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IVD EN

Real SARS CoV-2/Flu/RSV
Test for detecting and differentiating:
Coronaviruses SARS-CoV-1/2, Influenza A/B viruses, and respiratory syncytial A/B viruses

FOR PROFESSIONAL USE ONLY
PLEASE READ THESE INSTRUCTIONS CAREFULLY BEFORE USING THE TEST

INTRODUCTION

- Intended use

The Real SARS CoV-2/Flu/RSV test is designed to detect and qualitatively differentiate the following viruses using the RT PCR Real Time method: coronavirus SARS-CoV1/2, Influenza virus (Flu A/B) and respiratory syncytial virus (RSV A/B), in samples from individuals with symptoms of infection in respiratory tracts.
This involves using primers and probes that can amplify and detect a region of the E gene, specific to sarcovirus-type coronaviruses (SARS-CoV-1 and SARS-CoV-2), a region of the M gene, specific to Influenza viruses (Flu A/B), and a region of the N gene, specific to the respiratory syncytial viruses (RSV A/B).
Real SARS CoV-2/Flu/RSV has been validated for use with respiratory samples, such as nasopharyngeal swabs, nasal swabs, oropharyngeal swabs, saliva and nasopharyngeal aspirates, as well as with nucleic acids extracted from those samples.
The product is intended for specialised users as a means of support when diagnosing individuals suspected to have a viral respiratory infection. The clinical symptoms caused by the SARS-CoV-2, Influenza, and RSV viruses can be similar.
The product can be used for any indication involved with virus detection: initial diagnosis, infection monitoring, and contact control.
Take also under consideration other parameters such as symptoms or clinical history when giving the final diagnosis.

General information

COVID-19 (acronym for "*coronavirus disease 2019*") is an infectious disease caused by the SARS-CoV-2 virus, a sarcovirus-type coronavirus first detected in the Chinese city of Wuhan, Hubei province, in December 2019.
The Influenza virus, which causes flu, tends to appear during winter months, with incidence highest during a 5-month period, before completely disappearing with the onset of spring.
The respiratory syncytial virus (RSV) tends to appear with the onset of autumn and remains during the winter months. Its incidence is highest for around a 5-month period, before completely disappearing with the onset of spring.
The infection caused by these viruses courses with similar respiratory symptoms (fever, fatigue, aching muscles). Some cases can become serious and even prove fatal, but differentiating them is important as both treatment and management differ.

·Intended test population

This product is intended for use on the general population, as any individual is susceptible to infection by the following respiratory viruses: SARS-CoV-2, Flu or RSV.
The product is mainly intended for use with respiratory samples from patients with symptoms of viral respiratory infection compatible with the following infections: COVID-19, Flu, or RSV infection.

Disease or infection incidence among the intended test population

The SARS-CoV-2 sarcovirus has caused a worldwide pandemic since December 2019. The incidence is constantly changing, with major variations depending on the season, geographical location, and vaccination completion status. Since its appearance and up to March 2022, over 471 million infected people have been reported, with total mortality now surpassing 6 million.
The Influenza and RSV seasons during the years of onset of the SARS-CoV-2 pandemic have been very unusual, particularly during 2020-2021. This was because the viruses remained at a very low level of activity. This is probably due to the range of social and public health measures implemented in different countries to reduce SARS-CoV-2 transmission.
On examination of pre-pandemic data and according to the WHO, the global average for seasonal Flu is around 600 million cases, with 3 million cases of severe disease (0.5%) and 250,000-500,000 deaths per year (0.04-0.08%).
RSV is a highly contagious virus that can cause major bronchiolitis and pneumonia epidemics, affecting all age groups, but young children in particular. As with the Flu, its effects are markedly seasonal as it only presents during the winter months. It is estimated to cause around 64 million infections and 160,000 deaths each year worldwide.

