RSV & hMPV RT-PCR Kit

Multiplex real-time RT-PCR kit for the direct, qualitative detection and differentiation of RSV and hMPV from human nasal/throat swabs and bronchoalveolar lavage (BAL) samples.

For Research Use Only. Not for Use in Diagnostic Procedures.

Catalog Number: 86-RSVHU-100
Size: 100 determinations
Version: 2019-11-13 - ALPCO 1.0

INTENDED USE

The RSV & hMPV RT-PCR kit is a multiplex real-time RT-PCR assay for the direct, qualitative detection and differentiation of respiratory syncytial virus (RSV A/B) and human metapneumovirus (hMPV 1-4) from human nasal/throat swabs and bronchoalveolar lavage (BAL) samples. For Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE TEST

The RSV & hMPV multiplex real-time RT-PCR assay is for the direct, qualitative detection and differentiation of RSV and hMPV from human nasal/throat swabs and BAL samples. Detection takes place in one-step real-time RT-PCR formatwhere the reverse transcription (RT) and the subsequent PCR take place in the same reaction tube. The isolated RNA is transcribed into cDNA using a reverse transcriptase. The specific gene fragments for RSV (F gene) and hMPV (F glycoprotein) are then amplified using real-time RT-PCR.

The amplified target sequences 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 fluorescence signal which is detected by the optical unit of a real-time PCR instrument. The fluorescence signal increases with the quantity of formed amplicons. The RSV & hMPV RT-PCR Kit contains an Internal Control RNA (ICR) as an internal control of the sample preparation procedure and to determine possible PCR inhibition.

REAGENTS PROVIDED

Reagents provided in the kit are sufficient for 100 determinations.

Table 1. Reagents Provided

Kit Code	Reagent	Amount		Lid Color
1	Reaction Mix	2x	1050 uL	yellow
2	Enzyme Mix	1x	80 uL	red
R	Internal Control RNA	2x	1700 uL	brown
N	No Template Control	1x	450 uL	white
P	Positive Control	1x	200 uL	blue

STORAGE INSTRUCTIONS

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 After the expiration date, the quality guarantee is no longer valid.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

MATERIALS REQUIRED BUT NOT PROVIDED

The RSV & hMPV RT-PCR kit has been tested using the following extraction platforms and PCR instruments. If using another PCR instrument, refer to the settings for the tested instruments as a starting point and modify as necessary.

Table 2. Extraction Platforms and PCR Instruments

Extraction platform	
ALPCO	DNA/RNA Extraction Kit
Promega	Maxwell® RSC
Real-time PCR instrument	
Roche	LightCycler® 480II
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q (Use only 0.1mL reaction vials)

- Sterile swab collection system (e.g., eSwab®, Copan Diagnostics Inc.)
- Extraction platform if extraction not performed manually
- Real-time PCR instrument
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with a rotor for the reaction vials
- Vortex mixer
- Pipettes (0.5 - 20 uL, 20 - 200 uL, 100 - 1000 uL)
- Filter tips
- Powder-free disposal gloves
- PCR Grade water (bioscience grade, nuclease-free)
- Color Compensation Kit IV (if running the LightCycler® 480II)

WARNINGS and PRECAUTIONS

- Good laboratory practices must be used and the instructions for carrying out the test must be strictly followed.
- Do not mix reagents or coated microtiter strips from kits with different lot numbers.
- Do not pipet samples or reagents by mouth. Avoid contact with bruised skin or mucosal membranes.
- When handling reagents or samples, wear appropriate safety clothing (appropriate gloves, lab coat, safety goggles) and wash 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 must be disposed properly after use. Please refer to the relevant local and national regulations for disposal.

PREPARATION OF SAMPLES

Nasal / Throat Swabs

A commercially available nucleic acid extraction kit (e.g., DNA/RNA Extraction Kit; ALPCO) or nucleic acid extraction platform (e.g. Maxwell® RSC; Promega) is recommended for RNA preparation from nasal and throat swabs. It is recommended to use the amount of medium specified by the manufacturer in the nucleic acid extraction kit or nucleic acid extraction system and that the extraction is performed according to the manufacturer's instructions.

