STEAM DISINFECTION : ANTIMICROBIAL EFFICACY

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Cleaning, disinfection, steam, biofilm, antimicrobial efficacy

SUMMARY

The tests presented in this study were carried out to evaluate the cleaning and biocidal efficacies of a steam cleaning-disinfection procedure (SANIVAP method) according to three experimental protocols. The first study evaluated the cleaning efficacy of the SANIVAP procedure against surfaces artificially contaminated with a complex test soil. The second test series consisted in evaluating biocidal efficacy of the SANIVAP method according to a standardized carrier test. The third one allowed to evaluate this procedure against bacterial biofilms formed on inert support. The results obtained demonstrate that the SANIVAP method presents a good cleaning efficacy against the complex test soil and the bacterial biofilm coupled to an efficient biocidal activity against vegetative bacteria, mycobacteria, yeasts and fungi as well as against bacteria present within biofilms.

Introduction

The biocidal properties of water vapor have been widely described and make steam the most common sterilization process. Steam as a source of heat has many effects on the viability of microorganisms (denaturation of proteins, nucleic acids deconstruction, destabilization of cell walls and membranes ...) and its spectrum of activity is considered to be very wide.

Steam is typically used within closed environment that are capable of withstanding pressures required for sterilization but its direct use on surfaces remained far less widespread. Bibliographic data, however, show that the properties of steam can be usefull in areas as diverse as the food industry for decontamination of carcasses of freshly slaughtered beef or pork (1, 2, 3), laundry or for cleaning floors and surfaces. Nevertheless, its use in health, except in autoclave remained anecdotal except for some washer-disinfectors using steam for the disinfection of bedpans and urinals (4).

The company SANIVAP has developed and offers for professional areas, including health area, a cleaning / disinfection process for surfaces (room, operating room, ...) or medical equipment (incubator, surgery table , ...) based on the use of steam at high temperature (150 ° C) and pressure (77.55 psi). The principle of a method using only steam, which remove soils and would lead to the destruction of microorganisms by heat produced, is particularly interesting. Nevertheless, there is few scientific data which evaluate the benefits and efficacy of such methods for cleaning floors and surfaces in hospitals aera, and many questions persist about the risk of contaminants dispersion by steam under pressure (5).

The aim of the study presented below was to evaluate the cleaning and antimicrobial activities of a procedure for cleaning / disinfection using steam (SANIVAP) against different types of tests soils and contamination and to compare them, whenever possible, to those of a standard cleaning procedure based upon the application of a detergent / disinfectant for surfaces. These tests should also give us some informations about the aerosolization of contaminants. Three studies were conducted.

3 kinds of tests were performed. The first one was conducted to assess purely the cleaning efficacy of the SANIVAP procedure with surfaces contaminated with a complex test soil. The second was to evaluate the biocidal efficacy of the steam generator SANIVAP using a methodology based upon four European standards used to evaluate the biocidal activities of antiseptics and disinfectants: EN 14561:2007 (6), NF EN 14562: 2006 (7) and pr EN 14563:2004 (8). The third series of tests was done to verify the results previously obtained against a soil known to be particularly resistant to detergents and chemical disinfectants: bacterial biofilms formed on inert support.

MATERIAL AND METHOD

Evaluation of the cleaning efficacy:

A laminate, rough, sterile surface is artificially contaminated with a complex test soil according to one of the method suggested in ISO / TS 15883-5: 2006 (9) to evaluate the cleaning efficacy of instrument washer-disinfectors [100 ml of defibrinated sheep blood (BioMérieux 55822), 2g of hog mucin (Sigma M-2378) and 100 ml of egg yolks] (10, 11) and 1 ml of a suspension of *Staphylococcus aureus* CIP 4.83 containing 10^7 CFU / ml.

After contamination, the tests supports are submitted to the cleaning / disinfection procedures tested and the efficacy of each procedure is evaluated by determining after each treatment, the number of viable bacteria and the amount of residual proteins remaining on the surface of the test support.

