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Microbiological quality of laboratories works stations: impact of a system of saturated dry spray steam Running title. Laboratory safety and system of sanitation

ABSTRACT

Hygiene and sanitation in laboratories are some important focus for the well-being of scientists and workers. Due to the observed lack of these notions, a new sanitation system has been tested to overcome the limitations of traditional methods about risks of biological contamination. It was the Polti Sani System. For this work, some American Type Culture Collection and Institute Pasteur of Strasbourg reference strains have been used. The methodology used in this study was, firstly, to evaluate the biocides activities of dry saturated steam Nebulizer System which is the Sani System. Secondly, the count of the total aerobic mesophilic bacteria in the laboratory working environment before and after disinfection was done. Finally, the Adenosine Triphosphate from the differents surface types before and after disinfection was quantified. Based on the results and the experimental conditions adopted, Sani System reduced more than five logarithmic orders, the bacterial rate tested after a time of contact of 30 seconds. The log reduction achieved over the mushrooms with the same contact time is greater than four logarithmic orders. The antimicrobial activity was observed both in the environment and on inert supports made of glasses, Plexiglas or tiles with an average reduction rate of 99.13%.

This study showed that the Sani System associated with ATP-metry can be successfully used to quickly check the hygiene standards on surfaces or lab environment. It is a real challenge in terms of quality, efficiency and safety for the laboratories.

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Keywords: Bio-contamination, Disinfection, Saturated steam, Bioluminescence.

1. INTRODUCTION

Environment as air, surfaces and water faced a permanent but variable microbiological contamination variable in time and space. Microorganisms belong to the environmental saprophytic floras. It also comes from commensal or pathogenic floras of people [1]. Vectors of contamination, these environmental compartments disseminate microorganisms at some distance and insidiously contribute to progressive contamination of various inert supports.

There are many examples of bioburden that have hit the headlines, whether in the hospital, pharmaceutical or food area. Epidemiological data confirm the reality of these risks. Described infections are almost due to bacterial causes and in 13% of cases; it is associated with exposure to aerosols [2].

Causing human and material damage, aerobiocontamination is a fundamental problem which affects many sectors (food, commercial, pharmaceutical, health care, cosmetics, biotechnology, controlled environments and cleanrooms). Biocontamination is a biological contamination which may have an adverse effect on the product (false positives), the staff or the patient with regard to health facilities.

Faced with this ubiquitous contamination, personnel safety, protection of handled products can only be considered in a controlled microbial environment [3]. It is thus necessary to assess the risk of contamination and to manage them appropriately and consistently. The control of bioburden in areas at risk relates to compliance with preventive measures but also on physical, chemical and microbiological tests and finally on correctives measures. The traditional sanitation methods relate to the use

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of disinfectant solutions, sometimes with high chemical content, which come in contact with the support surfaces or heat or radiation [4].

The limitations of these methods are generally related to the difficulty in reaching the interstices or penetrate the rough surfaces and uneven, it does not provide a total cleaning of the contaminated area. In addition, these disinfectants have a risk of irritation or hypersensitivity due to their chemical composition. Moreover, traditional chemical disinfectants are not suitable for all types of surface and the phenomena of natural and acquired resistance to disinfectants were observed in bacteria [5]. In addition, it requires a manual contact with the surfaces to sanitize. Technological advances in microbiology and sanitation, combined with the new requirements of work safety, product safety and environmental protection led to a complete revision of disinfection procedures. Thus, some new products such as aerosols and complex synthetic compounds appeared.

Among these procedures, the steam was usually used within controlled enclosures which are capable of withstanding the pressures required for sterilization but its direct use on surfaces is not yet a reality in the world [6]**.** The principle of such method is particularly interesting because it uses a simple steam and leads to a destruction of microorganisms by the way of generated heat without pollution.

Within the overall framework of risk prevention, specifically the reduction of avoidable proportion of airborne infections and also to overcome the resistance problems due to the use of traditional disinfectants, this study aimed to assess the microbiological quality of the lab working environment by using a nebulizer system of dry saturated steam.

2. MATERIAL AND METHODS

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2.1. Material

The material used was composed of microbial reference strains, experimental equipment disinfection system (nebulization of saturated steam Sani System) luminometer, culture media and reagents, glasswares and consumables.

2.1.1. Microbial strains reference

The antibacterial and antifungal activities of the disinfectant was *in vitro* evaluated on ATCC (American Type Culture Collection) and IP (Institute Pasteur of Strasbourg) strains namely: *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* IP 4872 and *Aspergillus Niger* ATCC 16404.