- Characteristics of the virus and its infection

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 characteristic spiked crown seen around the surface of the virus.
Six species are known to cause human diseases: four viruses (229E, OC43, NL63, and HKU1) that cause common cold symptoms, and other two that cause more severe complications, such as pneumonia, bronchitis, bronchiolitis, Middle East respiratory syndrome (MERS), and 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. Case numbers increased rapidly and the World Health Organization declared a pandemic. It produces symptoms similar to the Flu, which include fever, cough, dyspnoea, myalgia, and fatigue. A sudden loss of smell and taste has also been observed. Severe cases are known for producing pneumonia, acute respiratory distress syndrome, sepsis, and septic shock, leading to death for around 3% of infected persons. As of today, there is no specific treatment; the main therapeutic measures involve providing symptom relief and maintaining vital functions.
Influenza viruses belong to the Orthomyxoviridae family, their genome comprises eight single-stranded RNA segments that codify 11 or 12 viral proteins. Influenza viruses are classified as A, B, and C types, with the first two causing most infections among humans.
Influenza A viruses are divided into subtypes based on two proteins on the virus' surface: hemagglutinin (H) and neuraminidase (N). The Influenza A virus subtypes currently circulating more commonly among people are: A (H1N1) and A (H3N2). Influenza B viruses, on the other hand, are classified into two genetically and antigenically distinct lineages: B/Yamagata and B/Victoria.
The symptoms associated with a Flu virus infection range from mild respiratory disease to severe pneumonia. These can prove fatal in some cases, whether due to the Influenza virus itself or a secondary bacterial infection in the lower respiratory tract. In some cases, it can also cause a wide range of non-respiratory complications that affect the heart, central nervous system, and other organ systems.
The respiratory syncytial virus (RSV), a member of the Pneumoviridae family (previously Paramyxoviridae), with two strains (subgroups A and B), is an enveloped virus whose genome comprises a nonsegmented negative-sense single-strand RNA (−ssRNA).
The clinical signs of infection by RSV vary from mild disease of the upper respiratory tract or an ear infection, to serious and potentially fatal conditions of the lower respiratory tracts. The most common form among breastfed infants infected by RSV is bronchiolitis, although pneumonia can be observed.

PRINCIPLE OF THE TEST

The Real SARS CoV-2/Flu/RSV 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-1/2, Flu A/B and RSV A/B.
Whether using a direct respiratory sample or nucleic acids extracted from the respiratory sample, the Real SARS CoV-2/Flu/RSV kit procedure involves two steps:
* If using a direct respiratory sample: a1) respiratory sample lysate and b) reverse transcription, amplification and detection of fluorescence (RT-PCR)
* If using nucleic acids: a2) nucleic acid extraction and b) reverse transcription, amplification and detection of fluorescence (RT-PCR)

a1) Respiratory sample lysate
The kit includes a lysis reagent, which in conjunction with heat treatment, helps to split the virus and release genetic material for its detection using RT-PCR.

a2) Nucleic acid 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/Flu/RSV 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 gene from SARS-CoV-1/2, of gen M from Flu A/B and N gene from RSV A/B 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 from SARS CoV-1/2 is performed on the ROX (or Texas channel, in the absence of the former), of M gene from Flu on the FAM channel, of N gene from RSV on the Cy5 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).

MATERIALS INCLUDED IN THE KIT

Real SARS CoV-2/Flu/RSV		Kit 48 test	Kit 96 test
Master Mix	MASTER MIX CoV/Flu/RSV	0.53 ml	1.04 ml
Probes/Primers	PRIMER CoV/Flu/RSV	0.265 ml	0.525 ml
Positive control	POS. CON.	1 Unit	1 Unit
Negative control	NEG. CON.	1 Unit	1 Unit
Solution of rehydration of controls	REHYD. SOL.	0.065 ml	0.065 ml
Lysis buffer	REAG LYSIS	1.1 ml	2 x 1.1 ml
Instructions for use	DO-09051045	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: 5.237.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 and its vials 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/Flu/RSV 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 stability of the reagents was confirmed by up to 5 freeze/thaw cycles.
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

The Real SARS CoV-2/Flu/RSV kit can be used for analysing both the direct respiratory sample and the nucleic acids extracted from those respiratory samples. Specifically, its use has been validated with the following specimens: nasopharyngeal swabs, nasal swabs, oropharyngeal swabs, aspirates and saliva.
When directly analysing nasopharyngeal/ oropharyngeal/ nasal swabs, the kit is only compatible for use when they are stored in non-inactivating transport media. **DO NOT use swabs in inactivating media**, as they affect the RT-PCR reagents.
When analysing nucleic acids (RNA or RNA+DNA), the kit is compatible with all types of swabs, whether stored in an inactivating or non-inactivating transport media.
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 and store under the medium manufacturer's recommended conditions.
In the case of saliva, directly collect 0.2-0.5 ml of saliva into a sterile tube or tube adapted for saliva sampling (eg SimplOFy, Oasis Diagnostics, USA). Sampling saliva is recommended first thing in the morning or after at least 1-2 hours of fasting.
Sample stability can depend on the transport medium in which they are conserved; please consult the manufacturer's usage instructions as required. Evaluations undertaken in Operon S.A. provided evidence for sample stability of up to at least 1 month when stored at 2-8 °C, -20 °C, or -70 °C. All the same, we recommend storing the samples at 2-8 °C if they are going to be tested within one day, or at -70 °C for longer storage periods.

Nucleic acid 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/Flu/RSV kit satisfactory results were observed with the QIAamp MinElute Virus Spin kit (Qiagen), Nucleic acid extraction kit (spin column) (XABT, Beijing Applied Biological Technologies Co, Ltd), Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega, in automatic extractor Maxwell).
Evaluations undertaken in Operon S.A. provided evidence for RNA stability of up to at least 1 month when stored at 2-8 °C, -20 °C, or -70 °C. All the same and whenever possible, we recommend storing RNA samples at -70 °C, or in its absence, at -20 °C.

