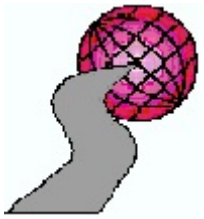


SILSOE RESEARCH INSTITUTE

UNIVERSITY OF BRISTOL

COMMERCIAL - IN - CONFIDENCE

CONTRACT REPORT

CR/1652/05/2963 Revised

REDUCING MICROBIAL CONTAMINATION FROM POULTRY TRANSPORT CRATES BY IMPROVED CLEANING AND DISINFECTION SYSTEMS BASED ON BETTER WATER USE

C H Burton and R T Whyte
Research Division, Silsoe Research Institute

V M Allen
Bristol University, Langford

D B Tinker
Formerly Silsoe Research Institute

June 2005 (with revisions February 2009)

Copy No:

Work carried out for:

Mrs Mary Howell
The Food Standards Agency
Room 315C Aviation House,
125 Kingsway,
London
WC2B 6NH

As part of the FSA project:

MO 1023
Reducing microbial contamination of poultry transport crates by improved cleaning & disinfection systems based on better water use

SUMMARY

Published work has established that crates used to transport poultry from farm to abattoir (poultry transport crates) are a source of contamination and cross contamination with respect to zoonotic pathogens, especially *Salmonella* and *Campylobacter*. Swabs taken from washed crates yield high counts (*eg*, up to10^7 cfu each of *Campylobacter*, coliforms and Enterobacteriaceae from the base of a single crate). Far from achieving a reduction in microbe numbers, existing washing processes have been shown to result in crates with higher surface counts of Camplyobacter compared to unwashed crates even if they are visibly clean.

The early stages of this project were given over to an extensive investigation into current practice followed in the UK poultry processing industry. The detailed report produced as main output of this part of the project confirmed that whilst good practice can achieve visually clean crates, similar improvements in microbiological standards were not assured. Crates largely free from debris is nonetheless an important criterion ahead of an adequate disinfection (either chemical or non-chemical means). However, the disinfection process is often poor or none existent.

The results of a series of factory and laboratory based studies led to the conclusion that the total removal of the developing biofilm on the crate surface was *not* essential in achieving a microbiologically clean crate. However, later factory-based trials indicated that crates, if well cleaned, could remain clean and be easier to clean. Related studies culminated in the design and construction of a test rig that would be used to evaluate a wide range of variations on the operation method and new techniques. The developed test rig for crate washing was used in the later stages of the project in a programme of factory trials. A total of six sets of trials were carried out which investigated distinct themes: series A - options based on variations of the existing crate washing system, series B - options based on water removal techniques, series C - chemical and none-chemical disinfection, series D - thermal and other rigorous methods, series E - combinations of the best methods, series F - ultrasonics and re-use of crates.

Of the techniques studied, none by themselves achieved a reduction in the microbe count on the surface of the crate by the target of four $\log_{10}$, units but several in combination could. In two cases, Enterobacteriaceae were reduced by more than 5 $\log_{10}$ units by a combination of methods. The five best techniques that were effective were: (a) the use of brushes, (b) using hot water (60 deg.C +) in soaking and spraying; (c) the use of hot water with detergents; (d) ultrasonics and (e), the use of high concentrations of certain disinfecting chemicals.

A number of parameters were adjusted but had little or no direct impact on microbial

contamination of transport crates. These included (a) increasing the soaking time, (b) including a pre-wash stage, (c) use of tepid or warm (40°C) soak temperature, (d) rinsing with clean, cold water, (e) removing excess water using vibration or air jets, (f) low level steam treatments and (g) exposure to ultraviolet light radiation. It is noted though that some of these methods such as the use of air jets did contribute to the general cleaning process and thus (indirectly) to the overall reduction of the microbial load on the crate surface.

The principal output from this study is a suggested code of good practice that would allow both for the improvement of existing plant and for further developments in the future.

COMMERCIAL - IN CONFIDENCE

CONTENTS

COMMERCIAL - IN CONFIDENCE

Appendices

1. State of the art report on crate washing
1a. Use of enterobacteriaceae counts for determining the likely efficiency of poultry transport crate decontamination
1b. Extracts from *Easyload Maintenance Manual* supplied by Anglia Autoflow Ltd.
1c. Presentation on the current technology for crate washing: its strengths and weaknesses.

2. Proposed code of good practice for the design and operation of crate washing processes for poultry transport crates.

3. Design and construction of a mobile test rig for washing poultry transport crates.
3a. Details of programme of factory based trials using the pilot scale test rig for crate washing.
3a. Full results from the programme of factory trials

4. Micrographs from studies of crate surfaces using scanning electron microscopy.

5. Micro-biological procedures used in this project.

1. INTRODUCTION

1.1 Contract arrangements

The work described here follows a contract drawn up between Silsoe Research Institute and The Food Standards Agency dated 17th September 2002. Although an equal partner, for the purpose of project management, Bristol University acted as a subcontractor to Silsoe Research Institute. Two further partners were formerly included in the capacity of consultants: Prof Geoff Mead (formerly of the Royal Vet College) and Dr Rob Davies (of the Veterinary Laboratories Agency). In addition the project was supported by several named industrial partners who participated in meetings and contributed to trials "in kind". These were: Anglia Autoflow (Diss), Faccenda Chicken (Brackley), PLC (Diss), Lloyd Maunders (Cullompton); Bernard Matthews (Norwich) joined the project midway through.

1.2 Background to the research

1.2.1 Contamination of crates used for live poultry transport

The use of reusable crates is commonplace throughout the food industry. However, the cleaning and disinfection operation is often not well understood, and systems may not give adequate performance for a variety of reasons. The operation can be difficult, needing to be done rapidly, and monitoring and quality control can be poor. The result is a high risk of microbial contamination of the trays; this is especially so in the poultry industry with the re-use of crates to bring successive batches of live birds (from a variety of farms) to a processing factory. In relation to the application of HACCP principles to poultry processing, the recycling of dirty transport crates between the processing plant and rearing farms poses a substantial risk of flock-to-flock transmission of pathogens including zoonotic. There is evidence that contamination of the skin and feathers of broilers with *Salmonella* or *Campylobacter* increases during transportation (e.g. Mulder 1995; Stern *et al* 1995; Line *et al* 1997). In addition, many flocks that are not apparently carriers of *Campylobacter* on the farm are externally contaminated with these organisms after transportation to the processing plant (e.g. Mead *et al* 1995). Mead *et al* also noted that routine cleaning of crates in the UK was inadequate. The problem is exacerbated by the need to clean crates rapidly.

Recent investigations carried out under MAFF funded project FS3301 have shown that *Salmonella* was not only isolated from a higher proportion of crates after automatic washing, but additional *Salmonella* serotypes were deposited on the crates. In most trials, *Salmonella* could be isolated from the tank used to pre-soak the transport crates throughout the processing period. Further studies under this project have shown that final sanitizer sprays in automatic washing systems had little impact on either the incidence of coliforms (faecal indicators) or the proportion of crates yielding *Salmonella* (Corry *et al*, 2001). Further studies, including the effect of crate washing, on *Campylobacter* contamination are being carried as part of an FSA funded project (BO3008). The operation of current poultry crate washing systems can leave the crates inadequately cleaned (often with dirt visible after cleaning) and the systems are a source of cross contamination as the water is used repeatedly. This is likely to have significant implications both

for food safety and animal health.

1.2.1 The crate cleaning process

The most common method used at present is based on soaking the crates in water, draining, then spraying with multiple jets of water (hot or cold and with or without cleaning chemicals) to remove dirt. The crates are often left to drain and sometimes sprayed with disinfectant. They are rarely dried as part of this process. Significant improvements to cleaning systems are only likely to be developed by undertaking a scientific review and investigation of microbial contamination of crates. The complete system, including module, truck body and on-farm handling systems need to be considered in the overall cleaning and disinfection strategy. The treatment of the base of the crate must also be considered.

The temptation to intensify the current cleaning process for the crates may even be counter-productive - for example, use of high velocity water sprays could lead to dispersion of microbes by aerosol formation. Indeed, the water itself may be a cause of the problem as crate-wash water provides ideal conditions for microbial growth (warmth, nutrients, dissolved oxygen and moisture) - the wet washed trays may be visually clean but may carry a higher microbial load than before washing. Chemical disinfection can lead to health risks to staff, contamination of the crated foodstuff and environmental problems and costs. Simply using more water will increase costs - both for supply and for disposal of an increased volume of effluent. Increasing water use implies recycling which is not without microbial risks (Rajkowski et al, 1996) but this approach is the best means of improving crate hygiene if adequate water treatment can be ensured. Re-use of water is possible for instance by effective thermal treatment with heat recovery to keep costs down. Other industries, such as water supply, effluent treatment and food and chemical processing, have continued to develop techniques for recycling water, improving heat exchangers and reducing costs. There is strong evidence that thermal treatment can readily meet hygienic standards without incurring excessive cost by the use of heat recovery (James et al, 1992; Turner et al, 1998).

A substantial re-think is thus required starting with a study to gain an understanding of the mechanisms by which microbes persist on crates to determine appropriate practical cleaning strategies to ensure their removal and destruction. Studies are needed to determine if microbes (including the pathogens of concern) form biofilms on crates or modules. Special techniques may be required to physically remove or chemically inactivate these attached microbes. This will require taking into account the machinery environment and its effects on the microbes of concern. The development of a revised processing approach and practical equipment package and operating procedures can then follow. The key strategy here is to provide an adequate, but not excessive, treatment thus enabling an efficient process. Equally important is a practical process that can fit into the space and the established operating procedures of the poultry processing company.

