Ministerio de Educación y Cultura

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INSTITUTO DE INVESTIGACIONES BIOLÓGICAS CLEMENTE ESTABLE

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Technical report for the validation of environmental monitoring using an air purification system based on non-thermal plasma technology (Trinum Plasma Uruguay).

Objective

To evaluate the air purification capacity in a controlled environment using the SIWÁ PREMIUM system.

Features

Air purifier with non-thermal plasma technology to eliminate viruses, including SARS-CoV-2, bacteria, spores, odors and volatile organic compounds in the environment up to 15 m^2.

Specifications

Descripción	Purificador de aire con tecnología de plasma no térmico
Presentación	Diseño limpio y robusto, fabricado en madera
Propósito	Control del ambiente, sanidad e higiene: Eliminación de virus, bacterias, esporas, olores y compuestos orgánicos volátiles en el ambiente
Modelo	SAP - 20P
Consumo Máximo	10 W
Consumo en espera	<1 W
Tensión de entrada	12 VDC

Material	Madera
Ventilador	2 x 5 CFM
Dimensiones	10 x1 10 x 17 cm
Peso	600 g
Tecnología	Plasma No - Térmico
Datos ADAPTADOR AC-DC	
Modelo	SMT-012-100 VP
Entrada	100-220VAC 50/60 Hz 3.0A
Salida	12 V / 3- 2.5 A
Certificaciones	Conforms UL STD.1012 CSA.C22.2 NO.107.1 ETL Listed Intertek

Design and methodology

The study was divided in two stages. Detection and elimination of total bacteria and detection and elimination of SARS-CoV-2. In the design of experiment 1, two air intake times (time zero and after 60 minutes of equipment operation) were established in two different environments. Sampling was established at 15 minutes for bacteria monitoring in a controlled environment (without people and low level of environmental contamination) and in a work office (with 5 people in normal work activity). For taking samples, we used the air intake system and specific plates for the cultivation of bacteria (growth in an oven at 37ºC for 24 and 48 hours. In experiment 2, synthetic DNA of SARS-CoV-2 was used in a controlled environment to evaluate the ability of the mentioned equipment to eliminate viral genomes. Inoculation was by direct spray (4 sprays with total viral load equivalent to 10^9 copies/µL) and sampling through filters positioned at the entrance and exit of the equipment at time zero and after 60 minutes in operation.

Extraction protocol for air samples.

For the detection of SARS-CoV-2, all filter samples conditioned in glass transport medium (400µL) and procedure controls were subjected to RNA extraction in an automated system (PurePrep 32). The extraction and purification of the viral RNA was performed using the MagMax Viral Pathogen Kit ™(Thermo Fisher Scientific - ref A42352), where each sample received an internal control for validation of the results of extraction and RT- qPCR.

SARS-CoV-2 RNA detection

All purified RNA samples and procedural controls were subjected to Real-Time PCR detection using the Coronavirus COVID-19 genesig® Real-Time PCR assay kit (Primerdesign™ Ltd, Ref. Z-Path-COVID-19-CE) in the Bio-Rad® CFX96 equipment. The system has a detection accuracy of 10 genome copies/µL reaction. Therefore, this concentration serves as the detection limit of the kit.

Results

Experiment 1. Controlled environment ($4m^2$) – clean room without presence of people with low air circulation and closed door.

Table 1. Colony-forming unit (CFU) count analysis – direct samples.

Plates	Zero Time	Final Time	Distance from the equipment	% Reduction relative
CFU (24 hours)	**16**	**1**	**15 cm**	**93,75%**
CFU (48 hours)	**22**	**1**	**15 cm**	**95,45%**

Table 2. Colony Forming Unit (CFU) count analysis – filter samples.

Plates	**Time zero**	Time zero **Post SARS-CoV-2**	**Final Time**
Air Inlet - UFC	**2**	**2**	**3**
Air outlet- UFC	**1**	**0**	**0/0**
% Reduction relative	**50%**	**100%**	**100%**

Table 3. SARS-CoV-2 removal analysis) – filter samples.

Samples	**Time (min)**	**Nº of copies inoculated**	**Viral Load relative**	**Result**
Filter 1 input T0	15	-	0	Nd*
Filter 2 Output T0	15	$4{,}2x10^{9}$	$5{,}2x10^{8}$	Detected
Post Output Filter 3 SARS-CoV-2	1	$4{,}2x10^{9}$	$5{,}2x10^{7}$	Detected
Filter 4 input	60	$4{,}2x10^{9}$	$5{,}2x10^{4}$	Detected
Filter 5 Output	60	-	0	Nd
Filter 6 Output	60	-	0	Nd
Negative control	-	-	0	Nd
Positive control	-	$1{,}67x10^{5}$	-	Detected

(*) Nd: Nondetectable

Experiment 2. Controlled environment ($8m^2$) – desk with work activity, flow between 3 and 5 people during the test and with air circulation relative to door opening.

Table 4. Colony Forming Unit (CFU) Count Analysis

Location of plates/ time of growth	**Zero Time (15 min)**	**Final Time (15 min)**	**Distance of the equipment**	**% Relative Reduction**
Window/bottom CFU (24 hours)	**5**	**1**	**1,5 m**	**80%**
Window/bottom CFU (48 hours)	**9**	**5**	**1,5 m**	**44,45%**
Closet/entrance CFU (24 hours)	**1**	**1**	**1,5 m**	**0,0%**
Closet/entrance UFC (48 hours)	**4**	**2**	**1,5 m**	**50%**
Filer/center (48 hours)	**7**	**0**	**30 cm**	**100%**

Comments and Conclusions

The results for the elimination of total bacteria in the two environments were considered satisfactory and agree with previous studies. For environment 1, the reduction in the number of CFUs was greater than 95% considering 48 hours of incubation. In this controlled environment, the incubation time did not significantly influence the results.

For room 2, the reduction was efficient and presented results consistent with the number of people and air circulation inside the desk with one (1) hour of operation. In the area with the lowest air circulation of the desk (near the window) the relative reduction ranged from 80 to 44% for 24 to 48 hours of incubation. In this environment, the circulation and renewal of air and the presence of people add a dynamic environmental condition, which hinders the elimination efficiency (bacteria, viruses, etc.) in the different conditions. But even if, the results in this experiment, allow demonstrating the ability to reduce bacteria in complex environments.

Considering the elimination of SARS-CoV-2, the results indicate the reduction of viral load from the first minutes of operation of the equipment. And considering the 60-minute operating time, the viral load is reduced to undetectable levels. It is important to note that the viral load used is high and compatible with environments contaminated with the presence of the virus.

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