

AMPLIRUN® TOTAL SARS-CoV-2/FluA/FluB/RSV CONTROL (SWAB)

REF

MBTC031



For *in vitro* diagnostic use

INTENDED PURPOSE

A panel of four purified respiratory viruses pooled, inactivated to render them non-infectious and formulated in viral transport medium. This reference is intended to validate and control sample processing, amplification and detection in nucleic acid assays based on the molecular identification of respiratory viruses, using the product as an external run control.

INTRODUCTION

Respiratory tract infections are a major cause of hospitalization. In humans, these infections are caused in a majority proportion by a heterogeneous group of viruses with similar clinical symptoms, which include influenza (Flu), parainfluenza, rhinovirus, adenovirus, respiratory syncytial virus (RSV), human metapneumovirus (hMPV) and coronavirus. Traditionally, influenza virus and RSV have produced annual outbreaks during the fall and winter months, both of which are highly seasonal and affect millions of individuals each year. Moreover, SARS-CoV-2 (SC2), severe acute respiratory syndrome coronavirus 2, was identified as the cause of an outbreak of respiratory illness first detected in Wuhan (China) in December 2019. WHO, on March 11th 2020 declared the disease produced by SARS-CoV-2, a pandemic.

KIT FEATURES

VIRCELL TOTAL CONTROL are lyophilized. It is necessary to reconstitute them before use (see "Preparatory treatment of the device" section).

VIRCELL TOTAL CONTROL are designed for single use, excess material should be discarded.

Nucleic acid detection requires an extraction step that releases DNA/RNA for amplification and detection.

MATERIALS PROVIDED

[1] VIRCELL TOTAL SARS-CoV-2/ FluA/FluB/RSV CONTROL (SWAB): 10 vials with a pooled of lyophilized respiratory viruses simulating a respiratory clinical sample. Each virus is in a concentration that ranges from 10000-25000 copies/vial. Batch concentration is provided in Certificate of Analysis.

Quantification validation was performed by real-time PCR.

Viral particles were purified from supernatants of infected cells by differential centrifugation (see Table 1). Viruses were inactivated, rendering them non-infectious, and diluted in viral transport medium containing cells obtained from epithelial human cell lines.

VIRUS	STRAIN	CELL-LINE
SARS-COV-2	Clinical isolate	VERO E6
INFLUENZA A H3N2	A/Perth/16/2009 (H3N2)	MDCK
INFLUENZA B	B/Brisbane/60/2008	MDCK
RESPIRATORY SYNCYTIAL VIRUS	9320	HEP-2

Table 1

Special materials required but not provided:

-Molecular Biology grade water.

-Additional extraction and detection kit.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCELL TOTAL CONTROL reconstituted: use it in the same day. Unused product should be discarded.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
5. Sterile tips with aerosol barrier are essential to prevent contamination.
6. Never pipette by mouth.
7. Do not use in the event of damage to the package.
8. Do not use the kit after expiration date.
9. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
10. Keep containers for samples and reagents closed while they are not being handled.
11. Handle in aseptic conditions to avoid microbial contaminations.
12. Dispose of unused reagents and waste in accordance with all applicable regulations.
13. Reagents in this kit contains inactivated microorganism and could include genetic material or substances of animal and/or human origin. Although the material is not infectious, it should be handled as potentially infectious. All material should be handled and disposed as potentially infectious. Observe the local regulations for waste disposal.
14. The glass elements contained in kits could cause physical damage in the event of break. Handle with care.
15. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

PREPARATORY TREATMENT OF THE DEVICE

1. Add 500 µl of Molecular Biology grade water to each vial [1] and mix until completely reconstituted. The cellular concentration will be approximately 35000 copies/ml once reconstituted.
2. Shake with vortex for 30 seconds to dissolve and homogenize completely.

ASSAY PROCEDURE

Follow diagnostic kit instructions treating TOTAL CONTROL in an identical manner to a clinical specimen using recommended amount for extraction and detection.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control (Q.C.) testing before batch release. Final Q.C. results for each particular lot are available.

