IVD EN

Real SARS-CoV-2

Test for the detection of *Coronavirus SARS-CoV-2*

INTENDED USE

The Real SARS-CoV-2 test is designed for the qualitative detection using the real-time PCR technique of SARS-CoV-2 Coronavirus, which causes CoVid19 disease. To obtain this, primers and probes are used that allow the amplification and detection of a highly specific region of the E gene, specific coronaviruses type sarcovirus (SARS-CoV-1 and SARS-CoV-2), and of a region of orf1ab gene, specific of SARS-CoV-2.

It is a product intended for specialized users to be used as an aid in the diagnosis of CoVid19 infection, caused SARS-CoV-2.

Take also under consideration other parameters such as symptoms or clinical history when giving the final diagnosis.

PRINCIPLE

Orthocoronavirinae, more commonly known as Coronaviruses, are a subfamily of single-stranded RNA viruses belonging to the Coronaviridae family. They get their name from a spiked crown characteristic seen around the surface of the virus and include phylogenetically similar virus genogroups with a helically symmetrical nucleocapsid with membrane, the virions of which can be between 50 and 200 nm in diameter. Its genetic material is the longest amongst RNA viruses, with genomes between 26 and 32 kilonucleotides.

The Orthocoronavirinae subfamily is subdivided into Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. The *Alphacoronavirus* genus includes subgroups 1a and 1b, the most representative members of which are the human coronaviruses HCoV-229E and HCoV-NL63. The *Betacoronavirus* genus includes several subgroups, the most prominent being subgroups 2a and 2b, which include human coronaviruses OC43, HKU1, SARS CoV and MERS CoV. The *Gammacoronavirus* genus includes all avian coronaviruses identified up to 2009. The 2019-20 pneumonia outbreak in Wuhan, China has led to the discovery of a new coronavirus, listed as SARS-CoV-2 by the WHO. To date, thirty-nine species of coronaviruses have been registered, several of them recently studied because until now they have not been previously identified in humans.

Coronaviruses are viruses that normally cause mild to moderate upper respiratory tract disease, such as the common cold, with symptoms such as a runny nose, cough, sore throat, and fever. Sometimes, however, they can also cause more serious illnesses, such as pneumonia, bronchitis, bronchiolitis, Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS).

COVID-19 (acronym of the English "*coronavirus disease 2019*"), is an infectious disease caused by the SARS-CoV-2 virus. It was first detected in the Chinese city of Wuhan, Hubei province, in December 2019 when a group of people were reported with pneumonia of unknown cause, mainly linked to shellfish wholesale market workers. The number of cases increased rapidly, reaching practically every country in the world in three months, first being declared a health emergency (30-01-2020) and then a pandemic (11-03-2020) by the World Health Organization. It produces symptoms similar to the flu, which include fever, cough, dyspnea, myalgia, and fatigue. Sudden loss of smell and taste has also been observed. In severe cases it is characterised by producing pneumonia, acute respiratory distress syndrome, sepsis and septic shock, causing death in around 3% of people infected. As of today, there is no specific treatment; the main therapeutic measures are to relieve symptoms and maintain vital functions.

In general terms, the methods for detecting the SARS-CoV-2 virus can be classified into three different strategies:

1) Detection of the genetic material of the virus (RNA contained in the nucleocapsid).
2) Detection of the virus as an individual entity, through the detection of viral antigens.
3) Detection of the antibodies generated in the infected host organism (serological test).

The Real SARS-CoV-2 kit is an "in vitro" diagnostic test, based on real-time PCR technology, for the qualitative detection and differentiation of the RNA specific to *SARS-CoV-2*.

The procedure of the Real SARS-CoV-2 kit consists of two steps:

a) RNA extraction

Reagents not included in the kit.
See "SAMPLES" section.

b) Reverse transcription, amplification and detection by probes labelled with fluorophores

The Real SARS-CoV-2 kit includes the reagents necessary to perform, in a single step, the reverse transcription of the RNA to its complementary DNA, the amplification of conserved and specific regions of the E and orf1ab genes from SARS-CoV-2 and its subsequent detection by using specific fluorescent probes.

