

## CERTIFICATE OF INVESTIGATION STUDY

### STUDY OF VALIDATION OF THE EFFICIENCY OF HAIER UVC GENERATOR ON SARS-COV-2 BY NO GLP VIRAL CLEARANCE STUDY (FIO)

Study number: 1198/01

Study report for:

**Sponsor: Qingdao Haier Air Conditioner General Co., LTD**

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Haier High-tech Industrial Park, Laoshan District  
Qingdao City, Shandong Province  
China

Submitted by:

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**In Haier conditions of use:**

**An inactivation and reduction titer of more 4.79 Log (99.998%) of Sars-Cov-2 in 1 hour was demonstrated in a 45L Box.**

Texcell

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**1. PERSONNEL INVOLVED IN THE PROJECT****From Texcell**



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 Bruno BOISSON  
 Test Facility Management




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 Date of signature

**Virology and Titration Group:**  
 Luck TY

**2. MATERIAL AND METHODS****2.1. VIRUSES (SPIKING TEST SYSTEM)****Sars-Cov-2**

INMI (Institut National des Maladies Infectieuses), reference Human 2019-CoV, strain 2019 nCoV/Italy-INMI

**2.1.1. Virus stock**

Main characteristics of Sars-Cov-2 virus are described in the table below:

<b>Virus</b>	<b>Sars-Cov-2</b>
<b>Family</b>	<i>Coronaviridae</i>
<b>Subfamily/Genus</b>	Coronavirus
<b>Host</b>	Human
<b>Genome</b>	single-stranded RNA
<b>Envelope</b>	Enveloped
<b>Size in diameter</b>	80-120 nm
<b>Resistance to physico-chemical treatment</b>	Medium
<b>Titration cells</b>	Vero Institut Pasteur, Laboratoire de Virologie Medicale

**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).  
Continuous cell line from kidney of an African green monkey.

### 3. EXPERIMENTS

#### 3.1. PROCESS DESIGN

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

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

Haier device

(A) Inactivation

- Virus slide glass preparation with SARS-COV-2
- Place virus slide glass and Haier device into a sealed system
- After the required incubation time (4h, 2h, 1h) the virus slide glass is removed
- virus slide glass in the test group and control group was eluted thoroughly with 10ml eluent, respectively
- Virus titration and calculation virus reduction factor R  
Process Duration (X) = 4h, 2h and 1

#### **Titration:**

Virus titration will be performed by end-point dilution assay (TCID<sub>50</sub>) for SARS-COV-2.

Samples obtained during the spiking experiments will be titrated immediately with the appropriate controls (depending of the Preliminary Assay result).

### 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<sub>2</sub> 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<sub>50</sub>/mL) is calculated using the Spearman-Kärber formula.

#### **\*Large Volume titration (LVT)**

LVT assay could be performed (at the sponsor 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<sub>2</sub> 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<sub>50</sub>/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<sup>5</sup> – 10<sup>7</sup> TCID<sub>50</sub>/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 (N2 and N3) 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  $\leq -70^{\circ}\text{C}$  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<sub>50</sub>/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  $\leq -70^{\circ}\text{C}$  until titration. These storage controls are prepared and titrated with the experiments generated samples

After titration, there is no storage effect on virus, when 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  $1\text{Log}_{10}$ .

**\*Viral interference assay(s)**

The evaluation of the potential interference of the lowest non-cytotoxic dilution of the sample on virus during the incubation period is carried out by comparison of the results obtained during the titration of the virus stock with medium, and the virus stock with diluted sample (final material/starting material and/or intermediate product).

The evaluation is carried out with the final material(s) (obtained from the mock run).

The results are used for the calculation of the reduction factor of the step.

The study of sample interference on virus titration is described as follows:

- First, the virus reference control (titration reference) at a low final concentration of approximately  $10^5$ - $10^7$  TCID<sub>50</sub>/mL, is titrated in the usual conditions i.e. serial 3-fold dilutions using medium as diluting medium
- In parallel, the same virus reference control is titrated by serial 3-fold dilutions using the lowest non-cytotoxic dilution of the sample to be tested as diluting medium.

The evaluation of the potential interference of the lowest non-cytotoxic dilution of the sample with the virus during the incubation period is carried out by comparison of the titers obtained in medium versus titers obtained in diluted sample.

For the defined non-cytotoxic dilution of the sample, there is no interference when the difference between the two titers is less than or equal to  $1\text{Log}_{10}$ . This non-cytotoxic and non-interfering dilution is then used for the calculation of the infectious titer of the sample.

**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<sub>50</sub>/mL (depending on virus), is used as reference control.

