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Microbiological quality control of a cage washing machine by means of a thin film culture system (Petrifilm™)

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Introduction

Effective cage washing in a washing machine is dependent on adequate water temperature, wetting of all surfaces, and the effect of detergent. Most cage washers make use of this concept in that the cage is exposed to a detergent that will act on fats and proteins, dissolving them and removing them from the surface. High pressure water scrubbing is often an important element. The process is facilitated by exposing the cage to high temperature. It is therefore important that all surfaces are exposed to water at high temperatures.

Cages are not sterilised, but should be expected to be disinfected.

A quality test of a washing machine will have to look at these factors. Temperature registration will indicate the degree of heat treatment that the cages can be exposed to. This does not give adequate information regarding the distribution of water and detergent in the machine. This can only be estimated by means of bacteriological surface counts taken in each cage. Such assays will indicate to what extent bacteria survive the rigours of a wash process.

Testing will make use of surface sampling. Bacteria on the cage surface can be collected and cultured. Reduction in non-specific total colony counts will indicate machine effectiveness. Few to no colonies should be recovered from the exposed surfaces. Similar systems are used in the food hygiene industry (*Anon* 1993).

This study has made use of a dry rehydratable medium (Petrifilm™) as an indicator of washing machine effect. Residual cage surface bacteria have been sampled and assayed quantitatively.

Non specific total colony counts have been used as a measure of surface decontamination.

Materials and Methods

Washing machine:

A Växjø stainless steel cabinet washing machine (Växjø Rostfritt model 490, Växjø Rostfritt, Växjø, Sweden) capable of washing 2 racks of cages simultaneously was tested. Each rack measured 137 cm long 180 cm high and 98 cm wide. Cages were loaded on both sides.

The washing programme is shown in fig. 1.

Two racks of cages were washed at the same time. A typical load is shown on fig. 2 The racks were numbered as rack 1 and rack 2. Each rack was loaded with up to eight plastic rabbit waste collecting pans or cage inserts (ScanBur model 10-NO8X, ScanBur ApS, Køge, Denmark), up to 4 on either side, or up 16 small rodent cages, 8 on either side. Varying numbers of MAKIII and MAKIV rodent cages (Techniplast, Milan, Italy), were loaded (depending on the daily production of dirty cages). Each rack side was designated as left and right.

Study one sampled bacterial counts before and after washing. Based on these results we concluded that further sampling was only necessary after washing.

Sampling strategy:

Five samples were taken from the inside of each cage. A typical sampling pattern is shown in fig. 2a and 2b.

Figure 1.
The temperatures (°C) in the washing chamber and main water tank (water and detergent) (Tank 1) and rinse/disinfection tank (tank 2) during two cycles (# 1 and #2) recorded on two separate days) are measured in comparison to manufacturers specification (washing chamber temperature). Measurements were taken each minute, starting from the start of a wash cycle.

- ✖ The temperature °C in tank 1 during cycle 1
- ◆ The temperature °C in tank 1 during cycle 2
- • The temperature °C in tank 2 during cycle 1
- ▼ The temperature °C in tank 2 during cycle 2
- ■ Represents the washing chamber temperature °C during cycle 1
- ▲ Represents the washing chamber temperature °C during cycle 2
- ❑ Represents the washing chamber temperature according to manufacturers specification
- **A** - Start phase
- **B** - Washing phase (detergent and temperature)
- **C** - Rinse
- **D** - Disinfection (hot water)
- **E** - Drying