For detection, the kit takes advantage of the 5' exonuclease activity of DNA polymerase. During amplification, the enzyme hydrolyses the probe that is attached to its complementary DNA sequence, thereby separating the fluorophore and quencher from the probe, leading to an increase in fluorescence signal proportional to the amount of target DNA.

The increase in fluorescence is monitored throughout the different amplification cycles in real-time with PCR equipment.

The kit includes the reagents necessary to perform the reverse transcription, amplification and simultaneous detection of a fragment of RNA from the human GAPDH gene, which is used as a valid internal control for taking samples, as well as reverse transcription and amplification.

Amplification detection of E gene is performed on the ROX (or Texas channel, in the absence of the former) of orf1ab gene on the FAM channel, and the internal GAPDH control on the HEX (VIC or JOE channel, in the absence of the former; depending on the Real Time equipment used, select the appropriate detection channel).

KIT CONTENTS

Real SARS-CoV-2		Kit 48 tests	Kit 96 tests
PCR mix	REAG PCRMIX COV	0.48 ml	0.925ml
Probes/Primers	REAG PRIMER COV2	0.275 ml	0.525 ml
Reverse transcriptase + TAQ	REAG HIGH-RT-TAQ	0.060 ml	0.110 ml
Positive control	COV-2 POS. CONTROL	1 Unit	1 Unit
Negative control	HUMAN NEG. CONTROL	1 Unit	1 Unit
Solution of rehydration of controls	REHYDRATION SOL.	0.065 ml	0.065 ml
Instructions for use	DO-09051041	1 Unit	1 Unit

1) The information about the composition of the reagents is indicated in the material safety data sheet of the product. You may request a copy of the MSDS through the email address msds@operon.es
2) Attention, the content of the kit guarantees a maximum of 4 total uses, with a minimum of 12 samples for analysis (48 tests format) or 24 samples (96 tests format).
3) This instructions for use apply to any commercial reference of the product: 5.233.XXX.88.000

MATERIALS NOT INCLUDED IN THE KIT

The following additional material is required when using the kit:

1. PCR microtubes compatible with real-time PCR equipment.
2. Micropipettes and micropipette tips (sterile or UV-irradiated and ideally with a filter)
3. Centrifuge.
4. Disposable powder-free gloves.

REQUIRED EQUIPMENTS FOR KIT DEVELOPMENT

The following thermocyclers have been succesfully used with the kit: CFX96 Real-Time PCR Detection System (BioRad); QuantStudio 5 Real-Time PCR System (Applied Biosystems); Gentier 96 Real-Time PCR (Tianlong) and Gentier 48 E Real-Time PCR (Tianlong).

PRECAUTIONS

1. Only use the reagents *in vitro*.
2. Strictly follow the instructions provided. Modifying any of the prescribed **steps or temperatures** may severely affect the test results.
3. This is a molecular biology product. Every required precaution to avoid reagent contamination and/or degradation of nucleic acids during the process must be observed. The user has to wear lab coat and gloves during all the process, changing them as frequently as needed, and maintain a deep cleaning of the working area. All the materials and reagents used must be free of ADN/ARN/DNAses/RNAses, etc. The use of filtered pipette tips is recommended for PCR preparation to prevent aerosol contamination.
4. A one-way workflow is recommended. It should start in the nucleic acid extraction area, followed by the pre-PCR area (reaction mixture preparation for PCR and addition of nucleic acid) and lastly the post-PCR area (amplification and detection). To maintain the unidirectional work flow, each of the areas must exclusively have all the necessary resources to carry out the technique (nuclease-free water, centrifuges, vortex, micropipettes, tips for micropipettes, vials, etc.).
5. Molecular biology procedures require trained staff to avoid the risk of incorrect results due to nucleic acid degradation or possible contamination in the amplification process.
6. Regular cleaning of the equipment used, mainly micropipettes and work surfaces, is recommended in order to minimise the risk of reagent contamination.
7. Store the kit components as indicated in the instructions.
8. Do not exchange components from kits with different lot numbers.
9. Do not use kit components after the expiration dates.
10. If the package is broken, the product can still be used providing none of the components have been damaged.
11. In case of breakage or alteration of the primary packaging, discard the test.
12. The used product should be discarded in compliance with current legislation.
13. Patient samples must always be treated as potentially infectious. Environmental and safety standards must be adhered to.
14. Do not discard the external box of the kit until its content has been totally used. The external box contains essential information regarding the EC marking and component lots.
15. Any serious incident related to the product must be reported to the manufacturer and to the competent authority of the Member State in which the user and/or the patient are established.

