

**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 different 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.

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

27 1. INTRODUCTION

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

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

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

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

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

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

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

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

72 **2. MATERIAL AND METHODS**

73 2.1. Material

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

77 2.1.1. Microbial strains reference

78 The antibacterial and antifungal activities of the disinfectant was *in vitro* evaluated on
79 ATCC (American Type Culture Collection) and IP (Institute Pasteur of Strasbourg)
80 strains namely: *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC
81 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853,
82 *Candida albicans* IP 4872 and *Aspergillus Niger* ATCC 16404.

83 2.1.2. Experimental disinfection equipment

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

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

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

97 **2.2. Methods**

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

103 **2.2.1. Evaluation of biocides activities of Sani System Polti**

104 The preparation of the microbial suspension was made according to AFNOR NF T
105 72-281 [7] where the dilution which gives a concentration of 10^6 germs per ml was
106 selected as microbial suspension. Biocidal activities of Sani System Polti have been
107 assessed by the test support [8; 9]. After contamination and drying, some slides
108 were treated by spraying of dry saturated steam in combination with sanitizer HPMed
109 for 30 seconds. Along with these tests, another slides contaminated by each
110 microorganism and untreated were maintained at room temperature (16°C)
111 throughout the tests for determining the initial contamination level brackets and
112 validation of experimental conditions.

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

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 / cm².

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:

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

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:

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

R is expressed in CFU/4h.

N₁, N₂, N₃, N₄, N₅ are the number of colonies counted for each of the five (05) plates seeded.

2.2.3. Enumeration of total aerobic mesophilic floras from lab surfaces

The samples were collected on three types of surfaces: glass, Plexiglas and tile (before and after 30 seconds of disinfection) using the technique of wet swab on an area of 10 cm². Ten samples were taken from the surface and 0.1 mL of each dilution sample was plated in duplicate using Tryptic Soy Agar [11]. After incubation at 30-35 °C for 72 h, the results expressed as CFU / ml of the initial product, were converted to counts per cm² using the following formula [12]:

$$N' = N \times 4 \text{ where:}$$

N': Number of CFU per cm².

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

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

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

2.2.5. Statistical analysis

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

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

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.

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 ($0-250 \text{ RLU/cm}^2$) [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

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

CONCLUSION

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

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%

NB: Composition: Nonionic surfactant <5%

Table II: Biocidal activities of HPMed sanitizer

	<i>E. hirae</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
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

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258 **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

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260 **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

time	2	18	9.1	0.2367	0.79
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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

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* : sd = standard deviation

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

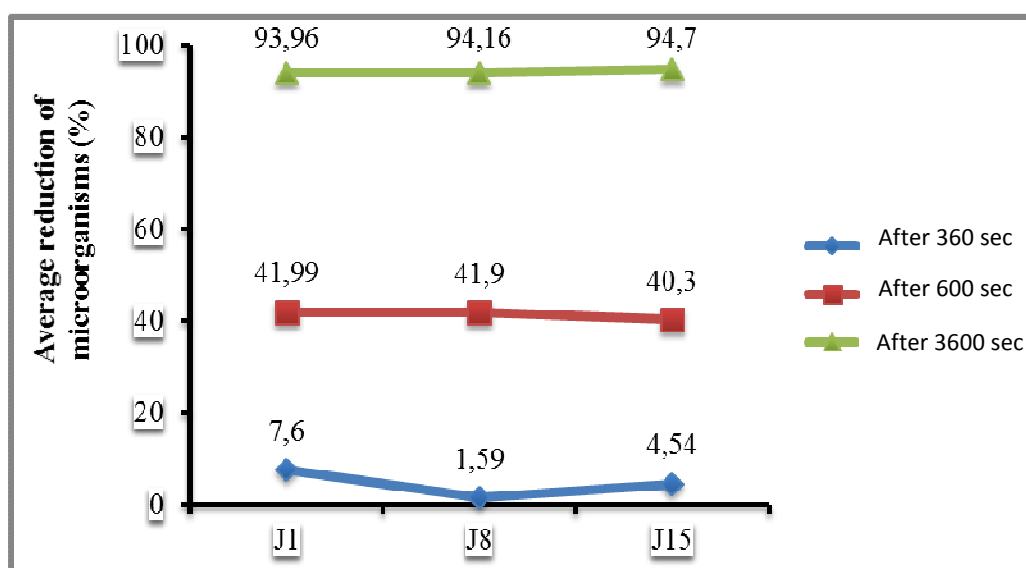


Figure 2: Evolution of the average rate of reduction of microorganisms by nebulization time

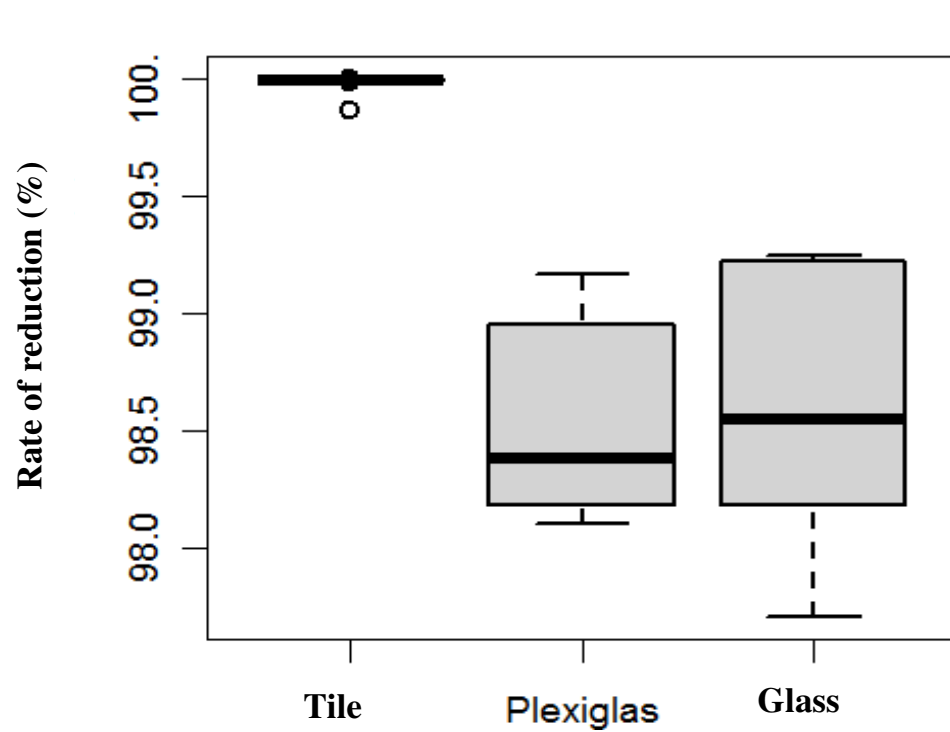


Figure 3: Distribution of the average rate of reduction of microorganisms by type of surface