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MOLECULAR BIOLOGY CENTRE SEVERO OCHOA

***TECHNICAL REPORT***

***DETERMINATION OF THE EFFECT OF THE DEVICE DuctFIT® AGAINST SARS-CoV-2***

**Purpose:**

Assessment of the ability of the device DuctFIT® of the company CleanAir to inactivate SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), the causative agent of the disease COVID-19 (Coronavirus disease 2019).

**Experiments performed at the MOLECULAR BIOLOGY CENTRE SEVERO OCHOA (Consejo Superior de Investigaciones Científicas [Spanish National Research Council]).**

**Researcher in charge:**

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**Research Professor of CSIC [Spanish National Research Council]**

**Group Immunity & Viromics**

**Signed by Antonio Alcamí**

**In Madrid, on 26 February 2021**

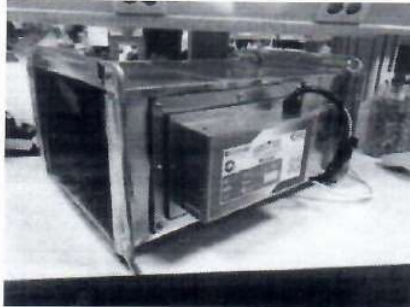
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### **DEVICE DuctFIT® included in a ventilation system**

The device consists of a unit DuctFIT® installed in an air conditioning pipe with a fan to simulate an equipment installed in an air conditioning system in a real environment.



*Photos: Device DuctFIT® used*

### **DESCRIPTION OF THE EXPERIMENT**

Establish the inactivation of SARS-CoV-2 exposed to a device DuctFIT® inside a microbiological safety cabinet. Exposure is performed at different periods of time (5 min, 15 min, 30 min, 1h and 3h), compared to a SARS-CoV-2 preparation that is not exposed to the effects of the device DuctFIT®. Experiment performed in a high biosafety level 3 (BSL3) laboratory.

Virus strain: SARS-CoV-2 MAD6, a Spanish reference isolation whose genomic sequence is identical to the SARS-CoV-2 Wuhan-Hu-1 isolation (GenBank MN908947), except for a silent mutation C3037T and two mutations resulting in amino acid changes, C14408T (protein nsp12) and A23403G (protein S).

Cell line: Vero E6 (ATCC No. CRL-1586), an epithelial cell line derived from a kidney of an African green monkey and susceptible to SARS-CoV-2. Grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). SARS-CoV-2 tests and infections are performed in DMEM 2% FBS.

Biological safety cabinets (BSC) adjacent to the BSL3 laboratory:  
BSC A, with DuctFIT® inside.  
BSC B, with DuctFIT® inside.

Switch on DuctFIT® 1 h before starting the exposure in BSC A.

Prepare the viral inoculum: 25 ml (5 ml SARS-CoV-2 MAD6 stock,  $5 \times 10^6$  pfu/ml).

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**Photos: Preparation of the carriers in the cabinet B**

Prepare carriers (P100 with surface treated for cell culture) in triplicate, 20 min prior to exposure on CSB B exposure in BSC B:

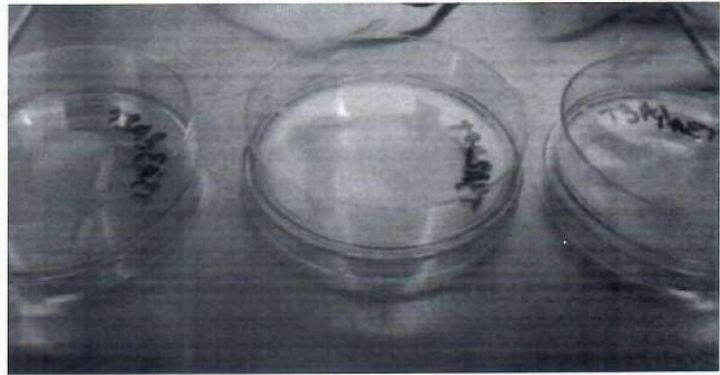
- 3 x C0
- 3 x T1A + 3 x T1B
- 3 x T2A + 3 x T2B
- 3 x T3A + 3 x T3B

Add 1.1 ml of inoculum (approx.  $10^6$  pfu) to each carrier and let dry with the lid open for 20 min.

After this period of time ( $t=0$ , C0):

3 x T1A + 3 x T2A + 3 x T3A: they are moved to BSC A.

3 x C0: they are collected in 2.2 ml DMEM 2% FBS, divided into 2 tubes and kept on ice.



**Photos: Arrangement of carriers in cabinet A during treatment, and appearance of the solution after 30 min.**

Different exposure times to DuctFIT®: triplicates are collected in 2.2 ml DMEM 2% FBS each, divided into 2 tubes and kept on ice until titration. After titration all samples are frozen at  $-80$  °C.

Titration of SARS-CoV-2 in 12 wells multiwell seeded two days before with Vero E6 cells, with 90% confluence at the time of titration. 250  $\mu$ l of inoculum from the prepared dilutions are added to the cell monolayer. After 2h of adsorption, the inoculum is removed and 1 ml of DMEM 2% FBS and 1% carboxymethyl cellulose are added. After 3 d of incubation at 37 °C, they are fixed (2% paraformaldehyde) and stained with methyl violet. The SARS-CoV-2 titre is determined based on the appearance of lysis plaques, and expressed in plaque forming units (pfu)/ml.