STORAGE

The components of the Real SARS-CoV-2 kit must be stored at -20 °C and protected from light. The kit can be shipped at 2-8 °C provided the shipping time is less than 1 week.

Repeated thawing and freezing of reagents should be avoided as this could impact product performance.

The expiry date of all the reagents is printed on the label.

The expiration and storage conditions of the reagents once opened is the same as that of the original reagents.

The kit specifications are maintained provided the test is stored and manipulated in the indicated conditions.

SAMPLES

Respiratory samples

The test has been designed and validated for use with RNA (or nucleic acids, DNA+RNA) obtained from samples from the respiratory tract. Specifically, the kit has been validated with RNA extracted from nasopharyngeal and oropharyngeal swab samples, saliva and buccal smear.

For an initial diagnosis, the CDC (Centers for Disease Control and Prevention) recommends, whenever possible, testing samples from the upper respiratory tract (nasopharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and the lower respiratory tract (sputum, in the case of productive cough, bronchoalveolar washes and/or tracheal aspirate). In the selection of one or the other sample, the time elapsed since the onset of symptoms must be taken into account (as indicated by the SEIMC, Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica - Spanish Society of Infectious Diseases and Clinical Microbiology), from day 7 of the onset of symptoms the PCR should be done on samples from the lower respiratory tract).

For swabs, use only sterile, synthetic fibre swabs with a plastic rod. Do not use calcium alginate swabs or those with a wooden rod. Ensure to obtain sufficient sample, without causing bleeding. Immediately store the swab in a sterile tube with a virus-suitable transport method (e.g. UTM Universal Transport Medium, etc.).

For sputum, expectorate sputum directly into a sterile tube.

In the case of saliva, directly collect 0.5-1 ml of saliva into a sterile tube or tube adapted for saliva sampling (eg SimplOFy, Oasis Diagnostics, USA).

For nasal aspirates, nasopharyngeal aspirates/washes, bronchoalveolar wash, and tracheal aspirate, collect 2-3 ml of the sample in a suitable sterile tube.

Store the RNA samples at 2-8 °C if they are going to be used shortly after, or at -20 °C for longer periods of storage (up to one year).

RNA samples

For the extraction of RNA (or nucleic acids) use approved extraction procedures that have been shown to generate quality RNA, such as one of the following: the bioMérieux NucliSens systems, QIAamp Viral RNA Mini kit, QIAamp MinElute Virus Spin kit or Rneasy Mini kit (Qiagen), Roche MagNAPure Compact RNA isolation kit, Roche MagNAPure Compact Nucleic Acid isolation kit, Invitrogen ChargeSwitch Total RNA Cell Kit, etc (kits validated and recommended by the CDC).

The quality and concentration of the extracted RNA/nucleic acids essential for the kit to function correctly. Any inhibitors in the sample or a low RNA/nucleic acids concentration could limit the test's sensitivity.

During the kit's development, with the Real SARS-CoV-2 kit satisfactory results were observed with the QIAamp MinElute Virus Spin kit (Qiagen) kit and the Nucleic acid extraction kit (spin column) (XABT, Beijing Applied Biological Technologies Co, Ltd), Maxwell 16 Viral Total Nucleic Acid Purification Kit (in automatic extractor Maxwell), MagCore Viral Nucleic Acid Extraction Kit (in automatic extractor MagCore) and EZ Virus Mini kit v2.0 (in automatic extractor Biorobot EZ1).

Store the RNA samples at -70 °C, or at -20 °C if that is not possible.

