internal Control RNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control RNA 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 Internal Control RNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, we recommend to add 1 µl of the Internal Control RNA 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 RT-PCR profile

Reverse Transcription	10 min, 58 °C
Initial Denaturation	1 min, 95 °C
Cycles	45 Cycles
PCR Denaturation	15 sec, 95 °C
Annealing/Extension	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 and 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	RSV	465/510	**RIDA®GENE Color Compensation Kit I (PG0001) is required**
	ICR	533/580	
	hMPV	618/660	
Cepheid SmartCycler®	RSV	Kanal 1	**Check that the "Manual Thres. Fluor Units" for Channel 1 is set to 30.0 and for Channel 2 and 4 is set to 5.0***
	ICR	Kanal 2	
	hMPV	Kanal 4	
ABI 7500	RSV	FAM	**Check that passive reference option ROX is none**
	ICR	VIC	
	hMPV	Cy5	
Abbott m2000rt	RSV	FAM	**-**
	ICR	VIC	
	hMPV	Cy5	
Agilent Techn. Mx3005P	RSV	FAM	**Check that passive reference option ROX is none**
	ICR	HEX	
	hMPV	Cy5	
Qiagen Rotor-Gene Q	RSV	Green	**The gain settings have to be set to 5**
	ICR	Yellow	
	hMPV	Red	
Bio-Rad CFX96™	RSV	FAM	-
	ICR	VIC	
	hMPV	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) 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 5×10^3 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 Quality Assurance Certificate
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 (RSV) on the LightCycler® 480II

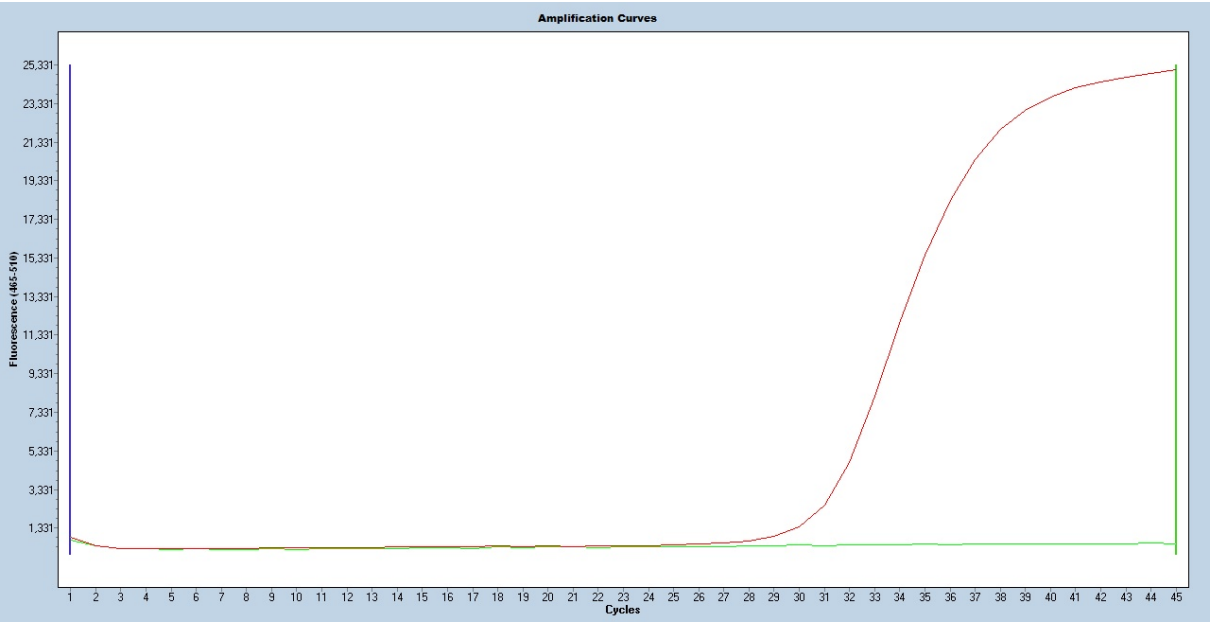
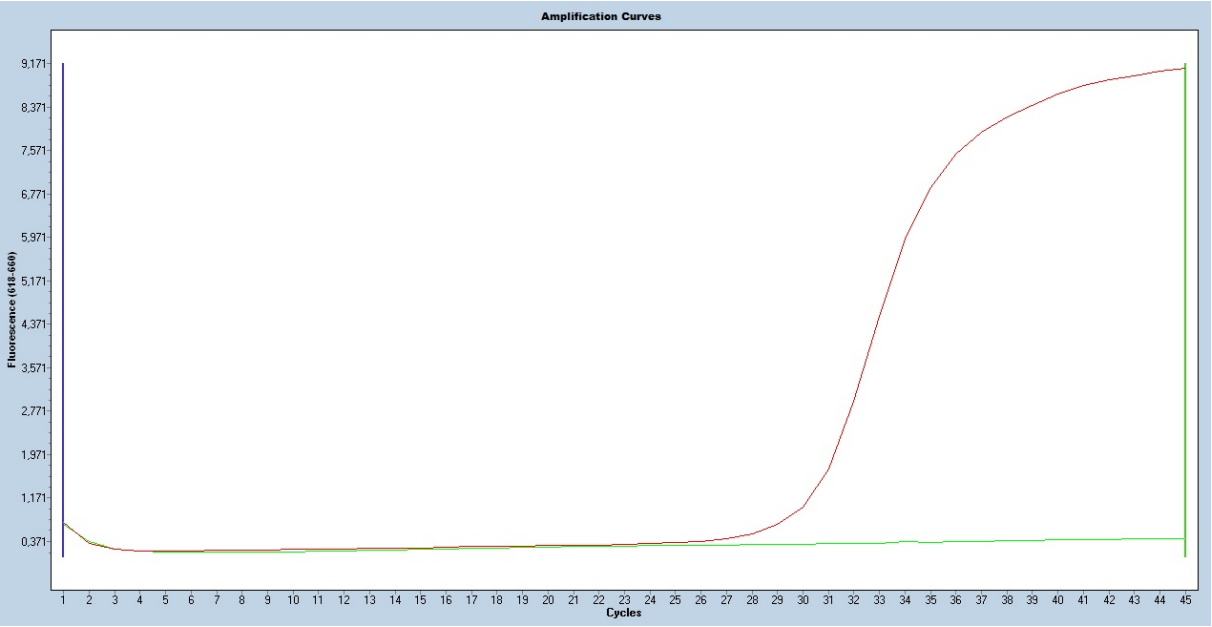


