Texcell

CERTIFICATE OF INVESTIGATIVE STUDY

VERIFICATION OF THE EFFICIENCY OF AIRZONE PURIFYING SOLUTION ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO)

Study number: **1284/01**
Study report for:

Sponsor: CORPORATION EMPRESARIAL ALTRA / AIRZONE

Sponsor Representative: Juan Antonio Bandera

Submitted by:

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In sponsor conditions of use:

A reduction titer of 3.46 log (99.965%) of Sars-Cov-2 was demonstrated after 2 hours in the box

A reduction titer of 1.19 log (93.543%) of Sars-Cov-2 was demonstrated after 8 hours and 1.91 log (98.769%) after 24 hours

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1. PERSONNEL INVOLVED WITH THE PROJECT

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2. MATERIALS AND METHODS

2.1. VIRUSES (SPIKING TEST SYSTEM)

- **Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2)**: (Istituto Nationale malattei infettive (INMI) "Lazzaro Spallanzani")

The human strain of SARS-COV-2 was obtained from a viral strain isolated in Italy at Istituto Nationale malattei infettive (INMI) from a sample collected on January 29, 2020. The virus identity has been confirmed by complete sequencing. The complete sequence was submitted to GenBank (SARS-Cov-2/INMI1-Isolate/2020/Italy: MT066156) and is available on the GISAID website (BetaVov/Italy/INMI1-isl/2020: EPI_ISL_410545) upon registration. 2019-nCoV/Italy-INMI1 strain was used as a virus source and propagated on vero cells as described in the journal articles listed below:

Capobianchi MR, Rueca M, Messina F, Giombini E, Carletti F, Colavita F, Castilletti C, Lalle E, Bordi L, Vairo F, Nicastri E, Ippolito G, Gruber CEM, Bartolini B. Molecular characterization of SARS-CoV-2 from the first case of COVID-19 in Italy. Clin Microbiol Infect. 2020 Jul;26(7):954-956. doi: 10.1016/j.cmi.2020.03.025.

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DGJC, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MPG, Drosten C. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020 Jan;25(3). doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
Ogando NS, Dalebout TJ, Zevenhoven-Dobbe JC, Limpens RWAL, van der Meer Y, Caly L, Druce J, de Vries JJC, Kikkert M, Bárcena M, Sidorov I, Snijder EJ. SARS-coronavirus-2

replication in Vero E6 cells: replication kinetics, rapid adaptation and cytopathology. J Gen Virol. 2020 Jun 22. doi: 10.1099/jgv.0.001453.

Park WB, Kwon NJ, Choi SJ, Kang CK, Choe PG, Kim JY, Yun J, Lee GW, Seong MW, Kim NJ, Seo JS, Oh MD. Virus Isolation from the First Patient with SARS-CoV-2 in Korea. J Korean Med Sci. 2020 Feb 24;35(7):e84. doi: 10.3346/jkms.2020.35.e84.

Chu H, Chan JF, Yuen TT, Shuai H, Yuan S, Wang Y, Hu B, Yip CC, Tsang JO, Huang X, Chai Y, Yang D, Hou Y, Chik KK, Zhang X, Fung AY, Tsoi HW, Cai JP, Chan WM, Ip JD, Chu AW, Zhou J, Lung DC, Kok KH, To KK, Tsang OT, Chan KH, Yuen KY. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. Lancet Microbe. 2020 May;1(1):e14-e23. doi: 10.1016/S2666-5247(20)30004-5.

Main characteristics of SARS-COV-2 virus are described in the table below:

Virus	SARS-COV-2
Name	Severe Acute Respiratory Syndrome-Related Coronavirus 2
Family	*Coronaviridae*
Subfamily / Genus	*Orthocoronavirus*
Size in diameter	90-110 nm
Genome	One molecule of single-stranded RNA
Envelope	Enveloped
Strain	2019-nCoV/Italy-INMI1 strain
Production standard operating procedure (SOP)	**TE1129**
Titer of the virus stock used for spiking experiments	≥ 6 Log_{10} $TCID_{50}$/mL (50 % tissue culture infective dose per milliliter)
Titration assay and cells	Virus titer is determined by end-point dilution titration assay on Vero cells (operating procedure **TE1088**).

2.2. CELLS (TITRATION TEST SYSTEM)

(Freshney R.I., 1989, ATCC)

The cells are grown in accordance with Texcell's operating procedures **TE1002**, **TE3001** and **TE3011**.

- **Vero cells**: (Institut Pasteur, Medical Virology Laboratory) (Simizu B. and Terasima T. 1988; Simizu B. et al., 1967).

  Isolated from C. aethiops kidney on 27 Mar 1962. Vero cells are a lineage of cells used in cell cultures. The 'Vero' lineage was isolated from kidney epithelial cells extracted from an African green monkey (Chlorocebus sp.; formerly called Cercopithecus aethiops, this group of monkeys has been split into several different species). The lineage was developed on 27 March 1962, by Yasumura and Kawakita at the Chiba University in Chiba, Japan. The original cell line was named "Vero" after an abbreviation of verda reno, which means "green kidney" in Esperanto.

  This is a cell line with the hypodiploid chromosome count. The modal chromosome number was 58 occurring in 66% of cells. In most cells, over 50% of the chromosomes in each cell complement belonged to structurally altered marker chromosomes. Normal A3, A4, B4, and B5 were absent; B2, B3 and B7 were occasionally paired; and B9, C1 and C5 were mostly paired. The rate of cells with higher ploidies was 1.7%. Other chromosomes were

mostly present in single copy. These cell lines from ATCC (CCL-81, CRL-1587, CRL1586), are used for the propagation, assay and isolation of numerous viruses like coronaviruses. This line is a clone from Vero 76. Vero E6 cells are known to show some contact inhibition, and are often suitable for propagating viruses that replicate slowly and was therefore used to isolate the strain of SARS-COV-2 virus used in this study. Virucidy assays are performed using either the Vero cell line from ATCC CCL-81 or from Institut Pasteur (IP)

2.3. MEDIUM

Vero cells:
Dilution medium: DMEM 2% glutamine, 1% gentamicine
Cell Culture medium: DMEM 4% SVF, 2% glutamine, 1% gentamicine
Titration medium: DMEM 2% glutamine, 1% gentamicine

3. EXPERIMENTS

3.1. PROCESS DESIGN

As requested by the sponsor the process below will be evaluated as one step in simplicate:

PART 1: Spiking (1 time points: 2 hours)	**AIRZONE PURIFYING SOLUTION**	**Gauze** ▪ Put the ionization system in the Plexiglas box ▪ Spike the gauze with SARS-COV-2 at a ~ 1/10 ratio at room temperature ▪ Place the Gauze in the box ▪ Start time duration in 2 hours ▪ Take sample	Process total duration : 2 hours = sample	Process Temperature = 20°C ± 5°C
PART 2: Spiking (2 time points: 8 and 24 hours)	**AIRZONE PURIFYING SOLUTION**	**Gauze** ▪ Put the full system in the working house ▪ Spike 1 gauzes with SARS-COV-22 at a ~ 1/10 ratio at room temperature ▪ Place the gauze at 1.8-1.9 metter in the workhouse ▪ Start time duration in 8 and 24 hours ▪ Take samples	Process total duration : 8 and 24 hours = 2 samples	Process Temperature = 20°C ± 5°C

3.2. TITRATIONS

Virus titration will be performed by end-point dilution assay (TCID50) for SARS-COV-2. Samples obtained during the spiking experiments will be stored at <-70°C and titrated following storage with the appropriate controls (depending on the Preliminary Assay results).

4. INFECTIVITY METHODS

4.1. TITRATION ASSAY

The samples are titrated according to operating procedure **TE1088**.

4.1.1. Principle of titration

The titration method is a quantitative assay in which the virus titer measurement is based on the detection of virus production in the infected cells, by observation of a specific cytopathic effect.

***Serial dilutions titration**

Briefly:

Test sample is diluted with medium by serial 3-fold dilutions (eight replicates are performed for each dilution) across the 96 well plate (sample dilution plate).

Each well from the "sample dilution plate" is then inoculated on the corresponding well of a new plate (sample titration plate).

Cell suspension is added to each well of the "sample titration plate" and the plates are then incubated at appropriate temperature with or without CO_2 atmosphere (depending on viruses).

After a period of incubation allowing viral replication and infection of adjacent cells, depending on viruses,

- wells with foci are counted after infection by observation under inverted light microscope.
- or a stain overlay (crystal violet) is added and wells are examined for cytopathic effect. The infected wells show up as clear areas whereas the non-infected wells are stained.

The infectious titer expressed as 50% tissue culture infective dose per milliliter ($TCID_{50}$/mL) is calculated using the Spearman-Kärber formula.

***Large Volume titration (LVT)**

LVT assay could be performed (at the sponsor's request) in order to improve the detection limit of the assay or the titer of the tested sample. Cells in 96 well plates or flasks (number according to desired sensitivity) are inoculated with a large volume of the lowest non-toxic and non-interfering dilution of the sample.

Cell suspension is then added to each well of the "sample titration plate" and the plates are incubated at appropriate temperature with or without CO_2 atmosphere (depending on viruses).

After a period of incubation allowing viral replication and infection of adjacent cells, depending on viruses,

- wells with foci are counted after infection by observation under inverted light microscope.
- or a stain overlay (crystal violet) is added and wells are examined for cytopathic effect. The infected wells show up as clear areas whereas the non-infected wells are stained.

The infectious titer expressed as 50% tissue culture infective dose per milliliter ($TCID_{50}$/mL) is calculated using the appropriate formula.

4.1.2. Titration assay controls

***Negative control N1** (cell reference control)
During titration assay, in each 96-well plate, 8 wells are prepared as cell reference control. These cells are prepared in the same conditions as those used for the titration of the samples generated during the viral clearance experiments except that they are inoculated with unspiked medium.

***Positive reference control** (virus reference control)
During each titration assay, a stock of each virus prepared at low final concentration of approximately $10^5 – 10^7$ $TCID_{50}$/mL (depending on virus) and used as virus reference control is titrated in the same conditions as those used for the titration of the samples generated during experiments.

4.1.3. Spiking experiments controls

***Cytotoxicity test control(s)**
The evaluation of the cytotoxic effect is carried out by visual observation under inverted light microscope. The quality of the cell monolayer (confluence, refringence, aspect) of the tested samples is compared with that of the cell reference control N1.
Negative controls (Nx) and each dilution of samples for which no total cytopathic effect is observed are evaluated for cytotoxicity by comparison with the cell reference control N1. Similar serial dilutions as those used for the titration assays are applied.
For a defined sample, the non-cytotoxic dilution is reached when no significant difference is observed in the 3-fold serial diluted sample compared with the cell reference control.

***Storage control(s)**
The evaluation of the effect of the storage conditions on virus in the tested sample during the storage at ≤ -70°C is carried out by comparison of the titers obtained in medium and in diluted sample after storage.
This evaluation is carried out with the starting material(s), and/or the final material(s) obtained from the Mock run when the generated samples are stored until titration.

The study is described as follows:

- The starting material and/or the final material is diluted at a defined dilution, and then spiked with virus (positive control or virus stock) at a low final concentration of approximately 10^3-10^4 $TCID_{50}$/mL.
- In parallel, medium is also spiked with the same virus (positive control or virus stock) to reach the same concentration as the starting material and/or final material samples.

Both storage controls samples are then stored at ≤ -70°C until titration. These storage controls are prepared and titrated with the samples generated from the experiments.

After titration, there is no storage effect on virus, if the difference between the two titers (titer in medium compared with titer in starting and/or final material(s)) is less than or equal to $1Log_{10}$.

4.2. ACCEPTANCE CRITERIA OF THE TITRATION ASSAY

During each titration assay, a virus stock prepared at low final concentration of approximately $10^5 – 10^7$ $TCID_{50}$/mL (depending on virus), is used as a reference control.

The titration assay is retained when:

- the cell reference control (N1) for each titration plate conforms to the expected result,
- the infectious titer of the virus reference control obtained is in the expected range.

4.3. DETERMINATION OF THE VIRAL TITER

(Schwartz D., 1993; Kaplan M. and Koprowski H., 1973)
Three situations may be predicted concerning the calculation of the viral titer.

Case	Subcase	Titer (T)
Infectious particles detected ≥ 12.5% positive wells/total tested wells		$T = T_{SK}$
Few infectious particles detected < 12.5% positive wells/total tested wells	$T_{MaxL} > dl$	$T = T_{MaxL}$
	$dl > T_{MaxL}$	$T = dl$
No infectious particles detected 0% positive well/total tested wells		$T < dl$

with:

T = titer retained for the calculation of the reduction factor
T_{SK} = infectious titer using the simplified Spearman-Kärber formula (Section 2.3.1)
T_{MaxL} = infectious titer using the Maximum likelihood estimation (Section 2.3.2)
dl = detection limit using the Poisson formula with 95% precision (Section 2.3.3)

4.3.1. Calculation of the $TCID_{50}$ using Spearman-Kärber formula

The $TCID_{50}$ is evaluated by quantitative assay and defined as the virus dose capable of infecting 50% of the inoculated cultures. The viral titer, T, expressed as the 50% tissue culture infective dose per milliliter ($TCID_{50}$/mL), is defined by its mean value, m(T), and its confidence interval, m(T) can be calculated according to the following formula:

$$m(T) = \frac{1}{Vo}\ 10^{m(a)}$$

with v_o = volume per replicate
"a" is also defined by its mean, m(a), and its standard deviation, S(a).