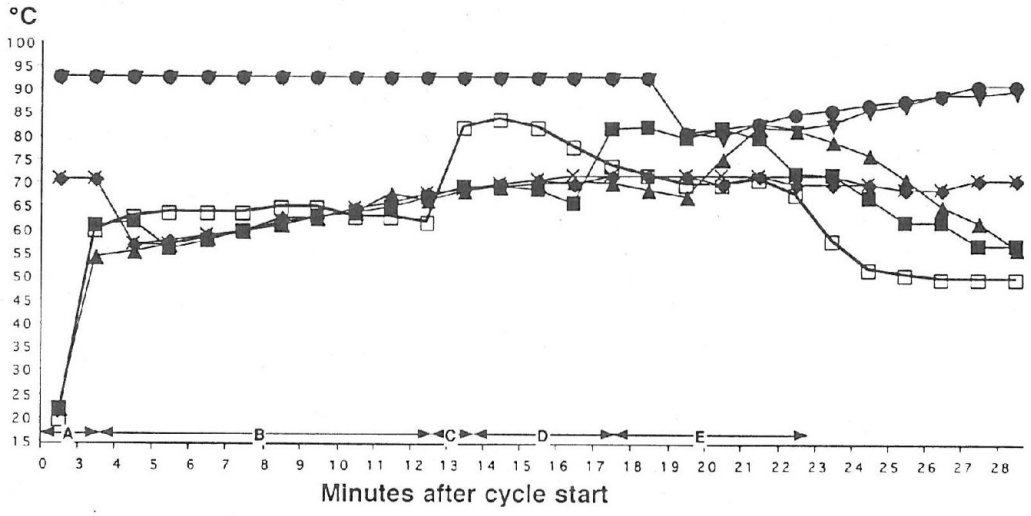


Figure 2a.
Sampling of plastic rabbit cages. Each rack was loaded with 8 cages, 4 on each side. Five samples (1, 2, 3, 4 and M (middle)) were taken from 2 cages on either side.

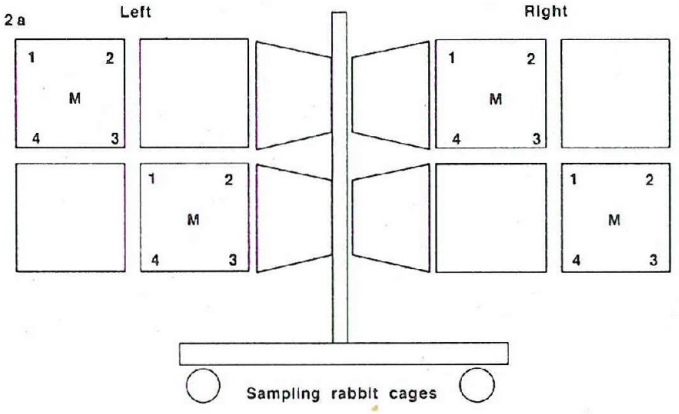
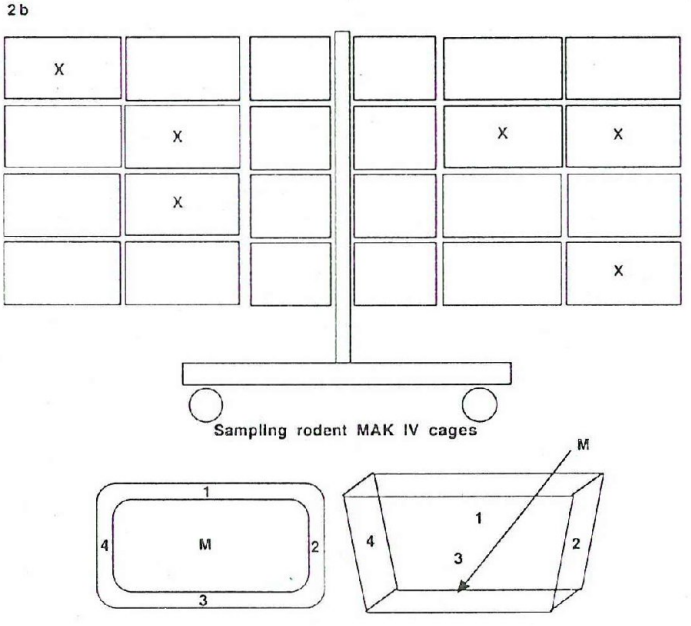


Figure 2b.
Sampling of MAK III and IV small rodent cages. A typical load consisted of 8 cages on each side of the rack. At least three cages were sampled on each side. Five samples were taken from the cages (1, 2, 3, 4 taken on the inner side wall, 5cm from the bottom and M (middle)). The cage is seen from the top and from the side.



