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FINAL REPORT

Bactericidal and fungicidal activity of dry steam

SUMMARY

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1. Objective

The aim of the activities described below was the evaluation of the efficacy of the treatment with dry steam in the reduction of bacterial and fungal contamination of surfaces.

2. Methods

2.1 EU regulation

The current European regulation on chemical disinfectants and antiseptics was taken as reference to realise the *in vitro* trials described below. Table 1 summarise current EU standards.

Table 1 – EU regulation on chemical disinfectants and antisepticals

BASIC TESTS		
Standards	Characteristics	Reference organisms
EN 1040:2005	Bactericidal activity	<i>Pseudomonas aeruginosa</i> ATCC 15442 <i>Staphylococcus aureus</i> ATCC 6538
EN 1275:2006	Fungicidal activity	<i>Candida albicans</i> ATCC 10231 <i>Aspergillus niger</i> ATCC 16404
EN 14347:2005	Sporicidal activity	-
MEDICAL AREA (*)		
Standards	Characteristics	Reference organisms
prEN 13727:2003	Bactericidal activity	
EN 14348:2005	Mycobactericidal and tuberculocidal activity	
(*) Standards refer to the disinfection of surfaces		
VETERINARY AREA (*)		
Standards	Characteristics	Reference organisms
EN 1656:2000	Bactericidal activity	
EN 1657:2005	Fungicidal activity	
EN 14204:2004	Mycobactericidal	
En 14349:2007	Bactericidal activity	
(*) Standards refer to the disinfection of surfaces		
FOOD, INDUSTRIAL (breweries, beverage and soft drink industry, dairies, cosmetics), DOMESTIC AND INSTITUTIONAL AREA		
Standards	Characteristics	Reference organisms
EN 1276:2000	Bactericidal activity	<i>Pseudomonas aeruginosa</i> ATCC 15442 <i>Escherichia coli</i> ATCC 10536 <i>Staphylococcus aureus</i> ATCC 6538 <i>Enterococcus hirae</i> ATCC 10541 <i>Salmonella typhimurium</i> ATCC 13311 <i>Lactobacillus brevis</i> DSM 6235 <i>Enterobacter cloacae</i> DSM 6234
EN 1650:2000	Fungicidal activity	<i>Candida albicans</i> ATCC 10231 <i>Aspergillus niger</i> ATCC 16404
EN 13697:2006	Bactericidal and fungicidal	

	activity	
(*) Standards refer to the disinfection of surfaces		

Table 1 lists the basic rules as well as those specifically applied to the different areas.
 Standards considered in the present report, referred to food industry, have been highlighted in green.

2.2 Analytical protocol

Microbial cultures used for the trials described in the present report are listed in Table 1. They were prepared following the indications of the standards highlighted in green.

Treated surface were composed of polymeric material similar to the surfaces used in food industry.

Size of treated surface were cm 80 x cm 50 (total surface 4.000 cm²).

1.000.000.000 (1 billion) living cells of each tested microorganism was uniformly distributed on the polymeric surface, previously three times washed with sterile saline solution, ethyl alcohol and sterile distilled water, respectively, to remove any environmental contamination.

Following the distribution of the microorganism, the surface was treated with dry steam (technical and physical treatment were previously defined by the customer, as described in the Technical annex).

Samples were taken, directly on the surface and on the condensation liquid, after the treatment by using Copan pads.

Pads were then inserted in selective culture medium and incubated for 48 hours at the optimal growth temperature of the tested microorganism.

The number of colony forming units (CFUs) developed following incubation represented the number of residual living cells after dry steam treatment.

3. Results

Detailed results for all of the trials are included to the present report (analytical reports) and their list is reported in the Technical annex. Table 2 summarises the results of reduction of bacterial and fungal activity following dry steam treatment.

