RIDA®GENE Flu

REF PG0505

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1. Intended use

For *in vitro* diagnostic use. The RIDA®GENE Flu test, performed on the Roche LightCycler® 480II, is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of influenza virus RNA (influenza A, influenza B, and H1N1v) in untreated human nasal/throat swabs from persons with signs and symptoms of acute respiratory infection.
The RIDA®GENE Flu test is intended to support the differential diagnosis of influenza virus infections in patients with symptoms of respiratory infection in connection with other clinical and laboratory findings.
Negative results do not rule out infection with influenza virus and should not be used as the sole basis for diagnosis.
The product is intended for use by professionals working in hospital laboratories, reference laboratories, private laboratories, or public laboratories.

2. Summary and explanation of the test

Also called the flu, influenza is one of the most significant infectious respiratory diseases, and it is caused by influenza viruses.
Worldwide three to five million people contract influenza every year, and approximately 290,000 to 650,000 people die from the illness. The annual influenza epidemics can have major impacts on the health care system and the economy.[1]
In the 2018/19 season, estimates put the number of influenza-related doctor's visits in Germany at about 3.8 million. The number of influenza-related hospitalizations from primary care practices was estimated at 18,000 cases.[2]
The Working Group on Influenza (AGI) of the Robert Koch Institute estimates there are between one and seven million influenza-related doctor's visits each year. During a severe wave of the flu, like in the 2012/13 and 2017/18 season, there were an estimated 30,000 influenza-related hospitalizations and 20,000 to 25,000 deaths. In contrast, mild seasons (like 2013/14) see only an estimated 3,000 hospitalizations, and influenza-associated excess mortality is not detected.[2,3]
Influenza viruses are RNA viruses belonging to the family Orthomyxoviridae and are divided into subtypes A, B, and C. Characteristic for influenza viruses is their high variability of the surface antigens hemagglutinin (HA) and neuraminidase (NA) due to mutations (antigenic drift). The influenza types A and B cause the annual flu epidemics, while infections with influenza C viruses cause only mild illness.
Epidemiologically, influenza A viruses are the most important due to their diversity: They were responsible for three pandemics in the 20th century, as well as the majority of flu epidemics. The majority of influenza A infections in humans are caused by the subtypes H1N1 and H3N2. In addition to the antigenic drift resulting from mutation, the mixing of a human and nonhuman influenza A strain can create new influenza A subtypes (antigenic shift), which can trigger a pandemic. The influenza A subtype H1N1 is associated with past and potentially new flu pandemics (e.g., the Spanish flu in 1918/19 and the swine flu in 2009). Today this influenza A subtype is

called H1N1v. Influenza viruses are transmitted by droplets and aerosols. The incubation period is one to four days. Clinical symptoms are severe, mainly respiratory tract illnesses accompanied by cough and high fever. A sudden onset of symptoms is characteristic. In serious disease courses, pneumonias and bacterial superinfections can occur, which can be fatal especially to the elderly and children.[4]

3. Test principle

The RIDA®GENE Flu test is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of influenza viruses (influenza A, influenza B, and influenza A subtype H1N1v). Detection is done in a one-step real-time RT-PCR format: reverse transcription (RT) and subsequent PCR take place in one reaction vial. In the process, the isolated RNA is transcribed into cDNA with the help of a reverse transcriptase. The specific gene fragments for influenza A (M protein gene), influenza B (NP gene), and influenza A subtype H1N1v (H1 gene) are then amplified using real-time RT-PCR.

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, 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 device. The fluorescent signal increases with the quantity of formed amplicons. The RIDA®GENE Flu test contains an Internal Control RNA (ICR) to be able to control sample preparation and/or any potential PCR inhibition.

4. Reagents provided

Table 1: Reagents provided (The reagents provided in the kit are sufficient for 100 determinations.)

Kit code	Reagent	Amount		Lid color
1	Reaction Mix	2x	1050 µl	Yellow
2	Enzyme Mix	1x	80 µl	Red
R	Internal Control RNA	2x	1700 µl	Brown
N	No Template Control	1x	450 µl	White
P	Positive Control	1x	200 µl	Blue

5. Storage instructions

- 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 to 8 °C).
- Repeated freezing/thawing of up to 20 times does not have an impact on the test properties (if necessary, create aliquots after the first thaw and refreeze reagents immediately).
- Cool all reagents appropriately during PCR preparation (2 °C to 8 °C).

