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THE NATIONAL CENTER FOR HOSPITAL HYGIENE

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STATENS
SERUM
INSTITUT

prevention and control
of infectious diseases
and congenital disorders

REPORT

Test on

JIMCO OZ2000

Compact Cleaner

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Report on tests with UV-generated ozone at Statens Serum Institut

This report refers to tests performed in the period 1 April 2000 to 2 May 2000. The tests were performed for JIMCO, Ellehaven 4, Rudkøbing.

Aim

The aim with the tests was to establish the effect of ozone on the bacteria *Listeria monocytogenes* and *Salmonella typhimurium*, specifically on surfaces where these bacteria typically are to be found in the food industry. To simulate a surface in the food industry pieces of stainless steel material were inoculated with a known quantity of culture. Thus, testing was aimed at eliminating the mentioned bacteria on surfaces.

The principle of the tests

In the tests the bacterial suspensions with an identified inoculum of known quantity were placed on a plate of stainless steel material and exposed to the action of ozone in respectively 1, 2, 3 and 4 hours.

Likewise, controls without ozone exposure were included to verify a possible reduction or growth on the plates before the exposure. The two tests were compared and the killing effect of ozone on the microorganisms was computed.

Beside the pure cultivation tests on steel plates, additional similar tests were carried out, with applied cultures protected by human albumin to copy a situation where blood residues or other growth promoting materials are found on e.g. a working table.

With the exception of the preliminary tests, all tests were carried out as double tests and all dilution series were performed in duplicate.
In this report all initial counting numbers are quoted in cfu pr. ml. (Colony Forming Units).

Equipment
JIMCO OZ2000 Compact Cleaner
Timer
Room no.109, building 11A

Test strains
Salmonella typhimurium
Listeria monocytogenes - serogroup 1

Growth media and reagents

1. Human albumin 5%
2. Natrium chloride dissolution 0,9%(salt water)
3. TGY agar plates
4. Blood agar plates 5%

Apparatus and utensils (sterile)

Flow hood (LAF) SSI no. 40631/29549
Vortex mixer
Hockey sticks
Stainless steel plates
Latex gloves
Empty Petri dishes
15 mm sterile plastic tubes with lid
Sterile pipette tips 10-1000 µl
Bar pipettes 1 ml + 10 ml
Incubators 36°C± 2
Pipettes, calibrated

Description of the test

1. The test was carried out in room 109, building 11A (room dimensions: 285 cm x 293 cm x 239 cm. Total 19,95 m^3.) The JIMCO OZ2000 Compact Cleaner was placed 110 cm over the surface of the table, on which the applied stainless steel plates were exposed.
2. At first the bacterial strains were grown, and counted initially in order to verify the concentration of the suspension. Then, dilution series until 10^{-8} were plated on agar and incubated.
3. A known quantity of microorganisms was placed on the stainless steel plates and dried at room temperature. Afterwards, the plates were exposed to the ozone. After having been exposed for a given period the steel plates were shaken up in sterile 0,9% natrium chloride dilution in order to measure the recovery percentage. Tests were carried out with and without the albumin load. All tests were performed as double tests (ref. Table 2).

The control tests were carried out following the same procedure as described above without the exposure to ozone. The control tests were not performed as double tests, except for the *Listeria* with albumin load (ref. Scheme 1).

Scheme 1: Control tests
The following strains have been exposed in a flow hood at room temperature.

Exposure ⇒	1 hour	2 hours	3 hours	4 hours
Listeria	x	x	x	x
Listeria with albumin	x	x	x	x
Listeria with albumin	x	x	x	x
Exposure ⇒	**1 hour**		**3 hours**	**4 hours**
Salmonella	x		x	x
Salmonella with albumin	x		x	x

The results of the control tests are shown in the Tables 1-5.

Scheme 2: Ozone tests
The following strains have been exposed in an ozone loaded test room at room temperature.

Exposure ⇒	1 hour	2 hours	3 hours	4 hours
Listeria				
Listeria				
Listeria with albumin				
Listeria with albumin				
Exposure ⇒				
Salmonella				
Salmonella				
Salmonella with albumin				
Salmonella with albumin				

The results of the ozone tests are shown in Table 6.