The titration assay is retained when:

- the cell reference control (N1) for each titration plate is conform 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<sub>50</sub> using Spearman-Kärber formula**

The TCID<sub>50</sub> 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<sub>50</sub>/mL), is defined by its mean value, m(T), and its confidence interval.

m(T) can be calculated according to the following formula:

$$m(T) = \frac{1}{V_0} 10^{m(a)}$$

with  $v_0$  = 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

$$m(a) = T_{SK} = -a_0 + \frac{k}{2} - k \sum_i p_i \quad \text{and} \quad S(a)^2 = k^2 \sum_i \frac{p_i(1-p_i)}{n_i - 1}$$

with:

- $a$  =  $\text{Log}_{10}$  of the titer relative to the test volume
- $a_0$  =  $\text{Log}_{10}$  of the reciprocal of the lowest dilution for which all wells are positive
- $k$  =  $\text{Log}_{10}$  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:

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

$$a^{\max} = m(a) + 2 S(a)$$

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

$$T^{\min} \leq T \leq T^{\max} \quad \text{with:}$$

$$T^{\min} = \frac{1}{V_0} 10^{a^{\min}}; \quad T^{\max} = \frac{1}{V_0} 10^{a^{\max}}$$

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  $\geq 12.5\%$  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.

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

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

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:

$$T_{MaxL} = (\text{Ln}(N/P) \times d \times (1000/v)) / \text{Ln}2$$

with:

$T_{MaxL}$  = titer relative to the test volume (TCID<sub>50</sub>/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 ≈ 0.69 = corrective factor to convert PFU/mL into TCID<sub>50</sub>/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):

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

$$\text{i.e.} \quad dl = \frac{-\ln(0.05)}{V_c d_c}$$

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  $\ln 2$  to be converted into TCID<sub>50</sub>/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),
- V<sub>t</sub> = 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<sub>i</sub> and DL<sub>f</sub> 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 Log<sub>10</sub> of the ratio of the virus load (L<sub>i</sub>) in the pre-treatment material (starting material) and the virus load (L<sub>f</sub>) 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, [R<sub>min</sub> ≤ R ≤ R<sub>max</sub>]. If R<sub>min</sub> and/or R<sub>max</sub> 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**.  $m(R_o)$  is defined as the  $\text{Log}_{10}$  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 ( $V_i$ )  $\pm$  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)
<b>Case 1</b>		R is evaluated according to the three subcases of Case 2, with $X_i = X_{cm}$
<b>Case 2</b> $m(R_o) \leq 1$	$m(x_f) < DL_f$	$R_t > \text{Log}_{10}\left(\frac{m(X_i)}{DL_f}\right)$ with $R_{min} \leq R_t \leq R_{max}$
	$X_i^{min} \geq X_f^{max}$	$m(R_t) = \text{Log}_{10}\left(\frac{m(X_i)}{m(X_f)}\right)$ with $R_{min} \leq R_t \leq R_{max}$
	$X_i^{min} < X_f^{max}$	$m(R_t)$ is calculated according to subcase 2 $R_t \approx 0$ if $R_{min}, R_t$ or $R_{max} < 0$ (negative value)
<b>Case 3</b> $m(R_o) \geq 1$	$m(X_i) < DL_i$	$R_t$ cannot be evaluated $R_o \geq \text{Log}\left(\frac{m(X_{cm})}{DL_i}\right)$ with $R_{min} \leq R_o \leq R_{max}$
	$m(X_i) \geq DL_i$	$R_t$ 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 $X_i = X_{cm}$ $m(R_o) = \text{Log}_{10}\left(\frac{m(X_{cm})}{m(X_i)}\right)$ with $R_{min} \leq R_o \leq R_{max}$

$X_i$  = 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  
 $R_{min} = R - 2 \sqrt{\text{var } Ti + \text{var } Tf}$   
 $R_{max} = R + 2 \sqrt{\text{var } Ti + \text{var } Tf}$   
 $\text{Var} = \text{Variance} = S(a)^2 = k^2 \sum_i \frac{p_i(1 - p_i)}{n_i - 1}$

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. TABLES OF RESULTS

Table 1: **STUDY OF EVALUATION OF THE EFFICIENCY OF HAIER DEVICES ON THE SARS-COV2 BY NO GLP VIRAL CLEARANCE STUDY (FIO)**

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	Definition
<b>Negative controls</b>						
<b>Positive controls</b>						
V1	Spiked (addition of virus stock) medium	TV1	5.71 $5.48 \leq T \leq 5.95$	NA	Virus stock control before process	
V2	Spiked (addition of virus stock) medium	TV2	5.36 $5.14 \leq T \leq 5.58$	NA	Virus stock control after 4 hours (old control)	
L1	Spiked (addition of virus stock) glass and recovery	TL1	5.95 $5.80 \leq T \leq 6.11$	NA	Virus stock recovery control before process	
L2	Spiked (addition of virus stock) glass and recovery	TL2	5.12 $4.90 \leq T \leq 5.34$	NA	Virus stock recovery control after 4 hours (old control)	

Infectious titers are expressed as  $\text{Log}_{10}$  50% tissue culture infectious dose per milliliter ( $\text{Log}_{10}$  TCID<sub>50</sub>/mL).