INTERFERING SUBSTANCES

Respiratory specimens may contain interfering substances that are known to inhibit PCR reactions. Most of these interfering substances will be eliminated during RNA/nucleic acid extraction. Any inhibitor remaining in the final RNA would be detected due to a lack of amplification of the control gene (GAPDH) during the PCR.

REAL SARS-COV-2 PROCEDURE

1.- Reconstitution of the dry positive and negative controls

To be carried out only on first use of the kit.

Attention: this reagent contains a high copy number of viral RNA as well as human RNA. It should be handled carefully in a cabinet dedicated exclusively to nucleic acid handling, to avoid contamination problems from PCR reactions.

Centrifuge the vial for 2 minutes at 6,000 x g.

Add 25 µl of rehydration solution to each one of the positive and negative control, give a centrifuge pulse, and allow them to rehydrate at room temperature for 15 minutes.

Vortex softly and centrifuge briefly at 6,000 x g.

Store at -70 °C, or at -20 °C if that is not possible, until use.

2.- Preparation of RT-PCR reaction

* Thaw the PCR reagents. Vortex and centrifuge briefly, to ensure that all contents are at the bottom of the tubes.
* Prepare the required PCR tubes based on the number of RNAs to be amplified.

Use vials suitable for the optical system of the Real Time equipment to be used. In addition to the sample vials, one vial will be required for the blank, one for the negative control and one for the positive control.

* Add to each PCR tube: 9 µl of PCRMIX + 5 µl of Primers/Probes + 1 µl of RT-TAQ.

If various RNA samples are to be amplified, prepare a common mixture with all the reagents is recommended to finally add 15 µl of mixture. For example:

No. PCRs	PCR MIX	PRIMER	RT-TAQ
1	9 µl	5 µl	1 µl
8	81 µl	45 µl	9 µl
16	162 µl	90 µl	18 µl

The mixtures containing all PCR reagents should always be prepared in excess to compensate for the loss of volume that takes place during the pipetting process.

Attention, to avoid unwanted contamination, it is recommended to carry out this preparation in a PCR workstation or clean area dedicated exclusively to the preparation of PCR reactions.

* Add 5 µl of RNA/nucleic acid to each tube (one sample per vial) and mix gently. In the case of a blank, add 5 µl DEPC-treated water; in the case of the negative control, add 5 µl of rehydrated negative control and in the case of the positive, add 5 µl of rehydrated positive control.

Attention, to avoid unwanted contamination, it is recommended to add the RNA/nucleic acids in a workstation dedicated exclusively to the manipulation of nucleic acids. It is recommended to follow the following order of addition: blank (1st), samples and positive control (always last).

* A blank (DEPC-treated water) and the negative and positive control included in the kit should always be included in each assay.
* Seal the vials with a cap suitable for the optical system of the Real Time equipment to be used.
* Centrifuge to eliminate possible bubbles that could affect the fluorescence reading.
* Insert the vials in the Real Time thermal cycler and proceed to amplification using the following parameters:

- Reaction volume= 20 µl.
- For thermal cyclers that operate with passive reference (such as Applied Biosystems 7500 Fast Real Time PCR System, Applied Biosystems StepOne Real Time PCR Systems, Startegene Mx3005P Real Time PCR System), check that the ROX passive control option is disabled.

* Amplification program:

Cycles	Step	Time	Temperature
1	Reverse transcription	20 min	48 ºC
1	Initial denaturation	2 min	95 ºC
45	Denaturation	5 sec	95 ºC
	Hybridization/Elongation (Fluorescence reading)	30 sec	60 ºC

Fluorescence data should be collected at the hybridization/elongation stage in the following channels:

ROX/TEXAS CHANNEL	FAM CHANNEL	HEX/JOE/VIC CHANNEL
E gene	Orf1ab gene	GAPDH (internal control)

NOTE: for basic information on the preparation and programming of the different real-time PCR instruments, see the user manual for each instrument.

3.- Interpretation of results

Once the reaction is complete, save and analyse the data with the proprietary software of the Real Time thermal cycler used and following the manufacturer's instructions.

The results of each target (E gene, orf1ab and GAPDH) must be analysed independently, adjusting the threshold value manually in each case. This threshold must be adjusted so that it is in the exponential phase of the fluorescence curve and above any background signal.

