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Test results of the Nocolyse sporicide activity against Clostridium difficile using the ASTM 2197:2002 test method (vapour phase)

Study done by :

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1. Summary of the test

Tested product: Nocolyse/Nocospray System
Manufacturer: Airel
Method used: Derivative of ASTM E 2197-02
Carriers: Polystyrene petri dishes
Testing temperature: Room temperature (22 ± 2 °C)
Dilution: None required
Four rooms (2 rooms per batch) were studied with 5 positions per room

Results:

Table I: Test results of the Nocolyse sporicide activity against *Clostridium difficile* using the ASTM 2197:2002 test method (vapour phase)

Organisms Tested	Positions	Room Numbers			
		310	311	304	305
C. difficile ATCC 43598	1	7.00	6.00	5.37	5.63
	2	6.25	5.65	5.43	5.60
	3	6.88	5.49	5.55	5.66
	4	6.78	5.43	5.72	5.65
	5	5.06	5.36	5.51	5.62
C. difficile NAP1A/027	1	6.52	5.00	5.60	5.55
	2	5.89	5.17	5.52	6.00
	3	6.00	6.10	6.10	5.52
	4	5.74	6.10	5.74	5.64
	5	4.97	4.86	5.64	4.80

As the testing was not done in the laboratory, closable petri dishes were chosen in order to facilitate the transportation of the inoculum to and from the hospital test site.

2. Introduction

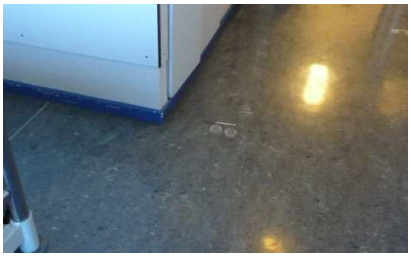
The objective of the study is to determine the efficacy, in a hospital milieu, of the Nocolyse/Nocospray Sytem against Clostridium difficile ATCC 43598 and NAP1A/027.

The Nocolyse/ Nocospray System consists of a portable plug-in machine (Nocospray) and a liquid hard surface disinfectant (Nocolyse).The disinfecting liquid Nocolyse is nebulized at 37ºC to form a dry vapour which is then propelled by the Nocospray turbine at 80m/sec permitting a rapid distribution throughout the target space.

The Microbiology testing was conducted at the Microbiology Laboratory of UCL by professor M. Delmee. The field testing was done at the Iris-Sud Hospital, Bracops in Bruxelles with the collaboration of doctor J.M. Hubrechts.

Test Method

The testing of the vapour was done in four standard hospital rooms with petri dishes of each strain located in five different positions. The population of each strain was counted on the carriers and compared to the control carriers not exposed to the Nocolyse/Nocospray System. The test was done using a derivative of the quantitative carrier test method, ASTM E 2197 (2002).



3. Test Product

Name: NOCOLYSE

Manufacturer: Airel, Champigny-Sur-Marne, France

Active Material: 6% solution of hydrogen peroxide.

Principal: Liquid Nocolyse is vaporized at 37°C into a dry vapour within the space to be disinfected.

4. Tested microorganisms

The microbial strains used for the testing are illustrated in Table II

Table II: Microbial strains utilized

Microorganisms	**Strains**	**Provider**	**Lot Numbers**
Clostridium difficile	ATCC 43598 (B+/A-)	Prof. M. DELMEE UCL	1470
Clostridium difficile	NAP1A/027	M. WARNY ACAMBIS	1067

5. Study Facility

The hospital test site was at the Iris Sud Hospital, Bracops in Bruxelles. Four standard hospital rooms were used for the test (refer to Annexe 1). The carriers were prepared and analyzed at the Microbiology Lab at the Catholique Louvain University.

Strains :

Two strains of *Clostridium difficile* were used, the Nord American epidemic strain NAP1A/027 and strain ATCC 43598 (strain B+/A-).

Preparation of the spores:

Inoculate 10 mL of Brain Heat Infusion broth (BHI) – reference BD BBL 211059 – with a colony of *Clostridium difficile* grown on a Columbia agar 24 hours (BD 017423). After 24 hours of anaerobic incubation, dilute the broth 50 times in BHI and allow the colonies to grow for 10 days anaerobically. Centrifuge to reduce 10 times.
Homogenize well and place 1 mL of the solution in an empty petri dish. Add 9 mL of absolute ethanol, mix well and let it evaporate slowly (laminar flow).

