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DE LA SAVOIE**

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**SYNTHESIS REPORT  
ASSAY N° 99-01  
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**STUDY OF THE SANITIZING EFFECTS  
(bactericidal, fungicidal)  
OF THE M 20 MACHINE realized by HYGITEC Company**

**Purpose :** As ordered by HYGITEC Company, the *Laboratoire Départemental d'Analyses Vétérinaires de la Savoie* has tested the hygienizing ability of a standard M 20 ozone machine which aim is to dry and disinfect work shoes (bactericidal, fungicidal effects).

**Testing procedure :** 5 micro-organisms, indicator of hygienic or medical risks, are used in the assay (see table I). For each one, 6 Wellington boots are contaminated with a concentrated pure micro-organism inoculum, dropped on their inside surface. Five of these 6 boots are submitted to a standard working cycle of the M 20 machine. The sixth contaminated boot is kept outside the machine to be used as positive no-treated control.

Sterility controls (no-contaminated shoes) are added either inside the M 20 machine, or beside the positive control, to prove the lack of exogenous contamination.

TEST STRAIN and Standard ref	CHARACTERISTICS	MICROBIAL CULTURE AND COLONY COUNT (C.C.)
<i>Candida albicans</i> : 1180.79 strain, Pasteur Institut	Inducing medical disorder, especially dermatomycosis	Medium : Potato Dextrose Agar Incubation : 30°C, C.C. at 48 h
<i>Staphylococcus aureus</i> : 53.154 strain, Pasteur Institut	Responsible for medical disorder, especially dermatitis	Medium : Chapman incubation : 37°C, C.C. at 24 et 48 h
<i>Pseudomonas aeruginosa</i> : A 22 strain, Pasteur Institut	ubiquitous pathogenic bacteria inducing nosocomial disorder	Medium : cetrimide agar incubation : 37°C, C.C. at 24 et 48 h
<i>Escherichia coli</i> : 54.127 strain, Pasteur Institut	Biological indicator for faecal pollution, which is the main risk in agriculture and food industry	Medium : 7Tergitol TTC lactose agar incubation : 37°C, C.C. at 24 et 48 h
<i>Bacillus cereus</i> : 78.03 strain, Pasteur Institut	Biological indicator for microbial resistance (spore forming bacteria)	Medium : Mossel incubation : 37°C, C.C. at 24 et 48 h

After standard treatment, the micro-organism burden is calculated for each germ and each boot, through colony counts of 6 ten-to-ten dilutions seeded on specific medium. Treated boots burden is compared with positive control boot burden. Colony count uncertainty is estimated as + 1/3 decimal logarithm.

## Results :

The following table summarizes the assay data, all the sterility controls being valuable :

	TESTED MICRO-ORGANISMS	Variation coefficient between the 5 tests (stand. dev. / mean)	Pathogenic burden of the positive control	average reduction (decimal log)	that means : reduction of pathogenic burden of :
90 minutes	<i>Candida albicans</i>	1,16	38 000	2,74	99,82 %
	<i>Staphylococcus aureus</i>	1,09	1 190 000	3,88	99,987 %
	<i>Pseudomonas aeruginosa</i>	0	1 060 000	> 6,03	100 %
	<i>Escherichia coli</i>	0	3 500 000	> 6,54	100 %
	<i>Bacillus cereus</i>	0	590 000	> 5,77	100 %

For *Bacillus cereus*, *Pseudomonas aeruginosa* et *Escherichia coli*, the standard working cycle of HYGITEC M 20 ozone machine eliminates all the germs at 90 mn.

Pour *Candida albicans* et *Staphylococcus aureus*, the average reduction is  $10^{-2,74}$  for the former and  $10^{-3,88}$  for the latter, compared to a no-treated boot..

## Conclusion :

This assay demonstrates an accurate hygienizing effect on internal surface of wellington boots, because of drying and ozone treatment during 90 mn.

Chambéry, 23-12-99

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