6. Reagents required but not provided

The RIDA®GENE Flu multiplex real-time RT-PCR was verified using the following combination of extraction platform and real-time PCR device:

Tab.2a: Necessary equipment (verified)

Extraction platform	
Promega	Maxwell® RSC
Real-time PCR devices	
Roche	LightCycler® 480II

Also, the RIDA®GENE Flu multiplex real-time RT-PCR test is compatible for use with the following extraction platform and real-time PCR devices:

Table 2b: Necessary equipment (compatible)

Extraction platform	
R-Biopharm	RIDA® Xtract
Real-time PCR devices	
R-Biopharm	RIDA®CYCLER
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500 Fast Dx
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: When using Rotor-Gene Q (QIAGEN), use only 0.1-ml reaction vials.

Should you have to use other extraction procedures or real-time PCR instruments, please contact R-Biopharm to check the compatibility at mdx@r-biopharm.de.

- Sterile swab collection system (e.g., eSwab® Amies medium, Copan Diagnostic Inc.)
- RIDA®GENE Color Compensation Kit IV (PG0004) when using LightCycler® 480II
- Real-time PCR consumables (plates, reaction vials, films)
- Centrifuge with rotor for reaction vials or plates
- Vortexer
- Pipettes (0.5 to 20 µl, 20 to 200 µl, 100 to 1,000 µl)
- Pipette tips with filters
- Powder-free disposable gloves
- PCR water (nuclease-free)

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.
- This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed.
- Always adhere strictly to the instructions for use 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.
- Ensure that the extraction, PCR preparation, and PCR are carried out in different rooms in order to avoid cross-contaminations.
- 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.
- Dispose of test kit once the expiration date has lapsed.
- Users are responsible for proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

For further details, see the safety data sheets (SDSs) at www.r-biopharm.com.

8. Collection and storage of samples

8.1 RNA preparation from nasal and throat swabs

A commercially available nucleic acid extraction kit (e.g., RIDA® Xtract (R-Biopharm)) or nucleic acid extraction system (e.g., Maxwell® RSC (Promega)) is recommended for RNA preparation from swabs. The manufacturer's instructions must be observed.

We recommend that you use the amount of medium specified by the manufacturer in the nucleic acid extraction of the nucleic acid extraction kit or nucleic acid extraction system, and that you follow the manufacturer's instructions.

The RIDA®GENE Flu 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 only as an inhibition control or as an extraction control for sample preparation and as an inhibition control.

If the Internal Control RNA is used only as an inhibition control, 1 µl of the Internal Control RNA must be added to the master mix (see Table 3).

If the Internal Control RNA is used as an extraction control for the sample preparation and as an inhibition control, 20 µl of the Internal Control RNA must be used during extraction. The Internal Control RNA should be added to the sample/lysis buffer mix and should **not** be added directly to the sample material. We recommend pipetting 1 µl of the Internal Control RNA to the RT-PCR mix of the negative control and positive control.

9. Test procedure

9.1 Master Mix preparation

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 balance out the pipette 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, mix thoroughly (except for the enzyme mix), and centrifuge for a short time. Always cool reagents appropriately during the work steps (2 °C to 8 °C).

Table 3: Example of the calculation and preparation of the master mix for ten (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 production of the master mix for ten (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 (vial/plate).

Negative control: Pipette 5 µl of the No Template Control into the pre-pipetted master mix.

Note: **If 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 RT-PCR mix of the negative control**.

Samples: Add 5 µl eluate to each pre-pipetted master mix.

Positive control: Pipette 5 µl of the Positive Control into the pre-pipetted master mix.

Note: **If 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 RT-PCR mix of the positive control.**

Seal the reaction vials or plates, briefly centrifuge at slow speed, and transfer into the real-time PCR device. Start RT-PCR according to PCR instrument set-up (see Table 5, Table 6).

9.3 PCR instrument set-up

9.3.1 Universal real-time PCR profile

Table 5: Universal real-time PCR profile for LightCycler® series and RIDA®CYCLER

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation Annealing/Extension	10 sec, 95 °C 15 sec, 60 °C
Temperature transition rate/ ramp rate	Maximum

Note: Annealing and extension take place in the same step.

Table 6: Universal real-time PCR profile for Mx3005P, ABI 7500 Fast Dx, Rotor-Gene Q, and CFX96™

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 30 sec, 60 °C
Temperature transition rate/ ramp rate	Maximum

Note: Annealing and extension take place in the same step.