The viral titer can be calculated using Spearman-Kärber (SK) formula (Payment P. and Trudel M. 1993).

*This method is applicable firstly, in situations where cytopathic effect is observed, ranging from 0% to 100% of positive replicas per dilution in a same titration plate.
m(a) = T_{SK}, and S(a) is calculated using the following simplified Spearman-Kärber formula

$$m(a) = T_{SK} = -a_o + \frac{k}{2} - k\sum_i p_i \quad \text{and} \quad S(a)^2 = k^2 \sum_i \frac{p_i(1-p_i)}{n_i - 1}$$

with:

- a = Log_{10} of the titer relative to the test volume
- a_o = Log_{10} of the reciprocal of the lowest dilution for which all wells are positive
- k = Log_{10} of the dilution factor
- p_i = proportion of positive wells at the non-cytotoxic dilution d_i / r_i
- r_i = number of positive wells at the non-cytotoxic dilution d_i
- n_i = number of replicates at the non-cytotoxic dilution d_i.

With a 95% precision, the confidence interval of "a" is the following:

$$a^{min} \leq a \leq a^{max} \text{ with: } a^{min} = m(a) - 2\,S(a)$$
$$a^{max} = m(a) + 2\,S(a)$$

With a 95% precision, the confidence interval of the titer T is the following:

$$T^{min} \leq T \leq T^{max} \text{ with:}$$
$$T^{min} = \frac{1}{Vo}\,10^{a^{min}} \; ; \; T^{max} = \frac{1}{Vo}\,10^{a^{max}}$$

The dilutions of the samples retained for the calculation of the infectious titers are those for which no cytotoxicity is observed.

*Secondly, when less than 100% but ≥ 12.5% of positive replicas per dilution is obtained for the lowest non-cytotoxic dilution tested, the virus titer is calculated assuming that the sample contains sufficient virus to infect 100% of tested wells at the previous serial dilution (worst-case).

***Total virus load**

The viral load L is defined by its mean value, m(L), and its confidence interval.

$$m(L) = \frac{m(T)V_t}{c}$$

$$\text{Lmin} \leq \text{L} \leq \text{Lmax}: \quad L^{min} = \frac{T^{min}V_t}{c} \text{ and } L^{max} = \frac{T^{max}V_t}{c}$$

with:

- c = concentration factor of the ultracentrifugation (c = 1 when the samples are not ultracentrifuged).
- V_t = total volume of the sample during the scaled down process.

4.3.2. Large Volume Titration assay: Maximum Likelihood estimation

During LVT assay, when few positive wells are detected (< 12.5% of all tested wells), viral titers (T_{MaxL}) in samples are calculated according to Maximum Likelihood estimation (Agut H., Calvez V., Barin F, 1997) as follows:

$$T_{MaxL} = (Ln\ (N/P) \times d \times (1000/v)) / Ln2$$

with:

T_{MaxL} = titer relative to the test volume ($TCID_{50}$/mL)
N = number of all tested wells
P = number of negative wells
d = non-cytotoxic dilution factor of the sample
v = tested volume per well
$Ln2 \approx 0.69$ = corrective factor to convert PFU/mL into $TCID_{50}$/mL.

When significant positive wells are detected (>12.5% of all tested wells) viral titers (T_{MaxL}) in samples are calculated using the Spearman-Kärber formula as described above.

***Total virus load**

The viral load L is defined by its mean value, $m(L_{MaxL})$.

$$m(L_{MaxL}) = \frac{m(T_{MaxL})V_t}{c}$$

with:

c = concentration factor of the ultracentrifugation (c = 1 when the samples are not ultracentrifuged).
V_t = total volume of the sample during the scaled down process.

4.3.3. Detection limit of titration assay

When a sample contains a low concentration of infectious virus and only a fraction of the sample is tested for titration, there is a probability that the result of the tested fraction will be negative due to random (and unequal) distribution of the virus throughout the sample.

The detection limit, **dl**, for the titration assay corresponds to the lower theoretical titer which results in the detection of one infectious particle in one of the replicates performed. Since the infectious particle would be detected in a volume V_c (mL) of the dilution d_c, the detection limit, dl, is calculated with a 95% precision according to the Poisson formula (Löwer J., 1991):

$$p(95\%) = e^{-dl\ [V_c\ d_c]} = 0.05$$

$$\textbf{i.e.} \qquad \mathbf{dl} = \frac{-\ln(0.05)}{V_c\ d_c}$$

with:

V_c = $[v_o\ n_c]$
v_o = volume per replicate
n_c = number of replicates at non-cytotoxic dilution d_c
dl = detection limit

The non-cytotoxic dilution of the samples for which no positive wells are detected is then retained for the calculation of the infectious titer using the Poisson formula.
The infectious titers calculated with the Poisson formula are expressed as PFU/mL and are divided by ln2 to be converted into $TCID_{50}$/mL.

***Detection limit of the viral load of a whole fraction**
The detection limit, DL, is the minimal viral load which could be theoretically detected in the total volume of a fraction belonging to the scaled down process.

DL is calculated according to the following formula:

$$DL = \frac{dl\ V_t}{c}$$

with:

c = concentration factor of the ultracentrifugation (c = 1 when the samples are not ultracentrifuged),
Vt = total volume of the sample during the scaled down process,
dl = detection limit for a titration assay.

The detection limit for the pre and post-treatment material are DL_i and DL_f respectively.

5. REDUCTION FACTOR CALCULATION

In accordance with the regulatory documents, the virus reduction factor (R) of an individual purification or inactivation step is defined as the Log10 of the ratio of the virus load (Li) in the pre-treatment material (starting material) and the virus load (Lf) in the post-treatment material (final material) which is ready for use in the next step of the process.

R is defined by its mean, m(R), and its confidence interval, [Rmin ≤ R ≤ Rmax]. If Rmin and/or Rmax is < 0 (negative value), then m(R) will present as ≈ 0 without confidence interval.

Different cases have to be considered related to the effect of the pre-treatment material on the virus.

