RIDA®GENE SARS-CoV-2

REF PG6815

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1. Intended use

For *in vitro* diagnostic use. The RIDA®GENE SARS-CoV-2 test, which will be performed on the Roche LightCycler® 480II, is a multiplex real-time RT-PCR for the direct qualitative detection of coronavirus (SARS-CoV-2) RNA from human throat and nasopharyngeal swabs of people with symptoms of a respiratory infection.
The RIDA®GENE SARS-CoV-2 test is designed to support the differential diagnosis of SARS-CoV-2 infections in patients with symptoms of respiratory infection in conjunction with other clinical and laboratory findings.
Negative results do not rule out infection with SARS-CoV-2 and should not be used as the sole basis for diagnosis.
The product is intended for use by professional users in hospital laboratories, reference laboratories, private laboratories or state laboratories.

2. Summary and explanation of the test

At the end of December in the Chinese metropolis of Wuhan, numerous cases of pneumonia of unknown cause occurred.[1] At the beginning of January, Chinese authorities identified a new type of corona virus (SARS-CoV-2) as the cause.[1] The disease caused by SARS-CoV-2 is officially named COVID-19 („Corona Virus disease 2019“) and is transmissible from person to person.[2]

Worldwide, 3,925,815 cases have been reported (as of May 10, 2020).[3] The initial cases in Germany were confirmed at the end of January 2020.[4] In Germany 169,575 cases have been reported (as of May 11, 2020).[4,5] The WHO declared an international health emergency on January 31, 2020.[1,5]

The original WHO guidelines recommended a three-target strategy.[6] When this guideline was prepared, there was insufficient sequence information for SARS-CoV-2, so that the sequence ranges published by the WHO in the individual target gene regions were too non-specific for reliable detection of SARS-CoV-2 RNA. In order to increase the specificity of the tests for SARS-CoV-2, a three-target strategy was pursued accordingly. On 17 January 2020, this three-target strategy was reduced to a two-target strategy because the third target had insufficient sensitivity.
On 17 March 2020, the WHO issued interim guidance for the diagnosis of suspected cases of coronavirus disease (COVID-19), caused by SARS-CoV-2, with regard to the requirements for molecular biological laboratory tests, which states: “In areas where the COVID-19 virus is widely spread, a simpler algorithm might be adopted in which, for example, screening by real-time RT-PCR of a single discriminatory SARS-CoV-2 target (single-target strategy) is considered sufficient.”

In developing the RIDA®GENE SARS-CoV-2 real-time RT-PCR assay, R-Biopharm AG has followed the single-target strategy right from the start. Our goal was to establish sensitive and specific detection of SARS-CoV-2 with an optimised workflow, i.e. without any further necessary confirmation. This was possible even at this point in

time because a large number of genome sequences of the novel coronavirus SARS CoV-2 were available. In the course of genome analyses, we were able to identify a region within the E gene which is highly specific to SARS-CoV-2 and which has high sensitivity for screening purposes. This region is located about 200 base pairs downstream of the non-specific region of the E gene of SARS-CoV-2 published by the WHO. In detecting this specific E gene region, we were thus able to reduce the detection of SARS-CoV-2 RNA to only one target.

3. Test principle

RIDA®GENE SARS-CoV-2 is a multiplex real-time RT-PCR for the direct qualitative detection of coronavirus (SARS-CoV-2) RNA from human throat and nasopharyngeal swabs of people with symptoms of a respiratory infection.
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 SARS-CoV-2 (E gene) are then amplified using real-time PCR. The amplified target sequences are detected using hydrolysis probes that are labeled at one end with a quencher 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 SARS-CoV-2 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 - 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).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE SARS-CoV-2 multiplex real-time RT-PCR test was verified with the following extraction platform and real-time PCR device combination:

Table 2a: Necessary equipment (verified)

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

Furthermore, the RIDA®GENE SARS-CoV-2 multiplex real-time RT-PCR test is compatible for use with the following extraction platforms and real-time PCR devices:

Table 2b: Necessary equipment (compatible)

Extraction platforms	
R-Biopharm AG	RIDA®Xtract
Roche	MagNA Pure 96[7]
Real-time PCR devices	
R-Biopharm AG	RIDA®CYCLER
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500 Fast Dx
Bio-Rad	CFX96™ Dx
QIAGEN	Rotor-Gene Q

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

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

- RIDA®GENE Color Compensation Kit IV (PG0004) when using LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with rotor for reaction vials or plates
- Vortexer
- Pipettes (0.5 - 20 µl, 20 - 200 µl, 100 - 1,000 µl)
- Pipette tips with filters
- Powder-free disposable gloves
- PCR water (nuclease-free)

7. Precautions for users

- 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 user instructions for 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 human respiratory samples

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

The RIDA®GENE SARS-CoV-2 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 for each reaction (see Table 4).