1.3 Project objectives and outline of the study

The overall objective was to review the existing methods of washing poultry crates and crate modules with the intention of understanding the underlying cleaning processes. This was to be followed by 1) proposing the best operating practices for existing systems and 2) developing system components to achieve a rapid, effective and efficient physical and microbial decontamination of poultry transport crates. Approaches would include (a) the effective use of (larger amounts of) wash water, (b) the addition of a drying stage and (c) more effective use of disinfectants.

Objective 1

To review current practices and produce a State of the Art report setting out the best operating regimes for existing equipment.

Objective 2

To establish the key conditions under which certain microbes can persist on poultry crates aas well as those conditions which lead to decontamination. To identify the best procedures that can achieve effective crate cleaning using physical and/or chemical methods with minimal residual microbes.

Objective 3

To produce a draft code of practice to enable existing equipment to be used in a more effective way with the application of relatively minor changes. The document will also set out the best approaches for improvements in the washing system.

Objective 4

To design and specify improved and novel cleaning processes including an efficient water re-use system; to verify the realistic quality of water achievable from laboratory scale trials and other available data. To review de-watering & drying options along with effective use of disinfectants.

Objective 5

To design and build a prototype cleaning system (test rig) based on the above findings, which will be used at the factory sites of industrial partners to handle samples of soiled crates removed from the production line.

Objective 6

To validate the recommendations in the code of practice and to evaluate a prototype system in terms of decontamination effectiveness, costs and practicalities (at the premises of participating poultry processing companies using actual contaminated crates).

Objective 7

To update the code of practice (objective 3) and demonstrate best practice for that based on existing technology; that based on the best practice achievable from modification of existing systems and indications for improvements to future systems.

Inevitably, there were some changes to the original plan as the project developed which reflected both new opportunities and changed ideas.

1.4 The division of the work in fulfilling project objectives

Under the project contract, the largest part of the research work was carried out by staff at Silsoe Research Institute (SRI) and at Bristol University Veterinary School (Bristol University). The broad division of duties was that the engineering inputs were covered by Silsoe Research Institute and the microbiology was provided by Bristol University. Crucial inputs were also made from the two project consultants and also from the participating industrial partners.

Objective 1

This culminated in a key interim document known as "the state of the art report on crate-washing" which is reproduced in Appendix 1. The background research was carried out jointly by UoB and SRI and comprised a series of factory visits and discussions on current practice. Key sections on the use of chemicals were contributed by the consultants and feedback from most project partners is noted. Discussion of the main issues arising is given in Section 2.1 of the report.

Objective 2

Several tasks made up this part of the project with SEM studies on biofilms being led by SRI (see Section 3) and persistence of bacteria on crates led by UoB (see Section 2.2). Related studies included the role of ultrasonics and of chemicals - both to dislodge and/or destroy the biofilms that had built up on the crates - the support from industrial partners was crucial in completing this work.

Objectives 3 and 7

The compilation of the first draft code of good practice for good crate washing was done by SRI - this was then developed by a continuous feedback from all project partners both within the project meetings and by written contributions outside the meetings. The final version is presented in Appendix 2.

Objectives 4 and 5

The task of designing and then building a test rig fell to staff at SRI. During the design concept

stage, important feedback from the industrial partners represented an important part of developing a practical piece of equipment that could meet the project demands. The design is given in Appendix 3 along with details of plant operation.

Objective 6

The programme of factory trials represented the largest single task of the project and encompassed most project partners at some time or other. The programme was led by SRI with all microbiological services led by UoB. Central to the success of this part of the project was the input from industrial partners both in practical terms and in reviewing the programme of work as it developed over the 8 months of trials. The programme of work is set out in detail in Appendix 3a. A full set of results from this work is given in Appendix 3b. Summaries and discussion are given in Section 4 of the report.

1.5 General acknowledgements

The original project proposal was put together (for SRI) by Colin Burton and Dave Tinker in collaboration with Dr Vivien Allen of Bristol University. In addition to these, large contributions were made to the project by Robin Whyte and Dave Wilkinson (SRI) and Jill Harris and Marie Lewis (Bristol University). Crucial contributions to the project were also made by John Bailey (Faccenda), Phil Slapp (PLC), David Wills (Anglia Autoflow) and Mary Howell (FSA). Special help was received from the consultants, Prof Geoff Mead and Dr Rob Davies.

Acknowledgement is also made of contributions from Robert Wills, Barry Landimore, Janet Corry, David Lanning, Chris Galer, Dean Burfoot, Jeremy Hall, Terry Stock, Roger Lovell, Robert Wills amongst many others.

This report was compiled by Colin Burton. Section 2.2 (and Appendix 5) was contributed by Dr Viv Allen. Section 3 (and Appendix 4) was contributed by Robin Whyte.

COMMERCIAL - IN CONFIDENCE

2. STUDIES ON CURRENT COMMERCIAL CRATE WASHERS

2.1 Review of current practice at poultry processors in the UK

2.1.1 Introduction

More than a dozen UK poultry processing plants were visited over the course of several months for the purpose of understanding the equipment currently used for the washing of poultry transport crates. A full report is given in Appendix 1; the main issues that formed much of the basis of the subsequent study are summarised in this section.

Crate washing systems in the UK are most commonly based around a system that involves a pre-wash booth, a soak tank, a main wash booth and a final disinfection/rinse stage. There is a large variation in crate washers in the UK reflecting (i) improvements achieved over recent years by the manufacturer, (ii) the importance given to the washing process and (iii) changes to the washing system implemented by the processing company itself. However, due to the limited use of water, it is unlikely, even with the best current crate washing systems, that a sufficient reduction in microbe counts (for example, 4 $\log_{10}$ units) will be achieved; the poorer systems fail even to achieve visually clean crates. Stipulating and enforcing the use of potable water is unlikely to achieve any significant reduction in microbe numbers on the crates with the current technology.

Potential improvements to the process fall into three categories: (i) those which can be easily implemented, (ii) changes to existing equipment requiring considerable investment and (iii) changes that are only possible with a new installation. The first group includes a range of items that come under the title "good housekeeping" as well as the general observation of best practice. This includes keeping the work area clean, avoiding spillages onto the floor, regular changing of water, more frequent cleaning of screens, inclusion of a rinse stage with clean water and effective disinfection. Use of a detergent in the soak tank and/or hot water are further options. It still remains likely that the sum of these changes may not be enough but a clear standard is lacking. Any reduction in microbe numbers by more than 2 $\log_{10}$ units would be welcome but fully effective cleaning and disinfection should reduce microbe numbers by 4-5 $\log_{10}$ units.

2.1.2 Use of hotter water in washing

The benefits of hot water may be best utilised by using it in conjunction with a counter-current flow; thus the hottest water (well over 70 deg.C) would be used in the final rinse with the drainage water contributing to warming the earlier stages. The draw back of increased microbial growth in warm water would be largely offset by the final wash which would effectively act as a disinfectant stage. Arrangements would still be necessary to contain the fog generated by hot water (especially in cold weather) and extraction equipment may be needed. Energy considerations based on warming a 10 kg plastic crate by 50 deg.C implies a heat load of one M.Joule per crate (specific heat capacity taken as 2000 J/kg.K). This is a third of a kilowatt hour or around 2p in terms of electrically supplied heat.

2.1.3 Reduction in water carry-over; better drainage

Crates, especially those with solid floors, have the capacity to carry dirty wash water to the next stage of the process. This can be as much as a litre of water representing a significant contamination of the next stage of the process. Thus drainage is an important consideration. This is especially important prior to any final disinfection stage where residual water will dilute the concentration of the applied disinfectant and organic debris may neutralise it. The removal of surplus water can be achieved by several means:

- Natural drainage: assisted by tipping the crate and even inverting it (in the case of the solid floor type). Sufficient time needs to be allowed to enable a large amount of residual water to drain away.
- Use of vibration: to enhance the water removal akin to "shaking dry". The attraction of this approach is that it is simple and involves only relatively minor changes. Drainage time is reduced as a result.
- Use of air jets: are more effective than vibration and they can impart some drying action. However, they can lead to the generation of aerosols and the process is only effective on surfaces reached by the air jet.
- Drying: only an option for the last stage of the process and one that normally requires at least some previous use of heat to enhance the process. Dry crates represent a greatly reduced risk of cross contamination; any disinfectant subsequently applied is also likely to be much more effective.

2.1.4 Better use of disinfectant

Spray jet evenness The use of spray heads that generate a fine even spray to evenly wet a larger area. The risk of blockage should be minimal so long as mixtures are made up with clean water but periodic checking will still be required. Many chemical manufacturers (along with the suppliers of spray equipment) provide extensive guidelines for such application.

Quantities of disinfectant needed A good spray does not by itself guarantee enough chemical or the total coverage of the crate. Quantities will depend on the residual microbial and other organic matter on the washed crate. Guidelines are available from chemical suppliers but these can be too general leaving the operator unclear of the correct amounts needed for crates. Furthermore, there is always the suspicion that recommended disinfectant quantities tend to suggest excessive use to promote sales (although the reverse may actually be the case with suppliers underestimating effective quantities to avoid appearing too expensive). It follows that better cleaning of the crates will have the benefit of a lower quantity of disinfectant being required to meet any set standard.

Deployment of spray jets Total coverage of the crate by disinfectant requires the deployment of sprays in a way that all surfaces are covered. In addition disinfectant wastage needs to be minimised and this is unlikely to be achieved with a single row of jets. A constraint to laying out jets is the need for the crates to move throughout the system without snagging on the nozzles. A moving nozzle system, although more complex, could enable an even application with minimal wastage

2.1.5 Improved treatment of recycled water

A major weakness in current crate washer designs is the inadequate treatment of water that is recycled around the process. The build up of suspended matter is inevitable along with the increase in bacteria numbers. The run-down screens used are cheap and simple in operation but they can only remove coarse particles (over 1mm). If kept clean, they remain a useful first step in treatment but further stages should be considered to improve water quality. This implies pulling water out of the system and passing it through a distinct treatment loop that meets a specific objective:

2.1.6 Use of sonication in the soak stage

The expected benefit from sonication is one of loosening up attached debris (including microbes) which suggests that it may be especially useful in the soak tank. Limitations with the technology include the tendency of plastics to absorb the energy (and thus negate its effect) and the importance of a gas-free water medium for good transmission. Published work (Mason and Lorimer, 2002) suggests that some destruction of bacteria can still be expected even in less than ideal conditions.