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

9.4 Detection channel setting

Table 7: Selection of appropriate detection channels

Real-time PCR device	Detection	Detection channel	Note
R-Biopharm RIDA®CYCLER	H1N1v	Green	-
	ICR	Yellow	
	Influenza B	Orange	
	Influenza A	Red	
Roche LightCycler® 480II	H1N1v	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	ICR	533/580	
	Influenza B	533/610	
	Influenza A	618/660	
Agilent Techn. Mx3005P	H1N1v	FAM	**Set the reference dye to none.**
	ICR	HEX	
	Influenza B	ROX	
	Influenza A	Cy5	
ABI 7500 Fast Dx	H1N1v	FAM	**Set the ROX passive reference dye to none.**
	ICR	VIC	
	Influenza B	ROX	
	Influenza A	Cy5	
Bio-Rad CFX96™	H1N1v	FAM	-
	ICR	VIC	
	Influenza B	ROX	
	Influenza A	Cy5	
Qiagen Rotor-Gene Q	H1N1v	Green	**The gain settings must be set to 5 (factory default) for all channels.**
	ICR	Yellow	
	Influenza B	Orange	
	Influenza A	Red	

10. 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 the correct results (see Table 8, Fig. 1, Fig. 2, and Fig. 3).

The Positive Control is present 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 8: 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	Not detectable

*[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 within in 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 specified values are not met, check the following things before repeating the test:

- Expiration date of the reagents used
- Functionality of the equipment being used
- Correct test procedure

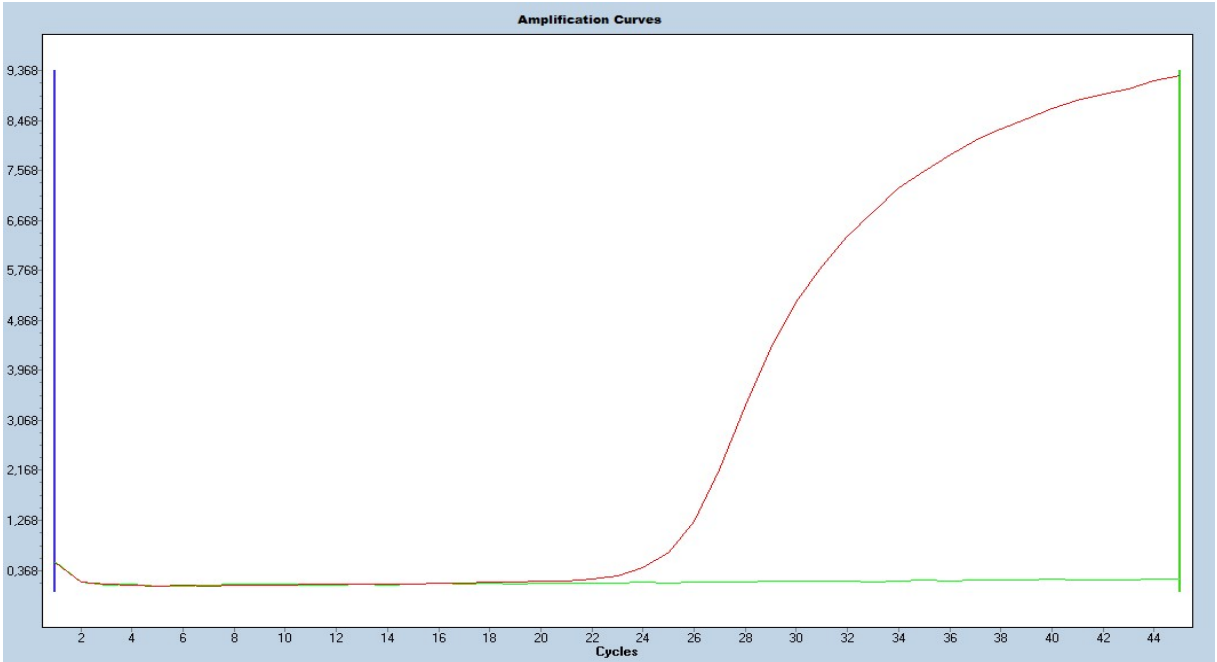

Fig. 1: Correct run of the (H1N1v) positive control (red) and negative control (green) on the LightCycler® 480II