INTERFERING SUBSTANCES

The effect of substances that may be present in the respiratory sample has been evaluated, with evidence for a lack of interference with the Real SARS CoV-2/Flu/RSV test by the following substances at the indicated concentrations: 4% capillary blood, 5 mg/ml mucin (Sigma), 1,5 mg/ml benzocaine (Hurricaine throat spray), 5% Normomar saline gel, 15% Disneumon pernasal (5 mg/ml phenylephrine), 10% Nebulicina (0.5 mg/ml oxymetazoline), 0.05 mg/ml mometasone furoate (Nasonex 50 µg spray), 5% Flixonase (fluticasone propionate 50 µg spray), 50 mg/ml Strepsil, 15% Cusicrom Fuerte (sodium cromoglycate 40 mg/ml), 5 mg/ml Osetalmivir (Tamiflu 30 mg/capsule), 4 µg/ml Tobramycin (Tobradex 3 mg/ml), 1 mg/ml amoxicillin (Amoxicilina Normon), 100 µg/ml Biotin, 10% Alkalol, 15% Propol 2, 2.2 mg/ml Ibuprofen Normon, 1 mg/ml Paracetamol Cinfa, 5 mg/ml Zanamivir, 1 mg/ml Ribavirin, 1 mg/ml acetylsalicylic acid (Aspirin 500 mg), 10% Budesonide Nasal Aldo-Unión.
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/Flu/RSV 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.- Sample lysate (only in case of direct respiratory sample testing)

* Allow the lysis reagent to thaw. Gently vortex mix and briefly centrifuge to ensure all the content is at the bottom of the tube.
* Add 20 µl of lysis reagent to a vial (for PCR, for example).
* Mix the sample well and add 20 µl to the lysis reagent. Gently mix by using the pipette and incubate: 5 min. at 95 °C + 5 min. at 4 °C (in a thermal cycler or thermal block for example). Store at 2-8 °C until required to add to the PCR reaction (in a chilled test-tube rack for example). Store at -20 °C prior to use if not testing immediately.

3.- RT-PCR reaction

* Thaw the PCR reagents and the samples for testing (if applicable). 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 and one for the positive control.
* Add to each PCR tube: 10 µl of PCRMIX + 5 µl of PRIMER.

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

No. PCRs	Master MIX	PRIMER
1	10 µl	5 µl
8	90 µl	45 µl
16	180 µl	90 µl

The mixtures containing all PCR reagents should always be prepared in excess to compensate for the loss of volume 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 nucleic acid/lysated sample to each tube (one sample per vial). For lysated direct samples, briefly mixing using a vortex or micropipette immediately prior to adding the sample is recommended to ensure it is homogeneous. In the case of a blank, add 5 µl DEPC-treated water; in the case of the negative, add 5 µl of rehydrated negative control; 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), negative control, samples and positive control (always last).
A blank (DEPC-treated water), positive and negative controls 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 briefly, insert the vials in the Real Time thermal cycler and proceed to amplification using the following parameters:
Reaction volume= 20 µl.
Amplification program:

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

For thermal cyclers that operate with passive reference, check that the ROX passive control option is disabled.

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

ROX/TEXAS	FAM	Cy5	HEX/JOE/VIC
E gene SARS -CoV1/2	M gene Flu A/B	N gene RSV A/B	GAPDH

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

4.- 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 (gen E gene, M gene, N gene and GAPDH) must be analysed independently, adjusting the threshold value manually in each case. This threshold must be adjusted so that it is above any background signal.
For the result to be valid, the acceptance criteria are the following:

Cq values	ROX/TEXAS (SARS CoV1/2)	FAM (Flu A/B)	Cy5 (RSV A/B)	HEX (GAPDH)
Blank	No signal	No signal	No signal	No signal
NEG CON	No signal	No signal	No signal	≤ 30
POS CON	≤ 35	≤ 35	≤ 35	≤ 35

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

RESULTS

The Real SARS CoV-2/Flu/RSV kit enables the detection and identification of the following viruses associated with respiratory tract infections: SARS-CoV-1/2, Flu A/B, and RSV A/B.
* 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: FAM (M gene from Flu A/B) / ROX (E gene from SARS CoV1/2) / Cy5 (N gene from RSV A/B).
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 this case, the internal control (HEX channel, GAPDH) should show a fluorescence growth curve that crosses the threshold with a Ct value of under 35 cycles.
* 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. Should the sample again produce an invalid result, repeating the sample collection is recommended (for direct sample testing) or the extraction process for nucleic acids, in case an inhibitor is present in the sample. In the case of nucleic acids, and if the result repeats after the new extraction, it could be repeated by eluting a smaller volume in order to rule out a low nucleic acid concentration problem.
* 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.