2.1.2. Experimental disinfection equipment

Disinfection equipment distributed by the company MIVA was a fogging system saturated vapor and a sanitizer. Sani System is an electro-medical device which delivers saturated steam at high temperature (180°C) associated with the sanitizing HPMed. Its uniqueness lies in the fact that the steam is brought to a high temperature in an expansion chamber.

HPMed is a co-adjuvant in sanitation vapor of Sani System. It consists of an alcohol-based solution of sodium metasilicate containing a nonionic surfactant, which helps the sanitizing activity of Sani System. HPMed represents an aid to sanitation carried out by superheated dry saturated steam delivered by the Polti Sani System. Table I shows the chemical composition of the sanitizer HPMed.

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A 3M luminometer clean-trace NG was used with 3M kits reagents for measuring contamination levels in samples of surface and can effectively monitor surface hygiene. The reagent kits were some dry swabs and Lucifer in-luciferase complex.

2.2. Methods

The methodology used was about the evaluation of the biocidal activities of the nebulization Sani System. Then, the numeration of total aerobic mesophilic germs in a working environment (air and hard surfaces) before and after disinfection was done. Finally, the Adenosine Triphosphate from the different types of surface was quantified before and after disinfection.

2.2.1. Evaluation of biocides activities of Sani System Polti

The preparation of the microbial suspension was made according to AFNOR NF T 72-281 [7] where the dilution which gives a concentration of 10^6 germs per ml was selected as microbial suspension. Biocidal activities of Sani System Polti have been assessed by the test support [8; 9]. After contamination and drying, some slides were treated by spraying of dry saturated steam in combination with sanitizer HPMed for 30 seconds. Along with these tests, another slides contaminated by each microorganism and untreated were maintained at room temperature (16℃) throughout the tests for determining the initial contamination level brackets and validation of experimental conditions.

After treatment by the Sani System, the slides (N' for the test and N for the untreated control) were transferred into 10 mL of neutralizer (polysorbate 80: 50 mL, sodium thiosulfate: 5 g, trypticase soy broth: qsp 500 mL). The erlemeyers containing the test slides were then subjected to stirring for 30 seconds and the number of viable microorganisms per mL of reaction medium was determined by successive decimal

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dilutions following by plating of each dilution on Trypticase Soy Agar (TSA). Each microorganism was tested three times. Seeded Petri dishes and controls were incubated under appropriate conditions for each microorganism. In fact, Tryptic Soy Agar was incubated at 35 ± 2 °C for 48 h in bacteriological incubator Memmert IBN400. Sabouraud agar with chloramphenicol was incubated at 25 ± 2 °C for 96 h in incubator Memmert IPP 400. After incubation, the colonies formed were counted using colony counter Stuart Scientific and the results were expressed in CFU / cm^2.

For each strain, the log decimal reduction between the number of microorganisms present on the slide before treatment (N) and after treatment (N') was calculated using the formula:

$$R = \frac{N}{N'} \text{ Where } \log_{10} R = \log_{10} N - \log_{10} N'$$

2.2.2. Numeration of mesophilic aerobic microorganisms suspended in the air

Mesophilic aerobic bacteria were collected and counted in the environment of three rooms before and after disinfection for respectively 360 seconds, 600 seconds and 3600 seconds. The method used was the sedimentation one on Petri dish [10]. Three samples were taken in each room and each sample was assayed five times. The time between two (02) samples was seven days. After cultivation staggered between 30-35 ° C for 48 h and 20-25 ° C for 96 h, the result of numeration R was expressed as follows:

$$R = \frac{(N_1+N_2+N_3+N_4+N_5)}{5} \text{ X } 2h$$

R is expressed in CFU/4h.

N_1, N_2, N_3, N_4, N_5 are the number of colonies counted for each of the five (05) plates seeded.

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141 **2.2.3. Enumeration of total aerobic mesophilic floras from lab surfaces**

142 The samples were collected on three types of surfaces: glass, Plexiglas and tile
143 (before and after 30 seconds of disinfection) using the technique of wet swab on an
144 area of 10 cm^2. Ten samples were taken from the surface and 0.1 mL of each
145 dilution sample was plated in duplicate using Tryptic Soy Agar [11]. After incubation
146 at 30-35 °C for 72 h, the results expressed as CFU / ml of the initial product, were
147 converted to counts per cm^2 using the following formula [12]:

148 $$N' = N \times 4$$ where:

149 N': Number of CFU per cm^2.

150 N: Number of CFU per ml of the original product.

151 **2.2.4. ATP-metry: measurement of residual ATP before and after disinfection**

152 Surface samples were collected (before and after 30 sec of disinfection) with dried
153 swabs (ATP test surface). Light emitted by the ATP test surface was measured and
154 the result was displayed on the screen of the luminometer.