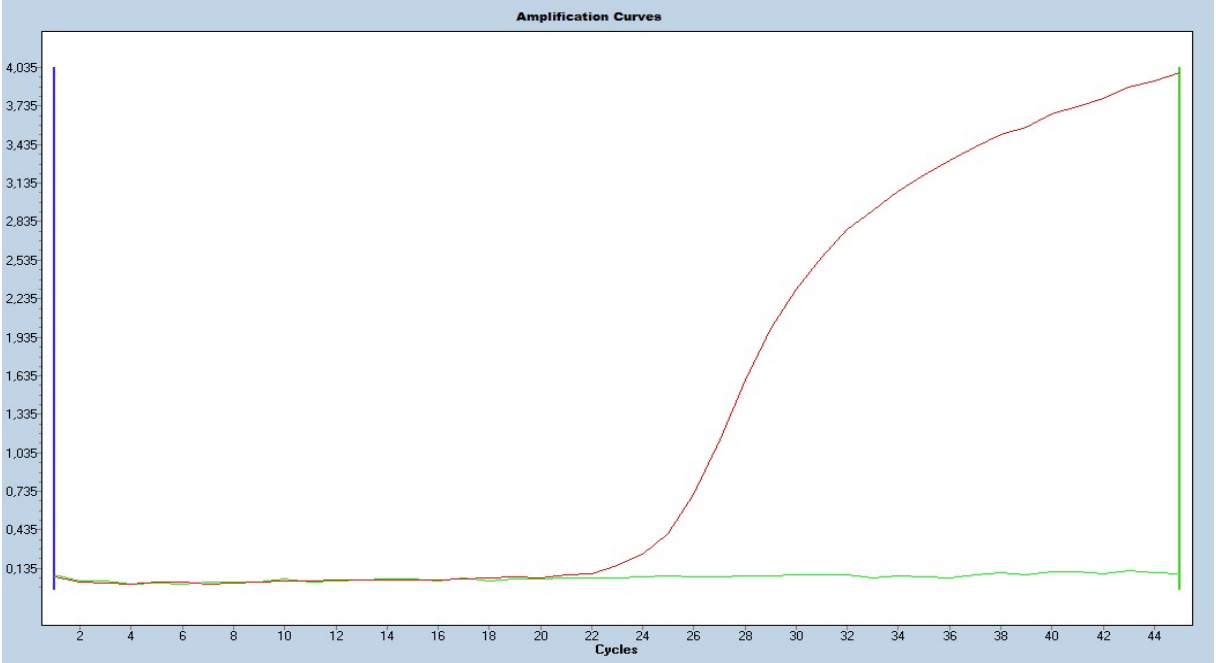

Fig. 2: Correct run of the (influenza B) positive control (red) and negative control (green) on the LightCycler® 480II

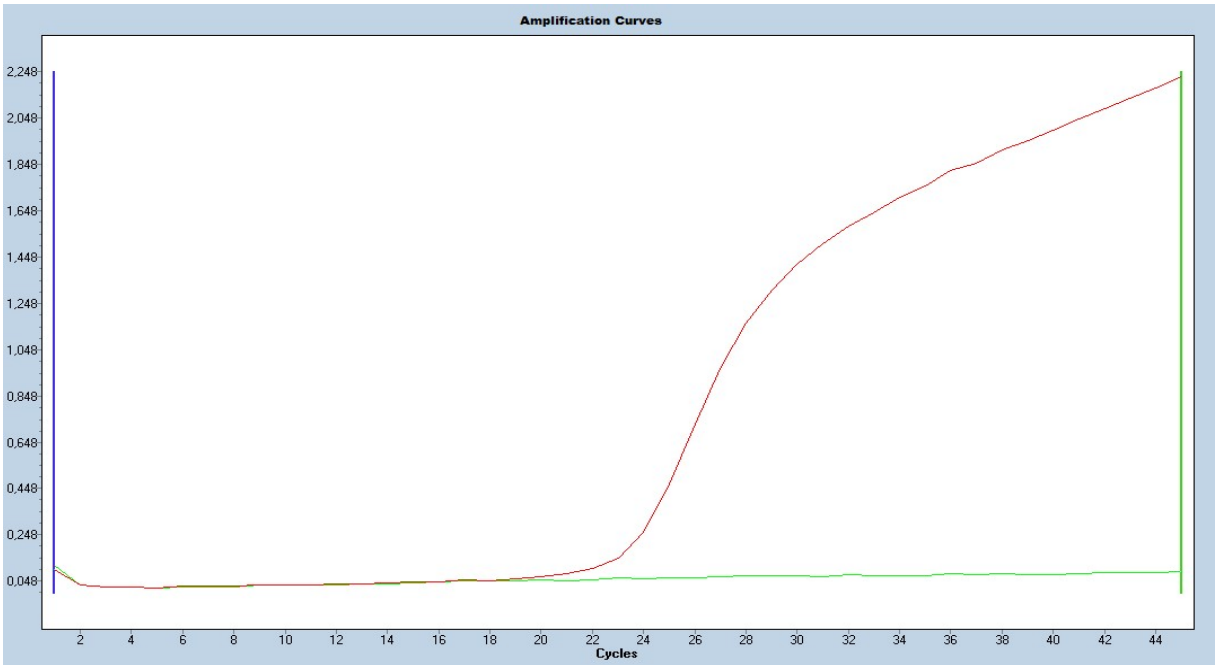

Fig. 3: Correct run of the (influenza A) positive control (red) and negative control (green) on the LightCycler® 480II

11. Sample interpretation

The result interpretation is done according to Table 9.