Flu A/B (FAM)	SARS CoV1/2 (ROX)	RSV A/B (Cy5)	GAPDH (HEX)	RESULT
+	-	-	+ or -	POSITIVE Flu
-	+	-	+ or -	POSITIVE CoV1/2
-	-	+	+ or -	POSITIVE RSV
+	-	+	+ or -	POSITIVE Flu and RSV
-	-	-	+	NEGATIVE
-	-	-	-	INVALID

QUALITY CONTROL

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

A blank that gives a positive result for either gene (E gene, M gene, N gene or GAPDH) in the analysis 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, results of Real SARS CoV-2/Flu/RSV kit 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 nasopharyngeal, oropharyngeal or nasal swabs, aspirates 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 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.
* Negative results do not exclude suffering from virus infection and should not be used as the sole basis for treatment or other patient management decisions. Collecting multiple samples (sample types and at various points over time) from the same patient may be necessary to detect the virus.

FEATURES / EXTERNAL AND INTERNAL EVALUATIONS

1. Diagnostic sensitivity and specificity concordance with the Clart PneumoVir kit (Genómica)

The presence of Flu and RSV in respiratory samples (nasopharyngeal aspirates) and nucleic acids extracted from those samples was evaluated using the Real SARS-CoV-2/Flu/RSV kits (respiratory sample and nucleic acid) and the Clart PneumoVir kit (only nucleic acid). This evaluation was performed in Operon's facilities (Cuarte de Huerva, Spain).

DIRECT SAMPLE (N = 62)		CLART® PneumoVir 2	
Influenza		Positive (46)	Negative (16)
Real SARS CoV2/ Flu/RSV	Positive (42)	42	0
	Negative (20)	4	16
Concordance Sensitivity: 42/46=91.3% (95%-CI: 79.8-96.5%) Concordance Specificity: 16/16=100% (95%-CI: 81.1-99.5%) Prevalence: 74.2% PPV: 100% NPV: 80% Efficiency: 93.6%			
RSV		Positive (13)	Negative (49)
Real SARS CoV2/ Flu/RSV	Positive (12)	12	0
	Negative (50)	1	49
Concordance Sensitivity: 11/12=92.3% (95%-CI: 67.1-98.3%) Concordance Specificity: 49/49=100% (95%-CI: 92.9-99.8%) Prevalence: 21% PPV: 100% NPV: 98% Efficiency: 98.4%			

NUCLEIC ACID (N = 153)		CLART® PneumoVir 2	
Influenza		Positive (62)	Negative (91)
Real SARS CoV2/ Flu/RSV	Positive (63)	61	2
	Negative (90)	1	89
Concordance Sensitivity: 61/62=98.4% (95%-CI: 91.5-99.6%) Concordance Specificity: 89/91=97.8% (95%-CI: 92.4-99.3%) Prevalence: 40.5% PPV: 96.8% NPV: 98.9% Efficiency: 98%			
RSV		Positive (84)	Negative (69)
Real SARS CoV2/ Flu/RSV	Positive (81)	80	1
	Negative (72)	4	68
Concordance Sensitivity: 80/84=95.2% (95%-CI: 88.4-98.1%) Concordance Specificity: 68/69=98.6% (95%-CI: 92.3-99.7%) Prevalence: 54.9% PPV: 98.8% NPV: 94.4% Efficiency: 96.7%			

2. Diagnostic sensitivity and specificity concordance with the BioGX Real Flu A, B and RSV kit for the BD Max system.

The presence of Flu and RSV in respiratory samples (nasopharyngeal aspirates) and nucleic acids extracted from those samples was evaluated using the Real SARS-CoV-2/Flu/RSV kits (respiratory sample and nucleic acid) and the BioGX Real Flu A, B and RSV kit for the BD Max system (only nucleic acid). This evaluation was performed in Operon's facilities (Cuarte de Huerva, Spain).

DIRECT SAMPLE (N = 80)		BioGX	
Influenza		Positive (64)	Negative (16)
Real SARS CoV2/ Flu/RSV	Positive (57)	57	0
	Negative (23)	7	16
Concordance Sensitivity: 57/64=89.1% (95%-CI: 79.2-94.6%) Concordance Specificity: 16/16=100% (95%-CI: 81.1-99.5%) Prevalence: 80% PPV: 100% NPV: 69.6% Efficiency: 91.3%			
RSV		Positive (16)	Negative (64)
Real SARS CoV2/ Flu/RSV	Positive (16)	16	0
	Negative (64)	0	64
Concordance Sensitivity: 16/16=100% (95%-CI: 81.1-99.5%) Concordance Specificity: 64/64=100% (95%-CI: 94,5-99.9%) Prevalence: 20 % PPV: 100 % NPV: 100 % Efficiency: 100 %			