***Case 1**

A studied manufacturing process step consists in the monitoring of a viral inactivation directly linked to a solution used during the process ("in process fraction", sanitization solution, etc.). In other terms, a putative virucidal effect associated with a solution is evaluated in a kinetics study.

***Case 2: Treatment reduction factor calculation m(R_t)**

The pre-treatment material has **no significant inactivating effect**, i.e. the mean reduction factor, **m(R_o) is lower than or equal to 1**. m(Ro) is defined as the Log_{10} of the ratio of the virus load in the medium (L_{cm}) and the virus load in the pre-treatment material (L_i).
In this case, the reduction factor of the step corresponds to the reduction factor of the treatment m(R_t).

***Case 3: Clearance factor calculation m(R)**

The pre-treatment material has **a significant inactivating effect** (excluding Case 1) i.e. the mean reduction factor, **$m(R_o)$ is higher than 1**.

- In a first approach, the clearance factor of the step, m(R), is calculated as the sum of the reduction factor associated with the initial inactivating effect, $m(R_o)$, and the reduction factor of the treatment, $m(R_t)$. The initial load is the virus load in medium (L_{cm}).

- In a second approach, the reduction factor of the treatment, $m(R_t)$ is calculated with the virus load in the pre-treatment material (L_i).

In practice,

- if the volume of post-treatment material (V_f) = volume of pre-treatment material (V_i) ± 5% (v/v), the reduction and clearance factors are calculated with viral titers and then X = T.
- Otherwise, the reduction and clearance factors are calculated with viral loads and then X = L.

CASES	SUBCASES	EVALUATION OF THE REDUCTION FACTOR R_t AND CLEARANCE FACTOR (R)
Case 1		R is evaluated according to the three subcases of Case 2, with $X_i = X_{cm}$
Case 2 $m(R_o) \leq 1$	$m(x_f) < DL_f$	$R_t > Log_{10}\left(\frac{m(X_i)}{DL_f}\right)$ with $Rmin \leq R_t \leq Rmax$
	$X_i^{min} \geq X_f^{max}$	$m(R_t) = Log_{10}\left(\frac{m(X_i)}{m(X_f)}\right)$ with $Rmin \leq R_t \leq Rmax$
	$X_i^{min} < X_f^{max}$	$m(R_t)$ is calculated according to subcase 2 $R_t \approx 0$ if Rmin, R_t or Rmax < 0 (negative value)
Case 3 $m(R_o) \geq 1$	$m(X_i) < DL_i$	R_t cannot be evaluated $R_o \geq Log\left(\frac{m(X_{cm})}{DL_i}\right)$ with $Rmin \leq R_o \leq Rmax$
	$m(X_i) \geq DL_i$	R_t can be evaluated at sponsor request, otherwise $R = R_t + R_o$ is calculated R is evaluated according to the three subcases of Case 2 with $X_i = X_{cm}$ $m(R_o) = Log_{10}\left(\frac{m(X_{cm})}{m(X_i)}\right)$ with $Rmin \leq R_o \leq Rmax$

X_i = initial sample titre (Ti) or load (Li)
X_f = final sample titre (Tf) or load (Lf)
X_{cm} = culture medium titre (Tcm) or load (Lcm)
m(X) = mean titre or load
$Rmin = R - 2\sqrt{\text{var } Ti + \text{var } Tf}$
$Rmax = R + 2\sqrt{\text{var } Ti + \text{var } Tf}$

$$\text{Var} = \text{Variance} = S(a)^2 = k^2 \sum_i \frac{p_i(1-p_i)}{n_i - 1}$$

with:

- k = Log of the dilution factor
- p = proportion of positive wells at the non-cytotoxic dilution d / r
- r = number of positive wells at the non-cytotoxic dilution d
- n = number of replicates at the non-cytotoxic dilution d.

6. SUMMARY OF RESULTS

TABLE 1 PART 1: EVALUATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" TO INACTIVATE THE SARS-COV2 AS A FOR INFORMATION ONLY (FIO) VIRAL CLEARANCE STUDY

TABLE 1.1: CONTROLS

Sample		Infectious Titer (T)			Reduction factor evaluation (R)	
code	Definition	code	Mean titer [m(T)] and confidence interval		Mean reduction factor m(R) and confidence interval	Definition
Negative controls						
N1	Starting material	TN1	Not cytotoxic 10 fold diluted		NA	Negative control in the starting material
Positive controls						
V1	Spiked (addition of virus stock) medium	TV1	5.71	$5.48 \leq T \leq 5.95$	NA	Virus stock control
V2	Spiked (addition of virus stock) medium during experiments	TV2	5.71	$5.44 \leq T \leq 5.98$	NA	Virus stock hold control
L1	Spiked (addition of virus stock) Gauze and recovery	TL1	5.83	$5.61 \leq T \leq 6.05$	NA	Virus recovery control in gauze
L2	Spiked (addition of virus stock) Gauze during experiments and recovery	TL2	5.00	$4.76 \leq T \leq 5.24$	NA	Virus recovery hold control control in gauze

Infectious titers are expressed as Log_{10} 50% tissue culture infectious dose per milliliter (Log_{10} $TCID_{50}$/mL).

TABLE 1 PART 1(CONTINUED): EVALUATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" TO INACTIVATE THE SARS-COV2 AS A FOR INFORMATION ONLY (FIO) VIRAL CLEARANCE STUDY

TABLE 1.2: CONTROLS							
Sample		**Infectious Titer (T)**			**Reduction factor evaluation (R)**		
code	Definition	code	Mean titer [m(T)] and confidence interval		Mean reduction factor m(R) and confidence interval		Definition
Comparison of positive controls							
V1	Spiked (addition of virus stock) medium	TV1	5.71	$5.48 \leq T \leq 5.95$	NA	NA	Virus stock control
V2	Spiked (addition of virus stock) medium during experiments	TV2	5.71	$5.44 \leq T \leq 5.98$	~ 0.00	Not significant	Experiment effect on virus control (comparison TV1 and TV2)
L1	Spiked (addition of virus stock) Gauze and recovery	TL1	5.83	$5.61 \leq T \leq 6.05$	~ 0.00	Not significant	Effect on virus in recovery control (comparison TV1 and TL1)
L2	Spiked (addition of virus stock) Gauze during experiments and recovery	TL2	5.00	$4.76 \leq T \leq 5.24$	= 0.83	Not significant	Experiment effect on virus in recovery control (comparison TL1 and TL2)

TABLE 1 PART 1(CONTINUED): EVALUATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" TO INACTIVATE THE SARS-COV2 AS A FOR INFORMATION ONLY (FIO) VIRAL CLEARANCE STUDY