2.1.7 Brushes

The use of brushes for poultry transport crates is limited because of crate design which includes many surfaces that are difficult to access. Nonetheless, brushes have been tried in at least one plant with limited success. The attraction lies with the mechanical removal of biofilm layers and where surfaces are readily accessible, this seems a reasonable expectation. Even if brushes can only reach some of the crate surfaces, their use may still be worthwhile as part of a system. Brushes used in a submerged location such as in the soak tank, might also be expected to achieve further benefit by causing liquid agitation. The extent of cleaning by brushing will increase with the number used but this will also require a much more sophisticated machine. The simplest system would be a single rotary brush that could only clean the base of the crate; further brushes could include the sides but reaching the inside of the crate would require a more elaborate system. In addition to the investment costs implied would be the periodic replacement cost of the brushes.

2.1.8 Steam drying and disinfection options

The use of steam in food production always appears to be a relatively costly process in relation to many other operations and its use would also require containment of the fogs produced. However, it does allow for a chemical free disinfection such as might be achieved in a steam tunnel. The likelihood of total disinfection of all surfaces is greatly increased over chemical sprays in that all surfaces will be in contact with the vapour and thus potentially heated by the condensation formed. It will be important that the temperature of the surface of the crate reaches 70 deg.C or more and is held at this level for at least a minute to ensure a large reduction in microbe numbers. This suggests that the crate should be in the steam tunnel for more than this period of time implying a length similar to the soak tank unless the crates can be turned on their side to save space. An alternative approach might be to steam treat the crates once reinserted into

their module.

The poor conductivity of the plastic should minimise steam consumption - only the surface would be heated possibly equating to no more than 10-20% of the crate volume. Assuming this implies a running cost of less than 0.5 pence per crate depending on efficiency. Larger amounts of steam could enable some drying of the crates on the basis that it (i) enhances drainage and (ii) enables subsequent evaporation on entering a cooler, dryer, environment outside the steam tunnel. The latter is not unlike the drying of domestic crockery on a draining-board after washing in hot water - the local air around the hot object is warmed with a rapid fall in relative humidity (RH) which increases evaporation. The extent of this benefit remains to be evaluated as does the potential shortening of the crate life as a result of including thermal cycles in the cleaning process.

2.1.9 Use of UV in the final stages

The use of UV light for microbe destruction is already well established in other industries such as potable water treatment. Illumination of the crate surface at the appropriate level could be expected to similarly reduce microbe numbers. The method does have the attraction of easy deployment implying little more than the fitting of a row of lamps around the final stages of crate washing with some shielding for operator protection. However, there are possible drawbacks to such an approach including:

- UV light will only penetrate transparent surfaces; it will not effect microbes "protected" by debris remaining on the crate surfaces after cleaning. It is essential that the crate is visually clean and well drained prior to UV treatment;
- the light application would need to be maintained for a period of time implying a series of lamps possibly set up in a treatment tunnel;
- any surface in shadow will be untreated; for poultry crates, this may mean a high proportion of the surfaces unless many lamps are used to illuminate from many directions;

2.2 Microbiological studies on factory-washed crates

2.2.1 Estimation of crate hygiene using visual and microbiological assessment

A series of trials were carried out using washed crates at a poultry processing plant to investigate the correlation between visual cleanliness and microbial numbers using washed crates. This was undertaken to explore the feasibility of screening washing and disinfection treatments by visual scores only. The most promising could then be assessed further using microbiological examination. Results are presented in Tables 2.1 and 2.2. Table 2.1 shows that there was little difference between washed and unwashed crates with differing types of contamination. Table 2.2 shows that there was little correlation between visual cleanliness, as assessed by amount (in grams of faecal matter), and total aerobic and Enterobacteriaceae numbers recovered from the crate base using the swabbing technique described in Appendix 5. Therefore both visual and microbiological assessments were carried out in the factory trials.

Table 2.1: To assess the numbers of APC and Enterobacter in mist and dry faecal matter and on crates with biofilms and clean crates (log_{10} cfu/g and log_{10} cfu/10cm^2)

Sample number	Type of sample	APC log_{10} cfu/g	Entero-bacteriaceae log_{10} cfu/10cm^2
	Unwashed crates		
A1	Moist soft faecal matter	7.98	7.89
A2	Dried on faecal matter	8.74	5.60
A3	Dried on faecal matter	7.54	3.23
A4	Moist soft faecal matter	8.82	7.86
	Washed crates		
B1	Washed crate with black biofilm	8.75	5.60
B2	Washed crate with black biofilm	8.60	6.04
B3	Washed crate with black biofilm	8.69	5.87
B4	Washed crate with black biofilm	8.56	4.94
	'Clean' washed crate	8.19	5.70

Table 2.2: To decide suitable scoring system and enumerated microbial contamination on washed crates (score = g/crate base, counts =$\log_{10}$ cfu/crate base*)

Sample number (Not same samples as Table 2.1)	Visual score g/crate base	APC counts $\log_{10}$ cfu/crate base	Enterobacteriaceae counts $\log_{10}$ cfu/crate base
A1	3	8.10	6.70
A2	<1	7.81	6.63
A3	2	7.81	6.33
A4	1	8.18	6.87
A5	8	8.02	6.67
A6	<1	8.18	6.77
A7	<1	8.04	6.62
A8	1	7.98	7.23
A9	2	8.60	6.85
A10	1	8.25	7.12
A11	1	7.98	6.82
A12	<1	8.24	7.14

2.2.2 Comparison of crate hygiene at poultry processing plants from three different companies.

Crates were visually and microbiologically assessed using the methods described in Appendix 5 in three processing plants. In two plants, companies A & B, twelve crates were examined after the removal of birds while in the third company, C, due to the high degree of automation, it was not possible to remove the crates from the line after the birds were removed but only after the pre-wash stage. The crates were taken from two flocks. In Company B, 12 crates were examined after being held in the lairage for 12h after the final wash. The weather conditions overnight were damp.

Visual assessment of crate cleanliness

As in the preliminary trials there appeared to be no significant difference between visually clean crates and those with biofilms (old firmly adhering visible faecal matter) in the numbers of APC (total aerobic counts) and Enterobacteriaceae recovered. Therefore crates were scored in terms of grams of debris as estimated visually. Three scores were given per crate and these were the total amount of debris in grams on three sites, 1) total surfaces inside the crate, 2) the outside walls of the crate and 3) the outside base. As expected, there was a wide variation in the scores of unwashed crates although this diminished after washing especially on the outside of the crates (see Table 2.3).

Table 2.3: Range of scores on transport crates with median value in parenthesis (n=12)

Company	Processing stage	Site 1	Site 2	Site 3
A	Untreated	5-10 [7]	3-12 [6]	1-5 [2]
B	Untreated	2-20 [4]	0-10 [3.5]	0-10 [2]
C	Pre-washed	2-10 [4]	1-4 [2]	1-3 [1]
A	Final wash	0-2 [0.5]	0-2 [1]	0-2 [0]
B	Final wash	0-8 [4.5]	0-2 [0]	0-1 [0]
C	Final wash	0-2 [1]	0-3 [0]	0-2 [0]

Assessment of crate microbiological load

The microbiological load on the inside base of the crates was assessed since this surface posed the greatest risk of contamination to the birds. The base was sampled using four large dry cotton wool swabs (Medical Wire, Corsham MW104J), each wiping a quarter of the base. These were enumerated for APCs, Enterobacteriaceae and *Campylobacter* as described in the microbiological methods in Appendix 5. The overall microbial numbers on the crates were similar between the companies. There was little reduction in total aerobic numbers on the crate base following washing (see Table 2.4) although this was statistically significant (p=0.02) in the case of company C. In Company A and C there was a statistically significant reduction for Enterobacteriaceae (p=0.02 and <0.01) although it the case of company A this was less than one log. The *Campylobacter* numbers recovered throughout the studies were erractic and were probably related to the colonisation status of the flock rather than the washing procedures.

Table 2.4: Microbial numbers (mean $\log_{10}$ cfu/ base + StDev) recovered from the inside base of crates (n=12)

Company	Processing stage	APC mean $\log_{10}$ cfu/ base + StDev	Enterobacteriaceae mean $\log_{10}$ cfu/ base + StDev	*Campylobacter* mean $\log_{10}$ cfu/ base + StDev
A	Untreated	7.80 ± 0.37	6.87 ± 1.02	6.91 ± 0.85
B	Untreated	7.90 ± 0.73	7.56 ± 0.72	5.60*
C	Pre-wash (post)	8.06 ± 0.36	7.07 ± 0.72	4.11 ± 0.16
A	Final wash (post)	7.57 ± 0.37	6.06 ± 0.34	5.66 ± 0.01
B	Final wash (post)	7.93 ± 0.52	7.35 ± 0.62	2.93 ± 0.86
C	Final wash (post)	7.73 ± 0.33	5.96 ± 0.22	5.34 ± 0.06

* 1 crate sampled only

The numbers of APC, Enterobacteriaceae and *Campylobacter* on the 12 crates in Company B,

stored in the lairage for 12h after final wash appeared to be very stable as shown in Table 2.5.