Table 2 – Reduction of the microbial charge of the treated surface

Code	Microbial species	Strain	Sampling	Pre-treatment count (CFU/4.000cmq)	Post-treatment count (CFU/4.000cmq)	Quantification
08F09712	Controllo - superficie non trattata				< LQ (#)	0,00E+00
08F09719	<i>Pseudomonas aeruginosa</i>	ATCC 15442	surface	1,00E+09	< LQ	0,00E+00
08F09720	<i>Pseudomonas aeruginosa</i>	ATCC 15442	surface	1,00E+09	2,00E+01	2,00E+01
08F09715	<i>Escherichia coli</i>	ATCC 10536	surface	1,00E+09	< LQ	0,00E+00
08F09713	<i>Staphylococcus aureus</i>	ATCC 6538	condensation	1,00E+09	< LQ	0,00E+00
08F09714	<i>Staphylococcus aureus</i>	ATCC 6538	surface	1,00E+09	1,00E+01	1,00E+01
08F09716	<i>Enterococcus hirae</i>	ATCC 10541	surface	1,00E+09	2,00E+01	2,00E+01
08F09724	<i>Enterococcus hirae</i>	ATCC 10541	surface	1,00E+09	< LQ	0,00E+00
08F09725	<i>Enterococcus hirae</i>	ATCC 10541	surface	1,00E+09	< LQ	0,00E+00
08F09717	<i>Salmonella typhimurium</i>	ATCC 13311	surface	1,00E+09	4,00E+01	4,00E+01
08F09718	<i>Lactobacillus plantarum</i> (*)	DSM 6235	surface	1,00E+09	< LQ	0,00E+00
08F09721	<i>Candida albicans</i>	ATCC 10231	surface	1,00E+08	< LQ	0,00E+00
08F09722	<i>Aspergillus niger</i>	ATCC 16404	surface	1,00E+07	2,00E+01	2,00E+01
08F09723	<i>Aspergillus niger</i>	ATCC 16404	condensation	1,00E+07	< LQ	0,00E+00

(*) *L. plantarum* replaced *L. brevis*

(**) lance passage at 10 cm from the surface with max steam

(***) passage with ¾ steam

(#) below the detection limit

Results listed in Table 2 show a significant reduction (taking into account the detection limit of the method) of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus hirae* e *Lactobacillus plantarum*. Further decrease in the charge of *E. hirae* has been obtained by increasing steam flow and approaching it at 10 cm from the surface. Condensation liquid was found to contain, after steam treatment, 20 CFUs of *P. aeruginosa* and 10 CFUs of *S. aureus* on the surface of 4.000 cmq.

S. typhimurium showed residual 40 CFUs out of 1.000.000.000 CFUs initially distributed.

Graphs 1 and 2 summarise the results obtained about bactericidal and fungicidal activity of dry steam.

Figure 1 – Bactericidal efficacy of dry steam treatment

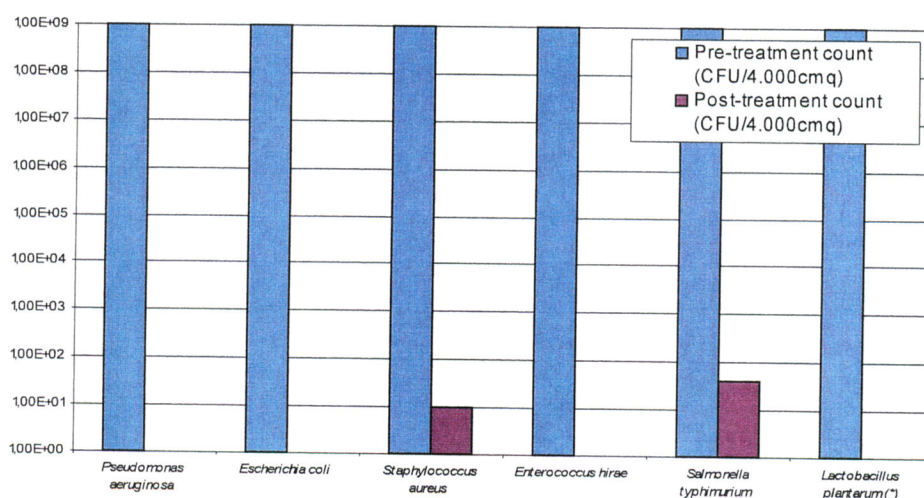
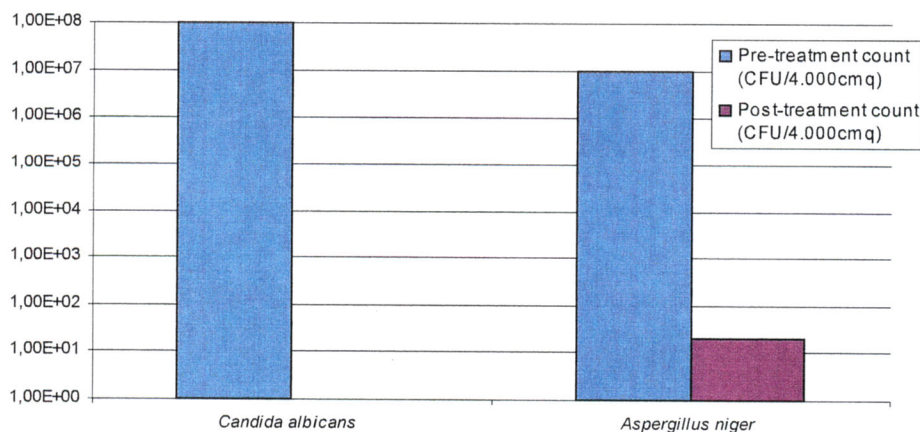