NUCLEIC ACID (N = 90)		BioGX	
Influenza		Positive (73)	Negative (17)
Real SARS CoV2/ Flu/RSV	Positive (68)	68	0
	Negative (22)	5	17
Concordance Sensitivity: 68/73=93.2% (95%-CI: 85-97%) Concordance Specificity: 17/17=100% (95%-CI: 82-99.6%) Prevalence: 81.1% PPV: 100% NPV: 77.3% Efficiency: 94.4%			
RSV		Positive (18)	Negative (72)
Real SARS CoV2/ Flu/RSV	Positive (18)	18	0
	Negative (72)	0	72
Concordance Sensitivity: 18/18=100% (95%-CI: 82.8-99.6%) Concordance Specificity: 72/72=100% (95%-CI: 95.1-99.9%) Prevalence: 20% PPV: 100% NPV: 100% Efficiency: 100%			

3. Diagnostic sensitivity and specificity concordance with the Xpert Xpresss Flu/RSV kit (GeneXpert)

The presence of Flu and RSV in respiratory samples (nasopharyngeal aspirates) and nucleic acids extracted from those samples was evaluated using the Real SARS-CoV-2/Flu/RSV kits (respiratory sample and nucleic acid) and the GeneXpert Xpert Xpresss Flu/RSV kit (only nucleic acid). This evaluation was performed in Operon's facilities (Cuarte de Huerva, Spain).

DIRECT SAMPLE (N = 93)		GeneXpert	
Influenza		Positive (31)	Negative (62)
Real SARS CoV2/ Flu/RSV	Positive (32)	30	2
	Negative (61)	1	60
Concordance Sensitivity: 30/31=96.8% (95%-CI: 84-99.3%) Concordance Specificity: 60/62=96.8% (95%-CI: 89.1-99%) Prevalence: 33.3% PPV: 93.8% NPV: 98.4% Efficiency: 96.8%			
RSV		Positive (32)	Negative (61)
Real SARS CoV2/ Flu/RSV	Positive (31)	29	2
	Negative (62)	3	59
Concordance Sensitivity: 29/32=90.6% (95%-CI: 75.9-96.6%) Concordance Specificity: 59/61=96.7% (95%-CI: 88.9-99%) Prevalence: 34.4% PPV: 93.6% NPV: 95.2% Efficiency: 94.6 %			

NUCLEIC ACID (N = 90)		GeneXpert	
Influenza		Positive (31)	Negative (59)
Real SARS CoV2/ Flu/RSV	Positive (32)	30	2
	Negative (58)	1	57
Concordance Sensitivity: 30/31=96.8% (95%-CI:84-99.3%) Concordance Specificity: 57/59=96.6% (95%-CI: 88.5-99%) Prevalence: 34.4% PPV: 93.8% NPV: 98.3% Efficiency: 96.7%			
RSV		Positive (33)	Negative (57)
Real SARS CoV2/ Flu/RSV	Positive (33)	32	1
	Negative (57)	1	56
Concordance Sensitivity: 32/33=97% (95%-CI:84.9-99.3%) Concordance Specificity: 56/57=98.3% (95%-CI:90.8-99.6%) Prevalence: 36.7% PPV: 97% NPV: 98.3% Efficiency: 97.8%			

4. Diagnostic sensitivity and specificity concordance with the Multiple Real Time PCR kit for detection of 2019-CoV (XABT).

The presence of SARS-CoV-2 in respiratory samples (oropharyngeal swabs, nasopharyngeal swabs, nasal swabs, and saliva) and nucleic acids extracted from those samples was evaluated with the Real SARS-CoV-2/Flu/RSV kits (respiratory sample and nucleic acid) and the Multiple Real Time PCR Kit for detection of 2019-CoV (XABT, Beijing Applied Biological Technologies Co, Ltd; only nucleic acid). This evaluation was performed in Operon's facilities (Cuarte de Huerva, Spain).

DIRECT SAMPLE (N = 446)		XABT	
SARS-CoV-2		Positive (241)	Negative (205)
Real SARS-CoV-2/ Flu/RSV	Positive (234)	230	4
	Negative (211)	11	200
	Invalid (1)	0	1
Concordance Sensitivity: 230/241=95.4% (95%-CI: 92-97.4%) Concordance Specificity: 200/204=98% (95%-CI: 95.1-99.2%) Prevalence: 54.2% PPV: 98.3% NPV: 94.8% Efficiency: 96.6%			
NUCLEIC ACID (N = 602)		XABT	
SARS-CoV-2		Positive (276)	Negative (326)
Real SARS-CoV-2/ Flu/RSV	Positive (275)	274	1
	Negative (326)	2	324
	Invalid (1)	0	1
Concordance Sensitivity: 274/276=99.3% (95%-CI: 97.4-99.8%) Concordance Specificity: 324/325=99.7% (95%-CI: 98.3-99.9%) Prevalence: 45.9% PPV: 99.6% NPV: 99.4% Efficiency: 99.5%			

5. Diagnostic sensitivity and specificity concordance with the "Allplex 2019-nCoV assay" (Seegene) kit

The presence of SARS-CoV-2 in respiratory samples (nasopharyngeal and oropharyngeal swabs) and nucleic acids extracted from those samples was evaluated with the kits "Allplex 2019-nCoV assay" (Seegene, only nucleic acid) and "Real SARS-CoV-2 Flu RSV" (Operon, respiratory sample and nucleic acid). This evaluation was performed in Operon's facilities (Cuarte de Huerva, Spain).

