TECHNICAL REPORT D202202041

Customer: ORUM International S.r.l.
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Sample Type: *AIRBIO virus Sampler*

LabAnalysis Code: FD-22-000592

PURPOSE

The purpose of the test was to confirm the capacity of the AIRBIO Virus Sampler to collect viruses (such as SARS-CoV-2) from the air. This sampler consists of a command unit connected to a sampling device with a conical shape that contains a liquid in which the virus present in the air should be captured and subsequently detected through Real-Time PCR approach (RT-PCR).

In this way it was possible to evaluate the AIRBIO -Virus sampler:

- Recovery: a precise amount of SARS-CoV-2 genomic copies (GC) was loaded in the sampling device. A simulation of an air sampling has been performed and the residual amount of GC was measured. The comparison between GC content before and after the simulation was used to estimate AIRBIO Virus Sampler Recovery.
- Retention Efficiency: a precise amount of SARS-CoV-2 genomic copies (GC) was loaded in the ampoule of an aerosol-generating machine coupled with AIRBIO Virus sampler. Air sampling was then performed and the amount of GC collected by the sampling device was measured. The comparison between GC content in the aerosol-nebulizer device and GC content in the AIRBIO Virus Sampler was used to estimate the Retention Efficiency of the latter.

SAMPLER IDENTIFICATION

AIRBIO Virus Sampler

LabAnalysis internal code: FD-22-000592-002237

Air Sampler principle.

The environmental air is aspirated into the collecting liquid inside a transparent conic container. The virus are separated from the air and collected in the liquid. The liquid is then analized by PCR to evidentiate the virus presence.

Material

EQUIPMENT

- AriaMX Real Time PCR system (Agilent)
  Internal Code: 6652
  Internal qualification: SOP-P-QUAL-043
  Next qualification: 2022/07
- Microcentrifuge
  Internal code: 9879
- Vortex
  Internal code: 4604
- Aerosol-Nebulizer (suitable for nebulization rate 0.3 mL/min, with mass median aerodynamic diameter (MMAD) of approx. 3 um)

REAGENTS

- Virus DNA/RNA purification kit (Generon)
  Code: MNP027-1E, Lot number: 0120935, Expiration date: 2022/09
- VETFinder kit Real Time PCR (Generon)
  Code: PMB00C_M2, Lot number: 012-21, Expiration date: 2022/06
- Heat inactivated 2019 Novel Coronavirus (ATCC)
  Code: VR-1986HK, Lot number: 70035039, Expiration date: 2022/06
- SARS-Related Coronavirus 2 (ZeptoMetrix)
  Code: 0810587CFHI, Lot number:325828, Expiration date: 2023/02
- Intype IC-RNA (Generon, Indical Bioscience)
  Code: IC289970, Lot number: F202000062, Expiration date: 2022/05
- PBS tablets (VWR)
- Code: E404, Lot number: 20C0656294; Expiration date: 2023/03
- Ethanol (Carlo Erba)
- Code: 414631, Lot number: V0D0592200; Expiration date: 2023/09
- Isopropanol (PanReac)
- Code: 221090.1612, Lot number:0002010059; Expiration date: 2024/01

PROTOCOL

Experimental set-up:

AIRBIO Virus sampling device was filled with 50 mL PBS and coupled with an aerosol-nebulizer. Titrated virus was added directly to the sampling device or in the aerosol-generating device. AIRBIO Virus Sampler was then activated for 10 minutes at 100 L/min flow. At the end of the treatment, aliquots were collected for the subsequent steps of RNA extraction and RT-PCR, to detect SARS-CoV-2 virus. Viral reference materials distributed by ATCC with a certified genome copies/ul content has been used to build reference curves useful to quantify the number of viral RNA copies in the samples (GC).

RNA extraction:

300 uL of the liquid to be analyzed were collected and subjected to extraction, accordingly to manufacturer's instructions. This kit exploits DNA/RNA adsorption on silica-based micro-spin columns. Briefly, the following steps were followed:

- Up 300 ul of the samples were transferred in a microcentrifuge tube, adding 500 uL GLX buffer, 10 ul IC-Type RNA (internal extraction control) and 20 uL Proteinase K;
- Samples were mixed by vortex for 30 sec and incubated at room temperature for 5 minutes;
- The surnatant (SN) was transferred in a RC2 column inserted in the collection tube, and centrifuged 1200 rpm for 1 minute;
- Flowthrough was discarded, 500 ul PD Buffer were added and centrifuged 12000 rpm for 1 minute;
- Flowthrough was discarded, 700 ul PW Buffer were added and centrifuged 12000 rpm for 1 minute, for 2 times;
- Flowthrough was discarded and columns were centrifuged again at 12000 rpm for 2 minutes;
- Columns were left for 5 minutes at room temperature to allow ethanol evaporation;
- Columns were placed in a new collection tube, 100 ul RNAse-free water was added and incubated 2 minutes at room temperature;
- Samples were eluted by centrifugation at 12000 rpm for 2 minutes.