Bronchoalveolar Lavage (BAL)

A commercially available nucleic acid extraction kit (e.g., DNA/RNA Extraction Kit; ALPCO) or nucleic acid extraction platform (e.g. Maxwell® RSC; Promega) is recommended for RNA preparation from BAL fluid. It is recommended to use the amount of sample specified by the manufacturer in the nucleic acid extraction kit or nucleic acid extraction system and that the extraction is performed according to the manufacturer's instructions.

Addition of Control RNA

The RSV & hMPV RT-PCR kit contains an Internal Control RNA that detects PCR inhibition, monitors reagent integrity, and confirms that nucleic acid extraction was sufficient. The Internal Control RNA can either be used as PCR inhibition control or as both an extraction control for the sample preparation procedure and a PCR inhibition control.

If the Internal Control RNA is used only as a PCR inhibition control, 1 uL of the Internal Control RNA should be added to the Master Mix for each reaction (see Table 4). If the Internal Control RNA is used as an extraction control for the sample preparation procedure and as a PCR inhibition control, 20 uL of the Internal Control RNA must 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. It is also recommended adding 1 uL of the Internal Control RNA to the negative control and positive control PCR Mix.

MASTER MIX PREPARATION

Calculate the total number of PCR reactions (sample and control reactions) needed. One positive control and one negative control must be included in each test run. It is recommended adding a 10% volume overage to compensate for imprecision in pipetting (see Tables 3 and 4). Thaw, mix gently and centrifuge briefly the Reaction Mix, the Enzyme Mix, the Positive Control, the No Template Control, and the Internal Control RNA before using. Keep reagents appropriately cold during working step (2-8°C).

Table 3. 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% overage)
1	Reaction Mix	19.3 uL	212.3 uL
2	Enzyme Mix	0.7 uL	7.7 uL
	Total Volume	**20 uL**	**220 uL**

Table 4. 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% overage)
1	Reaction Mix	19.3 uL	212.3 uL
2	Enzyme Mix	0.7 uL	7.7 uL
R	Internal Control RNA	1.0 uL	11 uL
	Total Volume	**21 uL**	**231 uL**

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

PREPARATION OF THE PCR MIX

1. Pipette 20 uL of the Master Mix into all reaction wells (tubes or plate wells).

2. Add 5 uL of the No Template Control to the negative control well.

Note: If the Internal Control RNA is being used as extraction control for the sample preparation procedure and as PCR inhibition control, add 1 uL of the Internal Control RNA to the negative control well.

3. Add 5 uL of eluate to the sample wells.

4. Add 5 uL of Positive Control to the positive control well.

Note: If the Internal Control RNA is used as extraction control for the sample preparation procedure and as PCR inhibition control, add 1 uL of the Internal Control RNA to the positive control well.

5. Cover tubes or plate. Briefly centrifuge at slow speed, then transfer into the real-time PCR instrument. The RT-PCR reaction should be started according to the PCR instrument set-up (see Tables 5 and 6).

PCR INSTRUMENT SET-UP

Table 5. Universal Real-time PCR Profile for LightCycler®

Reverse Transcription	10 min, 58°C
Initial Denaturation	1 min, 95°C
Cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	10 sec, 95°C 15 sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum

Table 6. Universal Real-time PCR Profile for Mx3005P, ABI7500, Rotor-Gene Q, and CFX96™

Reverse Transcription	10 min, 58°C
Initial Denaturation	1 min, 95°C
Cycles	45 cycles
PCR Denaturation PCR Annealing / Extension	15 sec, 95°C 30 sec, 60°C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and Extension occur in the same step

Note: The universal real-time PCR profile can also be used for DNA assays if ALPCO DNA and RNA real-time PCR assays are combined in one run.