DIRECT SAMPLE (N = 71)		Allplex 2019-nCoV assay	
SARS-CoV-2		Positive (24)	Negative (44)
Real SARS CoV2/ Flu/RSV	Positive (25)	25	0
	Negative (46)	2	44
Concordance Sensitivity: 25/27=92.5% (95%-CI: 76.1-97.8%) Concordance Specificity: 44/44=100% (95%-CI: 92.2-99.8%) Prevalence: 38% PPV: 100% NPV: 95.7% Efficiency: 97.2%			
NUCLEIC ACID (N = 71)		Allplex 2019-nCoV assay	
SARS-CoV-2		Positive (27)	Negative (44)
Real SARS CoV2/ Flu/RSV	Positive (27)	27	0
	Negative (44)	0	44
Concordance Sensitivity: 27/27=100% (95%-CI: 87.9-99.7%) Concordance Specificity: 44/44=100% (95%-CI: 92.2-99.8%) Prevalence: 38% PPV: 100% NPV: 100% Efficiency: 100%			

6. Diagnostic sensitivity and specificity concordance with the "Allplex Respiratory Panel" (Seegene) kit

The presence of Flu and RSV in respiratory samples (nasopharyngeal swabs) and nucleic acids extracted from those samples was evaluated with the kits "Allplex Respiratory Panel" (Seegene, only nucleic acid) and "Real SARS-CoV-2 Flu RSV" (Operon, respiratory sample and nucleic acid). This evaluation was performed by the Microbiology Service of the Donostia University Hospital.

DIRECT SAMPLE (N = 112)		Allplex Respiratory	
Influenza		Positive (53)	Negative (59)
Real SARS CoV2/ Flu/RSV	Positive (53)	53	0
	Negative (59)	0	59
Concordance Sensitivity: 53/53=100% (95%-CI: 93.4-99.8%) Concordance Specificity: 59/59=100% (95%-CI: 94-99.9%) Prevalence: 47.3% PPV: 100% NPV: 100% Efficiency: 100%			
RSV		Positive (53)	Negative (59)
Real SARS CoV2/ Flu/RSV	Positive (50)	50	0
	Negative (62)	3	59
Concordance Sensitivity: 50/53=94.3% (95%-CI: 84.7-98%) Concordance Specificity: 59/59=100% (95%-CI: 94-99.9%) Prevalence: 47.3% PPV: 100% NPV: 95.2% Efficiency: 97.3%			

NUCLEIC ACID (N = 116)		Allplex Respiratory	
Influenza		Positive (56)	Negative (60)
Real SARS CoV2/ Flu/RSV	Positive (56)	56	0
	Negative (60)	0	60
Concordance Sensitivity: 56/56=100% (95%-CI: 93.7-99.9%) Concordance Specificity: 60/60=100% (95%-CI: 94.1-99.9%) Prevalence: 48.3% PPV: 100% NPV: 100% Efficiency: 100%			
RSV		Positive (53)	Negative (63)
Real SARS CoV2/ Flu/RSV	Positive (53)	53	0
	Negative (63)	0	63
Concordance Sensitivity: 53/53=100% (95%-CI: 93.4-99.8%) Concordance Specificity: 63/63=100% (95%-CI: 94.4-99.9%) Prevalence: 45.7% PPV: 100% NPV: 100% Efficiency: 100%			

7. Diagnostic sensitivity and specificity concordance with the LightMix® SarbecoV E-gene plus EAV control (Roche) and the VIASURE SARS-CoV-2 Real Time PCR Detection Kit (Certest).

The presence of SARS-CoV-2 in respiratory samples (nasopharyngeal swabs) and nucleic acids extracted from those samples was evaluated with the kits LightMix® SarbecoV E-gene plus EAV control (Roche, only nucleic acid) and VIASURE SARS-CoV-2 Real Time PCR Detection Kit (Certest, only nucleic acid) and "Real SARS-CoV-2 Flu RSV" (Operon, respiratory sample and nucleic acid). This evaluation was performed by the Microbiology Service of the Donostia University Hospital.

