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
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2. Identification of the aerial disinfection system

Product: MICROBECARE™ 70-2
Batch: L091902 A
Expiry date: Not precised
Date of receipt: 09/01/2020
Internal 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 m³

Promotor: MICROBECLEAN

Storage conditions: Ambient 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: 32m³
- 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 manufacturer, 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
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 2.10^5 cell/ml.
  - Incubate for 48 or 72 hours at 36 °C ± 1 °C under 5% CO₂ ± 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:

\[ \text{lgDIC50} = \text{negative logarithm of the highest concentration of virus} - [(\text{Sum of % affected to each dilution}/100 - 0.5) \times (\text{lg dilution})] \]
6- Results

Virus suspension 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).

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<tr>
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<th>Degree of cytopathogenic effect (log)</th>
<th>Logarithmic reduction</th>
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<tbody>
<tr>
<td><strong>Sensitivity of cells to virus</strong></td>
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<tr>
<td>- With treatment (S1)</td>
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<tr>
<td>Carrier 1</td>
<td>8.40</td>
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<tr>
<td>Carrier 2</td>
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<tr>
<td>Average</td>
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<tr>
<td>- Without traitement (S2)</td>
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<td>Carrier 1</td>
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<td><strong>Efficiency for suppression of disinfectant activity</strong></td>
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<tr>
<td>- With treatment (D1)</td>
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<td>Average</td>
<td>8.33</td>
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<tr>
<td>- Without traitement (D2)</td>
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<td>Carrier 1</td>
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<td><strong>Test control</strong></td>
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<tr>
<td>Average</td>
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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, leads 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.