Table 9: Sample interpretation

Detection of				
H1N1v	**Influenza B**	**Influenza A**	**ICR**	**Result**
positive	negative	negative	**positive/ negative**	**Invalid***
negative	**positive**	negative	**positive/ negative**	**Influenza B detectable**
negative	negative	**positive**	**positive/ negative**	**Influenza A detectable**
positive	**positive**	negative	**positive/ negative**	**Influenza B detectable, detection invalid for influenza A H1N1v***
positive	negative	**positive**	**positive/ negative**	**Influenza A H1N1v detectable**
negative	**positive**	**positive**	**positive/ negative**	**Influenza A and influenza B detectable**
positive	**positive**	**positive**	**positive/ negative**	**Influenza A H1N1v and influenza B detectable**
negative	negative	negative	**positive**	**Target gene not detectable**
negative	negative	negative	**negative**	**Invalid**

* Also refer to Section 12, Item 9.

A sample is positive if the sample RNA and the Internal Control RNA show an amplification signal in the detection system.

A sample is also positive if the sample RNA shows an amplification signal, but no amplification signal can be seen for the Internal Control RNA in the detection system. Detecting the Internal Control RNA is not necessary in this case because high amplicon concentrations can result in a weak or absent signal of the Internal Control RNA.

A sample is negative if the sample RNA does not show an amplification signal, but an amplification signal is visible for the Internal Control RNA in the detection system. Inhibition of the PCR reaction can be ruled out by the detection of the Internal Control RNA.

A sample is invalid if the sample RNA and the Internal Control RNA do not show an amplification signal in the detection system. There are PCR inhibitors in the sample, or an error occurred during the extraction process. The extracted sample should be diluted 1:10 with PCR water and re-amplified, or the isolation and purification of the sample should be improved.

12. Limitations of the method

1. This test is intended only for nasal and throat swabs.
2. Improper specimen sampling, transport, storage, and handling or a viral load below the test's analytical sensitivity can lead to false negative results.
3. The presence of PCR inhibitors can lead to non-evaluable results.
4. 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 Flu.
5. As with all PCR-based *in vitro* diagnostic tests, extremely low concentrations of the target sequences under the limit of detection (LoD) can be detected. The results obtained are not always reproducible.
6. A positive test result does not necessarily indicate the presence of organisms capable of reproduction. A positive result indicates that the corresponding target genes (H1 gene, NP gene, M protein gene) are present.
7. This test differentiates only influenza A subtype H1N1v. Other influenza subtypes are not differentiated.
8. This test cannot be used to detect influenza C viruses.
9. If a new variant of the H1N1v subtype is present with a mutation in the primer and probe binding sites for influenza A, this can result in a positive signal in the H1N1v channel, but a negative signal in the influenza A channel. This result can be interpreted as a positive result for influenza A H1N1v.

13. Performance characteristics

13.1 Analytical sensitivity

The RIDA®GENE Flu multiplex real-time RT-PCR test has a detection limit of ≥ 50 RNA copies/reaction for H1N1v, influenza B, and influenza A.

Figures 4, 5, and 6 below show dilution series of H1N1v, influenza B, and influenza A (each with 5 x 10^5 to 5 x 10^1 RNA copies/reaction) on the LightCycler® 480II.