SARS-CoV-2		SarbecoV E-gene plus	
NUCLEIC ACID (*N = 118*)		Positive (52)	Negative (66)
Real SARS CoV2/ Flu/RSV	Positive (52)	52	0
	Negative (66)	0	66
Concordance Sensitivity: 52/52=100% (95%-CI: 93.3-99.8%) Concordance Specificity: 66/66=100% (95%-CI: 94.6-99.9%) Prevalence: 44.1% PPV: 100% NPV: 100% Efficiency: 100%			
DIRECT SAMPLE (*N = 116*)		Positive (51)	Negative (65)
Real SARS CoV2/ Flu/RSV	Positive (31)	50	0
	Negative (62)	1	65
Concordance Sensitivity: 50/51=98% (95%-CI: 89.8-99.5%) Concordance Specificity: 65/65=100% (95%-CI: 94.6-99.9%) Prevalence: 44% PPV: 100% NPV: 98.5% Efficiency: 99.1%			

SARS-CoV-2		VIASURE SARS-CoV-2	
NUCLEIC ACID (*N = 118*)		Positive (52)	Negative (66)
Real SARS CoV2/ Flu/RSV	Positive (52)	52	0
	Negative (66)	0	66
Concordance Sensitivity: 52/52=100% (95%-CI: 93.3-99.8%) Concordance Specificity: 66/66=100% (95%-CI: 94.6-99.9%) Prevalence: 44.1% PPV: 100% NPV: 100% Efficiency: 100%			
DIRECT SAMPLE (*N = 116*)		Positive (51)	Negative (65)
Real SARS CoV2/ Flu/RSV	Positive (31)	50	0
	Negative (62)	1	65
Concordance Sensitivity: 50/51=98% (95%-CI: 89.8-99.5%) Concordance Specificity: 65/65=100% (95%-CI: 94.6-99.9%) Prevalence: 44% PPV: 100% NPV: 98.5% Efficiency: 99.1%			

DETECTION CAPABILITY

The analytical sensitivity, **or the test limit**, is defined as the minimum amount of RNA that can always be detected with the test. The limit of the test was defined by analysing a series of dilutions in triplicate of an RNA preparation of the different viruses for detection, using three product batches.

The results led to the conclusion that the analytical sensitivity of the Real SARS CoV-2/Flu/RSV product is:

ARN Control	Flu A	Flu B	RSV A	RSV B	CoV 2
Copies per PCR	62.5	15.62	7.8	15.62	31.25

Control RNA: Twist - H1N1 (2009), Ref.103001; H3N2, Ref.103002, Influenza B, Ref.103003, SARS-CoV-2, Ref.102024, RSV ATCC-Strain:18537, Ref. VR-1580DQ™ and A2, Ref. VR-1540DQ.

On the other hand, the detection capability of the test was evaluated by spiking of negative samples of the different specimens compatible with the test with the WHO international standard for SARS-CoV-2 (ref. NIBSC 20/146), for Flu from ATCC: the A strain Wisconsin/67/2005 (ref. VR-1881) and B strain Florida/07/04 (ref. VR-1804) and for RSV: RSV A 2000/3-4 (ref. NR-28530) and RSV B1 (ref. NR 4052) from Bei Resources.

The following tables show the results:

RNA specimen	Flu A	Flu B	RSV A	RSV B	CoV 2
Oropharyngeal	500	2,000	0.25	75	1,000
Nasopharyngeal	500	2,000	0.25	25	1,000
Nasal	500	2,000	0.1	25	1,000
Nasopharyngeal aspirate	1,000	20,000	0.5	500	NA
Saliva	1,000	20,000	0.25	75	4,000

Sample specimen	Flu A	Flu B	RSV A	RSV B	CoV 2
Oropharyngeal	2,000	8,000	1	250	8,000
Nasopharyngeal	2,000	8,000	2.5	250	8,000
Nasal	2,000	10,000	2.5	250	8,000
Nasopharyngeal aspirate	8,000	40,000	10	1,500	NA
Saliva	4,000	80,000	1	250	40,000

LoD units: Flu $CEID_{50}$/ml; RSV $TCID_{50}$/ml and SARS-CoV 2 IU/ml.

SPECIFICITY

The presence of cross reaction with the following micro-organisms was ruled out by spiking mixes of negative respiratory samples with quantified cultures of the different microorganisms:

* Bacteria/yeasts: *Bordetella holmesii, Bordetella parapertussis, Bordetella pertussis, Candida albicans, Haemophylus Influenzae, Haemophylus parahaemolyticus, Haemophylus parainfluenzae, Klebsiella pneumoniae, Legionella pneumophila, Moraxella catarrhalis, Moraxella lacunata, Mycoplasma tuberculosis, Neisseria gonorrhoeae, Neisseria meningitidis, Neisseria subflava, Proteus vulgaris, Serratia marcescens, Staphylococcus haemolyticus, Staphylococcus saprophyticus, Staphylococcus aureus, Streptococcus A, B, C, D, F and G, Streptococcus mitis, Streptococcus mutans, Streptococcus oranlis, Streptococcus pneumoniae, Streptococcus salivarius, Streptococcus sanguinis, Pseudomonas aeruginosa, Chlamydophila pneumoniae and Mycoplasma pneumoniae.*
* Virus: coronavirus *OC43*, *NL63*, *229E* y *MERS*, *the parainfluenza viruses* (HPIV) 1, 2, 3, 4A and 4B, *Adenovirus*, *Enterovirus*, *Metapneumovirus*, *Rhinovirus*.