For this, a sterile stainless steel funnel is applied to the test surface and 10 ml of sterile liquid recovery (Polysorbate 80: 5ml; NaCl: 8.5034 g 1000 ml distilled water) or 10 ml of sodium dodecyl sulfate (1% m / V) are poured into the funnel respectively for the evaluation of the number of viable bacteria or dosing of the proteins present on the surface of the support after treatment.

The test medium is scraped evenly, using a sterile pipette and the solution is then collected and transferred to a sterile tube for analysis. Microbiological enumerations are made from the 10 ml of recovery solution by dilution / inclusion in counting agar (tryptic soy, Biomerieux 51044). The concentration of proteins in the 10 ml of SDS collected is determined by a colorimetric assay (MicroBCA. Optima Interchim). The results are expressed as the number of colony forming units (CFU) per cm ² and micrograms of protein per 20 cm ².

At the same time, a series of control is initiated to verify the viability of the test microorganism into the test soil and validate the experimental conditions to obtain a homogeneous and reproducible contamination of the test support.

Three cleaning and disinfection procedures are tested and compared:

- "SANIVAP " Procedure consisting to one passage of steam using the brush (Prototype SANIVAP, 2250 Watts) without followed by wiping with a microfiber.
- "SANIVAP + PROVAP" Procedure including a spray of PROVAP detergent followed by one passage of steam using the brush without windshield followed by wiping with a microfiber.
- Reference procedure using a detergent / disinfectant for surfaces (immersion of the sterile microfiber in a 0.5% (v / v) solution of detergent / disinfectant, spin, double passages of the impregnated microfiber by returning after each passage).

Evaluation of biocidal activities:

A test suspension of microorganisms is prepared in a tryptone-salt mixture (Biomerieux 42076) as recommended by NF EN 14561: 2007 and EN 14562: 2006 for the bacterial strains (*Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541) or fungal strains (*Candida albicans* ATCC 10231 and *Aspergillus Niger* ATCC 16404) and sterile distilled water as recommended by the draft of the standard pr EN 14563: 2004 for mycobacteria strains (*Mycobacterium terrae* CIP 104321 and *Mycobacterium avium* CIP 105415).

The exact number of viable organisms in each test suspension is controlled by successive tenfold dilutions and inclusion or spreading a sample of each dilution in the counting medium: trypticase soya agar (Biomerieux 51044) for bacteria, mycobacteria 7:10 agar (Difco) for mycobacteria and Malt extract for yeast and fungi.
The conditions of incubation are adapted to each microorganism:

- 36±2 °C for 48 hours for bacteria.
- 30±1 °C from 48 to 96 hours for yeast and fungi.
- 36±2 °C for 21 days for mycobacteria.

1 ml of interfering substance [Bovine Albumin (USBiological A1310.05): 0.30 g, tryptone-salt (Biomerieux 42076): qs 100 ml] previously sterilized by filtration is added to 9 ml of the test suspension. After homogenization, 50 µl of the mixture were sampled and placed on a predefined area of 1 cm ² of the test support (glass carriers, 15mm x 60mm x 1mm, frosted on one side). Contaminated test supports were then kept at room temperature until completely dry.
After contamination and drying, the glass carriers are placed on a metallic support to maintain them stable during testing. The test surface is then submitted to two passages of the 40 cm brush steam in contact with the tested surface (SANIVAP range SV, SV 3000, 3500 Watts, SANIVAP) without wiping with a microfiber. The speed of passage of the brush is about 50 cm / sec and the contact time with the inoculum is about of 2 x 1 second.
The effect of transfer inherent to the passages of the brush (forward and backward movement) on the metallic support around test support is evaluated using a swabbing method of the area (about 50 cm ²) surrounding the test surface (Nt). In parallel with these tests and for each microorganism, a contaminated test support is maintained at room temperature for the time required to perform the tests to determine the initial contamination level of microorganism (Nw) and validate the experimental conditions.
After treatment with the SANIVAP method (N) or for the validation of experimental conditions (Nw), the carriers were transferred to a tube containing 10 ml of neutralizing solution [Polysorbate 80 (P17 SIGMA-54): 50 ml, sodium thiosulfate (Sigma S85-03): 5g, saponin (SIGMA S79-00): 10g, lecithin (Sigma P53): 10g, tryptic soy broth (Biomerieux 42100): qsp500 ml] and approximately 1 ml of sterile glass beads 0.25 to 0, 5 mm diameter. The tubes containing the test supports are then vortexed for about 30 seconds and the number of viable microorganisms present per milliliter was determined by serial tenfold dilutions and inclusion into the counting medium.