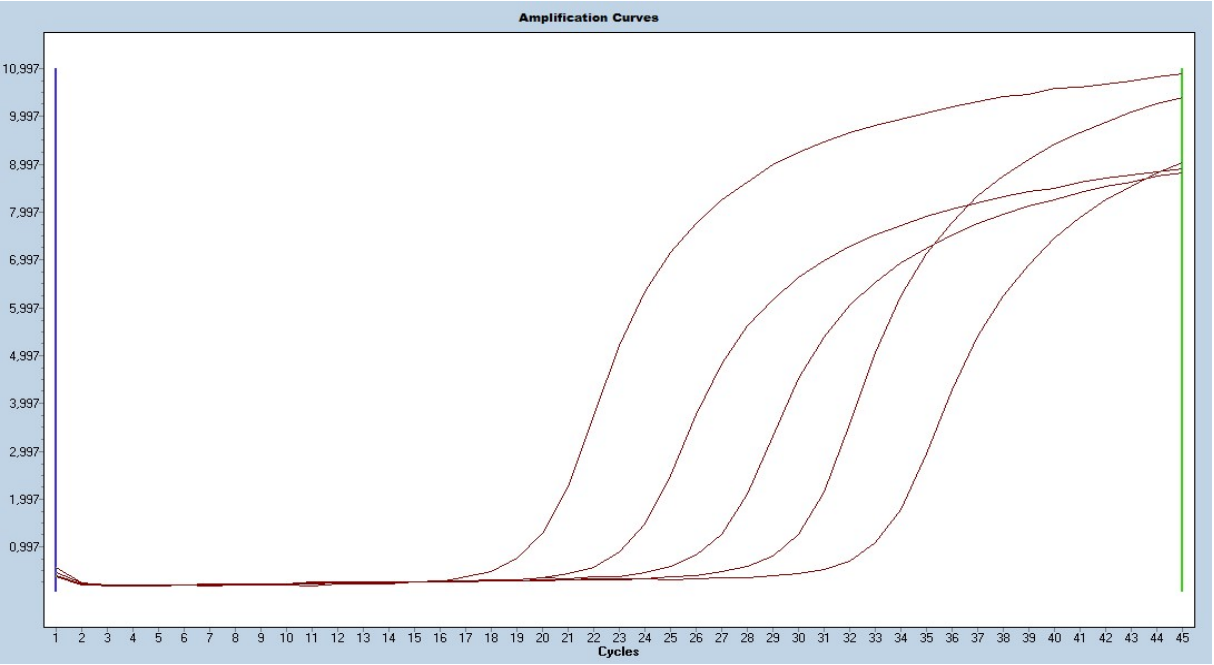

Fig. 4: Dilution series of H1N1v (5 x 10^5 to 5 x 10^1 RNA copies/reaction) on the LightCycler® 480II

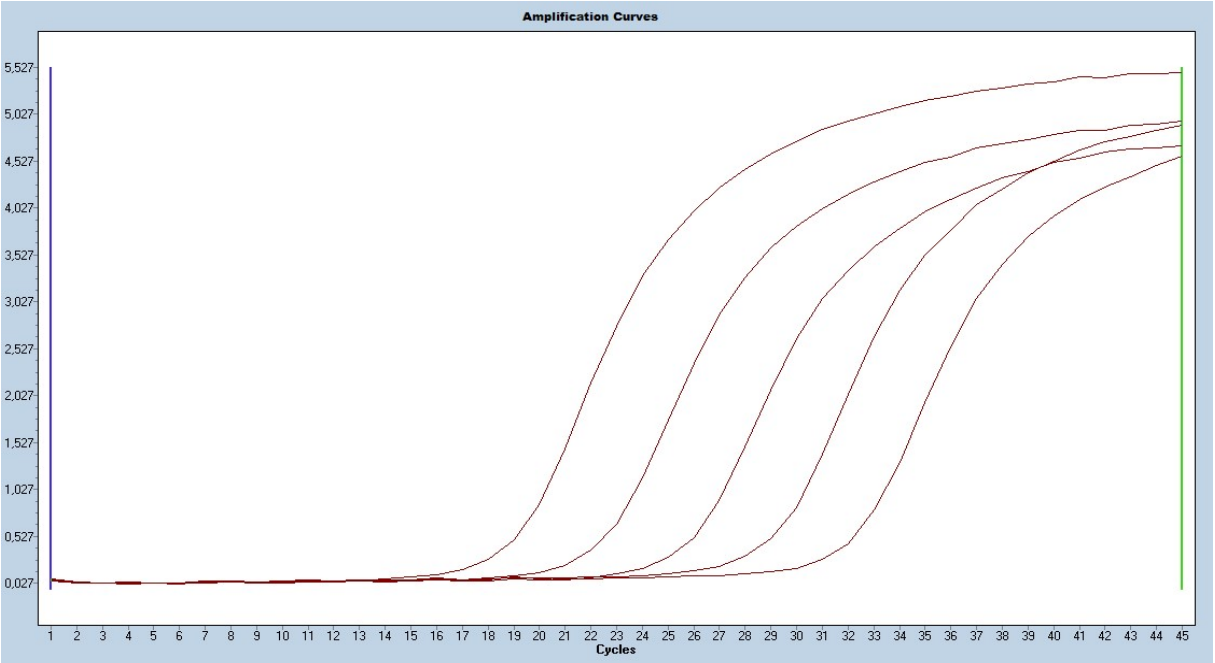

Fig. 5: Dilution series of influenza B (5 x 10^5 to 5 x 10^1 RNA copies/reaction) on the LightCycler® 480II