TABLE 1.3: SPIKING EXPERIMENTS									
Sample		**Vol**	**Infectious Titer (T)**			**Virus load (L)**			**Reduction factor evaluation (R)**
code	definition	(Vt) mL	code	Mean titer [m(T)] and confidence interval		code	Mean load [m(L)=m(T) x Vt]		Mean reduction factor m(R) and confidence interval [m(R)] = Log_{10} (L1) – Log_{10} (LS)
Positive control									
L1	Spiked (addition of virus stock) Gauze and recovery	1	TL1	5.83	$5.61 \leq T \leq 6.05$	LL1	5.83	$5.61 \leq T \leq 6.05$	Virus stock control in the Gauze
STUDY									
Samples collected, recovery and diluted: Clearance factor									
S	Gauze	1	TS1	2.37	$2.10 \leq T \leq 2.65$	LS1	2.37	NA	**R = 3.46**

Infectious titers are expressed as 50% tissue culture infectious dose per milliliter (Log_{10} $TCID_{50}$/mL). Viral loads are expressed as 50% tissue culture infectious dose (L

TABLE 2 PART 2: VERIFICATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" DEVICE ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO) AFTER 8 HOURS

TABLE 1.4: CONTROLS						
Sample		**Infectious Titer (T)**			**Reduction factor evaluation (R)**	
code	Definition	code	Mean titer [m(T)] and confidence interval		Mean reduction factor m(R) and confidence interval	Definition
Negative controls						
N1	Starting material	TN1	Not cytotoxic 10 fold diluted		NA	Negative control in the starting material
Positive controls						
V1	Spiked (addition of virus stock) medium	TV1	6.91	$6.75 \leq T \leq 7.06$	NA	Virus stock control
V2	Spiked (addition of virus stock) medium during experiments	TV2	6.79	$6.45 \leq T \leq 7.13$	NA	Virus stock hold control
L1	Spiked (addition of virus stock) Gauze and recovery	TL1	6.67	$6.51 \leq T \leq 6.83$	NA	Virus recovery control in gauze
L2	Spiked (addition of virus stock) Gauze during experiments and recovery	TL2	6.19	$5.92 \leq T \leq 6.46$	NA	Virus recovery hold control in gauze

Infectious titers are expressed as Log_{10} 50% tissue culture infectious dose per milliliter (Log_{10} $TCID_{50}$/mL).

TABLE 2 PART 2(CONTINUED): VERIFICATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" DEVICE ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO) AFTER 8 HOURS

TABLE 1.5: CONTROLS							
Sample		**Infectious Titer (T)**			**Reduction factor evaluation (R)**		
code	Definition	code	Mean titer [m(T)] and confidence interval		Mean reduction factor m(R) and confidence interval		Definition
Comparison of positive controls							
V1	Spiked (addition of virus stock) medium	TV1	6.91	$6.75 \leq T \leq 7.06$	NA	NA	Virus stock control
V2	Spiked (addition of virus stock) medium during experiments	TV2	6.79	$6.45 \leq T \leq 7.13$	= 0.12	Not significant	Experiment effect on virus control (comparison TV1 and TV2)
L1	Spiked (addition of virus stock) Gauze and recovery	TL1	6.67	$6.51 \leq T \leq 6.83$	= 0.24	Not significant	Effect on virus in recovery control (comparison TV1 and TL1)
L2	Spiked (addition of virus stock) Gauze during experiments and recovery	TL2	6.19	$5.92 \leq T \leq 6.46$	= 0.48	Not Significant	Experiment effect on virus in recovery control (comparison TL1 and TL2)

TABLE 2 PART 2 (CONTINUED): VERIFICATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" DEVICE ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO) AFTER 8 HOURS

TABLE 1.6: SPIKING EXPERIMENTS									
Sample		**Vol**	**Infectious Titer (T)**			**Virus load (L)**			**Reduction factor evaluation (R)**
code	definition	(Vt) mL	code	Mean titer [m(T)] and confidence interval		code	Mean load [m(L)=m(T) x Vt]		Mean reduction factor m(R) and confidence interval [m(R)] = Log_{10} (L1) – Log_{10} (LS)
Positive control									
L1	Spiked (addition of virus stock) Gauze and recovery	1	TL1	6.19	5.95 ≤ T ≤ 6.43	LL1	6.19	5.95 ≤ T ≤ 6.43	Virus stock control in the Gauze
STUDY									
Samples collected, recovery and diluted: Clearance factor									
S1	Gauze 1	1	TS1	4.04	3.77 ≤ T ≤ 4.31	LS1	4.04	3.77 ≤ T ≤ 4.31	**R = 1.19 (93.543%)**

Infectious titers are expressed as 50% tissue culture infectious dose per milliliter (Log_{10} $TCID_{50}$/mL). Viral loads are expressed as 50% tissue culture infectious dose (Log_{10} $TCID_{50}$).

TABLE 2 PART 2: VERIFICATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" DEVICE ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO) AFTER 24 HOURS

TABLE 1.7: CONTROLS

Sample		Infectious Titer (T)			Reduction factor evaluation (R)	
code	Definition	code	Mean titer [m(T)] and confidence interval		Mean reduction factor m(R) and confidence interval	Definition
Negative controls						
N1	Starting material	TN1	Not cytotoxic 10 fold diluted		NA	Negative control in the starting material
Positive controls						
V1	Spiked (addition of virus stock) medium	TV1	7.74	$7.46 \leq T \leq 8.03$	NA	Virus stock control
V2	Spiked (addition of virus stock) medium during experiments	TV2	6.67	$6.51 \leq T \leq 6.83$	NA	Virus stock hold control
L1	Spiked (addition of virus stock) Gauze and recovery	TL1	6.79	$6.79 \leq T \leq 6.79$	NA	Virus recovery control in gauze
L2	Spiked (addition of virus stock) Gauze during experiments and recovery	TL2	5.60	$5.31 \leq T \leq 5.88$	NA	Virus recovery hold control in gauze

Infectious titers are expressed as Log_{10} 50% tissue culture infectious dose per milliliter (Log_{10} $TCID_{50}$/mL).