Results

Table 1

Preliminary tests			
Listeria med albumin	shaken up	initial counting	recovery
Time 1	1,20E+08	9,30E+07	129 %
Time 2	1,80E+08	9,30E+07	194 %
Time 3	8,50E+07	9,30E+07	91 %
Time 4	7,80E+07	9,30E+07	84 %
average			124 %

Additional tests

Table 2

Preliminary tests			
Listeria med albumin	shaken up	initial counting	recovery
Time 1	1,70E+09	9,00E+08	189 %
Time 2	1,81E+09	9,00E+08	201 %
Time 3	1,69E+09	9,00E+08	188 %
Time 4	1,30E+09	9,00E+08	144 %
average			181 %

Table 3

Preliminary tests			
Listeria without albumin	shaken up	initial counting	recovery
Time 1	1,90E+07	1,00E+09	1,9 %
Time 2	1,90E+07	1,00E+09	1,9 %
Time 3	1,40E+07	1,00E+09	1,4 %
Time 4	1,90E+07	1,00E+09	1,9 %
average			2 %

Table 4

Preliminary tests			
Salmonella med albumin	shaken up	initial counting	recovery
Time 1	1,00E+08	4,00E+08	25,0 %
Time 2	9,60E+07	4,00E+08	24,0 %
Time 3	7,30E+07	4,00E+08	18,3 %
Time 4	7,30E+07	4,00E+08	18,3 %
average			21,4 %

Table 5

Preliminary tests			
Salmonella without albumin	shaken up	initial counting	recovery
Time 1	3,20E+08	4,90E+08	65,3 %
Time 2	4,00E+08	4,90E+08	81,6 %
Time 3	2,70E+08	4,90E+08	55,1 %
Time 4	2,00E+08	4,90E+08	40,8 %
average			60,7 %