Test medium
Petrifilm™ (Food Diagnostics AS, Oslo Norway) was used. The film surface consist of a reconstitutable agar nutrient containing tetrazolium indicator dye. Reduction of the concentration of the dye results in red colonies. The medium gives an indication of total aerobic bacterial colony counts and is non specific with regard to bacterial species. The films were reconstituted according to manufacturers instructions. One ml PBS was placed in the centre of the dry gel film. A buffer spreader was pressed on the outer cover film and the gel activated. The films could be used one half hour after reconstitution. Unused films were stored for up to 7 days in the refrigerator.
Sampling was performed after the surface temperature had dropped and the cages were warm to the touch. Samples were taken by pressing the activated gel surface onto the plastic cage. The film remained in contact with the sampling area for approximately 3 seconds.
The films were incubated at 37°C in an incubator (Termaks Bergen, Norway) for 2 days.
Bacterial growth was seen as red colonies of different diameters. All colonies irrespective of colour intensity and size were registered.

Control:
PBS buffer used to reconstitute the film was tested each time.

Scoring:
Table 1 shows the colony counts and scoring system used to estimate degree of contamination.

Table 1.
Colony counts in relation to score.

Colony count	Score
<5	0
5-10	1
11-20	2
21-30	3
31-40	4
>41	5

Measurement of washing machine chamber temperature.
A Therm 2250-1 temperature recorder (Ahlborn Meß und Regelungstechnik, Germany) was used to measure the washing chamber temperature. The thermistor lead was passed through the door seal and located approximately 31,5cm. into the chamber and 168 cm above the bottom of the washing machine. This is equivalent to the temperature that could be expected at the level of the top row of cages.
Temperature was manually registered every minute during a complete washing cycle.
The temperature in the two water tanks (detergent and rinse) were recorded from the machine display panel.
Two cycles recorded with a 14 day interval.

Sampling Strategy.
The study was initiated by sampling a load before and after washing.
Occasional cages that showed residues were re-washed.
Post wash samples were taken after the first and second wash cycles.
Figure 2a shows the rack arrangement for sampling of rabbit cages or collecting trays, and fig 2 b for sampling of small rodent cages.
Samples were always taken from cages that appeared to be clean on visual examination .

Test sessions.
Table 2 shows the test sessions, numbers and types of cages tested.

Results.
Temperature measurement:
Figure 1 shows the results of two temperature measurements compared with the manufacturers specified temperature profile. The highest chamber temperature (82°C) was registered approximately 5 minutes later than specification. The maximum chamber temperature was however, in accordance with manufacturers specifications. At no time did the chamber temperature reach the maximum temperature of the water in tank 2 (disinfection rinse). The temperature in tank 2 fell somewhat as replacement water was added. This implies that cages are never rinsed at an optimal temperature

(92-95°C). The highest temperature cages were exposed to was 82°C.

Petrifilm cultures.
Table 2 shows the results of samples taken from rabbit and macrolon cages during 6 studies and 9 cycles.
Cultures taken before and after washing (study 1) revealed that prewash cages had a score of >5 while scores were reduced to 0 after washing. Samples taken from cages at varying positions on the rack revealed that cage placement was not significant, and all cages appeared to be disinfected during a wash cycle. At least one cage on each level was sampled. Figure 3 shows the appearance of culture films taken from cages before and after washing.

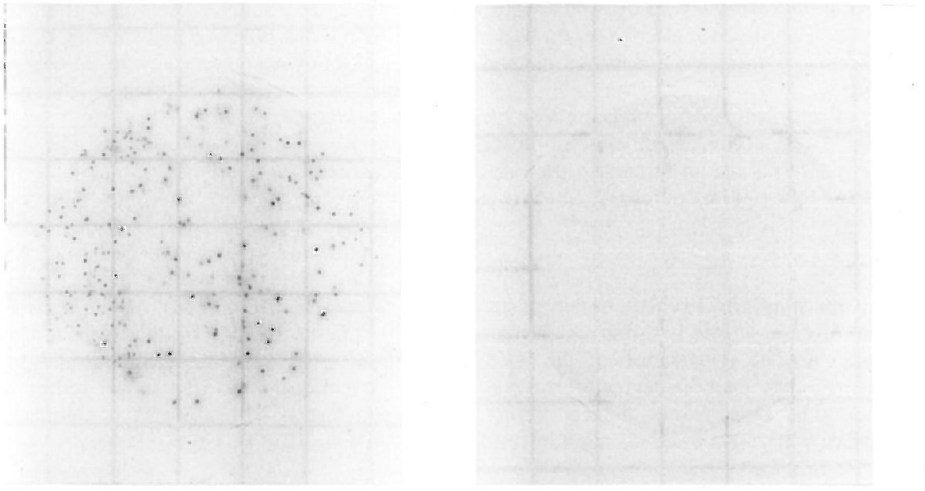
Table 2.