TABLE 2 PART 2 (CONTINUED): VERIFICATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" DEVICE ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO) AFTER 24 HOURS

TABLE 1.8: CONTROLS

Sample		Infectious Titer (T)			Reduction factor evaluation (R)		
code	Definition	code	Mean titer [m(T)] and confidence interval		Mean reduction factor m(R) and confidence interval		Definition
Comparison of positive controls							
V1	Spiked (addition of virus stock) medium	TV1	7.74	$7.46 \leq T \leq 8.03$	NA	NA	Virus stock control
V2	Spiked (addition of virus stock) medium during experiments	TV2	6.67	$6.51 \leq T \leq 6.83$	= 1.07	Not significant	Experiment effect on virus control (comparison TV1 and TV2)
L1	Spiked (addition of virus stock) Gauze and recovery	TL1	6.79	$6.79 \leq T \leq 6.79$	= 0.95	Not significant	Effect on virus in recovery control (comparison TV1 and TL1)
L2	Spiked (addition of virus stock) Gauze during experiments and recovery	TL2	5.60	$5.31 \leq T \leq 5.88$	= 1.19	Significant	Experiment effect on virus in recovery control (comparison TL1 and TL2)

TABLE 2 PART 2 (CONTINUED): VERIFICATION OF THE EFFICIENCY OF "AIRZONE PURIFYING SOLUTION" DEVICE ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO) AFTER 24 HOURS

TABLE 1.9: SPIKING EXPERIMENTS									
Sample		**Vol**	**Infectious Titer (T)**			**Virus load (L)**			**Reduction factor evaluation (R)**
code	definition	(Vt) mL	code	Mean titer [m(T)] and confidence interval		code	Mean load [m(L)=m(T) x Vt]		Mean reduction factor m(R) and confidence interval [m(R)] = Log_{10} (L1) – Log_{10} (LS)
Positive control									
L2	Spiked (addition of virus stock) Gauze during experiments and recovery	1	TL2	5.60	5.31 ≤ T ≤ 5.88	LL2	5.60	5.31 ≤ T ≤ 5.88	Virus stock control in the Gauze
STUDY									
Samples collected, recovery and diluted: Clearance factor									
S2	Gauze 2	1	TS2	3.69	3.40 ≤ T ≤ 3.97	LS2	3.69	3.40 ≤ T ≤ 3.97	**R = 1.91 (98.769%)**

Infectious titers are expressed as 50% tissue culture infectious dose per milliliter (Log_{10} $TCID_{50}$/mL). Viral loads are expressed as 50% tissue culture infectious dose (Log_{10} $TCID_{50}$).

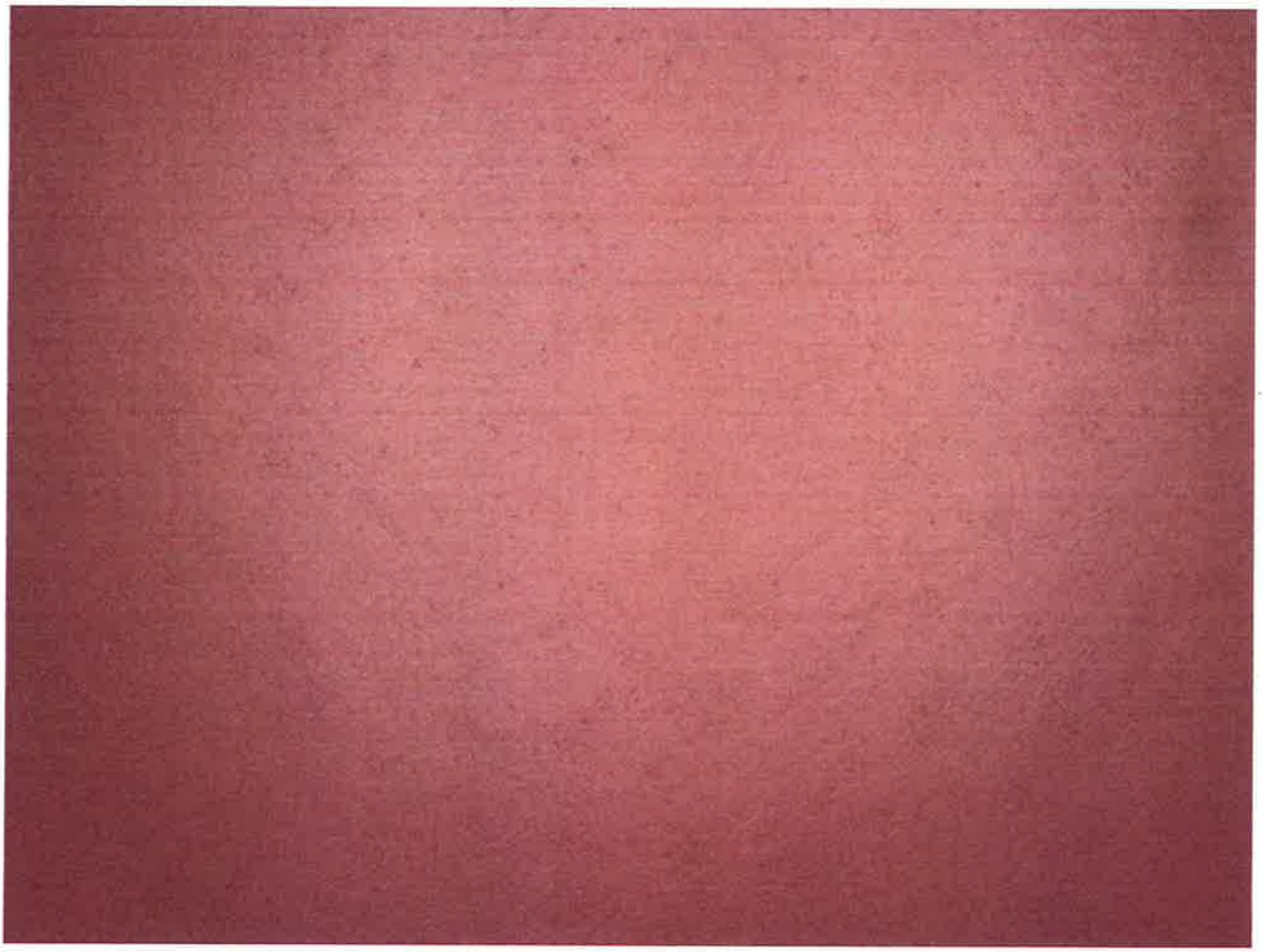
7. CONCLUSIONS

The resulted virus reduction factor (R) of the "Airzone Purifying Solution" device for the time tested (24 hours) in this study is 98.769% (1.91 log) due to the experiments duration in the Work House.

