FONdation pour le
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STUDY 20-2742

EVALUATION OF THE VIRUCIDAL ACTIVITY OF NON-POROUS SURFACES AGAINST HUMAN CORONAVIRUS 229 E ACCORDING TO THE METHODOLOGY OF STANDARD ISO 21702 (MAY 2019)

Client

NANOSELFCLEAN ltd (UK)
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Test laboratory

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I - TEST LABORATORY IDENTIFICATION

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II -SAMPLE IDENTIFICATION

- Product name:	PVC Vinyl Surface non treated
- Reference:	not communicated
- Batch:	not communicated
- Date of receipt:	Sept/09/2020
- Internal code:	20-2742-1
- Product name:	PVC Vinyl Surface treated
- Reference:	not communicated
- Batch:	not communicated
- Date of receipt:	Sept/09/2020
- Internal code:	20-2742-2
- Supplier:	NANOSELFCLEAN ltd
- Period of testing:	October 2020

III - TEST METHOD

III-1 VIRUS

Name:	Human Coronavirus 229 E
Origin:	ATCC
Reference:	VR-740
Supplier batch number:	58505270
Internal batch number:	SS-2-210920 (Passage 2)

II-2- Recipient cells

Name:	Vero
Origin:	ATCC
Reference:	CCL-81
Supplier batch number:	3372621
Internal batch number:	WCB-090708 (Passage 16)

IV -TEST CONDITIONS

- Contact times: 24 hours

- Test temperature: 25°C ± 1°C

V- TEST METHOD

V-1 Control of cytotoxicity

2.5 ml of neutralizing medium are added to 3 untreated and 3 treated samples. The samples are washed 4 times with the neutralizing medium.
A ten-fold serial dilution is made to check the absence of cellular cytotoxicity.

V-2 Control of the sensitivity of the cells to the virus and stopping the antiviral activity

2.5 ml of neutralizing medium are added to 3 untreated and 3 treated samples.
The samples are washed 4 times with the neutralizing medium. Then 1.98 ml of recovery medium are mixed with 20 µl of the virus suspension prepared at a concentration of 4 to 6 $.10^6$ TCID50/ml. After 30 min of 25°C incubation, tubs with virus solution are maintained in ice before titration.

V-3 Contact virus/surface

Each sample with a surface area of 5 cm x 5 cm (control and test samples) is placed in a sterile glass Petri dish.
- 400 µl of the viral suspension are deposited on each surface and spread over 16 cm2 using a 4 x 4 cm film to reduce desiccation of the inoculum.

V-4 Recovery of the viral film

After incubation, 3.6 ml of a neutralizing solution (frozen culture medium) are added to the samples in order to recover viable viruses.
The titration of the remaining viable viruses is then carried out immediately.

V-5 Viral Titer

The titration technique is indicated in the standard NF EN 14476 + A2 (July 2019).
A ten-fold serial dilution of the viral suspensions is made in the cell culture medium in neutral glass tubes in order to limit the phenomena of virus adsorption on the surfaces.
Titration is performed on 96-well microplates. Each dilution is transfer in 8 wells.

V-6 Viral load calculation

The assay is performed by the microplate method of suspension cells. The cytopathic effect is determined at least 4 days of culture.
The number of infectious units is estimated with the SPEARMAN-KÄRBER method by calculating the negative logarithm of the 50% limit point ($lgTCID_{50}$) using the following formula:
$lgTCID_{50}$ = Negative logarithm of the highest concentration of virus used - [(Sum of % assigned to each dilution/100 - 0.5) X (lg of dilution)]

The following tests are carried out 3 times.

VI- RESULTS

VI-1 Validation

VI-1-1 Control of cytotoxicity

No cytotoxicity was observed on the cells after contact of the culture medium with treated and untreated samples.

VI-1-2 Control of the sensitivity of cells to viruses and cessation of virucidal activity

The difference between the average titers (lg TCID50) of the neutralizing solution controls of and the sensitivity titers average of the treated and untreated surfaces must be less than or equal to 0.5 lg.