155 **2.2.5. Statistical analysis**

156 The results were analyzed regarding the disinfection time and the type of surface. To
157 evaluate the influence of the duration of disinfection and the type of surface on the
158 rate of reduction of microorganisms, the test of analysis of variance was used when
159 the conditions of normality and equal variances were met. Otherwise, the Kruskal
160 Wallis was preferred. Student's t test or Wilcoxon test was used to compare the
161 rates of reduction of microorganisms obtained for each factor. The significance level
162 was set at 5%.

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3. RESULTS AND DISCUSSION

3.1. Effectiveness of Sani System Polti

The table II presents the results of the effectiveness of Sani System Polti on microorganisms. It shows that the supports contaminated with different bacterial strains and treated with Sani System have no detectable microbial growth. The log reductions highlighted after 30 sec disinfection with dry saturated steam were all higher than those required in the NF EN 14561: 2007, EN 14562: 2006 standards [13; 14].

These results confirm those of [6] who have also obtained more than 5 log units reductions for bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae*) and reductions greater than 4 log units for *Aspergillus niger* and *Candida albicans*. Meanwhile, the log reductions obtained in this study for fungi differ from those published by [15] who highlighted, for a similar system, some reductions discounts greater than 5 log units for *Candida albicans*.

3.2. Numeration of mesophilic aerobic microorganisms in the air

The floras for the three rooms (S_1, S_2 and S_3) before and after disinfection are mentioned in the table III. More disinfection time increases, higher is the reduction of the number of microorganisms.

Microbial quantum in the three rooms (R_1, R_2, and R_3) after disinfection during 3600 seconds is respectively 02, 04 and 05 CFU/4h. All these rooms belong to Class B as recommended by limits about microbiological contamination (EU GMP, 2008) [16]**.** The average reduction of airborne microorganisms in three rooms after 360 sec, 600 sec and 3600 sec nebulization is shown in Figure 2. It shows that the rate of

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reduction of airborne germs in three rooms increases with duration of disinfection. Thus, the average reduction rate was 4.57% after 360 sec disinfection. This rate raised to 41.39% after treatment of 600 sec and reached 94.27% after 3600 sec of disinfection. The results of the analysis of variance of two factors are presented in the table IV. The duration of disinfection has a highly significant effect (p-value < 0.001) on the rate of reduction of microorganisms. This time would probably depend on the surface to be disinfected. The analysis of variance of two factors also indicates that the disinfecting time duration has a highly significant effect on the rate of reduction of airborne germs. Although, disinfection is a one-time treatment effect and does not persist over time (p-value = 0.79).

3.3. Numeration of mesophilic aerobic microorganisms on surfaces

The boxplot in Figure 3 shows the distribution of the average rate of reduction of microorganisms by type of surfaces. The average rate of reduction of microorganisms on glass and Plexiglas has a wide variation different from that of the tiles. The reduction rate corresponding to the average numbers were respectively 98.92 %, 98.51 % and 99.98 %. The system with saturated steam is effective on smooth surfaces. These results are similar to those of [16] who obtained a rate reduction between 98-100 % for smooth surfaces. Moreover, none of the treated surfaces have been damaged. No fundamental change in color, shape and general appearance has been noted.

Kruskal Wallis test shows that the nature of the surface significantly influences the rate of reduction of microorganisms after disinfection. Indeed, the rate of reduction of microorganisms after nebulization of ground (tiles) is significantly higher ($p < 0.001$) than the rate of reduction of the smooth surfaces.

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This result is contrary to the theory that the use of traditional methods of disinfection (towel) on surfaces of low roughness (shiny and well polished) provides a high rate of reduction [18]. The difference should be partly due to the nature of the microorganisms which are very sensitive to heat and sanitizer on the tiles. On the other hand, the fact that the vapours, unlike napkins, penetrate anywhere, even areas closer could explain such situation. By cons, there is no statistical difference between the rate of reduction of a glass surface and a Plexiglas surface ($p = 0.71$).