DETECTION CHANNEL SET-UP

Table 7. Selection of Appropriate Detection Channels

Real-time PCR Instrument	Detection	Detection Channel	Note
Roche LightCycler® 480II	RSV	465/510	Color compensation kit IV is required.
	ICR	533/580	
	hMPV	618/660	
Agilent Technologies Mx3005P	RSV	FAM	Set the reference dye to none.
	ICR	HEX	
	hMPV	Cy5	
ABI 7500	RSV	FAM	Set the ROX passive reference dye to none.
	ICR	VIC	
	hMPV	Cy5	
Bio-Rad CFX96™	RSV	FAM	-
	ICR	VIC	
	hMPV	Cy5	
Qiagen Rotor-Gene Q	RSV	Green	The gain settings must be set to 5 (factory default) for all channels.
	ICR	Yellow	
	hMPV	Red	

QUALITY CONTROL

Samples are evaluated using the analysis software of the respective real-time PCR device according to the manufacturer's instructions. Negative and positive controls must show correct results (see Table 8, Fig. 1, Fig. 2, Fig. 3) for a run to be valid. The Positive Control is present at a concentration of 10^3 copies/uL. It is used at a total of 5 x 10^3 copies in every PCR run.

Table 8. For a valid run, the following conditions must be met:

Sample	Assay result	ICR Ct	Target Ct
Positive Control	Positive	NA *[1]	See Quality Assurance Certificate
Negative Control	Negative	Ct > 20	Not detectable

*[1] *A Ct value for the ICR is not needed to obtain a positive result for the Positive Control*

If the positive control is not positive within the specified Ct range but the negative control is valid, all reactions need to be re-analyzed, including the controls.

If the negative control is not negative but the positive control is valid, all reactions need to be re-analyzed, including the controls.

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

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

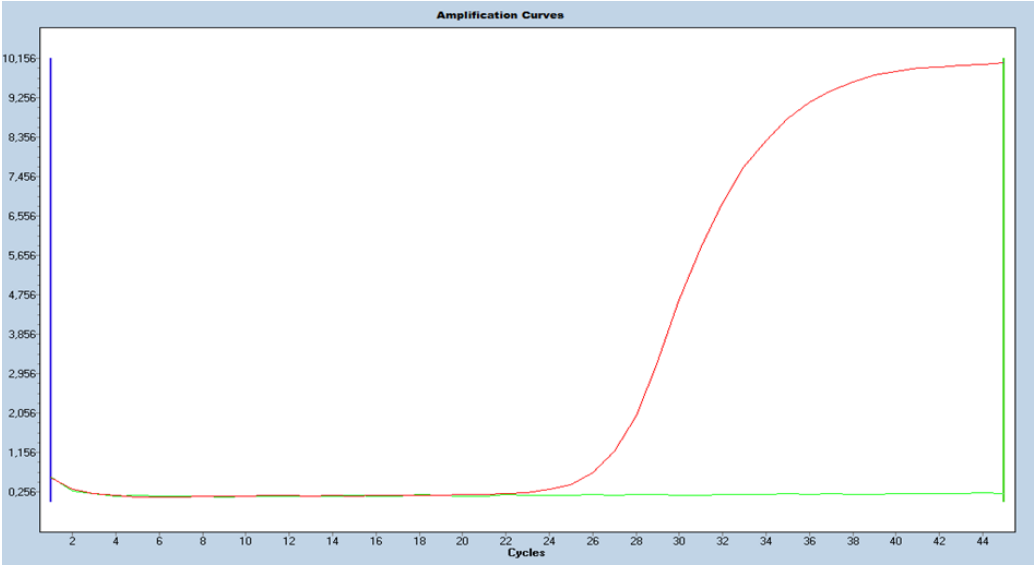

Figure 1: Correct run of the (RSV) positive control (red) and negative control (green) on the LightCycler® 480II