After incubation, the number of viable organisms were counted and results expressed in CFU / cm ². Three tests are performed for each microorganism. Finally, for each strain, decimal logarithmic reduction of the number of microorganisms present on the test support before treatment (Nw) and after treatment (N) is calculated using the formula:

$$R = \frac{Nw}{N} \text{ where } \log_{10}R = \log_{10}Nw - \log_{10}N$$

Evaluation of the efficacy against bacterial biofilms:

Pseudomonas aeruginosa CIP A22 biofilms are formed on an inert support (internal diameter Tygon tubing ® 6.4mm) as described in Annex F of ISO / TS 15883-5: 2006 (1). The tube portions contaminated by biofilm (approximately 10^8 bacteria / cm ²) are cut longitudinally to obtain semi-cylindrical surfaces of 4 cm long, 4 to 4.5 cm ². These tests supports were then submitted to different tested procedures (SANIVAP, SANIVAP + PROVAP and surface detergent / disinfectant).

The efficacy of each procedure is evaluated by determining after treatment:

- The number of viable bacteria still present on the inner surface of the tube Tygon ®. For that, after treatment, the Tygon ® tube portions are immersed in 10 ml of neutralizing solution [Lecithin (Sigma, P-5394): 2% (p / v) sodium thiosulfate (SIGMA, S-85 030): 5% (w / v) tween 80 (SIGMA, p-1754): 10% (v / v), histidine (Sigma, H-8000): 1% (w / v) trypticase soy broth (BioMérieux, 51019): qs 100 ml]. The tube is then cut longitudinally into four identical sections. The tube sections and neutralizing solution are then transferred into a test tube containing sterile glass beads and subjected to agitation for about 1 min (Vortex 2, 5 shake, Scientific Industries, Bioblock, FRANCE). Viable bacteria present in the mixture are then counted by dilution / inclusion in tryptic soya agar (Biomerieux 51044). After incubation for 24 to 48 hours at 36 ± 2 ° C, colonies were counted and results expressed as the number of viable bacteria per cm^2.
- The residual quantities of proteins and polysaccharides remaining on the inner surface of Tygon ® tubing. After treatment, a Tygon ® tube portion is transferred in 3 ml of sterile distilled water where the inner surface of theTygon ® tube was scrapped. The residual amount of polysaccharides and proteins on the inner surface of Tygon ® tube is then calculated from the concentration of proteins and polysaccharides measured in 3 ml of sterile distilled water using respectively the Lowry (12) and Dubois (13) method.

RESULTS

Evaluation of the cleaning efficacy:

Results presented in **Table I** and **Figure 1** show that the "SANIVAP " and " SANIVAP + PROVAP" procedures have comparable cleaning efficacy on the test soil and induce a reduction of about 98% of the concentration of proteins found on the surface of the test support. For both procedures the mean residual protein after application of the treatment is about 112 µg/20 cm.

