FONdation pour le
DEveloppement de la
REcherche
PHARmaceutique

Système de management de la qualité
Certifié ISO 9001

Toulouse, April 1st 2020

TEST REPORT N° 20-1441

STUDY 20 - 2616

Standard NF T 72-281 (November 2014)
Determination of virucidal activity for aerial surface disinfection processes
Human Coronavirus

Medical area

Clean condition / Obligatory conditions

Promotor	**MICROBECLEAN** **1 bis rue des Longrais** **35520 LA-CHAPELLE-DES-FOUGERETZ**
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

FONDEREPHAR - Faculté des Sciences Pharmaceutiques – 35, chemin des Maraîchers – 31062 TOULOUSE cedex 9 - Email : contact@fonderephar.com - www.fonderephar.com
Tél. +33 (0)5 62 25 68 60 - Fax. +33 (0)5 61 25 95 72 - Siret 328 649 538 000 27 - APE 7211Z - N° TVA : FR 80328649538 - Association Loi 1901 n° W313014489

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 :	**MICROBECARE ™ 70-2**
Batch :	L091902 A
Expiry date :	Not precised
Date of receipt :	09/01/2020
Inetrnal code :	**20-2616-1**
Active(s) substance(s):	Octadecylaminodimethyltrimethoxysilylpropylammonium CAS 27668-52-8 Isopropanol CAS 67-63-0
Device :	**VP200ES (VICTORY)**
Serial number :	24475
Date or receipt :	09/01/2020
Internal code :	**20-2616-2**
Device pressure (manual use) :	8 bar - nozzle 40µ
Distance device/carrier :	80cm
Waiting time :	60 minutes
Room volume :	30 to 150 m^3
Promotor :	MICROBECLEAN
Storage conditions :	Ambiant temperature, darkness
Period of testing :	March 2020

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-1-110214 (passage N°1)

Receiving cells

Origin :	ATCC
Designation :	VERO cells
ATCC reference:	CCL-81
Batch number ATCC:	3372621
Internal number Batch:	WCB-041113 (passage N°33)

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 disinfection system use

- **Room** :

Relative humidity: start of trial 51% - end of trial 48% (requirements 40 - 80%).
Temperature: start of trial 21.7°C – end of trial 21.3°C (requirements 18 – 22°C).
Test room volume: 32m^3

- **Carriers** :

The carriers were placed in a vertical position, towards the device.

- **Amount of disinfectant diffusion**

According to the VICTORY VP200ES device features indicated by the manufaturer, the 40µ nozzle corresponds to a 3,4 oz/minute product diffusion.

According to the Promotor specifications: diffusion in a single pass, with a distance device/carrier of: 80cm – waiting time 60 minutes

3-4 Interfering substance and culture media

-Interfering substance:	BSA fraction V at 0,3g/l (Batch N°287)
-Culture media:	EMEM 2% SVF (Batch N°2495)

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 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

Faculté des Sciences Pharmaceutiques – 35 Chemin des Maraîchers – 31062 TOULOUSE Cedex 09
Tél. 05 62 25 68 60 Fax. 05 61 25 95 72 Email. contact@fonderephar.com

The difference of titre 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 (1/4) 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 2.10^5cell/ml.
- Incubate for 48 or 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

Virus suspension title assay: lgDICT50 8.25

No cytotoxicity was observed on the carrier without treatment which has been pretreated with the aerial disinfection system according to treatment (assay conditions: 1/50).

	Degree of cytopathogenic effect (log)	Logarithmic reduction
Sensitivity of cells to virus		
- With treatment (S1)		
Carrier 1	8.40	
Carrier 2	8.55	
Average	8.48	Difference <1 lg.
- Without traitement (S2)		
Carrier 1	7.72	
Efficiency for suppression of disinfectant activity		
- With treatment (D1)		
Carrier1	8.40	
Carrier 2	8.25	Difference <0,5 lg.
Average	8.33	
- Without traitement (D2)		
Carrier 1	7.95	
Test control		
Carrier1	6.23	
Carrier 2	6.23	
Average	6.23	
Assay		
Support 1	2.10	
Support 2	2.10	4.13
Support 3	2.10	
Average	2.10	

7- Conclusion

According to the conditions of test for the standard NF T 72-281 (November 2014), the couple device/product: VICTORY VP200ES N° serial 24475/MICROBECARE™ 70-2 Lot L091902A (Exp. not precised), for a use in medical area under clean condition, leds to a virucidal activity against Human Coronavirus 229E (log reduction ≥ 4), after a treatment with the 40µm nozzle – distance 80 cm – waiting time 60 minutes.