Neutralization:

Even though a neutralization process was not necessary due to the rapid degradation of hydrogen peroxide vapour particles at a temperature of 37°C, a neutralization was done in order to follow lab procedures. A neutralization was done right after exposure of the carriers to the Nocolyse/Nocospray System with 10mL of Letheen broth (polysorbate 80, reference BD 268110).

Spore Count:

The number of spores of the control carrier and the exposed carrier were counted after successive dilutions of 10 in 10 of the neutralized solution in saline water. 200 µL of each dilution were spread on a TCCFA medium (taurocholate-containing medium). After an incubation period of 48 hours at a temperature of 35°C +/- 2°C, the number of colonies was counted and averaged.

6. Study Period

The test was done on the 28th of July 2009 at the Bracops Hospital.

Five petri dishes containing spores of the NAP1A/027 strain and the ATCC 43598 strain were placed in four different hospital rooms.

The control petri dish ATCC 43598 contained 30 000 000 spores/mL which equals to 1 265 823 spores per cm^2.
The control petri dish NAP1A/027 contained 10 000 000 spores/mL which equals to 421 940 spores per cm^2.

In order to accommodate the study, different volumes than the ones stated in the standard were used. The final number of cells was equal to the one stated in the ASTM standard.

7. Experimental Procedure

The testing done was based on a derivative of the ASTM E2197 :2002 standard.

Carriers :

The testing was done on 54mm diameter sterile petri dishes.

Culture Media and solutions :

Brain Heart Infusion broth (BHI) - (BD BBL 211059)
Columbia Agar With 5% Sheep Blood (BD017423)
Absolute ethanol
Letheen broth used with polysorbate 80 (BD 268110)
TCCFA medium (taurocholate-containing medium)

Preparation of the spores :

Inoculate 10 mL of Brain Heat Infusion broth (BHI) – reference BD BBL 211059 – with a colony of *Clostridium difficile* grown on a Columbia agar 24 hours (BD 017423). After 24 hours of anaerobic incubation, dilute the broth 50 times in BHI and allow the colonies to grow for 10 days anaerobically. Centrifuge to reduce 10 times.
Make sure to homogenize well and place 1 mL of the solution in an empty petri dish. Add 9 mL of absolute ethanol, mix well and let it evaporate slowly (laminar flow).

In order to accommodate the study, different volumes than the ones stated in the standard were used. The final number of cells was equal to the one stated in the ASTM standard.

Disinfection Testing

Disinfection testing took place at the Anderlecht Bracops Hospital under the supervision of the doctor J.M. Hubrechts, hygienist doctor of the institution.

5 dishes of each strain were located in various positions in each of the 4 rooms used for the testing. The rooms were standard hospital rooms with a $50m^3$ volume (refer to Annexe 2).

The petri dish covers were removed prior to disinfection with the Nocolyse/Nocospray System which was placed at a height of 40 cm (as seen in the picture below) giving a wide diffusion angle.

The room volume selector was set at seven times the volume of the room since experience has shown this factor to be effective in killing all microorganisms that have been previously evaluated using 350 mL of Nocolyse. Two lots of Nocolyse were tested (2 rooms per lot). One hour after the Nocospray stopped, neutralization was done using 10 ml of Letheen broth. The petri dishes were closed and sent to the UCL Faculty of Medicine where the analysis was done. The dishes were kept at 4°C during transportation.
Three control petri dishes were kept at the Laboratory (non- exposed to the Nocolyse/Nocospray System).

Spore Count :

The number of spores of the control carrier and the exposed carrier were calculated by successive dilutions 10 in 10 in the neutralized solution in saline water. 200 µL of each dilution were spread on a TCCFA medium (taurocholate-containing medium). After an incubation period of 48 hours at a temperature of 35°C +/- 2°C, the number of colonies was counted and averaged.

Calculating Log_{10} Reductions

The log_{10} reduction was calculated for each carrier in relation to the mean of the 3 controls.

Reduction on the Carrier n= $Log_{10}(T) - Log_{10}(N_n)$
T= mean of the number of colonies for the controls
N_n = number of colonies on the carrier n (n= 1 to 5). If $N_n = 0$, we add 1 in order to calculate the log.

The product Nocolyse reached the 6 log kill target and was able to eliminate 99.9999% of the organisms which were tested.