Table 2.5: Microbial numbers (mean $\log_{10}$ cfu/ base + StDev) recovered from the inside base of crates following 12h storage (n=8)

Company	Processing stage	APC mean $\log_{10}$ cfu/ base + StDev	Enterobacteriaceae mean $\log_{10}$ cfu/ base + StDev	*Campylobacter* mean $\log_{10}$ cfu/ base + StDev
B	Stored after wash	7.82 ± 0.64	6.79 ± 0.92	3.97 ± 0.51

In Company B low foaming alkaline detergent was added to the soak tank. This concentration was increased approximately 10-fold to monitor the effect. Although the numbers of Enterobacteriaceae were reduced to below detectable numbers (<2.70 $\log_{10}$ cfu/base), the numbers of APC on the crates were only reduced from $\log_{10}$ cfu/base 8.58± 0.46 to 5.96 ± 0.27. Using this level of detergent would not be practicable, due to cost and staff safety issues.

Numbers and types of microbes in re-cycled water and soak tank water
Some abattoirs use 'white' water, from offal flumes for the prewash and crate-soak stages. There is little information about the numbers and types of microbes present in this water prior to its use on the crates, but unpublished results during MAFF project FS3301 showed *Salmonella* sometimes to be present especially when using 'white' water, and numbers of coliforms per ml in the region 104 to 105. The microbiological quality of the water taken from the soak tank was similar whether white water or good quality bore-hole water was used. This is not surprising, since most of the dirt rinsed off the crates is faecal matter.

In the present study the microbial numbers recovered from the soak tank in Company B for APC, ranged from 6.58 $\log_{10}$ cfu/ml to 4.00 $\log_{10}$ cfu/ml depending on the concentration of detergent while the numbers of Enterobacteriaceae were below detectable numbers (<1.70 $\log_{10}$ cfu/ml). In Company A and C, on the other hand, these levels were considerably higher at 7.47 $\log_{10}$ cfu/ml ± 0.10 and 8.64 ± 0.48, 5.64 ± 0.24 and 6.72 ± 0.11 and 6.96 ±0.82 and 5.34 ± 0.06 for APC, Enterobacteriaceae and *Campylobacter*, respectively. However, this difference in numbers appeared to have little impact on the microbial load of the washed crates.

2.2.3 Comparison Between Cotton Wool Swabs and Sponges Used For Sampling

This study was to compare two methods for sampling purposes during the main factory rig trials. Twenty-four crates from a single flock were sampled using either one swab per quarter of the base and pooling the four swabs or a single sponge per base. The trial was repeated. The samples were processed as described in Appendix 5 and enumerated for APCs and Enterobacteriaceae. The bases of eight of the crates were sampled using either four swabs or a sponge for three consecutive times to ascertain the efficiency of the sampling technique based on the reduction in microbial numbers.

Overall sponges were found to be more effective at removing microbes from the crate surface by approximately 0.4 $\log_{10}$ cfu/base and this was statistically significant (p=0.03 and 0.01 for APCs and Enterobacteriaceae respectively). The mean numbers for the 24 crates sampled in the two

trials by sponging were 9.53 ± 0.70 $\log_{10}$ total aerobic cfu/base and 7.99 ± 0.56 $\log_{10}$ cfu /base for Enterobacteriaceae compared to 9.15 ± 0.42 and 7.60 ± 0.39 $\log_{10}$ cfu/base for the equivalent values using swabs. The greater efficiency of sponge technique was confirmed on the trials sampling three consecutive times on the same crate where there was over one $\log_{10}$ reduction in Enterobacteriaceae between the first and the third sample ($p<0.01$). Using swabs there was no significant difference in the microbial numbers recovered ($p>0.5$). These results are shown in Table 2.6.

Table 2.6: Microbial numbers (mean $\log_{10}$ cfu/ base + StDev) recovered from the inside base of crates (n=4)

Sample method	Order of sampling	APC mean $\log_{10}$ cfu/ base + StDev	Enterobacteriaceae mean $\log_{10}$ cfu/ base + StDev
Sponge	1	9.57 ± 0.21	7.90 ± 0.22
	2	9.17 ± 0.22	7.22 ± 0.42
	3	8.90 ± 0.25	6.82 ± 0.34
Swabs	1	9.15 ± 0.53	7.30 ± 0.34
	2	8.75 ± 0.47	6.92 ± 0.68
	3	8.57 ± 0.53	6.95 ± 0.68

2.2.4 Effect of Temperature of Soak-tank Water on Microbial Counts from Transport Crate Bases

In one processing plant the temperature of the soak tank water was raised to investigate if higher temperature reduced the microbial load or improved visual cleanliness. Three trials were carried out, each at three temperatures using 12 crates per temperature. The first trial used 37°C, 50°C and 60°C and the remaining two used 44°C, 55°C and 60°C. There was a progressive reduction in microbial numbers recovered from the crates with increasing water temperature in the first trial ($p=<0.001$). However, in the second and third trials there was no progressive reduction despite further increases in temperatures (Table 2.7) with no *Campylobacter* or Enterobacteriaceae being recovered from the soak tank water at 60°C. The crates, however, in the automated washing system pass through the soak tank in 17s with totally submersion for 14s. .

Table 2.7: Microbial numbers (mean $\log_{10}$ cfu/ base + StDev) recovered from the inside base of crates (n=12)

Trial	Processing stage	Temp deg.C	APC mean $\log_{10}$ cfu/ base + StDev	Entero-bacteriaceae mean $\log_{10}$ cfu/ base + StDev	*Campylobacter* mean $\log_{10}$ cfu/ base + StDev
1	Unwashed		8.53 ± 0.29	7.09 ± 0.48	4.24 ± 1.03
	Final wash (post)	37	7.81 ± 0.76	5.56 ± 0.51	3.25 ± 0.49
	Final wash (post)	50	7.64 ± 0.69	5.08 ± 0.76	2.97 ± 0.27
	Final wash (post)	60	6.80 ± 0.60	4.20 ± 0.86	2.60 ± 0.40
2	Unwashed		7.86 ± 0.28	7.30 ± 0.35	2.40 ± 0.29
	Final wash (post)	44	7.66 ± 0.38	5.75 ± 0.40	3.27 ± 0.53
	Final wash (post)	55	7.51 ± 0.43	5.67 ± 0.46	3.61 ± 0.31
	Final wash (post)	60	7.57 ± 0.29	5.06 ± 0.26	3.60 ± 0.20
3	Unwashed		8.09 ± 0.53	7.33 ± 0.47	3.06 ± 0.72
	Final wash (post)	44	7.03 ± 0.44	5.34 ± 0.33	4.05 ± 0.17
	Final wash (post)	55	7.78 ± 0.41	5.97 ± 0.52	3.85 ± 0.28
	Final wash (post)	60	7.28 ± 0.36	5.43 ± 0.74	4.41 ± 0.34

2.2.5 Crate washing laboratory trials using a pilot sonication tank

Methods Preparation of crate pieces and inoculation

Sections of crate (approx. 270 x 150mm) with a rectangular pattern of 25mm squares on their surface were submerged in the wash tank of the crate washing facility of a poultry plant. After one day, the sections were removed and returned to the laboratory. The sections were maintained in the condition that they left the tank i.e. there were variable amounts of debris stuck to the surface (bedding, feathers, etc.)

Treatments

Warm tap water containing either detergent Hyperclene-DBV at 5% or CB10 at 5% v/v was added to the sonicator tank. The temperature was set to either 35ºC, 55ºC or 58ºC and a digital thermometer used to check the accuracy of the setting. Prior to crate treatment, sonication was switched on for 1 min followed by thorough stirring to de-gas the solution. This was repeated several times.

- The crate pieces were washed by rinsing under a gently running cold water tap which removed most of the 'debris'.

- A section of crate was then hung on a wire cradle and lowered into the tank with the cradle resting on the top of the tank (the top row of squares, A as shown in Figure 2.1 below, was not immersed). This immersion was timed for 30s.

- The crate piece was then raised out of the water one row of squares at a time for a further 30s. This was repeated so that each row of squares (A, B, C & D) was immersed for 0, 30s, 60s and 90s respectively.

- This procedure was repeated with a second section of crate but at each time point was subjected to sonication, ie. each 'row' sonicated for 0, 30s, 60s or 90s.

- Following treatment, swabs were used to remove bacteria from the surface of each pair of squares. The swabs were each transferred into 10ml of MRD and vortex mixed prior to enumeration via the Miles - Misra technique on VRBG and PCA plates and colonies identified as described in Appendix 5.

A1	A1	0 secs		A2	A2		A3	A3
B1	B1	30 secs		B2	B2		B3	B3
C1	C1	60 secs		C2	C2		C3	C3
D1	D1	90 secs		D2	D2		D3	D3

Figure 2.1: location of sampling squares on section of crate studied

The treatment time was extended to 120s for the 35ºC and 45ºC trials.

Results

Overall there was little difference in bacterial reductio recorded between CB10 and Hyperclean. However as can be seen in the following Figures 2.2 and 2.3 there was a significant difference between sonication and immersion alone with a progressive reduction in numbers of both APCs and Enterobacteriaceae with time and temperature.