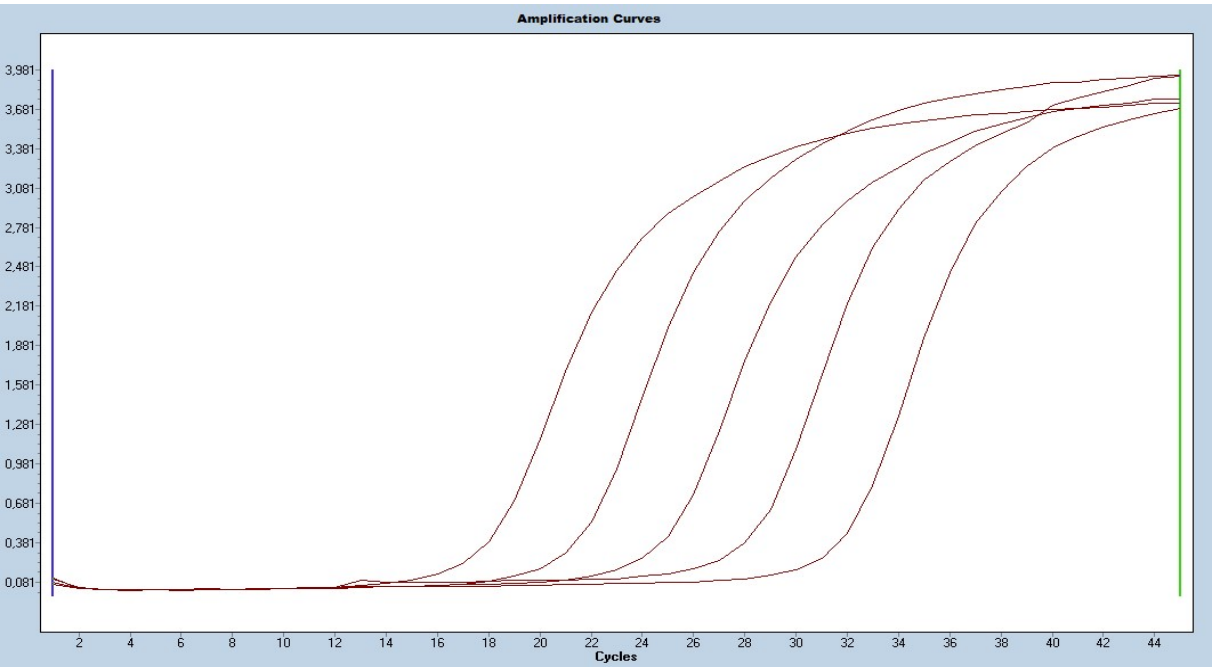

Fig. 6: Dilution series of influenza A (5 x 10^5 to 5 x 10^1 RNA copies/reaction) on the LightCycler® 480II

The limit of detection of the overall process depends on the specimen matrix, the RNA extraction, and the RNA concentration.

13.2 Analytical specificity

The RIDA®GENE Flu multiplex real-time RT-PCR is specific for H1N1v, influenza B, and influenza A. No cross-reactivities with the following species were detected (see Table 10).

Table 10: Cross-reactivity testing

Acinetobacter baumannii strain 5377	-	*Escherichia coli* (O6)	-	Human RSV strain 9320	-
Adenovirus 1, Human, Adenoid 71 strain	-	*Escherichia coli* (O157:H7)	-	Human rhinovirus genogroup A	-
Adenovirus 4	-	*Enterobacter cloacae*	-	*Klebsiella pneumoniae* strain MGH 78578	-
Adenovirus 7, Human, strain Gomen	-	Enterovirus type 71, strain 2003 isolate	-	*Lactobacillus plantarum*	-
Adenovirus 31	-	*Haemophilus influenzae*	-	*Legionella pneumophila* subsp. Pneumophila	-
Adenovirus 34	-	Herpes simplex virus 1 strain McIntyre	-	*Moraxella catarrhalis*	-
Adenovirus 37	-	Herpes simplex virus 2 strain MS	-	*Mycoplasma pneumoniae* strain FH of Eaton Agent	-
Aspergillus terreus	-	Human coronavirus 229E	-	*Neisseria meningitidis* strain FAM18	-
Bordetella parapertussis strain 12822	-	Human coronavirus OC43	-	*Proteus vulgaris*	-
Bordetella pertussis Tohama 1	-	Human coxsackievirus A2, strain Fleetwood	-	*Pseudomonas aeruginosa*	-
Candida albicans	-	Human coxsackievirus B4	-	*Serratia marcescens*	-
Chlamydophila pneumoniae	-	Human cytomegalovirus	-	*Streptococcus pneumoniae* strain NCTC 7465	-

