Pharmacia&Upjohn

MB

Antec International

March 21, 2000

Att: Mark Blackwell /
whom it might concern

Re: Biofilms report

University of Maryland trial

Please inform which products were used in the enclosed trial as well as who made it! Otherwise there is no use in referreing to it when discussing with our customers.

Best regards

Pharmacia & Upjohn
Animal Health AB

Tiina Wasberg

Encl. 3 pages

Pharmacia & Upjohn Animal Health AB
Postiosoite PL 31 FIN-20781 Kaarina Finland
Katuosoite Apilakatu 10 FIN-20740 Turku Finland
Puhelin/Telephone (02) 243 7600 National +358 2 243 7600 International
Telekopio/Telefax (02) 243 7660 National +358 2 243 7660 International
Kotipaikka Kaarina Kaupparek.no, 697.119

Report on the efficiencies of various disinfectants

Several commercially available disinfectants that fall into the groups of chlorine, quaternary ammonium compounds, acid groups, and phenols were tested for their efficiency against *Salmonella* species that are more frequently isolated from the poultry transport cages.
The five different species used in the tests are

1. *Salmonella typhimurium* (O group:B)
2. *Salmonella thompson* (O group:C_1)
3. *Salmonella hadar* (O group:C_2)
4. *Salmonella berta* (O group:D)
5. *Salmonella johannesburg* (O group:R)

Galvanized steel, a common material with which the poultry transport cages are constructed, was used to test for the efficiency of the disinfectants. In order to simulate the existing field conditions in the transport cages, disinfectants were first tested on galvanized steel (GS) surfaces contaminated with organic material and then on GS surfaces containing biofilms of *Salmonella*. A practical time of exposure, i.e., less than two minutes, was adopted in testing the efficiency of the disinfectants.

The bacteria was grown in brain heart infusion (BHI) broth overnight in a shaking incubator. Organic material in the form of chicken faecal slurry mixed with the 5 species of *Salmonella* constituting a microbial concentration of about 5 x 10^8 cfu/ml was seeded onto a 4" circle marked on a 5" x 5" GS surface and was allowed to dry for 45 minutes. Swabs were taken from drying time to determine the bacterial population that would be present on the surface. The bacterial population was estimated as colony forming units (cfu) through serial dilution and spread plate method. Then the disinfectant at the recommended concentration by the manufacturers, was applied evenly on the same 4" circular area on the GS surface. After two minutes of reaction time, the surface was swabbed to determine the number of colony forming units present on the surface after the treatment. Based on the results, those disinfectants that effectively reduced the bacterial load by at least 99% were chosen and tested on surfaces contaminated with biofilm containing *Salmonella*

The chicken transport cages when not properly cleaned will contain thick slimy layer in which bacteria could be buried. This layer is called a biofilm and is formed by the bacteria as a protective covering. For developing biofilms in the laboratory, a 15 ml-culture well was filled with 5 ml of enriched tryptic soy broth (TSB) and inoculated with 10 µl of each of the 5 *Salmonella* species. One galvanized steel chip of ¾" X ¾" was placed in the inoculated media and incubated to develop the biofilm on the GS chips. For biofilms of more than 2 days, the media was replaced without disturbing the formation of biofilm once in every two days. Since the scanning electron microscopic pictures of the biofilm surface revealed that the spread of bacteria was very scarce on 1- and 2-day biofilm surfaces, the disinfectant compounds were tested starting from 3-day biofilms.

To determine the bacterial population in biofilm, the GS chip in the broth culture was carefully removed not to disturb the surface and gently washed with phosphate buffer saline (PBS) to remove planktonic cells. The surface was then swabbed and analyzed for bacterial disinfectant solution at the same concentration as tested in the previous step. After 2 minutes of treatment time, the GS chip was removed from the disinfectant solution and swabbed to determine the bacterial population.

The efficiency of the disinfectants in the presence of a biofilm were tested by applying the selected disinfectants on 3-day old biofilms. Those disinfectants that reduced the bacterial load by at least 99% on the 3-day old biofilm surface were tested on 4-day old biofilm surfaces. The results are tabulated below. Each value reported is the average of at least two trials.

Product	Condition of the surface	Concen-tration	Contact time (min)	Bacterial population (cfu/ml)			Percent Reduction (B-C)/B* 100%
				Before Treatment		After treatment C	
				After drying A	After 45min drying B		
A	Surface Contamination	0.25%	2	$7.2x10^7$	$2.97x10^6$	$2.23x10^5$	92.49
			5	$7.2x10^7$	$2.97x10^6$	$1.92x10^5$	93.54

			10	$7.2x10^7$	$2.97x10^6$	$1.42x10^5$	95.22
		0.5%	2	$7.2x10^7$	$2.97x10^6$	$1.6x10^6$	46.2
			5	$7.2x10^7$	$2.97 x 10^6$	$7.75x10^4$	97.39
			10	$7.2x10^7$	$2.97x10^6$	$1.12x10^6$	62.29
		1%	2	$5.2x10^8$	$1.73x10^5$	$1.6x10^5$	7.51
			10	$5.2x10^8$	$1.73x10^5$	$1.14x10^4$	93.41
			30	$2.73x10^8$	$6.2x1-^4$	$1.35x10^3$	97.82
		2%	2	$5.2x10^8$	$1.73x10^5$	$9.35x10^3$	94.60
			10	$5.2x10^8$	$1.73x10^5$	$2.22x10^6$	
B	Surface Contamination	250 ppm	2	$2.49x10^8$	$2.25x10^7$	$1.67x10^6$	92.58
	3-day biofilm	500 ppm	2	$3.71x10^4$		0	92.58
	4-day Biofilm	500 ppm	2	$4.28x10^7$		0	100
Virkon S	Surface Contamination	2%	2	$3.14x10^6$	$3.92x10^5$	0	100
	3-day biofilm	2%	2	$3.71x10^4$		0	100
	4-day biofilm	2%	2	$4.28x10^7$		0	100
C	Surface Contamination	0.8%	2	$2.49x10^8$	$2.25x10^7$	$2.56x10^4$	99.89
	3-day biofilm	0.8%	2	$3.71x10^4$		$2.8x10^3$	92.45
D	Surface Contamination	0.8%	2	$2.49x10^8$	$2.25x10^7$	$2.1x10^3$	99.99
	3-day biofilm	0.8%	2	$3.71x10^4$		$9x10^2$	97.57
E	Surface Contamination	0.2%	2	$2.49x10^8$	$2.25x10^7$	$5.74x10^6$	74.44
F	Surface Contamination	0.2%	2	$2.49x10^8$	$2.25x10^7$	$1.6x10^6$	92.89
G	Surface Contamination	0.48%	2	$2.49x10^8$	$2.25x10^7$	$1.62x10^7$	99.28
	3-day biofilm	0.48%	2	$3.71x10^7$		$2x10^2$	99.46
	4-day biofilm	0.48%	2	$1.98x10^7$		$8.8x10^5$	95.56
H	Surface Contamination	3%	2	$1.91x10^8$	$3.17x10^7$	$7.5x10^5$	97.63
	3-day biofilm	3%	2	$3.71x10^4$		$2.5x10^3$	93.26
I	Surface Contamination	6%	2	$1.91x10^8$	$3.17x10^7$	$4.04x10^6$	87.26
J	Surface Contamination	1.6%	2	$1.91x10^9$	$3.17x10^7$	$2.33x10^7$	26.50
K	Surface Contamination	0.5%	10	$2.58x10^8$	$6.15x10^7$	$8.15x10^3$	99.99
	3-day biofilm	0.5%	10	$7.15x10^3$		$1x10^2$	98.60