FONdation pour le DEveloppement de la REcherche PHARmaceutique

Toulouse, January 6th 2023

STUDY 21-2920

TEST REPORT N° 23-1991

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

Medical area

Clean condition / Additional conditions

Promotor	**Company registration SIREN 448974253** **829 rue Marcel Paul** **94500 CHAMPIGNY SUR MARNE** **FRANCE**
Test Laboratory	**FONDEREPHAR** **Faculté des Sciences Pharmaceutiques** **35 Chemin des Maraîchers** **31062 TOULOUSE cedex 9** **France**

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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 :	**Formula N-1**
Batch :	A160421SB
Expiry date :	04/2023
Date of receipt :	April/21/2021
Internal code :	21-2920-2
Device :	**PX-00**
Serial number	109Z099

Concentration of disinfectant in the room: 5 mL/m^3

One treatment - recovery of the discs after 120 minutes waiting at the end of the diffusion.

Promotor :	Company registration SIREN 448974253
Storage conditions:	Ambiant temperature
Period of testing:	May 2021
Active Substance:	Hydrogen peroxide (6%)

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-2-280520 (passage N°2)

Receiving cells

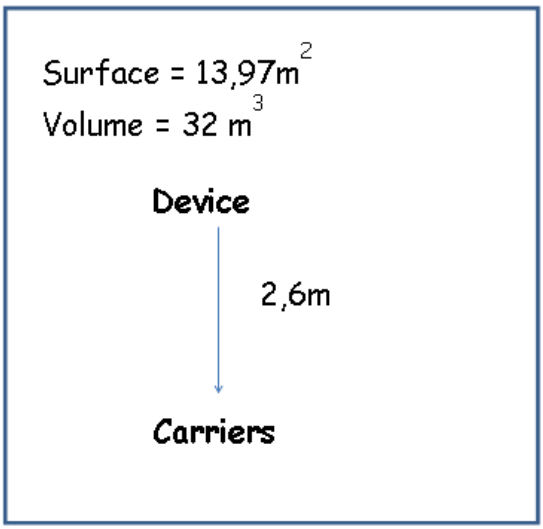
Name	Vero cells
Origin :	ATCC
ATCC reference:	CCL-81
Batch number ATCC:	3372621
Internal number Batch:	WCB-090708 (passage N°22)

3-2 Carriers

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

3-3 Conditions of use of the device/product

- **Room** :



Relative humidity : start of test 54% - end of test 51% (requirements 40 - 80%).
Temperature : start of test 20.1°C - end of test 18.5°C (requirements 18 – 22°C).
Test room volume: 32m^3

- **Carriers placement** :

Distance between the device and the carriers: 2,6m.
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°390)
-Culture media:	EMEM (Batches N°2842 and 2840)

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°C±1°C
- The cells are centrifuged at 1600trs/min for 10 min and resuspended in culture media
- The virus is diluted from 1/10 to 1/10 on a 96-well microplate (10 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 title reduction between cells treated by the solution S and cells treated by PBS shall be < 1 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 (1/10) 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 2.10^5cell/ml.
- Incubate for 72 hours at 36 ° C ± 1 ° C under 5% CO_2 ± 2%.
- The viral cytopathic effect is read by using an inverted microscope

The estimated of infectious unite is determined by method KARBER-SPAERMAN calculating the negative logarithm of 50% endpoint (lgDIC50) by the following formula:

lgDICT50 = negative logarithm of the highest concentration of virus - [(Sum of% affected to each dilution/100 - 0.5) X (lg dilution)]

6- Results

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

Test 05/07/2021 Start : 20,1°C / 54% RH End : 18,5°C / 51% RH	Degree of cytopathogenic effect (lgDICT50)	Logarithmic reduction
Sensitivity of cells to virus **- With treatment (S1)** Carrier 1 Carrier 2 Average **- Without traitement (S2)** Carrier 1	 7.25 7.50 7.38 7.38	Difference <1 lg.
Efficiency for suppression of disinfectant activity **- With treatment (D1)** Carrier1 Carrier 2 Average **- Without traitement (D2)** Carrier 1	 6.88 7.00 6.94 7.13	Difference <0,5 lg.
Test control Carrier1 Carrier 2 Average	 6.88 6.75 6.82	
Assay Support 1 Support 2 Support 3 Average	 2.50 2.38 2.250 2.38	**4.44**

7- Conclusion

According to the conditions of test for the standard NF EN 17272 (April 2020), the couple device/product: Diffuser PX-00 serial number 109Z099 / Product Formula N-1 Batch A160421SB, in clean condition, for use in medical area, shows a virucidal activity against Human Coronavirus 229 E, after treatment at 5 mL/m^3 and 120 minutes of contact (reduction 4.44 lg).

These results are only valid for the product tested and apply to the sample as received.