Neutralizing solution control

- Control 1 : lg $TCID_{50}$ = 3.75
- Control 2 : lg $TCID_{50}$ = 3.50
- Control 3 : lg $TCID_{50}$ = 3.50

lg TCID50 neutralizing solution control average = 3.58

Control Sensitivity of untreated surfaces

- Control 1 : lg $TCID_{50}$ = 3.50
- Control 2: lg $TCID_{50}$ = 3.75
- Control 3 : lg $TCID_{50}$ = 3.63

lg $TCID_{50}$ Sensitivity of untreated surfaces average = 3.63

Titer neutralizing solution average - Sensitivity of untreated surfaces average = -0.05

Difference ≤ 0.5 lg (verification valid)

Test Sensitivity and cytotoxicity of treated surfaces

- Control 1 : lg $TCID_{50}$ = 3.63
- Control 2 : lg $TCID_{50}$ = 3.50
- Control 3 : lg $TCID_{50}$ = 3.25

Average lg TCID50 Control Sensitivity = 3.46

Titer neutralizing solution average - Sensitivity of treated surfaces average = 0.12

Difference ≤ 0.5 lg (verification valid)

VI-1-3 T0 control

- Control 1 : lg $TCID_{50}$ = 6.00
- Control 2 : lg $TCID_{50}$ = 6.13
- Control 3 : lg $TCID_{50}$ = 6.00
-

lg $TCID_{50}$ T0 average =

$$\frac{\text{Maximum viral title - Minimum viral title}}{\text{Average of the 3 viral titles}} = 0.01$$

The titer (lg DICT50) of the 3 tests at T0 must be homogeneous
Maximum viral titer - Minimum viral titer / Average of the 3 viral titer ≤ 0,2.

TCID50 average /ml = $1{,}10.10^7$

Average $TCID_{50}$ /ml = $10^{\text{average log10 DCICT50}} \times 10$

Infectivity titer ($TCID_{50}/cm^2$) = $\frac{TCID_{50}/ml * \text{Volume de récupération (4ml)}}{\text{Surface (16 cm}^2\text{)}} = 2.7\ 10^6$

Infectivity titer at T0 ($TCID_{50}/cm^2$) must be between $8.94\ 10^5$ and $4.46\ 10^6$

VI-2 Tests

Control 24 hours :

- Control 1 : lg $TCID_{50}$ = 5.75
- Control 2 : lg $TCID_{50}$ = 6.00
- Control 3 : lg $TCID_{50}$ = 5.88

lg $TCID_{50}$ T0 average = 5.88

Average $TCID_{50}$ /ml = $7.59\ 10^6$

Average $TCID_{50}$ /ml = $10^{\text{average log10 DCICT50}} \times 10$

Infectivity titer ($TCID50/cm^2$) = $\frac{TCID50/ml * \text{Recovery volume (4ml)}}{\text{Surface (16 cm}^2\text{)}} = 1.89\ 10^6$

Infectivity titer at contact time 24 hours ($TCID50/cm^2$) must be greater than $2.21\ 10^3$

Test T 24 hours

- Assay 1 : lg $TCID_{50}$ = 3.13
- Assay 2 : lg $TCID_{50}$ = 3.00
- Assay 3 : lg $TCID_{50}$ = 2.88

lg $TCID_{50}$ Assay average = 3.00

R = lg TCID50 24 hours average - lg TCID50 Test 24 hours average = 2.88 lg

VII-CONCLUSION

According to the methodology of the ISO 21702 standard (May 2019), contact of the surface PVC Vinyl treated with the strain of Human coronavirus 229 E induces a reduction of the viral titer of 2.88 lg at the contact time 24 hours.

The treatment of the surface PVC Vinyl treated induces a reduction of the viral load of 99.87 % at the 24 hours contact time.