If the Internal Control RNA is used as an extraction control for sample preparation **and** as an inhibition control, 20 µl of the Internal Control RNA must be used for each sample 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 adding 1 µl for each reaction of the Internal Control RNA to the PCR mix of the negative control and the positive control.

For more information on the collection, storage and transport of samples, see WHO Interim guidance, 2 March 2020 https://apps.who.int/iris/handle/10665/331329.[6]

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). Before using the Reaction Mix, thaw the Enzyme Mix, Positive Control, No Template Control, and Internal Control RNA, mix thoroughly and centrifuge for a short time. Always cool reagents appropriately during 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 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 the RT-PCR mix of the negative control.**

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

Positive control: Add 5 µl of the Positive Control to 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 the 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 PCR according to PCR instrument set-up (see Table 5, Table 6, Table 7).

9.3 PCR instrument set-up

9.3.1 Universal real-time PCR profile

Table 5: Universal real-time RT-PCR profile for LightCycler® 480II 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 RT-PCR profile for Mx3005P, ABI7500 Fast Dx, Rotor-Gene Q, and CFX96™ Dx

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	Comment
R-Biopharm AG RIDA®CYCLER	SARS-CoV-2	Green	**-**
	ICR	Yellow	
Roche LightCycler® 480II	SARS-CoV-2	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	ICR	533/580	
Agilent Technologies Mx3005P	SARS-CoV-2	FAM	**Set the reference dye to none.**
	ICR	HEX	
ABI 7500 Fast Dx	SARS-CoV-2	FAM	**Set the ROX passive reference dye to none.**
	ICR	VIC	
Bio-Rad CFX96™ Dx	SARS-CoV-2	FAM	**-**
	ICR	VIC	
Qiagen Rotor-Gene Q	SARS-CoV-2	Green	**The gain settings must be set to 5 (factory default) for all channels.**
	ICR	Yellow	

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).
The Positive Control comes at a concentration of 10^3 copies/µl. It is used in a total quantity of 5×10^3 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.

The positive and negative controls are valid when they meet the conditions specified in the table. The Ct range for the positive control is specified on the Quality Assurance Certificate included with the product. If one of the two controls does not meet the conditions for a valid run, all the reactions need to be re-analyzed, including the controls.

If the specified values are not met, check the following before repeating the test:

- Expiration date of the reagents used
- Functionality of the devices used
- Correct test procedure

11. Sample interpretation

The results interpretation is done according to table 9.

Table 9: Sample interpretation

Detection of		
SARS-CoV-2	**ICR**	**Result**
positive	**positive/ negative**	**SARS-CoV-2 detectable**
negative	**positive**	**Target gene not detectable**
negative	**negative**	**Invalid**

SARS-CoV-2 is detectable if the sample RNA and the Internal Control RNA show an amplification signal in the detection system.

SARS-CoV-2 is also detectable if the 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.

SARS-CoV-2 is not detectable if the RNA shows no amplification signal, but an amplification signal can be seen 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.

The detection limit of the RIDA®GENE assay, using the LightCycler® 480II, is > 50 copies/reaction. Samples with a CT value of > 35 are within the detection limit. It should be noted that the detection limit of the RT-PCR depends on the sample matrix, the Cycler and the RNA extraction and can vary accordingly. We therefore recommend that these samples be assessed as inconclusive, examine the individual curves for sigmoidity and requesting a corresponding follow-up sample taking into account the clinical symptoms. The appropriately trained personnel are responsible for assessing these samples.

12. Limitations of the method

1. This test is intended only for human respiratory samples.
2. Improper specimen sampling, transport, storage, and handling or a pathogen 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 SARS-CoV-2.
5. As with all PCR-based *in vitro* 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 viable organisms. A positive result indicates that the target gene (E gene) is present.
7. This assay should be performed according to GLP (Good Laboratory Practice). The user must follow the manufacturer's instructions closely when performing the test.

13. Performance characteristics

13.1 Precision

The precision of the RIDA®GENE SARS-CoV-2 real-time PCR test was determined for the following observation levels.