3.4. ATP-metry

The average of different values taken by the luminometer before and after disinfection of different types of surfaces (tiles, glass and Plexiglas) and the average reduction are shown in Table V. The average rate of reduction of the relative amount of light into surfaces of tiles is 94.23% against 93.85% for the glass surfaces and 90.56% for Plexiglas. The average of RLUs for glass surfaces, plexiglas and tiles were respectively 37.7, 68.4 and 171.5. These values are well below the threshold of acceptability in terms of safety (0-250 RLU/cm^2) [19]**.** The rate of disinfection on glass and tile is significantly higher than the rate of disinfection in the plexiglas ($p < 0.001$). However, there is no statistically significant difference between the rate of reduction of microorganisms on the tile and glass ($p = 0.73$). Although, whatever the disinfected surface, the reduction rate estimated by ATP-metry method was highly significant ($p < 0.001$).

It is often wrongly assumed that the results obtained by Relative Light Unit (RLU) should demonstrate a direct correlation with the numeration on petri dish for the same sample. The amount of ATP present in microbial cells can vary greatly depending on the strain and one CFU may correspond to one or more microorganisms. In addition, the bacteria need to grow with food waste so even if in

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236 theory, it is possible to make a correlation between RLU and CFU, in practice,
237 however it is impossible to determine the exact origin of ATP (bacteria, yeast, mold,
238 food residue). The most frequent case is a combination of multiple factors. Thus the
239 ATP-metry is not a direct indicator of the presence of bacteria. Nevertheless, it is the
240 fastest and easiest technology to determine the potential for bacterial growth.
241 Therefore, it is an indicator of the actual surface level of hygiene. There is no easier
242 or faster else way to determine a problem. Thus, with the ATP-metry, it is possible
243 to implement corrective actions upstream to avoid any problem of non-compliance.

244 **CONCLUSION**

245 Microbial contamination is a concern for many industries. Therefore, research and
246 development in the field of disinfection are continuously evolving. Methods that can
247 reduce or eliminate microbial growth with less or no injury for the operator and the
248 environment are preferred. This is the case of disinfection using dry saturated steam
249 Sani System which presents many advantages for sanitation despite the cost. In
250 view of these advantages, it is quite possible to consider other applications in the
251 food and medical areas where food and nosocomial infections are not negligible.
252 Associated with ATP-metry, Sani System is then a real challenge in terms of quality,
253 efficiency and safety.

254 **Table I**: Composition of disinfectant HPMed

Ingredients	Metasilicate sodium	Carbonate sodium	Ethoxylate alcohol	Ethyl alcohol
Percentage	0.1-1%	0.1-1%	0.1-1%	5-10%

255 NB: Composition: Nonionic surfactant <5%

256 **Table II:** Biocidal activities of HPMed sanitizer

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	E. hirae	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
Untreated Supports (N)	$1.2\ 10^6$	$1.5\ 10^6$	$1.4\ 10^6$	$1.2\ 10^6$	$1.0\ 10^6$	$1.1\ 10^6$
Treated Supports (N')	<1	<1	<1	<1	20	30
log reduction (R)	> 6.07	> 6.17	> 6.14	> 6.07	4.69	4.56

Table III: Presence of mesophilic aerobic microorganisms in the three rooms

		R_1 (CFU/4h)			R_2 (CFU/4h)			R_3 (CFU/4h)		
		J1	J8	J15	J1	J8	J15	J1	J8	J15
After 360 sec	Not disinfected rooms	28	24	34	36	42	48	44	52	58
	Disinfected rooms	26	24	32	32	40	46	42	52	56
	% reduction	7.14	0	5.88	11.11	4.76	4.16	4.54	0	3.44
After 600 sec	Not disinfected rooms	36	32	44	66	58	64	80	86	84
	Disinfected rooms	18	18	24	34	28	34	58	60	60
	% reduction	50	43.75	45.45	48.48	51.72	46.87	27.5	30.23	28.57
After 3600 sec	Not disinfected rooms	34	38	36	74	70	68	88	92	90
	Disinfected rooms	2	2	2	4	4	4	6	6	4
	% reduction	94.11	94.73	94.44	94.59	94.28	94.11	93.18	93.47	95.55

Table IV: Analysis of variance of two factors

	Degree of liberty	Sums of squares	Mean squares	F statistic	**p-value**
Nebulization Time	2	36594	18297	476.5011	**<0.001**

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time	2	18	9.1	0.2367	**0.79**

Table V: Average rate reduction RLUs by area

Surfaces	before disinfection RLU	after disinfection RLU	Average reduction RLU_S % (sd*)	p-value
Tiles	2437.5	171.5	94.23 (3.17)	<0.001
Glass	603.8	37.7	93.85 (1.40)	<0.001
Plexiglass	719.9	68.4	90.56 (1.13)	<0.001