COMMERCIAL - IN CONFIDENCE

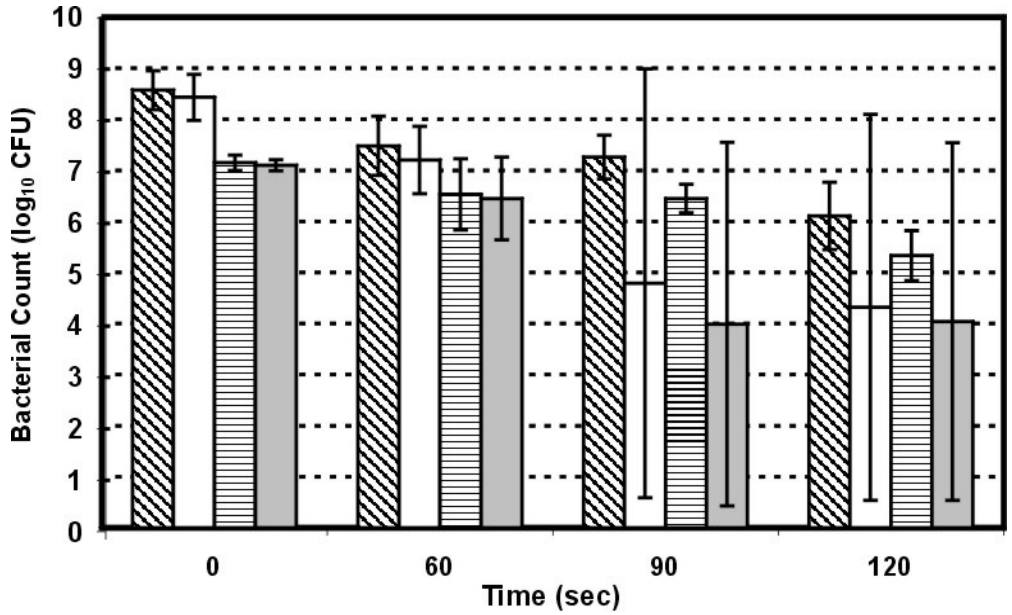


Figure 2.2: Comparison of immersion and sonication for removing bacteria from crate surface using 5% CB10 at 35oC. Diagonal shading - APC1; white - APC2; horizontal shading - Entero 1; grey - Entero 2.

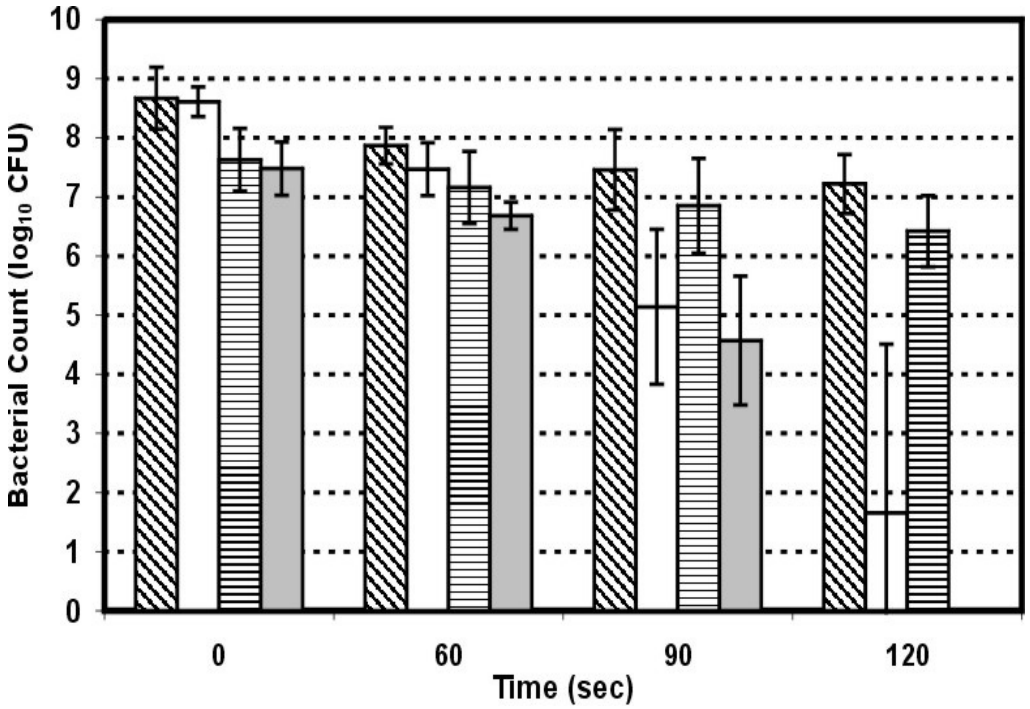


Figure 2.3: Comparison of immersion and sonication for removing bacteria from crate surface using 5% CB10 at 45oC. Diagonal shading - APC1; white - APC2; horizontal shading - Entero 1; grey - Entero 2

3. INVESTIGATION INTO THE DEVELOPMENT AND REMOVAL OF BIOFILMS ON CRATES

A limited series of studies were carried out where small 20 mm x 20 mm sections of the base of poultry transport crates were subjected to a number of treatments that could be useful in achieving low microbiological contamination. Both closed and open floor crates were examined. Following the treatment, the sections of the crate were examined by scanning electron microscope (SEM) at the Institute of Ophthalmology, London, to assess whether there was a physical effect of the treatment on the deposits of material on the crate surface.

Sections of clean, unused closed floor crate were examined initially and compared sections taken from a used, washed closed floor crate. The section from the clean crate (Appendix 4, Figures 1 and 2) shows no surface bacteria but fungal hyphae, salt crystals and possibly pollen grains were seen. The used, soiled crate showed the existence of a bio-film matrix (Appendix 4, Figures 3 and 4), which, at high magnification (Appendix 4, Figure 5), showed (possibly encapsulated) bacteria existing on the matrix.

3.1 The use of abrasion and ultrasonic technologies

Trials were carried out to study the effect of high pressure washing and ultrasonic treatment onthe bio-film formed on the surface of poultry transport crates. Small sections of used, open floor crate were prepared, immersed in a crate wash soak tank for 2 days to build up the bio-film before subjecting them to either pressure washing or ultrasonics. Untreated sections, and sections immersed in the soak tank only, acted as controls. The treated sections were examined using SEM and in the case of the sections that were treated with pressure washing, also by transmission electron microscopy (TEM).

3.1.1 Effect of pressure washing

The sections of soak tank treated crate were mounted on a plastic base and passed through the pressure washer at a speed of 41 mm/sec. The crate sections were subjected to sprays at 3, 6, 9 and 12 bars in a 45 degree fan pattern, 110 mm from nozzles.

The main findings by SEM were a) the pressure spraying did not dislodge the bio-film matrix from the crate material surface, b) the bio-film matrix varied in thickness across the sample surface, c) the spray was able to remove surface objects partially embedded in the bio-film matrix (Appendix 4, Figure 6) and d) although the bio-film matrix was not dislodged from the crate surface, the sprays appeared to disrupt the surface of the bio-film in matrix places.

The samples examined by SEM were embedded in resin and the crate material prised away from the resin bed. The underside of the bio-film matrix was then "stained" with osmium before being embedded in a second resin layer. Thin sections were then cut and examined by TEM (Appendix 4, Figure 7). Findings from this work were that a) the bio-film matrix did not readily separate from

the crate material and would leave material adhering to the crate material surface, b) the bio-film matrix varied in depth, from around 100 nm to many micrometers and c) it was difficult to identify objects within the bio-film matrix.

3.1.2 Effect of ultrasonics.

A small experimental ultrasonic bath was used to expose small sections of open and closed floor poultry transport crates to ultrasonics. As for the pressure washing treatment the small sections of crate pieces were treated by immersion a crate wash soak tank at a conventional poultry processing plant, for 2 days. Surfactant was added to the water filling the ultrasonic bath, which was heated to 50°C. The water was then degassed for approximately 30 minutes. The crate pieces were immersed in the bath for 0, 30 and 60 seconds and exposed either ultrasonic or no ultrasonic ttreatment.

A certain amount of penetration and smoothing of the bio-film matrix surface (Appendix 4, Figure 8) seemed to be evident resulting from exposure to ultrasonics, although the bio-film matrix was not removed from the surface of the crate sections. On the older, closed floor crates evidence of salt build up was suggested (Appendix 4, Figure 9). This probably forms part of the of the bio-film matrix seen earlier in the TEM slides.

3.1.3 Physical abrasion

Physical abrasion of the crate section surface using cotton swabs was attempted. Examination of the results by SEM (Appendix 4, Figure 10) suggested that this had little effect on the bio-film matrix on the crate surface.

3.2 The use of chemical methods to remove biofilms from crates

It was considered that an acid wash to dissolve the salt build up might form an effective means of loosening the bio-film matrix from the crate surface. Crates are normally immersed in an alkaline soak tank that will not dissolve salt build-up. An intermittent acid wash treatment might help promote bio-film matrix removal by the cleaning methods tried earlier i.e. pressure washing and ultrasonics.

As before, small sections of used, open and closed floor crate were prepared, immersed in a crate wash soak tank for 2 days to build up the bio-film matrix before being sent to Holchem Ltd. and Johnson Diversey Inc. for chemical treatment. Untreated sections, and sections immersed in the soak tank only, acted as controls.

The treatments of the crate sections by Holchem were 1% v/v solutions for 15 and 30 seconds of a) Chlorosan at 50°C, b) TWS at 60°C and c) Holphos, an acid treatment at 60°C. The treatments of the crate sections by Johnson Diversey were 1% v/v solutions at 16°C and 50°C for 90 seconds

by a) caustic based detergent, b) caustic based detergent containing low foam wetting agents, and c) neutral detergent.

The results of SEM analyses of the crate sections that underwent treatment showed that the bio-film matrix remained on the surface of the poultry transport crate material for all the chemical treatments applied. The appearance of the bio-film matrix appeared to differ slightly after treatment when compared with the control sections (Appendix 4, Figure 11). A number of the treatments resulted in the layers of the bio-film matrix appearing to have been eroded. It was considered that the bio-film matrix was made up of a stable aggregate of polysaccharides and mineral salts, making it difficult to attack chemically.

Salt deposits on the crate surfaces were not removed by all the chemical treatments applied (Appendix 4, Figure 12).