The scores of 6 studies, involving 75 cages and 10 washing cycles.

Study #	Cycle nr.	Nr. tested	Type of cage tested	Score 0	Score 2	Score 3	Score 4	Score 5
1	Pre	8	MAK IV					8
	Post	8	MAK IV	8				
		1	PBS Control	1				
2	1	4	Rabbit collecting tray	2				2
		1	PBS Control	1				
3	1	2	Rabbit collecting tray	2				
	2	3	Rabbit collecting tray					3
		7	MAK IV					7
		1	PBS Control	1				
4	1	7	MAK III,IV	7				
		3	Rabbit collecting tray	2	1			
		1	PBS Control	1				
5	1	3	Rabbit collecting tray	3				
		2	MAK IV	2				
	2	2	MAK III,IV	2				
		3	Rabbit collecting tray	2	1			
		1	PBS Control	1				
6	1	6	Rabbit cage insert	5		1		
	2	11	MAK IV	11				
		1	PBS Control	1				

Figure 3.

Petrifilm ™ cultures from cages before and after washing. The cultures have been taken from the same place in each cage.



We were careful to sample from the same sites in study 1. Results here reveal that the sample sites were disinfected.

Series 2 and 3 showed that bacterial colonies (score >5 - >100 colonies/ film) was detected in cages placed on the top and bottom of the rack. Subsequent investigation revealed that the rinsing pump had failed and that no hot water came through for final rinse and disinfection.

Scoring revealed that there was no bacterial growth on any surfaces when the machine operated normally (study 1, 4, 5 and 6). The sampling pattern was not significant and no growth was seen even when cages from different species were washed at the same time. Due to normal variation in the numbers of cages washed on any given day it was difficult to standardise the sampling procedure. We attempted however to sample at different sites thereby obtaining a representative sample over time.

The fortuitous pump breakdown was immediately reflected in significant colony growth. Cages on the upper level were contaminated on the first cycle and one week later all cages were contaminated.

Discussion.

Contact bacteriology is used extensively in food and surface hygiene control. Agar contact plates are used to sample surfaces and colony growth reflects the degree of surface bacterial contamination. The Vivarium was not able to acquire contact plates. Furthermore contact plates have to be made freshly and do not store well. Petrifilm™ systems are available as dry plates with a long storage life. The films can be made on demand and used when needed.

Contact plate techniques are used in quantitative bacterial studies in dermatitis (*Williams et. al* 1990). Similar techniques are common in quality assurance in the food hygiene industry (*Anon* 1993).

The study was not able to evaluate water distribution in the chamber. Assuming that the distribution of water in the chamber was optimal, all cages should have been treated at this temperature. It is conceivable that cages were not rinsed optimally. In this case bacterial sampling will reveal the true state of surface contamination.

Comparison of samples taken before and after washing (study 1) revealed that the numbers of

bacterial colonies were reduced following washing. The presence of colonies in studies 2 and 3 were associated with machine breakdown. From this we conclude that Petrifilm™ can be used as a indicator of decontamination. Visual examination of cages did not reveal any residues on the surface of the cage. The presence of colonies on bacteriological testing is therefore a sensitive method of detection.

The use of reconstitutable dry rehydratable media (Petrifilm™) is a convenient way of ensuring machine quality. Since an error in a process can occur at any time routine testing will uncover such episodes.

Summary

Washing machine effectiveness is dependent on treatment of cage surface with hot water containing detergent. The temperature of the washing chamber will not reflect the degree of treatment of each individual cage. Bacteriological testing of cage surface will give a true indication of residual contamination. This study describes the use of rehydratable contact films to harvest and culture residual bacteria. The method registers numbers of colonies retrieved from the surface. It does not type the bacteria and is as such a non-specific method. The study reveals that the machine in question was effective and that no bacteria were found following a normal cycle. A machine breakdown was demonstrated before the mechanical cause was uncovered.

References

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Williams RE, AG Gibson, TC Aitchison, R Lever, RM Mackie. Assessment of a contact-plate sampling technique and subsequent quantitative bacterial studies in atopic dermatitis. British Journal of Dermatology. 1990, *123* (4), 493-501.