Chlamydia psittaci	-	Human metapneumovirus	-	*Streptococcus pyogenes*	-
Clostridium perfringens	-	Human parainfluenza virus 1 strain C35	-	*Streptococcus salivarius*	-
Corynebacterium diphteriae	-	Human parainfluenza virus 2 strain Greer	-	*Staphylococcus aureus*	-
Echovirus 11	-	Human parainfluenza virus serotype 3	-	*Staphylococcus epidermidis*	-
Epstein-Barr virus strain B95-8	-	Human parainfluenza virus 4a strain M-25	-		
Escherichia coli (O26:H-)	-	Human RSV strain Long	-		

13.3 Analytical reactivity

The reactivity of the RIDA®GENE Flu multiplex real-time RT-PCR was examined using different strains of influenza A and influenza B viruses (see Table 11).

Table 11: Analytical reactivity testing

Subtype	Strain	H1N1v	Influenza B	Influenza A
H1N1	Influenza A/Brisbane/59/2007	negative	negative	**positive**
H1N1v	Influenza A/Brisbane/02/2018	**positive**	negative	**positive**
H1N1v	Influenza A/Michigan/45/2015	**positive**	negative	**positive**
H1N1v	Influenza A/California/7/2009	**positive**	negative	**positive**
H3N2	Influenza A/Perth/16/2009	negative	negative	**positive**
H3N2	Influenza A/Brisbane/10/2007	negative	negative	**positive**
H3N2	Influenza A/ South Australia/34/2019	negative	negative	**positive**
H3N2	Influenza A/Texas/50/2012	negative	negative	**positive**

H3N2	Influenza A/Victoria/361/2011	negative	negative	**positive**
H3N2	Influenza A/Hong Kong/4801/2014	negative	negative	**positive**
H3N2	Influenza A/Singapore/INFIMH-16-0019/2016	negative	negative	**positive**
H3N2	Influenza A/Kansas/14/2017	negative	negative	**positive**
H3N2	Influenza A/ Switzerland/9715293/2013	negative	negative	**positive**
H7N9	Influenza A/Anhui/1/2013	negative	negative	**positive**
	Influenza B/Brisbane/60/2008/ Victoria lineage	negative	**positive**	negative
	Influenza B/Washington/02/2019/ Victoria lineage	negative	**positive**	negative
	Influenza B/Colorado/06/2017/ Victoria lineage	negative	**positive**	negative
	Influenza B/Wisconsin/1/2010/ Yamagata lineage	negative	**positive**	negative
	Influenza B/Massachusetts/ 2/2012/Yamagata lineage	negative	**positive**	negative
	Influenza B/Phuket/3073/13/ Yamagata lineage	negative	**positive**	negative

14. Version history

Version number	Section and designation
2013-12-10	Previous version
2020-09-30	General revision: 1. Intended use 2. Summary and explanation of the test 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. Sample interpretation 12. Limitations of the method 13. Performance characteristics 14. Version history 15. Explanation of symbols 16. References

15. Explanation of symbols

General symbols

	For in vitro diagnostic use
	Consult instructions for use
	Batch number
	Use before
	Store at
	Item number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Reaction Mix

Enzyme Mix

Internal Control RNA

No Template Control

Positive Control

16. References

1. World Health Organisation 2009, Fact Sheet N°211, Influenza (Saisonal) www.who.int/mediacentre/factsheets/fs211/en/index.html. Last accessed: 09.10.2020.
2. Robert Koch Institut RKI_Inluenzabericht_2018-19 https://edoc.rki.de/bitstream/handle/176904/6253/RKI_Influenzabericht_2018-19.pdf. Last accessed: 30.10.2020
3. Robert Koch Institut https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Influenza_saisonal.html. Last accessed: 09.10.2020.
4. World Health Organisation 2011, Manual for the laboratory diagnosis and virological surveillance of influenza.