For the result to be valid, the acceptance criteria are the following:

Ct values	ROX/TEXAS CHANNEL (E GENE)	FAM CHANNEL (orf1ab GENE)	HEX/JOE/VIC CHANNEL (GAPDH)
Blank	No signal	No signal	No signal
Negative control	No signal	No signal	≤ 30*
Positive control	≤ 35**	≤ 35**	≤ 30*

* usually, 20-25 Cts
** usually, 25-30 Cts

After confirming that the results obtained for the reaction controls are valid, the results for the different samples will be analysed.

RESULTS

* The result obtained is interpreted as **POSITIVE** if the reaction generates a fluorescence growth curve that crosses the threshold with a Ct value equal or under 40 cycles in the reading channel for ROX/TEXAS (E gene) and FAM (orf1ab gene).

The internal control of amplification (HEX/VIC/JOE channel, GAPDH) may or may not show a fluorescence growth curve, since the presence of a high amount of viral RNA could cause preferential amplification of it.

* The result obtained is interpreted as **NEGATIVE** if the reaction generates a fluorescence growth curve that crosses the threshold with a Ct value greater than 40 cycles, or if the reaction does not generate a fluorescence growth curve, in channels ROX/TEXAS (E gene) and FAM (orf1ab).

In this case, the internal control (HEX/VIC/JOE channel, GAPDH) should show a fluorescence growth curve that crosses the threshold with a Ct value of under 35 cycles.

* The result obtained is interpreted as **INCONCLUSIVE** if the reaction generates a fluorescence growth curve that crosses the threshold with a Ct value equal to or less than 40 cycles in only one of the two reading channels: the ROX/TEXAS channel (gene E) or FAM (orf1ab).
The internal control (HEX/VIC/JOE channel, GAPDH) should show a fluorescence growth curve that crosses the threshold with a Ct value of less than 35 cycles.
For **inconclusive results**, negative target results suggest:

- Sample at concentrations close to or below the detection limit of the test.
- A mutation in the corresponding target region.
- Presence of another type of sarcovirus (if only the E gene is present) or viral variant that could generate a cross reaction (if only the orf1ab gene is present).
- Other causes.

It is recommended to repeat the test and take into account the patient's history and other tests to establish the result.

* The result is interpreted as **INVALID** if a fluorescence growth curve is not generated in any of the measurement channels, or if the aforementioned positive or negative criteria are not met.

In the case of invalid samples, it is recommended to repeat the assay. If the sample gives another invalid result, it is recommended to repeat diluting the RNA sample to rule out potential inhibition issues. If it gives an invalid result again, it is recommended to repeat the RNA extraction process, with a smaller elution volume, to rule out the issue of low nucleic acid concentration.

* It is important to check the amplification profiles of each sample to confirm that the result obtained is correct and cannot be attributed to background or device noise generated during the reaction. All samples should show the typical sigmoidal amplification profile.

E gene	Orf1ab gene	GADPH	RESULT
+	+	+ or -	POSITIVE
+	-	+	INCONCLUSIVE
-	+	+	INCONCLUSIVE
-	-	+	NEGATIVE
-	-	-	INVALID

QUALITY CONTROL

It is necessary to confirm the correct functioning of the kit in every assay by amplifying a negative control (DEPC-treated water) and the negative and positive controls included in the kit.

A blank that gives a positive test for either gene (E gene, orf1ab gene or GAPDH) in the test or a negative that gives a positive result for the E gene and/or the orf1ab gene, in addition to the GAPDH gene, indicates a contamination problem. In this case, the process should be repeated, ensuring that the work area and equipment are adequately decontaminated and that extreme precautions are taken during the PCR.

A negative control that is negative for the GAPDH gene indicates either a negative control degradation problem or a failure to prepare the reaction. In this case, the process must be repeated.

A positive control that returns a negative result indicates a failure of the PCR reaction, either due to inadequate reaction preparation, failure of the reagents, or failure of the positive control. Before repeating the amplification process, make sure that all reagents have been stored correctly and have not expired.