Figure 2 – Fungicidal efficacy of dry steam



4. Conclusions

Trials described in the present report allowed to demonstrate that dry steam treatment (applied as previously established by the customer) was successful in reducing the microbial charge of surfaces deliberately contaminated with some bacteria and fungi. Significant reduction was detected for *P. aeruginosa*, *E. coli*, *E. hirae*, *L. plantarum*. *C. albicans* that were killed at levels lower than the detection limit of the method.

Other micororganisms, like *S. aureus*, *S. typhimurium* ed *A. niger*, were consistently reduced but the detected level of contamination post-treatment was higher than the detection limit (> LQ) of the applied method.

Anyway, count values measured after-treatment always revealed a reduction of contamination higher than those required by the European standards for chemical disinfectants and antisepticals that indicate a reduction in the total bacterial charge of at least 10^4 CFUs for fungicides and at least 10^5 CFUs for bactericides.

Piacenza, 07.07.2008

Signature *Marina Elli*

(Marina Elli, President of the Board of Directors)

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5. Technical annex

Trials were realised by the company Neutron S.p.A., Stradello Agazzotti 104, 41100 S. Maria di Mugnano, Modena (Italy).

Neutron S.p.A. released to AAT S.r.l. the corresponding analytical reports, included in the present document, that were used by AAT to elaborate tables , graphs and final remarks.

List of analytical reports included:

- 08F09712 – prelievo iniziale ante-trattamento sulla superficie lavata
- 08F09713 – prelievo post-trattamento con *Staphylococcus aureus* su liquido di trascinamento
- 08F09714 - prelievo post-trattamento con *Staphylococcus aureus* con passaggio su tutta la superficie
- 08F09715 - prelievo post-trattamento con *Escherichia coli* con passaggio su tutta la superficie
- 08F09716 - prelievo post-trattamento con *Enterococcus hirae* con passaggio su tutta la superficie
- 08F09717 - prelievo post-trattamento con *Salmonella typhimurium* con passaggio su tutta la superficie
- 08F09718 - prelievo post-trattamento con *Lactobacillus plantarum* con passaggio su tutta la superficie
- 08F09719 - prelievo post-trattamento con *Pseudomonas aeruginosa* con passaggio su tutta la superficie
- 08F09720 - prelievo post-trattamento con *Pseudomonas aeruginosa* su liquido di trascinamento
- 08F09721 - prelievo post-trattamento con *Candida albicans* con passaggio su tutta la superficie
- 08F09722 - prelievo post-trattamento con *Aspergillus niger* con passaggio su tutta la superficie
- 08F09723 - prelievo post-trattamento con *Aspergillus niger* su liquido di trascinamento
- 08F09724 - prelievo post-trattamento con *Enterococcus hirae* con passaggio su tutta la superficie
- 08F09725 - prelievo post-trattamento con *Enterococcus hirae* su liquido di trascinamento

Bactericidal and fungicidal efficacy of dry steam

Study sponsored by



Objective

Evaluation of the efficacy of dry steam treatment realised by menikini machines in order to clean surfaces contaminated by bacteria and fungi.

Reference

Trials were based on the European Regulation related to chemical disinfectants and antiseptics with the aim to evaluate the ability of dry steam to decrease bacterial and fungi contamination of surfaces.

List of EU rules:

EN 1040:2005

EN 1275:2006

EN 1276:2000

EN 1650:2000

Methods

One billion of colony forming units of each microorganism were uniformly distributed on a polymeric surface (as used in food industry), previously cleaned to remove any contamination. After the distribution of the test microorganism, the surface was treated with saturated dry steam. At the end of the treatment, bacteria on the surface and in the residual condensation liquid were sampled and lab analysed by sterile pads.