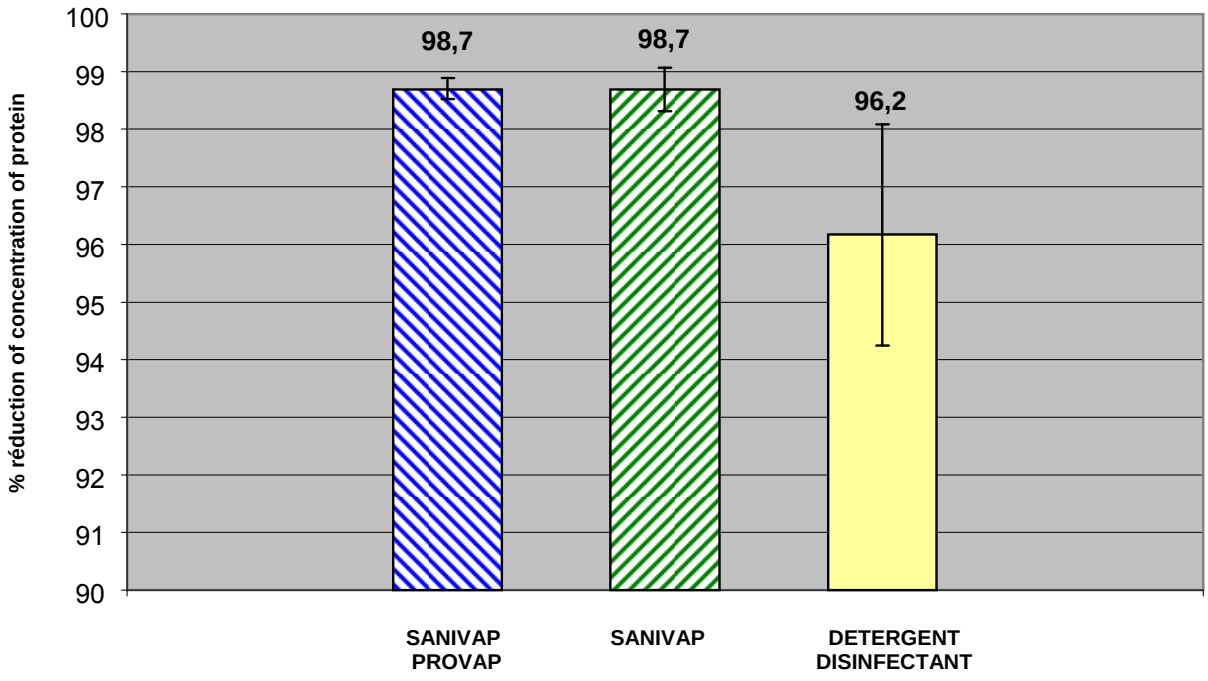
The application of the "DETERGENT-DISINFECTANT" procedure does not provide the same level of efficacy and the reduction of the protein concentration is slightly lower (96%) with a mean concentration of protein remaining after treatment of 327 µg/20 cm ².

These preliminary results are confirmed by the analysis of the bacterial counting which confirms that the "SANIVAP " and " SANIVAP + PROVAP" procedures induce a reduction of the number of viable bacteria initially present on the surface of the test support of at least 3.3 log units, the "DERTEGENT-DISINFECTANT of SURFACE " procedure only achieves a reduction of about 2.2 log surface unit.

Tableau I : Comparison of the efficacy of three cleaning / disinfection procedures against carrier tests contaminated with a complex test soil : values of residual concentrations of bacteria and proteins remaining on the test support after submitted them to the tested procedure

	PROCEDURE			
PARAMETERS	**CONTROL**	**DETERGENT-DISINFECTANT**	**SANIVAP PROVAP**	**SANIVAP**
Concentration of microorganisms (CFU/20cm²)	**7700** ± 1100	**127,8** ± 109,1	**2,7** ± 3,6	**8,0** ± 8,0
Concentration of protein (µg/20cm²)	**8550** ± 425,6	**327,6** ± 163,9	**112,1** ± 14,1	**111,8** ± 32,3

Figure 1: Comparison of the efficacy of the three cleaning/disinfection procedures against test surfaces contaminated with a complex test soil



A more detailed analysis of the data also shows a highly variability of results obtained after application of the procedure using the detergent-disinfectant for surfaces compared to the two procedures involving the SANIVAP generator.