COMMERCIAL - IN CONFIDENCE

4. PROGRAMME OF FACTORY-BASED PILOT RIG STUDIES

4.1 Overview of the equipment and the schedule

4.1.1 The crate-washing test rig

For the purpose of the factory-based trials, a mobile test rig was designed and built. Full details are given in Appendix 3 along with operation procedure.

The test rig was designed to clean crates one at a time following a wide range of cleaning regimes. These could follow the current washing practices (spraying, soaking, disinfection etc) or enable the exploration of new techniques such as vibration, steam treatment, sonication or irradiation by ultra-violet light. The principle in each case was a batch process that simulated a continuous cleaning. Thus if a crate were to receive a 15 second spray then a 15 second soak as it passed through a washing line, this would be simulated in the rig by 15 seconds of water spray with the crate moved to and fro followed by the filling of the unit with water to reproduce soaking. By keeping with a batch operation, the test system was both simpler and more versatile.

The main purpose of the test rig was to enable a wide range of conditions to be explored at the factory site in a methodical way. Trials could proceed independently of the commercial operation and only relied on the factory for services and a supply of freshly dirtied crates.

4.1.2 Procedures

The same protocol was used for each of the six visits made to Faccenda Chicken at Buckingham Road, Brackley in 2004 and 2005. This is set out in the first section of Appendix 3a and resulted from a series of project discussions. After some preparation work including commissioning at Silsoe and the provision of services to the lairage area where the rig was to be sited, the first set of trials took place in July 2004. Each visit lasted one week, the plant being delivered and set up on the Monday and removed on the Friday. Modifications to the plant in preparation for the next set of trials would then take place at the workshops at Silsoe ahead of the next set of trials.

The procedures for the microbiological measurements are given in Appendix 5.

4.1.3 Factory trial schedule

Full details of the six factory trials (labelled A to F) are set out in Appendix 3a.

The organisation of the factory trials followed a series of themes to allow both a systematic approach to the many parameters identified and to make best use of the available resources. The initial theme corresponding to the first visit (Trials A) was based around the current practices. This enabled a baseline for the programme of study to be established. Clean and "dirty" water was used the latter being taken from the soak tank of the commercial unit. Spray and soak times were

varied and the potential benefits of hot water explored. Of special interest were the sensitivity of two parameters on the effectiveness of the process: ***that a long enough soak time and pre-wash were both crucial to the effectiveness of the cleaning process.*** In the event, the results show that neither were found to be critical in a general process that was micro-biologically inadequate.

The main theme with the second series of trials (Trial B) moved onto the benefits of water removal equipment. The hypothesis was that residual water would carry a high microbial load and that its efficient removal would enable a more effective cleaning. Technical problems with the equipment mid-way through this trial meant that the trial had to be completed on a second visit a few weeks later.

The third trial (Trial C) was given over to the various options for disinfection. These included use of chemicals, ultrasonics, use of steam and ultra-violet light. The effectiveness of any of these methods could be expected to better if the crates were firstly well washed for example the presence of residue organic matter would be an especial problem with the application of chemical disinfectants. The fouling of a sonication tank by the build up of debris would also reduce process efficiency and necessitate a water cleaning cycle.

The fourth trial (Trial D) looked at more rigorous washing systems including drying, scrubbing, multiple washing and the use of a commercial try washer. In some of the trials, "excessive practice" was followed, such as the use of very large amounts of chemical disinfection. This was in order to establish conditions where effective cleaning would be achieved (both visually and micro-biologically).

The results from trials A to D were reviewed and the most promising treatments were studied in more depth in Trial E. This included the use of large amounts of chemical, brushing, hot water soaking, repeated washing and the use of ultrasonics (sonication).

The final trial (Trial F) was a short piece of work lasting just three days covering a series of outstanding experiments including the re-washing of crates sent back to the farm. This was achieved by a great deal of cooperation with factory and transport staff:- two modules of 12 crates were washed using an ultrasonic bath, clearly marked and sent back to the farm. With careful tracking, the same modules were identified on the next day as they were returned full of birds. These same crates were so re-washed and the cumulative benefit of a rigorous washing evaluated. The hypothesis was that if the crates were well washed, they would both remain cleaner and be easier to clean on each cycle. Conversely, poorly washed crates would spiral downwards and become progressively dirtier as they were inadequately cleaned on each passage through the factory.

4.2 Presentation of micro-biological results

4.2.1 Tabulated results

All the results of the many swabs taken of washed, un-washed and control-washed crates are set

out in Appendix 3b. These are organised according to the six sets of trials (A to F) and paired up with the visual assessments carried out at the same time. There were three types of microbe assayed: (a) total aerobes (PCA), (b) Enterobacteriaceae (VRBG) and, for some of the later trials, *Camplyobacter* (CCDA). The following summary tables are based on this information. Treatments with a change greater than 1 log have ben highlighted to ease appraisal of the results. Each day was treated as a single unit and unwashed crate results were combined from morning and afternoon sessions to be more representative and based on a larger sample.

4.2.2 Trial Series A

Below, laid out in Table 4.1 a summary of the microbiological results are presented. The full set of data is given in Appendix 3b. In all cases, these are relative values showing the difference to a control in the numbers of $\log_{10}$ units. Mean values for each set of crates are compared (a) to the mean value of the unwashed control (not given in this table) and (b) to the mean value for the relevant washed control. Control wash trials are denoted by "C" and experiments by "T". Positive values represent an improvement over the control.

Table 4.1: Summary of microbiological results for trial **series A**

Treatment	Relative to unwashed control* ($\log_{10}$)		Relative to washed control* ($\log_{10}$)	
	PCA	**VRBG**	**PCA**	**VRBG**
12/7 Monday				
C 15sec prewash,30sec soak,15 sec main wash; clean water	0.3	-0.1		
T 15sec prewash,5 mins soak,15 sec main wash; clean water	0.2	0.3	-0.1	0.4
13/7 Tuesday				
C 15sec prewash,30sec soak,15 sec main wash	-0.9	-0.3		
14/7 Wednesday				
C 15sec prewash,30sec soak,15 sec main wash	0.6	0.4		
T No prewash, 30sec soak, 60sec main wash	0.5	**-1.0**	-0.0	**-1.4**
T No prewash, 30sec soak, 5min main wash	**1.0**	**1.8**	0.4	**1.4**
T 5min prewash, 30sec soak, 5min main wash	0.8	**1.8**	0.2	**1.5**
15/7 Thursday				
C 15sec prewash,30sec soak,15 sec main wash	0.2	0.5		
T 15sec prewash, 5min soak (40 deg.C), 15sec main wash	0.6	**1.1**	0.3	0.5
T 15sec prewash, 30sec soak (40deg.C), 15 sec main wash	0.9	-0.1	0.7	-0.6
T 15sec prewash, 5min soak (60deg.C), 15sec main wash	**1.6**	**1.5**	**1.4**	1.0
16/7 Friday				
T No prewash, 1min soak, no main wash	**1.5**	**2.6**	-0.4	**-1.0**
C 15sec prewash,30sec soak,15 sec main wash	**1.9**	**3.6**		
T No prewash, no soak, 5min main wash	0.2	**3.0**	**-1.7**	-0.5

*proportional log change

Overall with the sole exception of the last trial done on 15/7 with a hot soak of 60°C, differences in microbial counts compared to unwashed and washed control were either small or insignificant. In other words, none of the parameters explored were critical. The exception was 16/7, where compared to unwashed, an improvement was seen in microbial counts. However, it is noted that there were only two unwashed controls of which one was especially dirty (see Appendix 3a).

On the 12/7, extending the soak time from 30 to 300 seconds achieved no improvement in microbiological terms. Leaving out the pre-wash (14/7) led to no improvement but increasing the main wash time (after soaking) from 15 to 300 seconds on the same day did achieve a reduction of 1.8 logs in Enterobacteriaceae numbers. Using warm (40°C) soak water on 15/7 achieved little improvement even with a longer soak time. When this temperature was increased to 60°C, both microbe groups fell by 1 to 1.4 $\log_{10}$ units.

4.2.3 Trial Series B

Table 4.2 summarizes the results from the second series of trials. Once again, there was very little improvement despite a wide range of techniques tried, these largely based on water removal. Relative to the control wash, nothing achieved more than one log reduction in either of the two microbe groups studied. The control washes themselves achieved up to one log improvement (eg 14/09) especially with the less resilient Enterobacteriaceae.

Crates with solid floors were used in this series of trials; one might have expected poorer results from greater difficulty in water removal. Water removal either with air jets or vibration rig was harder with the solid floor crates but in the event, this did not translate to higher microbe counts. The air jets did seem to remove solid debris contributing to a visually cleaner crate but this too did not improve the microbe counts by itself.

Most disappointing was the final trial on 7/10 where a hot water rinse was combined with vibration and air jets but without any perceivable reward.

