AES08
June 10, 2022

Practices for Environmental Monitoring and Detection of Airborne Virus in Public Indoor Settings and High Risk Hospital Wards

Roberto.Ligugnana@gmail.com

Roberto Ligugnana[1], Anna S. Klavins[2], Kirsten Spallino[2], Rianna Malherbe[2], Andre Hsiung[2], Marta Castrica[3], Francesca Stefano[3], Claudia M. Balzaretti[3], Laura Menchetti[4], Sara Bisi[5], Guido Premoli[5]
[1]Villa Cella Via Novara 89 20153 Milan, Italy, [2]Hardy Diagnostics, California, United States, [3]Department of Veterinary Medicine and Animal Sciences, University of Milan, Via dell'Univerisità 6, 26900 Lodi, Italy
[4]Department of Agricultural and Agri-Food Sciences and Technologies, University of Bologna, Viale Fanin 46, 40138 Bologna, Italy, [5]LabAnalysis, Via Europa 5 27041 Casanova Lonati, Italy

Abstract

Introduction: It is recognized by the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) that aerosol transmission in poorly ventilated indoor environments may play a significant role in viral exposure (1,2). Air monitoring is especially important if possible contamination by SARS-CoV-2 viral particles is expected. Hospitals and industries that are involved in the control of the pandemic may benefit from monitoring the air for SARS-CoV-2 to ensure customer and employee safety. In this study, the AIRBIO ONE RAPID-VIRUS (TRIO.BAS™) was evaluated for the recovery of RNA from heat-inactivated (HI) SARS-CoV-2 (ATCC).

Methods: HI SARS-CoV-2 was spiked into phosphate buffered saline (PBS) that was added into the AIRBIO collecting tube prior to sampling the air. 300µL of the collection liquid was sampled before and after sampling the air. Additionally, HI SARS-CoV-2 was spiked into an aerosol nebulizer and passed through the AIRBIO as a closed system. 300µL of the nebulizer liquid was sampled before passing it through the AIRBIO. In each test, air was sampled for 10 minutes at 100 liters per minute to obtain a sample of one cubic meter of air. After the air sample was processed with the nebulizer attached, 300µL of the collection liquid was obtained. The liquid samples were stored at 4°C for less than 24 hours prior to RNA extraction (Generon). After extraction, reverse transcriptase polymerase chain reaction (RT-PCR) was performed using the PMB00C_M2 kit (Generon) and the AriaMX Real Time PCR system (Agilent). The number of genomic copies per microliter (GC/µL) of the collecting liquid from the AIRBIO after the air sampling process was compared to the number of GC/µL in the initial viral load inoculated to the collecting liquid or aerosol nebulizer. After collecting the air sample in both tests, the percentage difference in GC/µL was calculated.

Results: The GC/µL recovery (air sampling with spiked collection liquid) was 71.2% and the GC/µL retention efficacy (nebulizer test) was 71,6%.

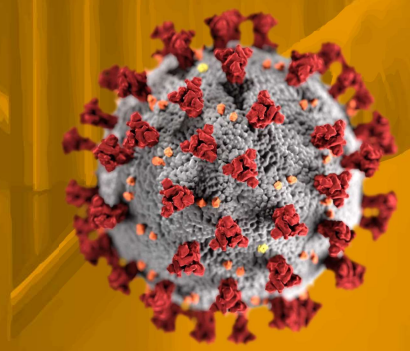
Conclusions: The method of air sampling for viruses using the AIRBIO ONE RAPID-VIRUS instrument was shown to recover and retain HI SARS-CoV-2 RNA in the collecting liquid but there was a reduction in the GC/µL for each test performed. Historically, air monitoring is limited to use by specific industries and manufacturing sectors. However, in the future, the use of virus air monitoring may become increasingly important for the future of public health safety and hospital environmental monitoring.

Introduction

The effects of indoor viral exposure during a pandemic are a concern for industries such as hospitals, health clinics, air travel, hospitality, theatres, convention centers, and other businesses and venues receiving large public populations. Small aerosols or droplets remain in the air longer and travel farther than larger droplets (>5 µm). If aerosols contain viruses in sufficient quantities, a susceptible person could inhale them and become infected. As more data about infectious dose becomes available, monitoring indoor air environments by quantitative analysis will become an important tool to provide data for risk assessments to evaluate viral exposure.

In regards to sampling time, viruses are present in the air at diluted concentrations and therefore the aspiration time should be several minutes with more than one cubic meter (1000 liters) of air. Air samplers should be positioned to the trajectory of the room airflow. A high air flow rate may degrade RNA virus during collection, thus the air flow rate should not exceed more than 100 liters/minute. The most common collection liquid for containing virus is Phosphate Buffered Saline. Samples should be stored at 4°C for not more than 24 hours and freezing the samples could be considered for long term storage. Aspirating parts (stainless steel connections and the container) should be decontaminated after each test by autoclave, using a 70% sterile ethanol immersion, or by hydrogen peroxide vapor.