Evaluation of the biocidal activities:

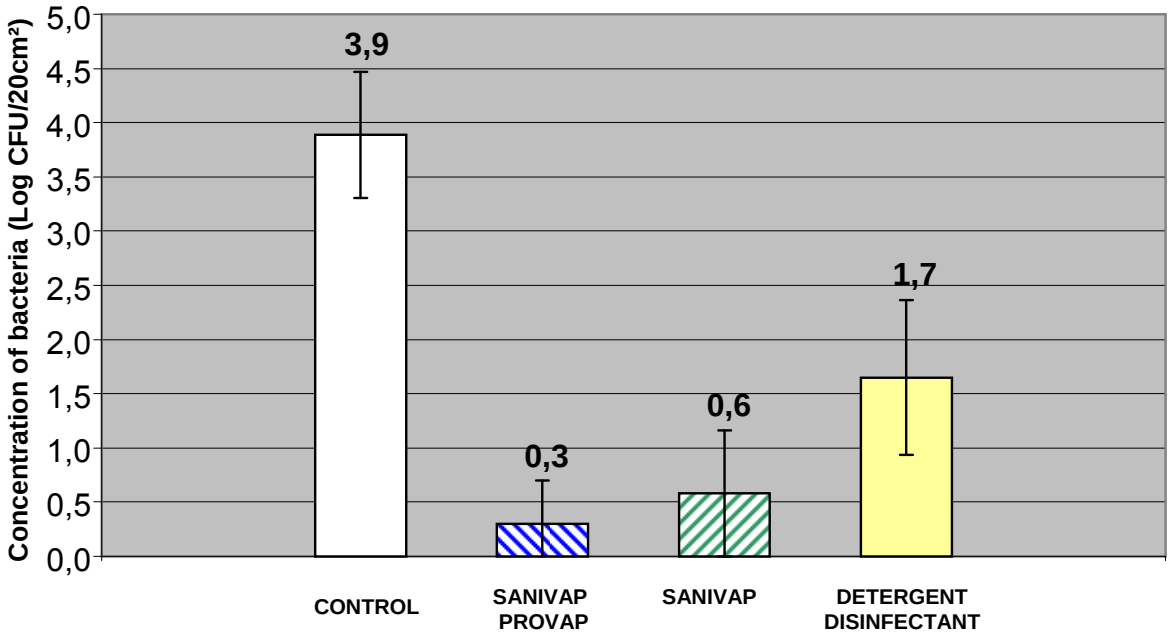
Tests carried out using a methodology based upon NF EN 14561:2007 (see **Table II**) show that the SANIVAP method, after contact of the 40 cm vapor brush with the test surface, induces a reduction of the number of viable bacteria deposited on glass carrier in the presence of interfering substances (clean conditions: 0.3 g / L albumin) to 5.8 log10 units for *Enterococcus hirae* ATCC 10541. These reductions are higher than respectively 6.0 log10 and 5.8 log10 units for *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6538

Table II : Determination of the biocidal activities of the steam method SANIVAP according to a test method based upon standards NF EN 14561: 2007, EN 14562 or draft pR EN 14563 decimal log reduction (R) between the number of viable microorganisms present on the test support before the treatment (Nw) and after treatment with the steam generator SANIVAP (N). Nt: number of viable microorganisms present on the surface surrounding of the initially contaminated area.