8. APPENDIXES

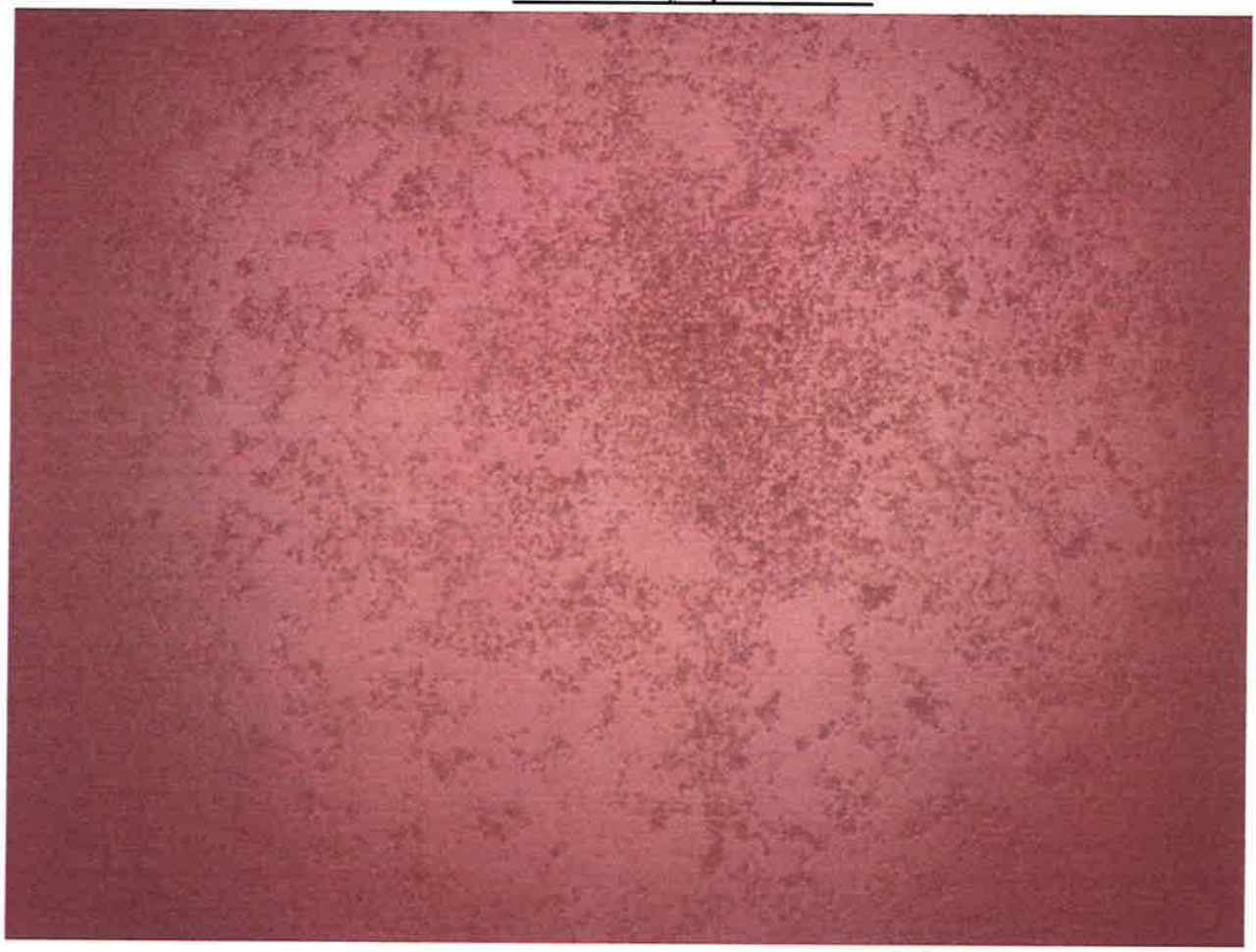
APPENDIX 1

Cells without cytopathic effect



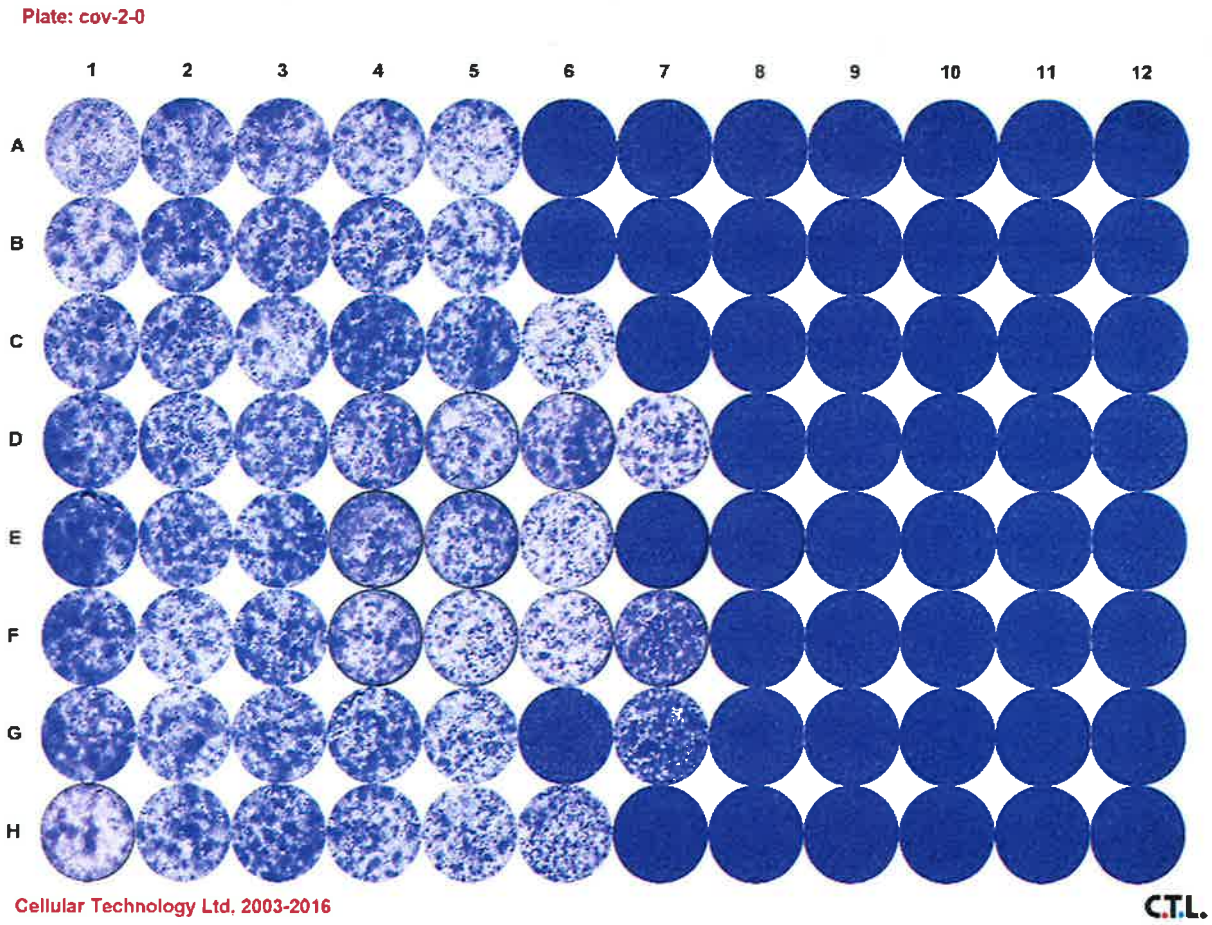
APPENDIX 1 (Cont'd)