TEST LIMITATIONS

* As with any other diagnostic test, the kit results must be interpreted by a healthcare professional, taking into account the patient's medical history, clinical symptoms and any other diagnostic tests available.
* The results obtained with this product depend on the proper collection, transportation, storage and processing of samples. To avoid erroneous results, it is necessary to pay particular attention to these stages and carefully follow the instructions provided with the products for the extraction and storage of the nucleic acids.
* This assay could be used with other types of samples, although it has only been validated with samples of nasopharyngeal, oropharyngeal or buccal swabs and saliva.
* There is the possibility of false negatives or of variable results when working with samples with a low number of copies of the target template, below the detection limit of the test (see sensitivity heading).
* Due to the high analytical sensitivity of the technique, there is a possibility of false positives due to cross-contamination with SARS-CoV-2 from positive samples with high viral load, with the positive control or with the same products of the amplification reaction. These phenomena can only be avoided with good laboratory practice and carefully following product instructions.
* A positive result does not necessarily indicate the presence of viable viruses and does not imply that these viruses are infectious or that they are the causative agents of clinical symptoms. However, a positive result may be indicative of the presence of the target viral sequences (*E* gene, orf1ab gene).
* Negative results do not exclude suffering from SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimal sample types and when maximum viral load levels are reached during SARS-CoV-2 infections have not been determined. Collecting multiple samples (sample types and at various points over time) from the same patient may be necessary to detect the virus. If diagnostic tests for other respiratory diseases are negative and the patient's clinical presentation and epidemiological information suggest a possible SARS-CoV-2 infection, then the result should be considered a false negative and further testing of the patient should be discussed.

FEATURES / EXTERNAL AND INTERNAL EVALUATIONS

The use of this test is restricted to professional users that are familiar with the methods used in Molecular Biology.

The sensitivity and diagnostic specificity of the test have been evaluated with samples of different origin:

Evaluation No 1:

Evaluation performed in the facilities of OPERON, S.A. (Cuarte de Huerva, Spain).

115 samples from nasopharyngeal smears were analyzed, using as reference technique the Multiple Real Time PCR Kit for detection of 2019-CoV (XABT, Beijing Applied Biological Technologies Co, Ltd).

The results obtained are shown below:

N = 115		2019-CoV (XABT)		
		Positive (65)	Negative (50)	Inconclusive (0)
Real SARS-CoV-2	Positive (64)	64	0	0
	Negative (49)	1	48	0
	Inconclusive (2)	0	2*	0

Concordance of Sensibility (64/65)= 98.5 %
Concordance of Specificity (48/48) = 100 %
PPV: 100 % NPV: 98 %

* Detection only of gene E by both techniques, XABT considers it negative, OPERON inconclusive. They are not taken into account for the calculations because they are inconclusive.

Evaluation No 2:

Evaluation performed jointly between the Department of Microbiology of Hospital San Jorge (Huesca, Spain) and the facilities of OPERON, S.A. (Cuarte de Huerva, Spain).

161 samples from nasopharyngeal smears were analyzed, using as reference technique the Allplex 2019-nCoV assay (Seegene).

The results obtained are shown below:

N = 161		Allplex 2019-nCoV (Seegene)		
		Positive (64)	Negative (94)	Inconclusive (3)
Real SARS-CoV-2	Positive (62)	61	1	0
	Negative (96)	3	92	1*
	Inconclusive (3)	0	1*	2*

Concordance of Sensibility (61/64)= 95.3 %
Concordance of Specificy (92/93) = 99 %
PPV: 98.4 % NPV: 96.8 %
* They are not taken into account for the calculations as they are inconclusive.

SENSITIVITY

We define **the test limit** as the minimum amount of RNA that we can always genotype or always detect with the test.

The test limit was evaluated by analyzing, by duplicate, serial dilutions of a SARS-CoV-2 virus RNA preparation (corresponding to the GeneBank sequence MN908947) with three batches of the product.

With the results obtained it was concluded that both the test limit of the Real SARS-CoV-2 product is 6.25 copies of RNA per PCR reaction (1.25 copies/µl in the nucleic acids sample).