Microorganisms	**Nw** (Nb. CFU/cm²)	**N** (Nb.CFU/cm²)	***R***	***Nt*** (Nb. CFU/50 cm²)
Pseudomonas aeruginosa ATCC 15442	$2{,}2.10^8$	$<1{,}4.10^2$	> 6,1	<1
Staphylococcus aureus ATCC 6538	$1{,}1.10^8$	$<1{,}4.10^2$	> 5,8	<1
Enterococcus hirae ATCC 10541	$6{,}3.10^8$	$9{,}3.10^2$	5,8±0,0	18
Mycobacterium terrae CIP 104321	$1{,}4.10^7$	$<1{,}4.10^2$	> 5,0	<1
Mycobacterium avium CIP 105415	$6{,}9.10^7$	$<1{,}4.10^2$	> 5,7	<1
Candida albicans ATCC 10231	$4{,}5.10^6$	$<1{,}4.10^2$	> 4,5	<1
Aspergillus niger ATCC 16404.	$1{,}4.10^6$	$<1{,}4.10^2$	> 4,0	<1
Bacillus subtilis CIP 52.62	$1{,}1.10^8$	$5{,}2.10^5$	2,3 ± 0,1	>150
Bacillus cereus CIP 105151	$2{,}5.10^7$	$6{,}2.10^6$	0,6 ± 0,0	>150

Results of tests performed with mycobacteria using a method based upon the draft standard pr EN 14563:2004, show that after a single treatment, the reduction of the initial microbial load is higher than 5.0 log 10 and 5.7log10 units for respectively *Mycobacterium terrae* CIP 104321 and *Mycobacterium avium* CIP 105415.

Figure 2 : Comparison of the efficacy of three cleaning / disinfection procedures against carrier test contaminated with a complex test soil : values of residual contamination levels after treatment



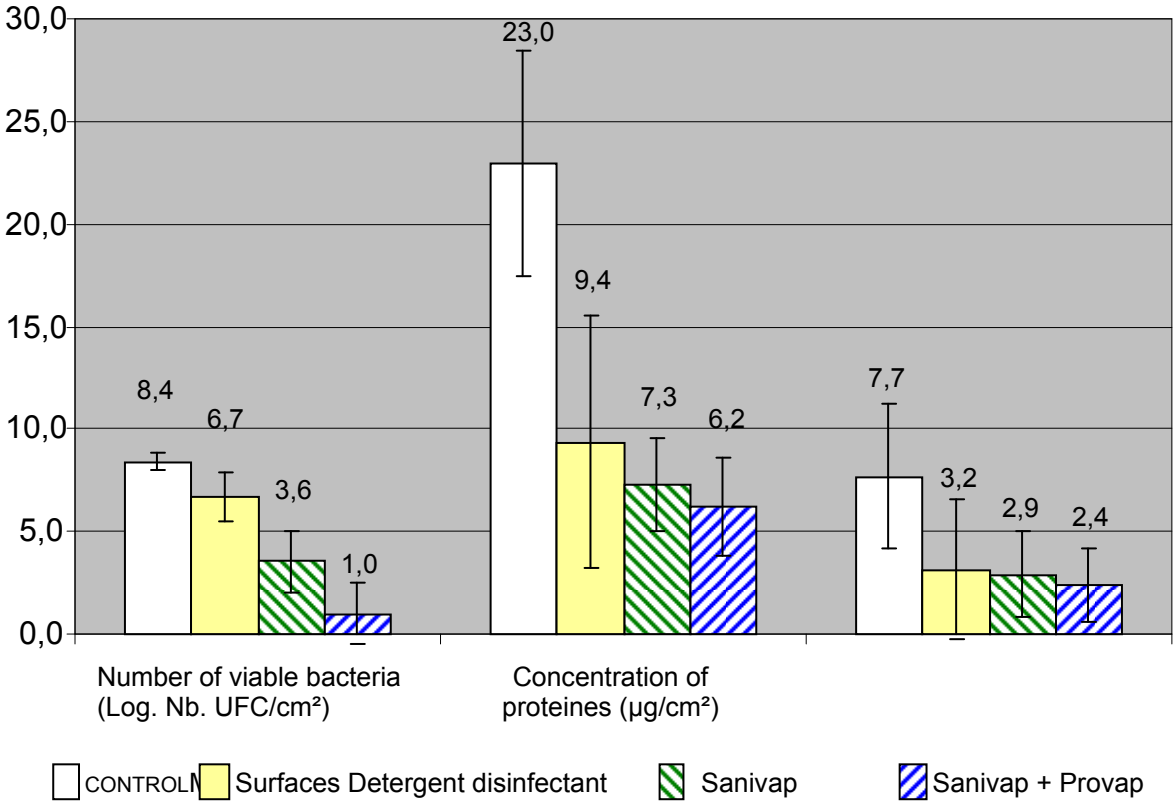
Tests carried out using a methodology based upon NF EN 14562: 2006 show that after two passages of the steam brush a reduction higher than respectively 4.5 and 4.0 log units when the test strains are *Candida albicans* ATCC 10231 and *Aspergillus Niger* ATCC 16404 was observed.
On the opposite, tests carried out with tests carriers contaminated with bacterial spores indicate that the steam treatment applied to the surface test induce only a small reduction of the initial microbial load (R = 2.3 ± 0.1 log10 for spores of *Bacillus subtilis* CIP 52.62 and R = 0.6 ± 0.0 for spores of *Bacillus cereus* CIP 105151).