8. Results

Table VII: Log Reduction Results for *Clostridium difficile* ATCC 43598

Rooms	Lot Numbers	Positions	Spores/mL	Spores/cm²	T	R. Log
310	060509 OS	1	0	0	3 E+07	7,00
		2	17	1	3 E+07	6,25
		3	2	0	3 E+07	6,88
		4	5	0	3 E+07	6,78
		5	262	11	3 E+07	5,06
311	220709 OS	1	30	1	3 E+07	6,00
		2	67	3	3 E+07	5,65
		3	97	4	3 E+07	5,49
		4	112	5	3 E+07	5,43
		5	132	6	3 E+07	5,36
304	060509 OS	1	127	5	3 E+07	5,37
		2	112	5	3 E+07	5,43
		3	85	4	3 E+07	5,55
		4	57	2	3 E+07	5,72
		5	92	4	3 E+07	5,51
305	220709 OS	1	70	3	3 E+07	5,63
		2	75	3	3 E+07	5,60
		3	65	3	3 E+07	5,66
		4	67	3	3 E+07	5,65
		5	72	3	3 E+07	5,62

T = 5,65 : mean of the spore count on non-exposed carriers (non-exposed to the Nocolyse/Nocospray System)
N = mean of the spore count on exposed carriers placed in different locations in the four rooms
R. Log = Reduction of the strain population by the disinfection system in comparison to the mean of the non exposed carriers (T)

Table VIII: Log Reduction Results for *Clostridium difficile* NAP 1A/027

Rooms	Lot Numbers	Positions	Spores/mL	Spores/cm²	T	R. Log
310	060509 OS	1	3	0	1	6,52
		2	13	1	1	5,89
		3	10	0	1	6,00
		4	18	1	1	5,74
		5	108	2	1	4,97
311	220709 OS	1	100	4	1	5,00
		2	68	3	1	5,17
		3	8	0	1	6,10
		4	8	0	1	6,10
		5	138	6	1	4,86
		1	25	1	1	5,60

T = mean of the spore count on non-exposed carriers (non-exposed to the Nocolyse/Nocospray System)
N = mean of the spore count on exposed carriers placed in different locations in the four rooms
R. Log = Reduction of the strain population by the disinfection system in comparison to the mean of the non exposed carriers (T)

9. CONCLUSIONS

A 6 log reduction was achieved for all 4 rooms and all 5 tested carriers for each pathogen tested.
For the ATCC 43598 strain, there was a reduction from 3 10^7 to +/- 7.6 10^1(76 spores remaining) which represents a log reduction of 5.78.
For the NAP1A/027 strain, there was a reduction from 1 10^7 to +/- 3.9 10^1(39 spores remaining) which represents a log reduction of 5.59.

For the ATCC 43598 strain, 99.99974% of the spores were eliminated (76 spores remaining out of the 30 000 000 spores). For the NAP1A/027 strain, 99.99961% of the spores were eliminated (39 spores remaining out of the 10 000 000 spores).

In order to accommodate the study, different volumes than the ones stated in the standard were used. The final number of cells was equal to the one stated in the ASTM standard.

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Responsable de l'étude

10. ANNEXE 1 : DESCRIPTION OF THE TEST ROOMS (310-311-304-305)

The four test rooms were identical (same dimensions and layout) and all located on the same floor (Bracops Hospital, B Building, 3rd floor):

Standard hospital room with 1 bed, night table, chair and table, small fridge and build in cupboard.

Height of the room: 2.70 m Volume of the room: 48,8 m³

The temperature and humidity of the room were recorded during the trial as well as the distances between the appliances in the room and the carriers.