Results

In vitro trials demonstrated that the treatment with saturated dry steam was effective in the reduction of bacterial and fungal contamination of surfaces

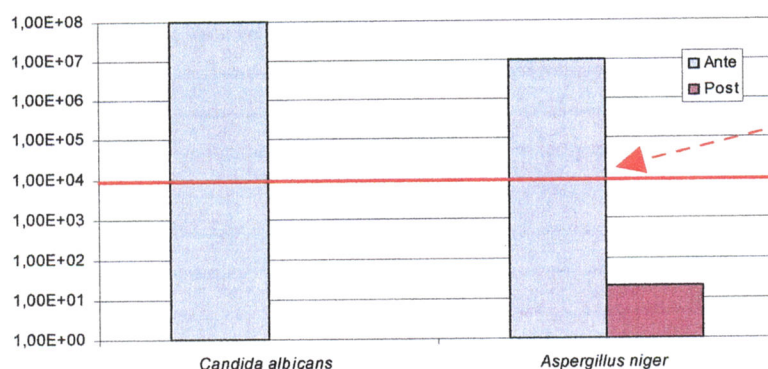
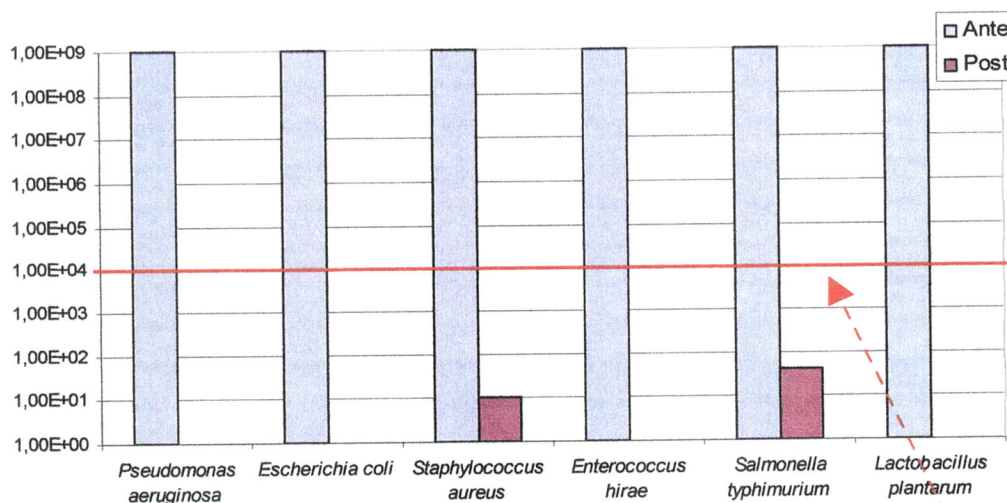
The analytical values of bacterial and fungal contamination after treatment were always below the levels indicated by the EU rules that refer to a decrease of 10^4 UFC for fungicides and 10^5 UFC for bactericides.

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Results and graphs at sh. 2 →

Results

Code	Microbial species	Strain	Sampling	Pre-treatment count (CFU/4.000cmq)	Post-treatment count (CFU/4.000cmq)	Quantification
08F09712	Surface check before treatment				< LQ (#)	0,00E+00
08F09719	<i>Pseudomonas aeruginosa</i>	ATCC 15442	surface	1,00E+09	< LQ	0,00E+00
08F09720	<i>Pseudomonas aeruginosa</i>	ATCC 15442	surface	1,00E+09	2,00E+01	2,00E+01
08F09715	<i>Escherichia coli</i>	ATCC 10536	surface	1,00E+09	< LQ	0,00E+00
08F09713	<i>Staphylococcus aureus</i>	ATCC 6538	condensation	1,00E+09	< LQ	0,00E+00
08F09714	<i>Staphylococcus aureus</i>	ATCC 6538	surface	1,00E+09	1,00E+01	1,00E+01
08F09716	<i>Enterococcus hirae</i>	ATCC 10541	surface	1,00E+09	2,00E+01	2,00E+01
08F09724	<i>Enterococcus hirae</i>	ATCC 10541	surface	1,00E+09	< LQ	0,00E+00
08F09725	<i>Enterococcus hirae</i>	ATCC 10541	surface	1,00E+09	< LQ	0,00E+00
08F09717	<i>Salmonella typhimurium</i>	ATCC 13311	surface	1,00E+09	4,00E+01	4,00E+01
08F09718	<i>Lactobacillus plantarum</i> (*)	DSM 6235	surface	1,00E+09	< LQ	0,00E+00
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08F09722	<i>Aspergillus niger</i>	ATCC 16404	surface	1,00E+07	2,00E+01	2,00E+01
08F09723	<i>Aspergillus niger</i>	ATCC 16404	condensation	1,00E+07	< LQ	0,00E+00



Reference values for
EU regulation

Complete results and reports are available Menikini S.r.l. and can be supplied to the customer following request