Table 6. The results of the tests with ozone

	A		B	C		D		E
Exposure	Initial counting Suspension		Corrections factor	Corrected initial counting A x B		cfu after exposure		Reduction C - D
	cfu	log	%	cfu	log	cfu	log	%
Listeria with albumin								
1 hour	$4x\ 10^8$	8.60	129	$5.16x10^8$	8.71	$1.30\ x\ 10^8$	8.11	74.8
1 hour	$4x\ 10^8$	8.60	129	$5.16x\ 10^8$	8.71	$1.50\ x10^8$	8.18	70.9
2 hours	$4x\ 10^8$	8.60	194	$7.76x10^8$	8.89	$1.50^8\ x10^8$	8.18	80.7
2 hours	$4x\ 10^8$	8.60	194	$7.76x10^8$	9.89	$1.20^8\ x10^8$	8.01	84.5
3 hours	$9x\ 10^8$	8,95	91	$8.19x10^8$	8.91	$4.50^8\ x10^8$	8.65	45.1
3 hours	$9x\ 10^8$	8,95	91	$8.19x10^{\ 8}$	8,91	$5.90^8\ x10^8$	8.77	28.0
4 hours	$9x\ 10^8$	8.95	84	$7.56x10^8$	8.88	$4.50^8\ x\ 10^8$	8.65	40.5
4 hours	$9x\ 10^8$	8.95	84	$7.56x10^8$	8.88	$5.50^8\ x\ 10^8$	8.74	27.2
Listeria without albumin								
1 hour	$4x\ 10^9$	9.60	1.9	$7.60\ x\ 10^7$	7.88	$6.30\ x\ 10^7$	7.80	98.43
1 hour	$4x\ 10^9$	9.60	1.9	$7.60\ x\ 10^7$	7.88	$4.10\ x\ 10^7$	7.61	98.98
2 hours	$4x\ 10^9$	9.60	1.9	$7.60\ x\ 10^7$	7.88	$2.20\ x\ 10^7$	7.34	99.45
2 hours	$4x\ 10^9$	9.60	1.9	$7.60\ x\ 10^7$	7.88	$3.20\ x\ 10^7$	7.51	99.20
3 hours	$7x\ 10^9$	9.60	1.4	$9.80\ x\ 10^7$	7.99	$2.70\ x\ 10^7$	7.43	99.61
3 hours	$7x\ 10^9$	9.60	1.4	$9.80\ x\ 10^7$	7.99	$2.70\ x\ 10^7$	7.43	99.61
4 hours	$7x\ 10^9$	9.60	1.9	$1.33\ x\ 10^8$	8.12	$3.50\ x\ 10^7$	7.54	99.50
4 hours	$7x\ 10^9$	9.60	1.9	$1.33\ x\ 10^8$	8.12	$3.00\ x\ 10^7$	7.48	99.57
Salmonella with albumin								
1 hour	$3x\ 10^8$	8.48	25.0	$7.50\ x\ 10^7$	7.88	$5.40\ x\ 10^7$	7.73	28.00
1 hour	$3x\ 10^8$	8.48	25.0	$7.50\ x\ 10^7$	7.88	$5.80\ x\ 10^7$	7.76	22.67
2 hours	$3x\ 10^8$	8.48	24.0	$7.20\ x\ 10^7$	7,86	$4.60\ x\ 10^7$	7.66	36.11
2 hours	$3x\ 10^8$	8.48	24.0	$7.20\ x\ 10^7$	7,86	$4.00\ x\ 10^7$	7.60	44.44
3 hours	$2.8x10^8$	8.45	18.3	$5.12\ x\ 10^7$	7.71	$5.90\ x\ 10^7$	7.77	-15.14
3 hours	$2.8x10^8$	8.45	18.3	$5.12\ x\ 10^7$	7.71	$5.40\ x\ 10^7$	7.73	-5.39
4 hours	$2.8x10^8$	8.45	21.4	$5.99\ x\ 10^7$	7.78	$4.20\ x\ 10^7$	7.62	29.91
4 hours	$2.8x10^8$	8.45	21.4	$5.99\ x\ 10^7$	7.78	$4.00\ x\ 10^7$	7.60	33.24
Salmonella without albumin								
1 hour	$2.4x\ 10^9$	9.38	65.3	$1.57x\ 10^9$	9.20	$1.60\ x10^8$	8.20	89.79
1 hour	$2.4x\ 10^9$	9.38	65.3	$1.57x\ 10^9$	9.20	$1.30\ x10^8$	8.11	91.70
2 hours	$2.4x\ 10^9$	9.38	81.6	$1.96x\ 10^9$	9.29	$9.30\ x10^7$	7.97	95.25
2 hours	$2.4x\ 10^9$	9.38	81.6	$1.96x\ 10^9$	9.29	$6.90\ x10^7$	7.84	96.48
3 hours	$2.4x\ 10^9$	9.38	55.1	$1,32x\ 10^9$	9.12	$1.00\ x10^8$	8.00	92.44
3 hours	$2.4x\ 10^9$	9.38	55.1	$1,32x\ 10^9$	9.12	$7.30\ x10^7$	7.86	94.48
4 hours	$2.4x\ 10^9$	9.38	40.8	$9.79x\ 10^8$	8.99	$6.20x\ 10^7$	7.79	93.67
4 hours	$2.4x\ 10^9$	9.38	40.8	$9.79x\ 10^8$	8.99	$7.50x\ 10^7$	7.88	92.34

References and conclusion

Salmonella typhimurium and *Listeria monocytogenes* were used for the testing of the bactericidal effect of ozone. These strains were considered to be relevant contaminants on surfaces in the food production industry.

The testing of the effect of ozone was planned according to the principles of the prEN 13697:1999, which is possibly going to be the Danish standard for quantitative surface testing of chemical disinfectants and antiseptics used in the food industry.

A complete standard, which precisely describes the requirements to this particular test, does not exist. PrEN 13697:1999 indicates that a bactericidal effect exists if the test results show a reduction factor of 10^4 or 99.99% both with albumin load as well as without albumin load.

The accomplished test of the product, JIMCO OZ2000 Compact Cleaner, demonstrated a reduction factor that did not exceed 2 logarithmic steps, which corresponds to a reduction factor of max. 10^2 or 99% reduction of the non-albumin loaded microorganisms.

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