The AIRBIO ONE RAPID-VIRUS instrument is specifically designed for total pathogen surveillance of bacteria, fungi, yeasts and viruses. The instrument consists of a command unit connected to a conical sampling device that holds a collecting liquid. The environmental air is aspirated into the collecting liquid and this allows for capture of viral particles. The liquid sample can subsequently be submitted for testing by rapid analytical test methods such as PCR. The purpose of these tests was to confirm the capacity of the AIRBIO Virus sampler to collect viruses (such as SARS-CoV-2) from the air.



Methods

To measure the viral recovery, the AIRBIO Virus conical collecting container was filled with 50 mL PBS spiked with HI SARS-CoV-2 (ATCC). After collecting a 300µL sample of the spiked PBS, the AIRBIO Virus sampler was activated for 10 minutes at 100 L/min flow. At the end of the test, another 300µL sample from the collection liquid was obtained.

To measure the viral retention efficacy, the AIRBIO Virus sampling device was filled with 50 mL PBS and coupled with an aerosol-nebulizer. HI SARS-CoV-2 was added directly to the ampoule of the aerosol nebulizer (suitable for nebulization rate 0.3 mL/minute with aerosol diameter 3 µm) coupled with the AIRBIO Virus sampler. 300µL of the sample spiked into the nebulizer was collected prior to running the test. The AIRBIO Virus sampler was activated for 10 minutes at 100 L/min flow. At the end of the test, a 300µL sample from the collection liquid was obtained.

Using an RNA extraction kit (Generon), all four 300µL samples were processed following the manufacturer's instructions for use. This kit exploits DNA/RNA absorption on silica-based micro-spin columns. After extracting the RNA, the samples were analysed by RT-PCR using the AriaMX Real Time PCR system (Agilent) and the VETFinder Reverse Transcriptase qPCR kit (Generon, PMB00C_M2). The PMB00C_M2 kit is composed of RT-PCR enzymes mix and 2 different oligo mixes for the detection of the 1) SARS-Cov-2 RdRp gene and 2) Intype IC-RNA (Internal control for the evaluation of RNA extraction efficiency). The reaction Mastermix was prepared following the manufacturer's instructions. Next, 20µL of Mastermix was added to a 5µL sample for each well and RT-PCR was started accordingly to the thermal profile described in Table 1. Appropriate negative and positive controls were added to the run together with reference virus extracts. RT-PCR provided an amplification curve from which Ct (cycle threshold) was determined. The RT-PCR was performed to detect SARS-CoV-2 virus GC/µL. The ATCC HI SARS-CoV-2 had a certified GC/µL content which was used to build reference curves to quantify the number of viral RNA copies in the samples.

Table 1. Thermocycler Reaction

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

Results

The Ct value and GC was plotted on the calibration curve of the reference virus suspension. The obtained GC number was corrected according to the applied analytical dilution factor. The percent recovery was determined by comparing the viral load in the collection liquid before and after the air sampling test. The viral RNA was mostly stable, but there was a 28.8% decrease in genomic copies after air sampling. This was possibly due to the unfiltered air degrading the viral RNA in the liquid.

Table 2. Calculated Recovery of the AIRBIO Virus Sampler

Viral load prior to air sampling	5.12×10^9 GC
Viral load after air sampling	3.64×10^9 GC
Recovery of AIRBIO Virus sampler	**71.2%**

The retention efficacy of the AIRBIO Virus sampler was slightly higher than the recovery rate when testing the AIRBIO Virus sampler with the nebulizer attachment. However, in this case there was still some RNA degradation after air sampling.

Table 3. Calculated Retention Efficacy of AIRBIO Virus Sampler

Viral load prior to nebulizing (total GC loaded into the aerosol nebulizer)	2.41×10^9 GC	R1
Non-nebulized viral load after air sampling (the GC not nebulized after the sampling)	1.25×10^9 GC	R2
Total GC nebulized (R1-R2)	2.29×10^9 GC	R3
Recovery of AIRBIO sampler (from Table 2)	71,2%	R4
Challenge viral load[1] prior to sampling (R3xR4/100)	1.63×10^9 GC	R5
AIRBIO sampler load after sampling	1.16×10^9 GC	R6
Retention Efficiency of AIRBIO Virus sampler ((R6/R5)x100)	71,6%[2]	R7

[1] Challenge was corrected taking into account the non-nebulized viral suspension remaining in the ampoule from aerosol-nebulizer and the Recovery obtained for the system.
[2] 71.6 derives from XLS rounding.

Conclusions

- Air sampling may be an effective method for certain industries to implement in order to assure the safety of the public and protect public health.
- The Recovery, the rate of SARS-CoV-2 genomic copies that the device is capable to retain after the sampling, was 71,2%.
- The Retention Efficiency, the rate of the SARS-CoV-2 genomic copies that the device is capable of collecting in a 1 m^3 air sampling, was 71,6%. This value was calculated taking into account the mentioned Recovery.
- Air flow in the collection liquid may degrade the viral RNA or DNA by approximately 28% if sampling up to a cubic meter of air. Thus, the amount of air sampled and the air flow rate should be taken into consideration when developing an air sampling plan for viruses. A validation may be necessary to ensure the viruses being tested for in the air are stable for the collection liquid used, the air flow rate applied, and the volume of air sampled.

References

(1) https://www.cdc.gov/media/releases/2020/s1005-how-spread-covd.html

(2) https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions

051122ss