Table 1 (continued): **STUDY OF EVALUATION OF THE EFFICIENCY OF HAIER DEVICES ON THE SARS-COV2 BY NO GLP VIRAL CLEARANCE STUDY (FIO)**

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	
<b>Comparison of positive controls</b>						
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	TV2	5.36	$5.14 \leq T \leq 5.58$	Not significant = 0.36	Experiment effect on virus in hold control (comparison TV1 and TV2)
L1	Spiked (addition of virus stock) glass and recovery	TL1	5.95	$5.80 \leq T \leq 6.11$	Not significant ~ 0	Experiment effect on virus in recovery glass control (comparison TV1 and TL1)
L2	Spiked (addition of virus stock) glass and recovery	TL2	5.12	$4.90 \leq T \leq 5.34$	Not significant = 0.83	Experiment effect on virus in recovery glass hold control (comparison TL1 and TL2)

Table 1 (continued): **STUDY OF EVALUATION OF THE EFFICIENCY OF HAIR DEVICES ON THE SARS-COV2 BY NO GLP VIRAL CLEARANCE STUDY (FIO)**

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 <sub>10</sub> (L1) – Log <sub>10</sub> (LS)		
<b>Positive control</b>									
L1	Spiked (addition of virus stock) glass and recovery	1	TL1	5.80 ≤ T ≤ 6.11	LL1	5.80 ≤ T ≤ 6.11	Virus stock recovery glass control		
<b>STUDY</b>									
<b>Samples collected, recovery and diluted: Clearance factor</b>									
S1	1 hour	1	TS1	1.16	NA	LS1	1.16	NA (no virus)	R > 4.79
S2	2 hours	1	TS2	1.16	NA	LS2	1.16	NA (no virus)	R > 4.79
S3	4 hours	1	TS3	1.16	NA	LS3	1.16	NA (no virus)	R > 4.79

Infectious titers are expressed as 50% tissue culture infectious dose per milliliter (Log<sub>10</sub> TCID<sub>50</sub>/mL). Viral loads are expressed as 50% tissue culture infectious dose (Log<sub>10</sub> TCID<sub>50</sub>).

**APPENDED**

**Cells without effect cytho pathogen**



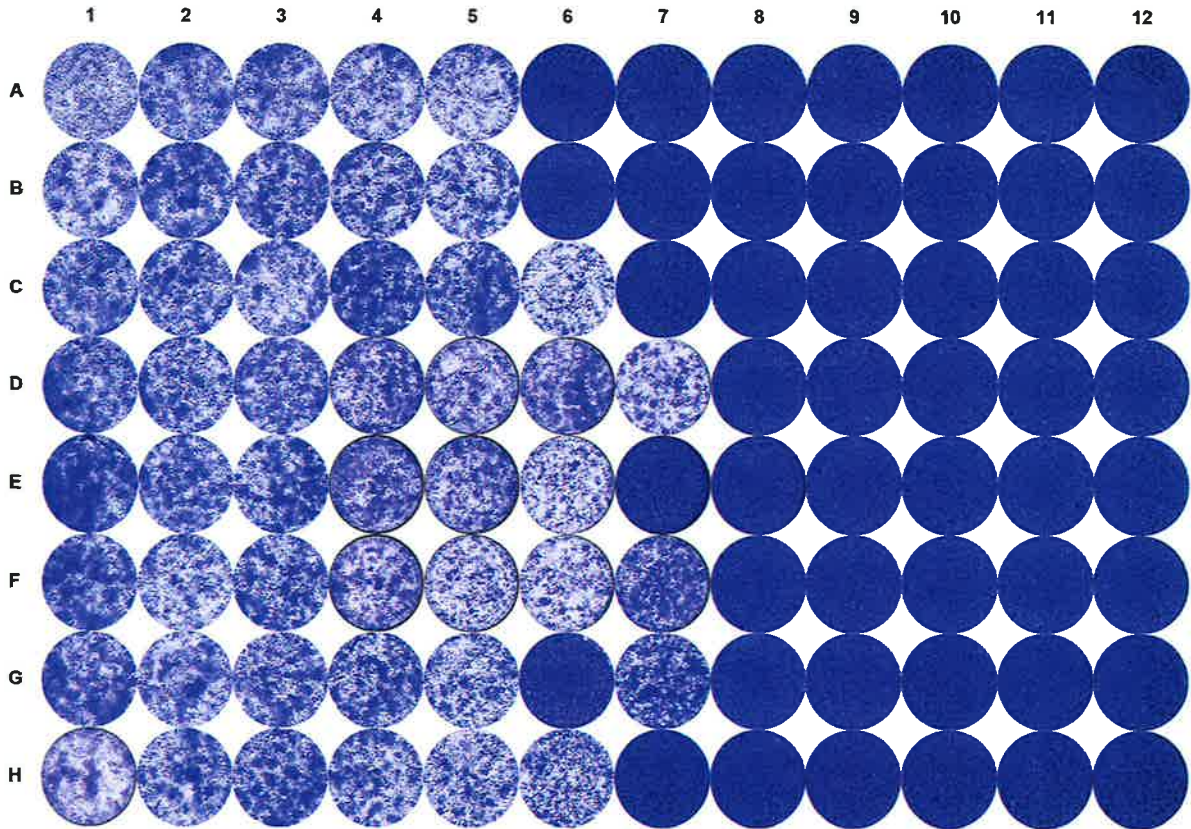


Cells with effect cytho pathogen



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

Plate: cov-2-0



Cellular Technology Ltd, 2003-2016

CTL.