COMMERCIAL - IN CONFIDENCE

Table 4.2: Summary of microbiological results for trial **series B**

	Treatment	Relative to unwashed control* (log_{10}) PCA	VRBG	Relative to washed control* (log_{10}) PCA	VRBG
	13/09 Monday				
C	15sec prewash,30sec soak,15 sec main wash	0.1	0.1		
T	5 minutes main wash	-0.1	0.1	-0.2	-0.0
	14/09 Tuesday				
C	15sec prewash,30sec soak,15 sec main wash	0.3	**1.1**		
T	15 sec prewash, 30 sec soak + 15 sec main wash/rinse with clean cold water	0.6	**1.2**	0.3	0.2
T	15sec prewash,30sec soak, I min air jet + 15 sec wash with clean cold water	0.5	**1.3**	0.2	0.2
T	15sec prewash,30sec soak + 15 sec rinse with clean hot water (55 - 60deg C).	0.4	**1.4**	0.1	0.3
	15/09 Wedneday				
C	15sec prewash,30sec soak,15 sec main wash	0.7	-0.5		
T	15sec prewash,30sec soak, 1 min airjet + 20 sec wash clean hot water 60 deg C	-0.7	-0.2	**-1.4**	0.4
	Solid base				
T	15sec prewash,30sec soak + 1 min airjet, 20 sec wash clean hot water 55 deg C	-0.1	0.1	**-1.7**	-0.5
	06/10Wednesday				
C	15sec prewash,30sec soak,15 sec main wash	0.6	0.2		
T	Stantard prewash, standard soak + 15 sec vibration & cold rinse	**1.0**	0.3	0.4	0.1
T	Standard prewash, standard soak + 15 sec vibration & hot rinse	**1.1**	0.7	0.5	0.4
T	Standard prewash, standard soak + 15 sec vibration + 60 secs air jet & hot rinse	0.4	0.5	-0.2	0.3
	07/10 Wednesday				
C	Standard prewash, standard soak	**1.1**	**1.1**		
T	Standard prewash, standard soak + 15 sec vibration + 60 secs air jet & cold rinse	**1.2**	**1.2**	0.1	0.1
	Solid base				
T	Standard prewash,& soak + 15 sec vibration + 60 secs air jet & hot rinse 60 deg C	**1.8**	**1.2**	-0.8	0.7

*proportional log change

4.2.4 Trial Series C

The third series of trials concentrated on the various disinfection options. The main results are summarised in Table 4.3 below, the full details being given in Appendix 3b. Relative to the

unwashed crates, control washing improved things by 1 to 1.5 log_{10} units which is slightly more than for the previous two trials. Relative to the controls, steam, UV light radiation, and sonication resulted with no further significant improvements. However, it is noted that the ulta-sonic bath may have been too cool (at 45°C) for an effective use of this particular technology.

Table 4.3: Summary of microbiological results for trial **series C**

	Treatment	Relative to unwashed control* (log_{10})		Relative to washed control* (log_{10})	
		PCA	VRBG	PCA	VRBG
	25/10 Monday				
C	15sec prewash,30sec soak,15 sec main wash	**1.5**	0.6		
T	Control wash then 120 secs steam	**1.4**	0.8	-0.1	0.2
	26/10 Tuesday				
C	15sec prewash,30sec soak,15 sec main wash	0.9	-0.1		
T	Contol wash; rinse with hot clean water; 250ml of 0.5% Virkon	**1.6**	**1.7**	0.7	**1.8**
T	Contol wash; rinse with hot clean water; 60 secs air + 250ml of 0.5% Virkon	**1.4**	**1.7**	0.5	**1.8**
T	Contol wash; rinse with cold clean water; 60 secs air + 250ml of 0.5% Virkon	**1.8**	**1.6**	0.8	**1.7**
	27/10 Wednesday				
C	15sec prewash,30sec soak,15 sec main wash	-0.3	**1.4**		
T	Contol wash; rinse with hot clean water; 60 secs air + 60 secs under UV lamps	0.4	0.3	0.6	**-1.1**
C	No US (soak only) - control	-0.2	**1.9**		
T	2 kW US	-0.3	**2.0**	-0.1	0.1
T	4 kW US	0.3	**2.3**	0.5	0.4
	28/10 Thursday				
C	15sec prewash,30sec soak,15 sec main wash	**1.2**	**1.2**		
T	Contol wash; rinse with hot clean water + 120 secs steam	0.6	**1.2**	-0.6	-0.0
T	Contol wash + 250ml of 0.5% Virkon	0.6	0.7	-0.5	-0.6
T	Contol wash; rinse with hot clean water + 250ml of 0.5% Virkon	**1.5**	**1.1**	0.4	-0.1
T	Unwashed control + 250ml of 0.5% Virkon	0.6	0.8	-0.5	-0.4

*proportional log change

The most effective disinfection method emerged as the application of an approved chemical, "Virkon S" in this case. 250 ml of 0.5% solution applied to the well cleaned crates on the 26/10 resulted in reductions of 1 to 2 log_{10} units of the microbe counts. However, this was not achieved a second time when repeated on 28/10.

4.2.5 Trial Series D

In the fourth series of trials, more rigorous methods were tried - even if not necessarily practical. The purpose was to establish that significant improvements in crate cleaning could be achieved.

COMMERCIAL - IN CONFIDENCE

The results are summarised in Table 4.4 below.

Table 4.4: Summary of microbiological results for trial **series D**

	Treatment	*Relative to unwashed control* (log_{10})*		*Relative to washed control* (log_{10})*	
		PCA	*VRBG*	*PCA*	*VRBG*
	22/11 Monday				
C	15sec,30sec clean soak (52deg.C) 15sec rinse hot (63deg.C)	**1.0**	**1.4**		
T	Control + 300 secs brushing then 20 sec rinse with hot water	**2.4**	**3.8**	**1.4**	**2.4**
	23/11 Tuesday				
C	15sec,30sec cold dirty soak,15 sec, hot (63deg.C) rinse	-0.1	**1.0**		
T	Control + 500ml Virkon at 1%	0.8	**1.4**	0.9	0.4
T	Control + 500ml Virkon at 2%	**2.1**	**3.6**	**1.7**	**2.4**
T	Control + 120 sec scrub, hot rinse + 500ml Virkon at 2%	**2.8**	**5.4**	**2.4**	**4.2**
	24/11 Wednesday				
C	15sec, 30sec cold soak,15 sec hot (63deg.C) rinse	0.4	0.7		
T	Control + 300 secs steam	0.7	**1.3**	0.3	0.6
T	Control + 300 secs blower	0.7	**1.7**	0.3	**1.0**
T	Control + 300 secs blower + 250 ml Virkon at 0.5%	0.6	**1.3**	0.2	0.6
T	70 secs in commercial tray cleaner (steam) "Oliver-Douglas"	**1.0**	**1.5**	0.6	0.8
	25/11 Thursday				
C	15sec,30sec hot dirty watersoak, 15 sec hot (63deg.C) rinse	**1.3**	**2.0**		
T	Control + 0.1% detergent in hot (50+) soak tank x1 wash	**2.7**	**3.2**	**1.4**	**1.1**
T	Control + 0.1% detergent in hot (50+) soak tank x2 wash	**3.6**	**4.1**	**2.3**	**2.1**
T	Control + 0.1% detergent in hot (50+) soak tank x3 wash	**2.6**	**4.2**	**1.3**	**2.2**
	26/12 Friday				
C	15sec,30sec cold dirty soak,15 sec hot (60deg.C) rinse	0.1	0.1		
T	Control + 0.1% detergent in soak tank x1 wash	-0.6	**-1.8**	-0.7	**-1.9**

*proportional log change

The techniques used included brushing, high concentrations of chemical disinfectant, drying, detergents and a commercial tray washer. On this occasion, significant improvements were at last achieved with a reduction of more than four log_{10} units (for Enterobacteriaceae) for a combined wash system on 23/11. Most reductions were around 2 log_{10} units with slightly less for the total aerobes. Only the drying and the use of a steam cleaner on 24/11 produced disappointing results with reductions of less than 1 log_{10} units.

Mechanical brushing produced consistently good results with reductions of 1.4 and 2.4 log_{10} units on top of an improvement made (relative to the unwashed control) of 1 to 1.4 by the hot control wash. The benefit of brushes is again seem on the last trials on 23/11. Virkon at 2% (a higher than recommended application) reduced the microbe numbers relative to the control by 1.7 and 2.4 log_{10} units for the aerobe and Enterobacteriaceae counts respectively. When the crates were brushed as well, this improvement increased further to 2.4 and 4.2 log_{10}.

The combination of a hot soak and 0.1 % detergent produced some benefit in a 1 to 2 $\log_{10}$ reduction but repeated washes of the same crates did not add to this improvement. When the crates were washed in the same water but cold the following day, no improvement was achieved. It was thus concluded that detergent only had a benefit in reducing microbe numbers in a hot system as one might expect from the general advice from the laundry industry.

4.2.6 Trial Series E

The penultimate series of trials, concentrates on the most encouraging methods achieved so far. The results are summarised in Table 4.5 below - these include measurements of *Camplyobacter* (CCDA). Once again, the benefits of brushing are evident in a more encouraging series of trials. On the specific trial on the 7/2, improvements ranging from 0.6 to 1.7 $\log_{10}$ units was achieved by brushing alone. When brushing was combined with the Virkon chemical disinfectant on 8/2 it is seen to improve the reduction in microbe numbers by an additional1 to 2 $\log_{10}$ units. A similar benefit is evident from results on 9/2.

The benefit of applying chemical disinfectant is again evident (see 8/2) with up to 2 $\log_{10}$ of improvement in numbers. However, this was by applying 500 ml per crate at 2% concentration. When this is reduced to 250 ml per crate at 1%, the benefit falls to below 1 $\log_{10}$ units of reduction.

On the last day of trials, ultrasonics were used and produced more encouraging results with reductions of up to 3.5 $\log_{10}$ units. However, it is noted that the control in this case was a simple cold water wash. The ultrasonic tank used hot (65 deg.C) water and it remains to be seen what contribution resulted from the immersing of the crate in a tank of hot water itself.