Fig. 2: Correct run of the positive and negative control (hMPV) on the LightCycler® 480II



11. Result interpretation

The result interpretation is done according to Table 7.

Tab. 7: Sample interpretation

Target genes			
F-Gene	**F-Glycoprotein**	**Internal Control RNA (ICR)**	**Result**
positive	negative	**positive/negative**	**RSV**
negative	**positive**	**positive/negative**	**hMPV**
negative	negative	**positive**	**Negative (Target genes are not detectable)**
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, nasopharyngeal swabs, throat washes and BAL.
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 RSV & hMPV 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.
7. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result is indicative for the presence of the target gene for RSV & hMPV.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE RSV & hMPV multiplex real-time RT-PCR has a detection limit of ≥ 50 RNA copies per reaction for RSV and hMPV, respectively (see Fig. 3, Fig. 4).

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

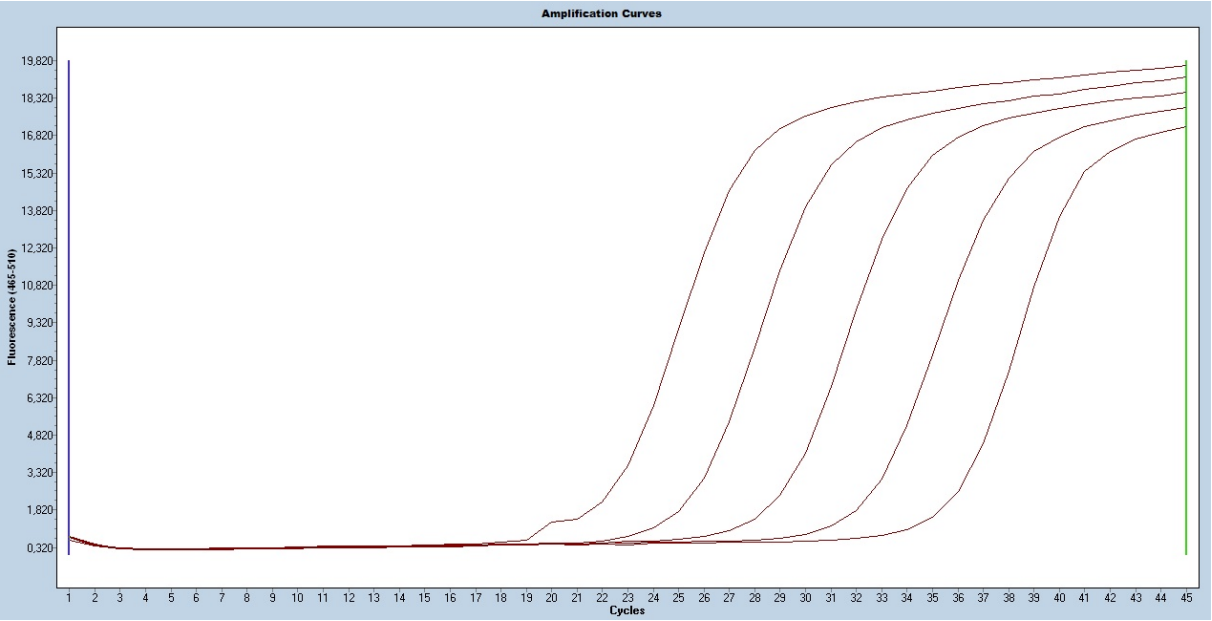
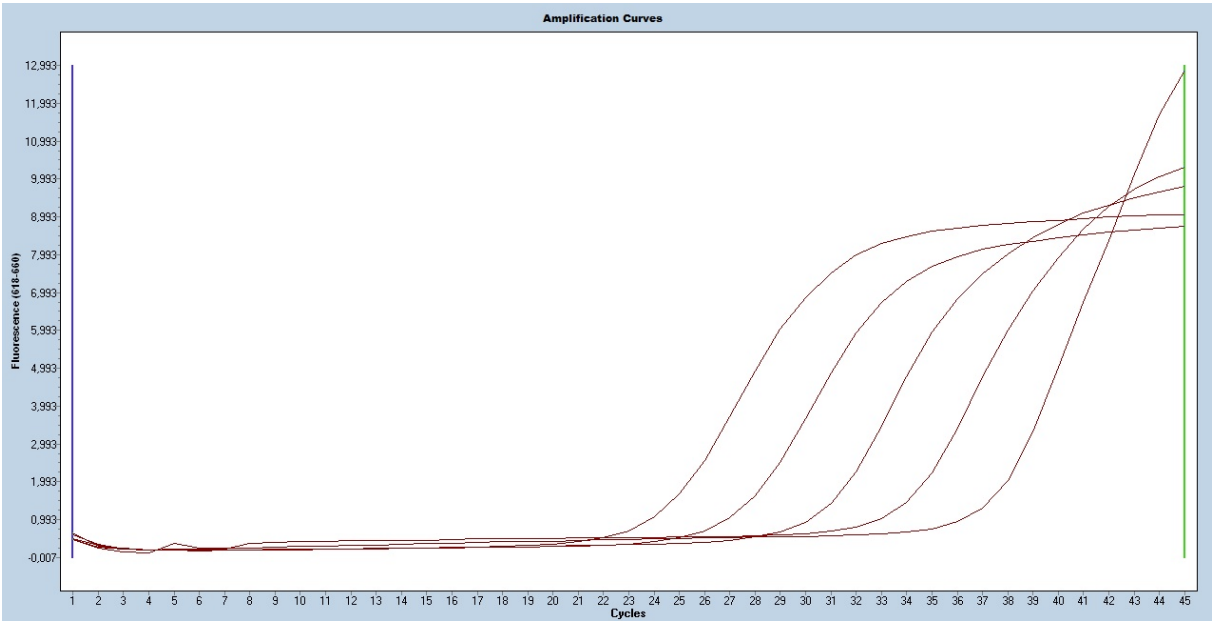