* : sd = standard deviation

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Figure 1. A nebulizer with dry saturated steam: Sani System Polti

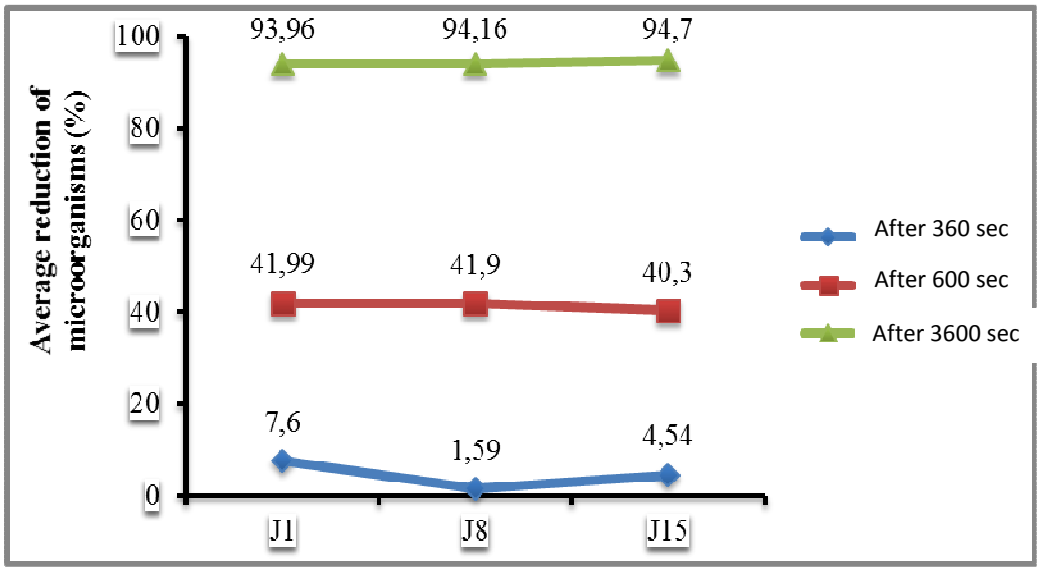

Figure 2: Evolution of the average rate of reduction of microorganisms by nebulization time

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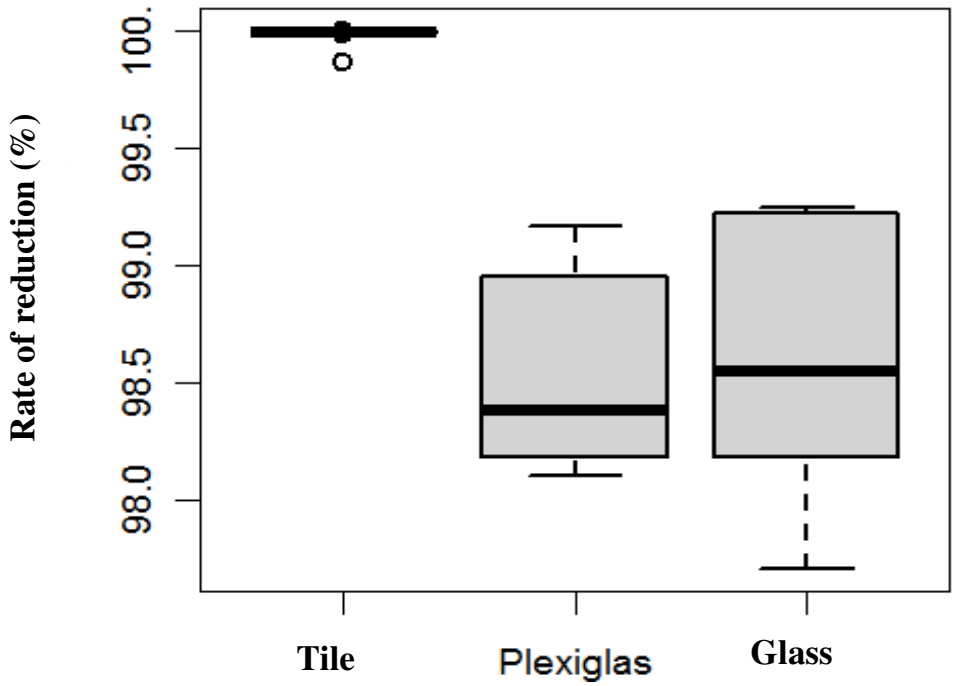

Figure 3: Distribution of the average rate of reduction of microorganisms by type of surface