Intra-assay precision: Determination of 5 control samples with 20 replicates each on the LightCycler® 480II under identical conditions.

Inter-Assay Precision: Determination of 5 control samples in duplicates over 10 working days by different operators under reproducible conditions.

Inter-Lot Precision: The tests for intra- and inter-assay precision are carried out on three different lots.

The variation coefficients obtained for the respective measurements with the RIDA®GENE SARS-CoV-2 real-time PCR test on the LightCycler® 480II were below 3 %.

13.2 Analytical sensitivity

13.2.1 Device detection limit

To determine the device detection limit, 20 replicates of a control sample (50 copies / reaction) were measured with the LightCycler® 480II. All replicas were positive.
The device detection limit is therefore 50 copies / reaction.

13.2.2 Limit of detection (LoD 95 %)

The detection limit of the RIDA®GENE SARS-CoV-2 real-time PCR test was determined in an external study using the MagNaPure 96 DNA and Viral NA Small Volume Kit and the LightCycler® 480II. The LoD 95 % determined here for the RIDA®GENE SARS-CoV-2 real-time PCR test is 4.3 copies / ml.[7]
The detection limit of the overall procedure depends on the sample matrix, the Cycler and the RNA extraction.

13.3 Analytical specificity

The RIDA®GENE SARS-CoV-2 real-time PCR test is specific for human SARS-CoV-2 from nasopharyngeal swab. Sequence matching using existing nucleotide databases (National Center for Biotechnology Information, NCBI) showed specificity for SARS-CoV-2 and no significant homology to other organisms.
Various organisms were also tested. No cross-reactivities to the following species were found (see Table 10):

Table 10: Cross-reactivity testing

Human Coronavirus 229E	-	Parainfluenza 1 (strain C35)	-	Enterovirus (Type 71)	-	*Streptococcus pyogenes*	-
Human Coronavirus OC43	-	Parainfluenza 2 (strain Greer)	-	RSV (strain Long)	-	*Bordetella pertussis*	-
Human Coronavirus NL63	-	Parainfluenza 3	-	RSV (strain 9320)	-	*Mycoplasma pneumoniae*	-
SARS	-	Parainfluenza 4 (b, strain CH19503)	-	Rhinovirus (Genogruppe A)	-	*Pneumocystis jirovecii*	-
MERS	-	Influenza A H1N1 Brisbane/59/07	-	*Chlamydia pneumoniae*	-	*Candida albicans*	-
Adenovirus 7, Human (strain Gomen)	-	Influenza A H3N2 Texas/50/12	-	*Haemophilus influenzae*	-	*Pseudomonas aeruginosa*	-
Adenovirus 1, Human (strain Adenoid 71)	-	Influenza B/Washington/02/2019	-	*Legionella pneumophila*	-	*Staphylococcus epidermidis*	-
Human Metapneumovirus	-	Influenza Virus B/Colorado/6/2017	-	*Streptococcus pneumoniae*	-	*Streptococcus salivarius*	-

13.4 Analytical reactivity

The analytical reactivity of the RIDA®GENE SARS-CoV-2 multiplex real-time RT-PCR was demonstrated using the proficiency test INSTAND e.V. RV 340059 (see Table 11).

Tab. 11: Analytical reactivity

SARS-CoV-2 RV 340059	+				

13.5 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 could be present in the corresponding samples due to widespread use in respiratory infections or widespread occurrence were examined (see Table 12).

Tab. 12: Interfering substances

Active ingredient / pharmaceutical	Concentration
Ethanol	5 % [v/v]
Guanidine hydrochloride	5 % [w/v]
Azithromycin	84 mg/mL
Mucine	60 µg/mL
Xylometazoline / nasal spray ratiopharm®	10 % [v/v]
Beclometasone dipropionate	10 % [v/v]
Paracetamol	10 mg/mL
Amoxicillin	1 mg/mL
Human blood	2 % [v/v]
Dihydrocodeine	10 % [v/v]

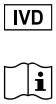
The substances listed in Table 12 showed no interfering effect at the concentrations tested.

14. Version history

Version number	Section and designation
2020-05-15	Previous version
2020-06-17	General revision: 2. Summary and explanation of the test 5. Storage instructions 11. Sample interpretation 13 Performance characteristics
2020-07-27	Revision of the spanish Instruction for Use 2. Summary and explanation of the test

15. Explanation of symbols

General symbols

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

Test-specific symbols

Not applicable

16. References

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