Fig. 4: Dilution series hMPV (10^5 – 10^1 RNA copies per µl) on the LightCycler® 480II



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

13.2 Analytical specificity

The RIDA®GENE RSV & hMPV real-time RT-PCR is specific for RSV and hMPV. No cross-reaction could be detected for the following species (see Tab. 8):

Tab. 8: Cross-reactivity testing

Adenovirus 1, Human, strain Adenoid 71	-	Herpes simplex virus 2 strain MS	-	Human Parainfluenza Virus serotype 3	-	*Streptococcus pneumoniae* strain NCTC 7465	-
Adenovirus 7, Human, Strain Gomen	-	Human Coronavirus 229E	-	Human Rhinovirus Genogroup A	-	Varicella Zoster Virus (Type B)	-
Bordetella parapertussis Strain 12822	-	Human Coxsackie B4	-	Influenza virus infectious A/PR/8/34	-		
Bordetella pertussis Tohama 1	-	Human Cytomegalovirus	-	*Klebsiella pneumoniae* strain MGH78578	-		
Epstein-Barr-Virus B95-8 strain	-	Human parainfluenza virus 1 strain C35	-	*Legionella pneumophila* subsp. Pneumophila	-		
Haemophilus influenzae Rd	-	Human parainfluenza virus 2 strain Greer	-	*Mycoplasma pneumoniae* Strain FH of Eaton Agent	-		
Herpes simplex virus 1 strain McIntyre	-	Human parainfluenza virus 4b strain CH19503	-	Neisseria meningitidis Strain FAM18	-		
Acinetobacter baumannii Strain 5377	-	*Campylobacter lari* subsp. Lari	-	*E. coli* (O6)	-	*Staphylococcus aureus*	-
Adenovirus	-	*Campylobacter upsaliensis*	-	*E. coli* (O157:H7)	-	*Staphylococcus epidermidis*	-
Adenovirus 40, Human, Strain Dugan	-	Candida albicans	-	*Enterobacter cloacae*	-	*Staphylococcus haemolyticus SM131*	-
Adenovirus 41, Human, Strain Tak	-	*Citrobacter freundii* NCTC 9750	-	*Enterococcus faecalis*	-	*Staphylococcus hominis* subsp. novobiosepticus R22	-
Aeromonas hydrophila	-	*Clostridium bifermentans*	-	Klebsiella oxytoca	-	*Vibrio parahaemolyticus*	-
Arcobacter butzleri	-	*Clostridium difficile*	-	*Proteus vulgaris*	-	*Yersinia enterocolitica*	-
Astrovirus	-	*Clostridium perfringens*	-	*Pseudomonas aeruginosa*	-		
Bacillus cereus	-	*Clostridium sporogenes*	-	Rotavirus	-		
Bacteroides fragilis	-	*Clostridium septicum*	-	*Salmonella enteritidis*	-		
Campylobacter coli	-	*Clostridium novyi*	-	*Salmonella typhimurium*	-		
Campylobacter jejuni	-	*Clostridium sordellii*	-	*Serratia liquefaciens*	-		
Campylobacter fetus subsp. Fetus	-	*E. coli* (O26:H-)	-	*Shigella flexneri*	-		

13.3 Analytical reactivity

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

Tab. 9: Analytical reactivity testing

Subtype	Strain	RSV	hMPV
A	Human Respiratory syncytial virus Strain Long	**positive**	negative
B	Human Respiratory syncytial virus Strain L9320	**positive**	negative

Explanation of Symbols

IVD	For *in vitro* diagnostic use
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
	Number of test
	Date of manufacture
	Manufacturer

Literature

1. Robert Koch Institut. Respiratorische Synzytial-Viren-Infektionen. RKI-Rategeber für Ärzte, Stand Mai 2011.
2. Thorburn K. Pre-existing disease is associated with a significantly higher risk of death in severe respiratory syncytial virus infection. Arch Dis. Child 2009, 94: 99-103.