



Toulouse, December 29th 2021

STUDY 20 - 2793

TEST REPORT N° 21-1803

Standard NF EN 17272 (Avril 2020)
Determination of virucidal activity for aerial surface disinfection processes
Human Coronavirus 229E

Medical area

Clean condition / Obligatory conditions

Promotor

OXY'PHARM
829 rue Marcel Paul
94500 CHAMPIGNY SUR MARNE

Test Laboratory

FONDEREPHAR
Faculté des Sciences Pharmaceutiques
35 Chemin des Maraîchers
31062 TOULOUSE cedex 9

Dr. Laila HADDIOUI
Assay Manager

Dr Jocelyne BACARIA
Quality Manager

1. Test Laboratory

Fondation pour le Développement de la recherche en Pharmacie (FONDEREPHAR)
Faculté des Sciences Pharmaceutiques, 35 chemin des Maraîchers 31062 Toulouse cedex 9,
France

2. Identification of the aerial disinfection system

Product : **NOCOLYSE FOOD**
Batch : A291020FD/1
Expiry date : 12/2022
Date of receipt : January/04/2021
Internal code : 20-2793-1

Device : **NOCOSPRAY**
Serial number : 172X731

Concentration of disinfectant in the room: 5 mL/m³
One treatment - recovery of the discs after 1 hour waiting at the end of the diffusion.

Promotor : OXY'PHARM
Storage conditions: Ambient temperature
Period of testing: December 2021
Active Substance: Hydrogen peroxide (7.9%)

3- Experimental conditions

3-1 Virus/Receiving cells

Virus

Name Human Coronavirus 229E
Origin : ATCC
ATCC reference: VR-740
Batch number supplier: 58505270
Internal number Batch: SS-210920 (passage N°2)

Receiving cells

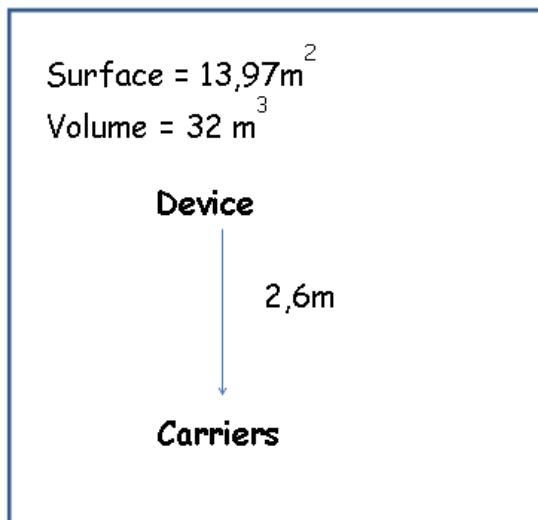
Name Vero cells
Origin : ATCC
ATCC reference: CCL-81
Batch number ATCC: 3372621
Internal number Batch: WCB-080205 (passage N°12)

3-2 Carriers

The selected tests surfaces are stainless steel discs, flats, corresponding to the requirements of paragraph 5.2.3.1 of the standard. The suppliers are MERCIER CLAUSSE.

3-3 Conditions of use of the device/product

- Room :



Relative humidity: start of test 64% - end of test 60% (requirement 50 - 70%).

Temperature: start of test 18.5°C - end of test 18.4°C (requirement 20°C±2°C).

Test room volume: 32m³

- Carriers placement :

Distance between the device and the carriers: 2,6m (Annex B - table B.1).

The carriers were placed at a height of 1.31m, in a vertical position, towards the opposite side of the device

3-4 Interfering substance and culture media

- Interfering substance: BSA fraction V at 0,3g/l (Batch N°437)
-Culture media: MEM (Batch N°2980)

4- Validations Protocol

4-1 Control of sensitivity of cells to virus

- Add one volume of solution S or PBS + one volume of cellular suspension at 2.10^5 cells/ml for one hour in water bath at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$
- The cells are centrifuged at 1600trs/min for 10 min and resuspended in culture media
- The virus is diluted from 1/4 to 1/4 on a 96-well microplate (15 dilutions)
- Add 100 μl of cell suspension treated (Solution S) or not treated (PBS control) to each well of the microplate
- Incubate for 72 hours

The difference of titre reduction between cells treated by the solution S and cells treated by PBS shall be $< 1 \text{ lg}$.

4-2 Control of efficiency for suppression of disinfectant activity

- Add 1 volume of BSA + 1 volume of virus suspension + 1 volume of solution S or distilled water
- Leave the mixture in the ice bath for 60 min at room temperature

5- Titration method

- Titrate the virus (method titration on cell in suspension) by following steps:
- Serial dilutions (1/4) are realized with culture medium in the glass tube
- Transfer 0,1 ml of each dilution into eight wells of a microplate plaque
- The last row of eight wells will receive 0,1 ml of culture medium (control untreated cells)
- Add 0,1 ml of cell suspension at 2.10^5 cell/ml .
- Incubate for 72 hours at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$ under $5\% \text{ CO}_2 \pm 2\%$.
- The viral cytopathic effect is read by using an inverted microscope

The estimated of infectious unite is determined by method KARBET-SPAERMAN calculating the negative logarithm of 50% endpoint (lgDICT_{50}) by the following formula:

lgDICT_{50} = negative logarithm of the highest concentration of virus - $[(\text{Sum of \% affected to each dilution}/100 - 0.5) \times (\text{lg dilution})]$

6- Results

Virus suspension titre assay: IgDICT50 = 8.13

No cytotoxicity was observed on the carrier without treatment which has been pretreated with the aerial disinfection system according to treatment.

	Degree of cytopathogenic effect (log)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1	7.00	
Carrier 2	7.13	
Average	7.07	Difference <1 lg.
- Without traitement (S2)		
Carrier 1	6.88	
Efficiency for suppression of disinfectant activity		
- With treatment (D1)		
Carrier1	6.38	
Carrier 2	6.63	
Average	6.50	Difference <0,5 lg.
- Without traitement (D2)		
Carrier 1	6.88	
Test control		
Carrier1	5.50	
Carrier 2	5.00	
Average	5.25	
Assay		
Support 1	0.88	
Support 2	1.00	
Support 3	0.88	
Average	0.92	4.33

7- Conclusion

According to the conditions of test for the standard NF EN 17272 (Avril 2020), the couple device/product: NOCOSPRAY N° serial 172X731/NOCOLYSE FOOD - Batch N° A291020FD/1 for a use in medical area under clean condition, leads to a virucidal activity against Human coronavirus 229E (log reduction ≥ 4), after treatment at 5 mL/m³ and 1 hour waiting time.