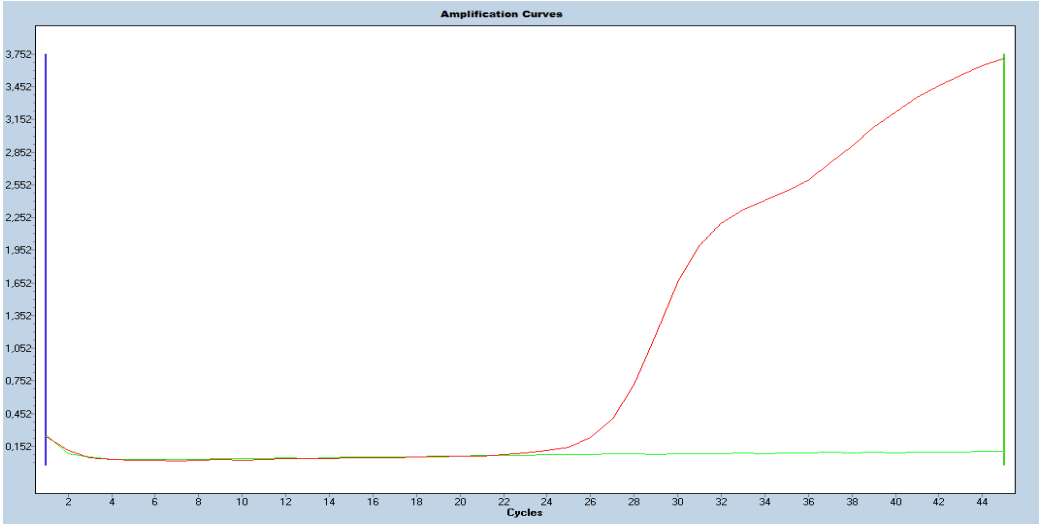

Figure 2: Correct run of the (hMPV) positive control (red) and negative control (green) on the LightCycler® 480II

RESULTS INTERPRETATION

The result interpretation is done according to Table 9.

Table 9: Sample interpretation

RSV	hMPV	ICR	Result
positive	negative	**positive/negative**	RSV detected
negative	**positive**	**positive/negative**	hMPV detected
positive	**positive**	**positive/negative**	RSV and hMPV detected
negative	negative	**positive**	Target gene not detectable
negative	negative	negative	**Invalid**

A sample is evaluated positive if the sample RNA and the Internal Control RNA show an amplification signal in the detection system. A sample is also evaluated positive if the sample RNA shows an amplification signal but none for the Internal Control RNA in the detection system. The detection of the Internal Control RNA is not necessary because high concentrations of the amplicon can cause a weak or absent signal of the Internal Control RNA.

A sample is evaluated negative if the sample RNA shows no amplification signal, but an amplification signal for the Internal Control RNA is visible in the detection system. An inhibition of the PCR reaction can be excluded by the detection of the Internal Control RNA.

A sample is evaluated invalid if the sample RNA and Internal Control RNA show no amplification signal in the detection system. The sample contains a PCR inhibitor or an error occurred during the extraction process. The extracted sample needs to be further diluted with PCR water (1:10) and reamplified, or the isolation and purification of the sample must be improved.

LIMITATIONS OF THE METHOD

1. This kit is For Research Use Only. It is not for use in diagnostic procedures.
2. Inappropriate specimen collection, transport, storage and processing or a pathogen load in the specimen below the test's analytical sensitivity can result in false negative results.
3. The presence of PCR inhibitors may cause invalid results.
4. Mutations or polymorphisms in primer or probe binding regions may affect new variants resulting in a false negative result with the RSV & hMPV RT-PCR kit.
5. As with all PCR based tests, extremely low levels of target below the limit of detection (LoD) may be detected, but results may not be reproducible.
6. A positive test result does not necessarily indicate the presence of viable organisms. However, a positive result indicates that the target genes (RSV and hMPV) are present.