Cells with cytopathic effect



APPENDIX 1

Plates of titration by TCID50/mL with Sars-Cov-2



TEST HOUSE
INSTALLATION & USER MANUAL

Versions Control			
Version	Elaborated by	Date	
v01	Juan (Taller)	11/05/2022	-
v02	Juan A. Bandera (Energía IAQ)	12/05/2022	

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Contents

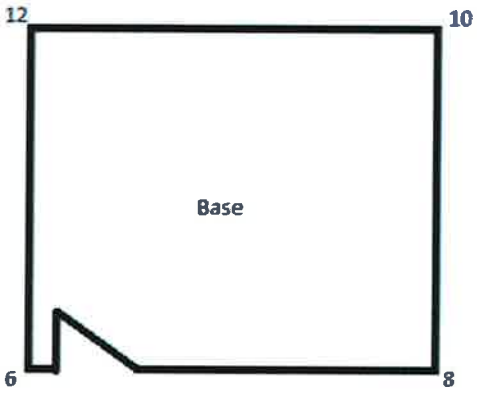
Introduction

This document contains the installation manual to build the test house with the materials provided by Airzone. The test house will be used for a SARS-CoV-2 virus inactivation test performed by Texcell in their facilities with Airzone ionization device to find the reduction value corresponding to a percentage value of viral inactivation caused by Airzone ionization device.

Installation steps

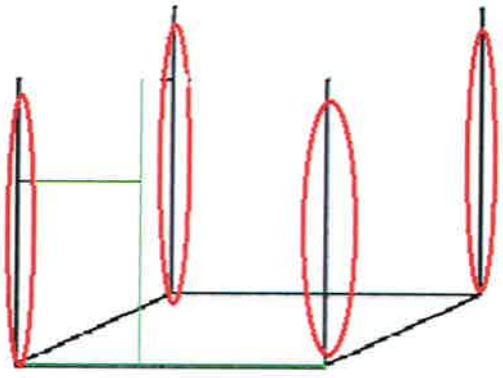
Step 1

Assemble the base frame. To do this you have to join the square bars with the corners, using the screws that come in the box with the help of an 8 mm socket spanner.



Step 2

Assemble the perimeter pillars **and** door frame.



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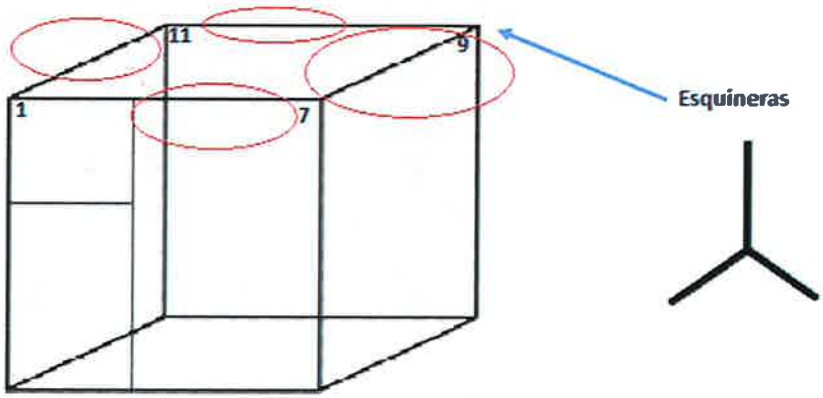
Step 3

Install the crossbars that join the perimeter pillars using the corner pieces (*esquineras*). Now, the main structure is completed.



Step 4

Place the door in its position and the white doorstops.

View of the door from the inside of the test house.

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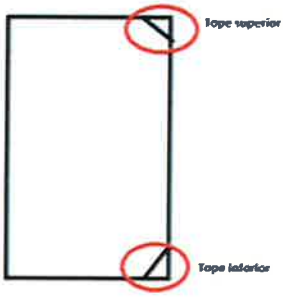
Upper doorstop (*Tope superior*)



Bottom doorstop (*Tope inferior*)

Step 5

Place and screw the ionization device module which consists of a fan, a damper where the Airzone ionization device is located and a grille. It also has an Airzone thermostat, but it is not connected to anything.

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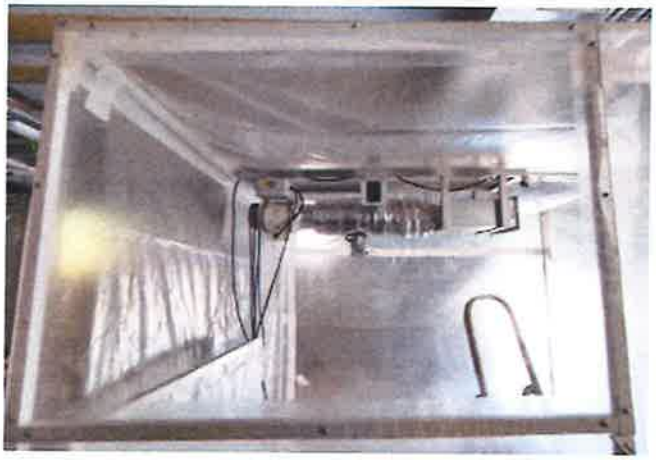
Step 6

Place the protective plastic, inserting it from above being careful not to break it and adjusting the corners correctly.

Step 7

Install the plexiglass window above the door and screw it.



User Manual

Once the installation is completed, then the power cable must be connected to the 220 V supply.

Both the fan and the ionization device are interconnected, so just one power cable is needed.

In order to start the fan and the ionization device, the switch on the grey box must be turned to the "12V+" sign on the left. Please, check the last picture.

The gauze with the SARS-CoV-2 virus must be placed 1.8 m from the ground and 10 cm from the plastic cover. **This information is to be confirmed before the test.**

Pictures of the Airzone ionization device which consists of a fan, a damper where the ionization device is located, a grille with its plenum, an electronic box and one Airzone Blueface thermostat.

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