SPECIFICITY

The absence of cross-reaction has been checked for the following pathogens:

Adenovirus	*Proteus vulgaris*	*Aracnobacterium aemolyticum*
Rhinovirus	*Haemophilus influenzae*	*Streptococcus pneumoniae*
RSV (A and B)	*Haemophilus parainfluenzae*	*Streptococcus mitis*
Influenza (A and B)	*Haemophilus aphrophilus*	*Streptococcus oralis*
Parainfluenza (types 1, 2, 3, 4)	*Haemophilus haemolyticus*	*Streptococcus salivarius*
Coronavirus (229E, OC43, NL63)	*Haemophilus parahaemolyticus*	*Streptococcus agalactiae*
Bocavirus	*Legionella pneumophila*	*Streptococcus saphrophyticus*
Enterovirus	*Legionella dumophi*	*Streptococcus sanguinis*
Metapneumovirus (A and B)	*Legionella micdadei*	*Streptococus A*
Moraxella catarrhalis	*Legionella longbechae*	*Streptococus B*
Moraxella lacunata	*Legionella maceachernii*	*Streptococcus C*
Bordetella pertussis	*Legionella bozemanii*	*Streptococcus D*
Bordetella parapertussis	*Legionella israelensis*	*Streptococcus G*
Bordetella holmesii	*Legionella cherri*	*Streptococcus aureus mec A –*
Bordetella bronchiseptica	*Yersinia enterocolitica*	*Streptococcus aureun mec A +*
Neisseria meningitidis	*Pseudomonas aeruginosa*	*Candida albicans*
Neisseria subflava	*Serratia marcescens*	*Mycoplasma pneumoniae*

HOOK EFFECT

The effect of the addition of increasing amounts of SARS-CoV-2 RNA on the analytical sensitivity of the Real SARS-CoV-2 kit has been evaluated. Thus samples were prepared with viral RNA concentrations (corresponding to the GenBank MN908947 sequence) between 10^6 - 1.6 x 10^3 copies/µl (5 x 10^6 - 8x10^3 copies/PCR) and were analyzed, in duplicate, with a batch of product.

For to the amounts tested, no inhibition of the virus-associated amplification signal (measured as an increase in Cts or a reduction in RFUs with increasing the amount of viral RNA) has been observed. Therefore, the existence of the Hook Effect is ruled out up to these concentrations.

INTRA-ASSAY PRECISION

The intra-assay precision has been studied by analyzing 5 replicates of a serial dilution curve of a RNA from SARS-CoV-2 (sensitivity curve) and real samples.

The analysis was performed by the same person and the results demonstrated a high intra-assay precision of the product, with relative standard deviations of the Ct values obtained below 10%.

INTER-DAY PRECISION

The inter-day precision was studied by analyzing 2 replicates of a serial dilution curve of a RNA from SARS-CoV-2 (sensitivity curve) and real samples over 5 consecutive days.

The analysis was carried out by the same person and the results demonstrated a high inter-day precision of the product, with relative standard deviations of the Ct values obtained below 10%.

INTER-LABORATORY PRECISION

The inter-laboratory precision has been studied by analyzing 2 replicates of serial dilutions of a rNA from SARS-CoV-2 (sensitivity curve) and real samples.

Each analysis was carried out by three different people, on the same day and using the same batch of product. To vary the conditions of use of the kit as much as possible, each operator used a different thermal cycler.

The kit showed good reproducibility by varying the operator and conditions, with relative standard deviations of the Ct values obtained below 10%.

INTER-LOT PRECISION

The inter-lot precision has been studied by analyzing 2 replicates, with three product batches, of serial dilutions of a rNA from SARS-CoV-2 (sensitivity curve) and real samples.

The analysis was carried out by the same person and throughout the same day, and the results showed a high inter-batch precision of the product, with relative standard deviations of the Ct values obtained below 10%.

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Expiration date

Lot number

For in vitro diagnostic use

This product complies with the requirements of Directive 98/79 / EC on in vitro diagnostic medical devices

Catalogue number

Please read pack inserts

Manufactured by

Contents sufficient for <n> tests

Store at

Caution

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