Samples taken by swabbing method do not show transfer of viable microorganisms from the test carrier to the surrounding area, except for tests performed with *Enterococcus hirae* ATCC 10541 for which a mean of 18 CFU /50 cm ² were detected. It is the same for bacterial spores, an amount of viable bacterial spores (> 150 CFU/50 cm ²) are transferred from the test carrier to the surrounding surfaces.

Evaluation of the efficacy against bacterial biofilm:

The comparison of the efficacy of the three tested procedures (**Figure 3**) shows that with a residual number of viable bacteria of about 10 CFU / cm ² ie a reduction of 7.0 log_{10} units, the "SANIVAP + PROVAP " procedure induces a higher reduction than the "SANIVAP " or the "detergent/disinfectant" procedures with respectively a reduction of 4.7 log10 and 1.7 log units).

Figure 3 : Evaluation of the cleaning and/or biocidal activity of three tested procedures against *Pseudomonas aeruginosa* CIP A22 : Mean number of viable bacteria (log,) present in the biofilm, mean concentrations of residual protein and polyssacharids on the inner surface of the Tygon® after submitted them to the three tested methods.



The comparison of the protein concentrations remaining on test carriers after application of the tested procedure show that the three procedures have a cleaning efficacy, inducing a reduction of protein higher or equal to 60 %. The "SANIVAP + PROVAP " method induces a higher reduction than the two others method, with a remaining concentration of 6,2 µg / cm ² and a reduction of 73%. The amount of

remaining proteins after application of the "SANIVAP " method (7.3 µg / cm ²) and after the "Detergent /Disinfectant " (9.4 µg / cm ²) lead respectively to a reduction of 68% and 60%.
Even if these values could be influenced by the fact that the amount of remaining polysaccharides on surface is quite low and variable, the results confirm that the cleaning efficacy of the "SANIVAP + PROVAP " method (reduction of 68.8%) is higher than the efficacy of the "SANIVAP " procedure (reduction of 62.3%) and than the Detergent-Disinfectant procedure (58.4% reduction).