RT-PCR:

SARS-CoV-2 detection was based on a commercial kit available from Generon. In particular, the kit PMB00C_M2 is composed of RT-PCR enzymes mix and 2 different oligo mixes for the detection of:

1) SARS-Cov-2 RdRp gene

2) Intype IC-RNA (Internal control for the evaluation of RNA extraction efficiency and exclude PCR inhibition).

Reaction Mastermix were prepared following manufactures's instructions, 20 uL of Mastermix were then added to 5 uL sample for each well, and PCR started accordingly to the following thermal profile:

Steps		T (°C)	Duration	Cycles
Reverse Transcription		55	10 min	1
Preheating		95	3 min	1
Amplification	Denaturation	95	15 sec	45
	Annealing/Extension+ Plate reading	58	30 sec	

Appropriate negative and positive control were added, together with reference virus extracts. RT-PCR provides an amplification curve from which Ct (cycle threshold) is determined, the latter value was plotted on the calibration curve of the reference virus suspension, plotting the number of genomic copies (GC) against Ct value. The obtained GC number was corrected accordingly to the applied analytical dilution factor.

RESULTS

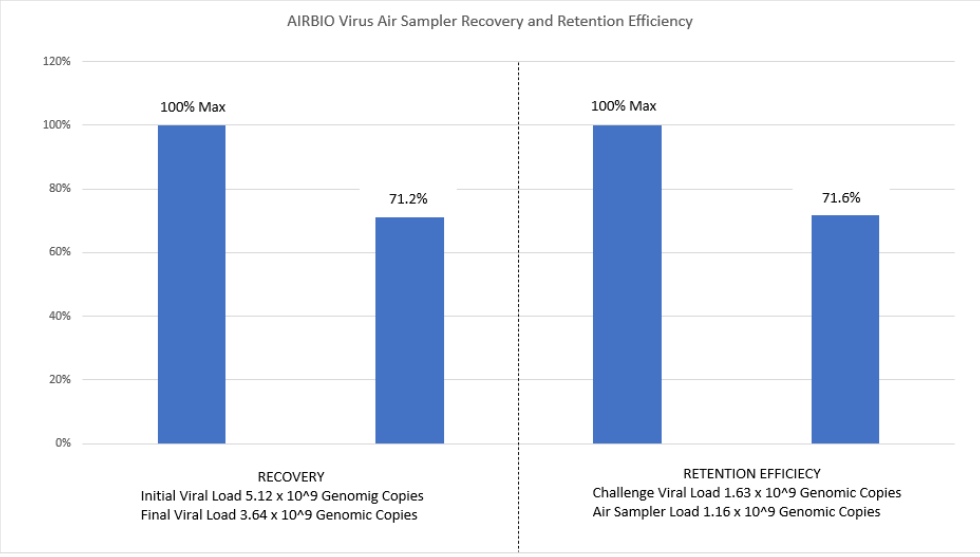
Initially, virus was directly loaded into the 50 ml of PBS solution in the sampling device. An air sampling was simulated switching on the device: 10 minutes at 100 L/min flow, to collect 1 m^3 of air. 300 ul of the 50 ml PBS solution were collected before and after the test and subjected to RNA extraction and RT-PCR. Results are presented in the following table:

Initial viral load	5.12 x 10^9 GC
Final viral load	3.64 x 10^9 GC
Recovery of AIRBIO Virus sampler	**71.2 %**

Subsequently, after an accurate wash of the entire system, virus was loaded in the ampoule of the aerosol-generating device coupled with AIRBIO Virus Sampler. The AIRBIO Virus device and the aerosol generator were activated and an air sampling was performed in the same condition as above: 10 minutes at 100 L/min flow. The two devices were coupled in such a way that all the aerosol produced was collected by the AIRBIO Virus sampling device. Appropriate aliquots of the liquid loaded in the ampoule of the aerosol-nebulizer and from the 50 ml of PBS solution in AIRBIO sampling device were collected and subjected to RNA extraction and RT-PCR. Results are presented in the following table:

Initial viral load	2.41 x 10^9 GC
Not-nebulized viral suspension	1.25 x 10^8 GC
Recovery of AIRBIO sampler	71.2 %
Challenge viral load*	1.63 x 10^9 GC
AIRBIO sampler load	1.16 x 10^9 GC
Retention Efficiency of AIRBIO sampler	**71.6 %**

* Challenge was corrected taking into account the not-nebulized viral suspension remaining in the ampoule from aerosol-nebulizer and the Recovery obtained for the system.



CONCLUSIONS

The Recovery and the Retention Efficiency of AIRBIO Virus Sampler were evaluated in a worst case scenario: the maximum sampling volume was considered (1 m^3). For this evaluation heat inactivated SARS-CoV-2 was used. Single test was performed.

The Recovery, the rate of SARS-CoV-2 GC (genomic copies) that the device is capable to retain after the sampling, is 71.2%.

The Retention Efficiency, the rate of the SARS-CoV-2 GC (genomic copies) that the device is capable of collect in a 1 m^3 air sampling is: 71.6%. This value was calculated taking into account the above mentioned Recovery.

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