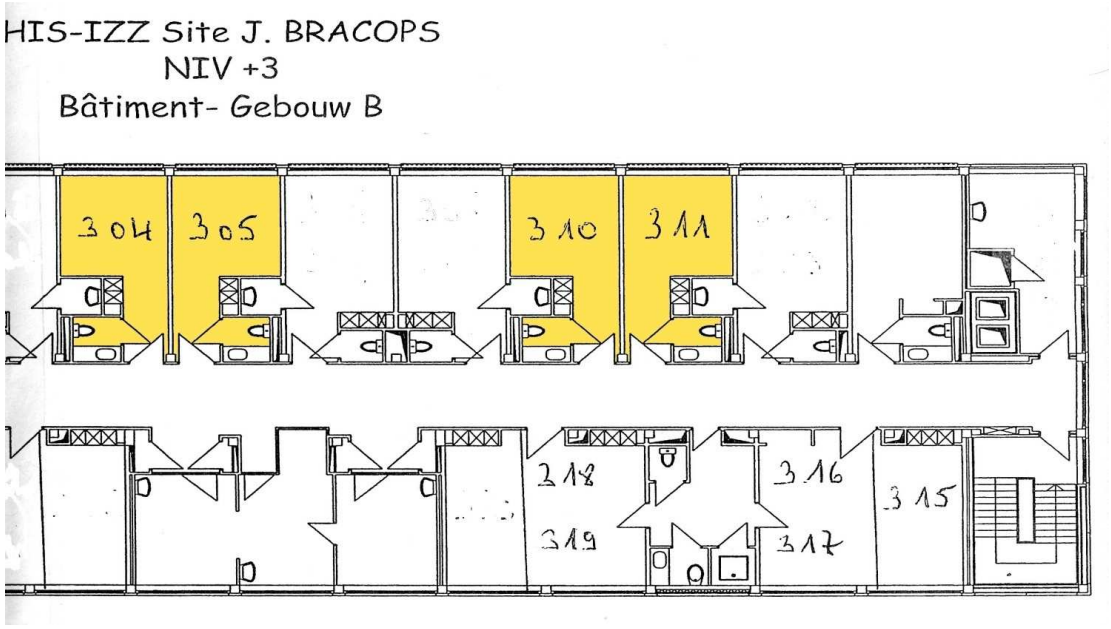
Washroom :

Seperate washroom **:** 6,6 m³

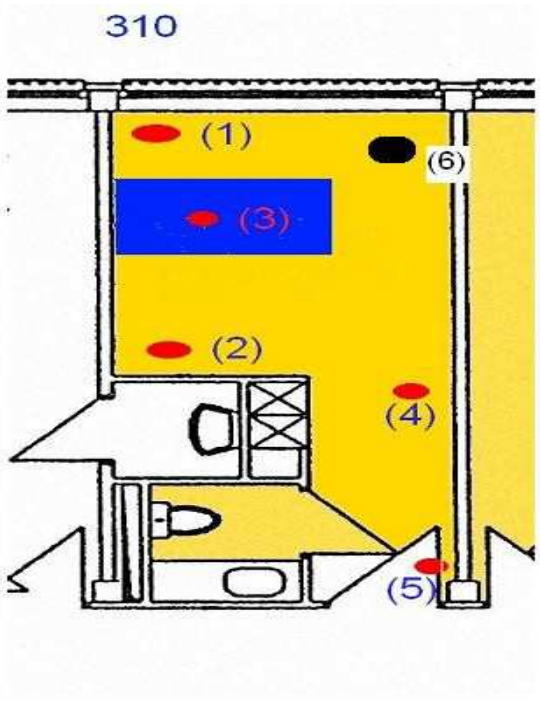
Hallway of the room :

Interior passageway: During the trial, the door towards the washroom was left open.

11. ANNEXE 2 : PLAN OF THE TEST ROOMS (310-311-304-305)



Location of the Carriers in the Room



Legend :

(1) left corner of the room beside the bed
(2) right corner of the room beside the bed
(3) in the middle of the room, on the bed
(4) beginning of the corridor in the room
(5) end of the corridor in the room
(6) Nocospray position (at a height of 40 cm)

12. ANNEXE 3: TESTING PARAMETERS

PARAMETERS	**ROOM 304**	**ROOM 305**	**ROOM 310**	**ROOM 311**
At time 0 (Before the treatment) :				
Time when the parameters were taken	12 :30	12 :40	11 :45	11 :55
Temperature	23.7°C	24.1°C	22°C	23.5°C
Humidity	46%	50%	49%	53%
Volume of the Room	50 m³	50 m³	50 m³	50 m³
Tested Product	Nocolyse	Nocolyse	Nocolyse	Nocolyse
Lot Number	060509 OS	220709 OS	060509 OS	220709 OS
Expiry Date	05/2011	07/2011	05/2011	07/2011
Time at the beginning of the treatment	12 :30	12 :40	11 :45	11 :55
Time at the end of the treatment	12 :52	13 :02	12 :08	12 :17
Programmed volume	350 m³	350 m³	350 m³	350 m³
Volume of the product used	350 ml	350 ml	350 ml	350 ml
At time 1 (After treatment with the Nocolyse/Nocospray System) :				
Temperature	25.2°C	25.4°C	25.6°C	26.5°C
Humidity	47%	46%	48%	47%

References

ASTM 2197:2002. Standard quantitative disk carrier test method for determining the bactericidal, virucidal, fungicidal, mycobactericidal and sporocidal activities of liquid chemical germicides. Doc