DISCUSSION

Evaluation of the cleaning efficacy:

With a residual concentration of protein below 6 µg / cm ², the cleaning efficacy of the two procedures using the SANIVAP method can be deemed to be satisfactory. These two procedures are more efficient and more reproducible than the method using a Detergent-Disinfectant for surfaces. Even if the much more important variations in efficacy observed for the detergent-disinfectant method are probably due to the density of the soil used in these tests, these show the difficulty in the experimental conditions described to reproduce for this kind of process the movement which induces the better efficacy. The good results obtained in these tests in terms of reduction of the bacterial load on test carrier does not confirm the fears raised by White LF (14) about the lower efficiency of steam against *Staphylococcus aureus.*

Evaluation of biocidal activities:

Under the experimental conditions described, the logarithmic reductions obtained for the tested microorganisms after application of the SANIVAP method, are all higher than those required in the standards EN 14561:2007, EN 14562:2006 and the draft standard pr EN 14563 (December 2004) to qualify the bactericidal, fungicidal and mycobactericidal activity of a chemical disinfectant used for instruments (surgical instruments, anesthesia equipment, endoscopes, etc..). These results confirm those published by A. Haas (15) in 1998 where with a similar method, logarithmic reductions obtained were higher than 5 log units for three microorganisms (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*) and for three types of support (tile, wood and carpet) after a processing of 5 seconds at a distance of 2.5 cm
The interpretation of the activity against carriers tests artificially contaminated with bacterial spores is quite difficult because, no type test according to step II (simulated used conditions tests or surface tests) has been developed in Europe to evaluate the sporicidal activity of disinfectant used in medical area.
However, based on the requirements of EN 14347 (16) results obtained are below the minimum required for a basic sporicidal activity (4 log reduction). Although even if these results show the limits of the SANIVAP method, it should be considered as unacceptable given that the sporicidal activity is required only in the case of terminal processing of critical medical devices introduced in sterile cavities

(17) while the method suggested by SANIVAP is mainly used for disinfection of floors and surfaces (biocleaning).

According to the requirements used by the French Society for Hygiene Hospital (SFHH), detergents and disinfectants used for floors and surfaces (18) must have a basic bactericidal and yeasticidal (*Candida albicans*) activity [EN 1040 (19) and EN 1275 (20)] and having a bactericidal activity in dirty conditions for 15 minutes contact time. Therefore, even if the methods used in this study (tests on glass support) are different from those used by the SFHH (suspension tests), the activities identified for the SANIVAP process can be deemed to be satisfactory.

Additional tests will nevertheless be performed to confirm, according to the requirements of SFHH, the bactericidal activity of the steam produced by the SANIVAP process in the presence of larger amounts of interfering substances (dirty conditions testing).

Even if they are not required, the evaluation of the fungicidal (with *Aspergillus Niger*) and mycobactericidal (*Mycobacterium avium* and *Mycobacterium terrae*) activities of the cleaning / disinfection SANIVAP method could be interesting for some risk areas activity.

The tests performed by swabbing the area surrounding the initially contaminated area show that the risk of dispersion of microorganisms outside the tested surface with steam under pressure cannot be excluded. However, the results show that this phenomenon is limited only to the microorganisms known to be less sensitive to heat, as spores of *Bacillus cereus* and *Bacillus subtilis* and to a lower extent *Enterococcus hirae.*

Evaluation of the activity against biofilm:

The results of test performed against biofilm confirm that the SANIVAP method is more efficient than the method using the detergent / disinfectant to remove the biofilm components and inactivate the viable bacteria. These results confirm the interest of this method seeing that the inactivation of bacteria within the biofilm is quite easy whereas the removal stay more difficult. (21)

CONCLUSIONS

Under the experimental conditions described, the results of tests performed show that the SANIVAP cleaning / disinfection method presents a good cleaning efficacy against complex tests soil and bacterial biofilms. This detersive activity is completed with a biocidal activity against bacteria, mycobacteria, yeasts and fungi including the adherent bacteria in biofilms. As stated by the French Society for Hospital Hygiene (22), the steam method presents all required efficacy for the cleaning and disinfection of floors and surfaces (biocleaning).

Finally, due to the many advantages of this method, it is quite easy to consider, if adapted devices are available, or specific protocol are described, other applications in medicinal area including the treatment of medical devices.

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