VALIDATION PROTOCOL FOR USERS

Refer to indications of additional extraction and detection kit.

INTERPRETATION OF RESULTS

Refer to indications of additional extraction and detection kit.

LIMITATIONS OF USE

1. This reagent is intended to be used with methods of human diagnostics. Other methods have not been verified.
2. This external run control does not substitute internal diagnostic kit controls.
3. Quantification conclusions cannot be drawn from a single point sample of known concentration. Precise clinical sample quantification could only be achieved by the standard curve method using a calibrator as MBC137 AMPLIRUN® SARS-CoV-2 RNA CONTROL, MBC029 AMPLIRUN® INFLUENZA A H3 RNA CONTROL, MBC030 AMPLIRUN® INFLUENZA B RNA CONTROL or MBC083 AMPLIRUN® RESPIRATORY SYNCYTIAL VIRUS (B) RNA CONTROL.
4. AMPLIRUN® TOTAL has not been designed to be used with a particular diagnostic kit coming from a certain manufacturer. It is used to validate and control sample processing, analysis and detection of a diagnostic laboratory functioning procedure.

PERFORMANCE CHARACTERISTICS

PRECISION

Real-time PCR analysis including 2 replicates of each vial, two runs per day (with different operators and different thermocyclers) for 20 days. The CV% of within-run precision, between-run precision, between-day precision and between-laboratory precision were analysed.

The results were as follows:

SARS-CoV-2

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Between-laboratory precision %CV
Vircell Total Control	1.6	1.5	0.5	2.3

CV: Coefficient of variation

Influenza A H3N2

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Between-laboratory precision %CV
Vircell Total Control	1.8	0.5	0.8	2.0

CV: Coefficient of variation

Influenza B

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Between-laboratory precision %CV
Vircell Total Control	2.1	1.6	1.0	2.8

CV: Coefficient of variation

Respiratory syncytial virus

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Between-laboratory precision %CV
Vircell Total Control	1.8	1.2	1.5	2.6

CV: Coefficient of variation

IDENTITY TEST

SARS-CoV-2

After extraction, PCR analysis was performed using an oligonucleotide pair specific for the identification of SARS-CoV-2, previously described in literature. The reactions produced a specific amplification.

Influenza A H3N2

After extraction, PCR analysis was performed using an oligonucleotide pair specific for the identification of Influenza A H3N2, previously described in literature. The reactions produced a specific amplification.

Influenza B

After extraction, PCR analysis was performed using an oligonucleotide pair specific for the identification of Influenza B, previously described in literature. The reactions produced a specific amplification.

Respiratory syncytial virus

After extraction, PCR analysis was performed using an oligonucleotide pair specific for the identification of respiratory syncytial virus, previously described in literature. The reactions produced a specific amplification.

QUANTIFICATION TEST

Quantification is based on Real-Time qPCR using the standard curve method. Real-time PCR analysis of 3 replicates of each vial. The concentration (Log copies/vial) is determined by interpolating the Ct value obtained for each replicate on the previously obtained standard curve performed with the corresponding quantification standard.

The results were as follows:

SARS-CoV-2

% Coefficient of variation: 0.5

Influenza A H3N2

% Coefficient of variation: 0.8

Influenza B

% Coefficient of variation: 1.7

Respiratory syncytial virus

% Coefficient of variation: 1.3

SYMBOLS USED IN LABELS



In vitro diagnostic medical device



Use-by (expiry date)



Store at x-y °C



Batch code



Catalogue number



Consult instructions for use



Reconstitute in <X> µl



Shipment temperature



Storage temperature



Manufacturer

BIBLIOGRAPHY

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3. Symis, M. W. et al. 2004. A sensitive, specific, and cost-effective multiplex reverse transcriptase-PCR assay for the detection of seven common respiratory viruses in respiratory samples. J Mol Diagn, 6(2), 125-31.
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5. Ye, F. et al. 2018. Analysis of influenza B virus lineages and the HA1 domain of its hemagglutinin gene in Guangzhou, southern China, during 2016. Virol J, 15(1), 175.
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Updates: New reference