The lack of cross reaction was confirmed for all cases by "in silico" analysis of every available sequence in GenBank.

HOOK EFFECT

The presence of a Hook effect was ruled out by adding increasing amounts of a positive RNA control for the different viruses to the RT-PCR reaction.

For Flu A, Flu B, and SARS-CoV-2, a control RNA from Twist Bioscience was used, up to 5 x10^6 copies/PCR.

For RSV A and B, a control RNA from ATCC was used, up to 3.25 and 1.65 x10^6 copies/PCR.

INTRA-ASSAY PRECISION

Analysing 5 replicates of 7 series of dilutions of a positive RNA control, 4 respiratory samples and their corresponding nucleic acids, a positive control, a negative control, and a blank with three different product batches, evidence was provided for a high intra-assay precision for the Real SARS CoV-2/Flu/RSV test, obtaining RSDs under 3% between the Cq values obtained in the different replicates.

INTER-DAY PRECISION

Analysing 2 replicates of 7 series of dilutions of a positive RNA control, 4 respiratory samples and their corresponding nucleic acids, a positive control, a negative control, and a blank over 5 consecutive days, evidence was provided for a high inter-day precision for the Real SARS CoV-2/Flu/RSV test, obtaining RSDs under 4% between the Cq values obtained in the different replicates.

INTER-LABORATORY PRECISION

Analysing 2 replicates of 7 series of dilutions of a positive RNA control, 4 respiratory samples and their corresponding nucleic acids, a positive control, a negative control, and a blank by 3 different operators and different equipment (micropipettes, cabinet, Real Time PCR thermal cyclers), evidence was provided for a high inter-laboratory precision for the Real SARS CoV-2/Flu/RSV test, obtaining RSDs under 6% between the Cq values obtained in the different replicates.

INTER-LOT PRECISION

Analysing 2 replicates of 7 series of dilutions of a positive RNA control, 15 respiratory samples and their corresponding nucleic acids, and a blank with three different product batches, evidence was provided for a high inter-batch precision for the Real SARS-CoV-2/Flu/RSV test, obtaining RSDs under 4% between the Cq values obtained in the different replicates.

BIBLIOGRAPHY

1. CDC. Interim guidelines for collecting, handling, and testing clinical specimens from persons under investigation (PUIs) for coronavirus disease 2019 (CoVid-1). Available from https://www.cdc.gov/coronavirus/ 2019-nCoV/lab/guidelines-clinical-specimens.html. Accessed February 2020.
2. CDC. Real-time RT-PCR panel for detection 2019-novel Coronavirus. Available from https://www.fda.gov/media/134922/download. Accessed March 2020.
3. Pang J. et al. "Potential rapid diagnostics, vaccine and therapeutics for 2019 novel Coronavirus (2019-nCoV): a systematic review". J. Clin. Med. (2020), 9, 623.
4. Chu D. et al. "Molecular diagnosis of a novel Coronavirus (2019-nCoV) causing an outbreak of pneumonia". Clin. Chem (2020), 66 (4), 549.
5. World Health Organization. Laboratory biosafety guidance related to coronavirus disease 2019 (CoVid-19). Available from https://apps.who.int/iris/handle/10665/331138. Accessed February 2020.
6. Food and Drug Administration (FDA). FAQs on diagnostic testing for SARS-CoV-2. https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2. Accesed March 2020.
7. Current performance of COVID-19 test methods and devices and proposed performance criteria. 16 April 2020. Working document of Commission services. European Commission.
8. Communication from the Commission: Guidelines on COVID-19 in vitro diagnostic tests and their performance. Brussels, 15.4.2020 C(2020) 2391 final. European Commission.
9. WHO information for molecular diagnosis of Influenza virus—update. www.who.int/influenza/gisrs_laboratory/molecular_diagnosis_influenza_virus_humans_update_201211.pdf Accessed September 2020.
10. CDC- Influenza https://www.cdc.gov/flu/about/viruses/types.htm
11.CDC-RSV Infection https://www.cdc.gov/RSV/index.html
12. Peaper DR, Landry ML.; Rapid diagnosis of Influenza: state of the art.; Clin Lab Med. 2014 Jun;34(2):365-85.
13. van Elden LJ et al. "Applicability of a real-time quantitative PCR assay for diagnosis of respiratory syncytial virus infection in immunocompromised adults".*J Clin Microbiol*. 2003;41(9):4378-4381.
14. F. de-Paris *et al*. Optimization of one-step duplex real-time RT-PCR for detection of Influenza and respiratory syncytial virus in nasopharyngeal aspirates. *Journal of Virological Methods* 2012; 186(1-2): 189- 192.
15.Max Q. Arens, et al. "Comparison of the Eragen Multi-Code Respiratory Virus Panel with Conventional Viral Testing and Real-Time Multiplex PCR Assay for Detection of Respiratory Viruses". J Clin Microbiol. 2010 Jul; 48(7): 2387–2395.

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