COMMERCIAL - IN CONFIDENCE

Table 4.5: Summary of microbiological results for trial **series E**

	Treatment	Relative to unwashed control* (as log_{10}) PCA	VRBG	CCDA	Relative to washed control* (as log_{10}) PCA	VRBG	CCDA
	7/2 Monday						
C	Control - 15s prewash,30s soak and 15s rinse in cold clean water	0.3	0.4	**1.1**			
T	Contol + 90s brushing	0.9	**2.1**	**1.9**	0.6	**1.7**	0.8
	8/2 Tuesday						
C	Control - 15s prewash,30s soak and 15s rinse in hot (55deg.C) clean water	**1.5**	**1.7**	0.9			
T	Control + 90s rotary brushing + 0.1% detergent + 2 washes + 500 ml Virkon @ 2%	**4.2**	**5.4**	**3.6**	**2.7**	**3.7**	**2.7**
T	Control (Tues am) + 90s rotary brushing + 0.1% detergent + 500 ml Virkon @ 2%	**4.6**	**5.3**	**3.7**	**3.1**	**3.6**	**2.7**
T	Control (Tues am) + 0.1% detergent + 500 ml Virkon @ 2%	**4.0**	**4.2**	**2.5**	**2.5**	**2.4**	**1.5**
	9/2 Wednesday						
C	Control - 15s prewash,30s soak and 15s rinse in cold dirty water	0.8	**1.1**	0.3			
T	Control + 500 ml Virkon @ 2%	**2.1**	**1.4**	**1.1**	**1.3**	0.3	0.9
T	Control (Wed am) + 250 ml Virkon @ 1%	**1.3**	**1.7**		0.5	0.6	
T	Control (Wed am) + 90 secs hand brushing + 250 ml Virkon @ 1%	**1.8**	**3.5**		0.9	**2.5**	
	10/2 Thursday						
C	Control - 15s prewash,30s soak in hot (55) dirty water; 15s rinse hot clean water	0.1	**1.2**				
T	Control + 500 ml Virkon @ 2%	**2.5**	**3.8**		**2.4**	**2.6**	
C	Control (as Thurs am)	**1.0**	**1.2**				
T	Control (Thurs am) + 500 ml Virkon @ 1%	**1.5**	**4.0**		0.5	**2.8**	
	11/2 Fri day						
C	Control - 15s prewash,30s soak cold dirty water, 30s brush,15s rinse cold clean water	0.1	**1.0**				
T	Control + 6mins US at 4kW at 65 deg.C (2% additive)	**2.7**	**4.4**	**2.5**	**2.6**	**3.4**	
T	Control + 3mins US at 4kW at 65 deg.C (2% additive)	**2.9**	**3.9**	0.8	**2.8**	**2.9**	
T	Control + 6mins US at 4kW at 65 deg.C (2% additive) but no brushing	**2.5**	**4.5**	0.9	**2.4**	**3.5**	

*proportional log change

4.2.7 Trial Series F

The final series of trials is summarised in Table 4.6 below.

COMMERCIAL - IN CONFIDENCE

Two pieces of work formed the main part of this trial: (a) a repeat of the ultrasonic work and (b) evaluation of the progressive benefit of repeated wash cycles. The crate wash rig was not used for these trials. Washed crates were removed from the commercial crate washing line. The control wash in this case represents washed crates removed from the line and simply soaked in the hot solution in the ultrasonic tank.

Table 4.6: Summary of microbiological results for trial **series F**

	Treatment	Relative to commercial washed control* (as log_{10})		
		PCA	VRBG	CCDA
	Expt FA1			
C	Control - commercial wash + 3mins at 60 deg.C	0.1	**1.8**	0.1
T	Commercial wash + 3mins at 60 deg.C with US	**2.3**	**3.2**	**2.8**
	Expt FA2			
C	Control - commercial wash + 3mins at 60 deg.C	**1.9**	**2.5**	0.0
T	Commercial wash + 3mins at 60 deg.C with US	**2.6**	**3.2**	**2.7**
	Expt FB			
T	Commercial wash + 1mins at 60 deg.C with US	0.4	0.3	0.7
	Expt FC			
C	Control - commercial wash + 30secs at 60 deg.C with US	**1.5**	**1.0**	0.8
T	Commercial wash + 60 s brushing + 30secs at 60 deg.C with US	**1.7**	**2.1**	**1.3**
	Expt FF1 (Recycled crates from A1)			
C	Control - commercial wash + 3mins at 60 deg.C	**2.4**	**4.5**	0.7
T	Commercial wash + 3mins at 60 deg.C with US	**2.5**	**4.5**	0.7
	Expt FF2 (Recycled crates from A2)			
C	Control - commercial wash + 3mins at 60 deg.C	**2.5**	**2.9**	0.7
T	Commercial wash + 3mins at 60 deg.C with US	**2.7**	**2.9**	0.7

*proportional log change

In both expt FA1 and its duplicate, expt FA2, the benefit of using 3 minutes sonication beyond the effect of simply applying heat is evident especially with respect to the reduction in *Campylobacter*. The additional benefit of the ultrasonic treatment is a further reduction of 1 to 2 log_{10} units for the total aerobe counts and the Enterobacteriaceae. Reducing the treatment from 3 to 1 minutes (in expt FB) and 30 secs (in expt FC) sees some of this improvement eroded. The use of brushing again achieves some benefit - again around 1 log_{10} unit or so.

The cleaned crates on recovery (having passed back to the farm for another batch of birds) indeed proved to be cleaner and easier to clean (expt FF1 and FF2). Even without the ultrasonic treatment, the control treatment reduced the microbe numbers by up to 4.5 log_{10} units - adding in the ultrasonic treatment brought little further improvement.

4.3 Presentation of visual cleanliness results

4.3.1 Tabulated results

For this piece of work, the main parameter for evaluating the crate washing lies with the microbe numbers found by swabbing the cleaned crates. However, some assessment of the visual cleanliness was also made to assist in evaluating the process. There is a general theory that disinfection processes (which ultimately reduce microbe numbers) can be expected to be more efficient if the crate is firstly well cleaned. The presence of residual debris can thus be expected to neutralise in part (or totally) the disinfection process that follows.

Visual cleanliness in this case was based on the estimates of the number of grams of debris on the crates before and after cleaning. Each of the five sides was assessed and scored a number of grams of debris. The effect of the cleaning process indicated by expressing the amount of debris after cleaning as a percentage of that present on the same crate before cleaning. Because the swabbing was done on the floor of the crate only, cleaning results are given as (a) for the total crate (all five sides, inside and out) and for the inside base or floor only. It is noted that the inside floor represents to most likely part of the crate for cross-contamination.

A totally cleaned crate would have a score of 0% on the scale used but any crate with a residual debris of below 20% after cleaning can be described as well cleaned. Where crates still have more than 50% of the debris remaining on them after cleaning, it would be reasonable to mark the process as poor. *It is noted that the evaluations in this series of studies is of crates passing through the rig and not of the equivalent commercial process*. A full set of results for each table that follows in given in Appendix 3b.

The highlighted values are those with less than 25% of the unwashed assessment AFTER treatment.

4.3.2 Trial series A

The visual assessment scores are set out in Table 4.7 below.

Table 4.7: Summary of assessments for the experiments that make up trial **series A**

	Treatment	*Relative to unwashed* Improvement Total After" as a % of "before"	Improvement Internal base After" as a % of "before"
	12/7 Monday		
C	15sec prewash,30sec soak,15 sec main wash; clean water	56	60
T	15sec prewash,5 mins soak,15 sec main wash; clean water	69	81
	13/7 Tuesday		
C	15sec prewash,30sec soak,15 sec main wash	57	58
	14/7 Wednesday		
C	15sec prewash,30sec soak,15 sec main wash	40	32
T	No prewash, 30sec soak, 60sec main wash	48	58
T	No prewash, 30sec soak, 5min main wash	26	**20**
T	5min prewash, 30sec soak, 5min main wash	**23**	**12**
	15/7 Thursday		
C	15sec prewash,30sec soak,15 sec main wash	47	34
T	15sec prewash, 5min soak (40 deg.C), 15sec main wash	42	32
T	15sec prewash, 30sec soak (40deg.C), 15 sec main wash	80	78
T	15sec prewash, 5min soak (60deg.C), 15sec main wash	38	34
	16/7 Friday		
T	No prewash, 1min soak*, no main wash	80	78
C	15sec prewash,30sec soak,15 sec main wash	38	34
T	No prewash, no soak, 5min main wash	32	**23**

The overall message here is similar to that for the corresponding evaluation of the effect on microbe counts - section 4.2.2. That is (a) altering many parameters such as soak time have little effect on the process and (b) the general cleaning is far from complete. Best results were seen for long main washes 14/7 (3rd and 4th) and 16/7 (3rd). The hot wash on 15/7 (4th) also gave better cleaning than average but on this occasion, it should be noted that the control was also relatively good.

4.3.3 Trial series B

The visual assessment scores are set out in Table 4.8 below. In this case, whereas there was little real improvement micro-biologically (see section 4.2.3), some visual cleaning benefits are revealed. This is especially the case for the crates cleaned by air-jet and rinsed with hot water (on 15/09 and 7/10). There was no equivalent improvement in the reduction in microbe numbers. The air-jets in particular were clearly very efficient in dislodging even quite firmly held dirt leaving a crate that looked cleaner. However, the limitations of "visual cleanliness" are also apparent in that the bacteria contamination can easily remain in invisible wet layers lying throughout the surface of the crate.

Table 4.8: Summary of assessments for the experiments that make up trial **series B**

	Treatment	*Relative to unwashed* Improvement Total After" as a % of "before"	Improvement Internal base After" as a % of "before"
	13/09 Monday		
C	15sec prewash,30sec soak,15 sec main wash	56	34
T	**5 minutes main wash**	47	39
	14/09 Tuesday		
C	15sec prewash,30sec soak,15 sec main wash	48	29
T	15 sec prewash, 30 sec soak + 15 sec main wash/rinse with clean cold water	46	**24**
T	15sec prewash,30sec soak, 1 min air jet + 15 sec wash with clean cold water	55	167
T	15sec prewash,30sec soak + 15 sec rinse with clean hot water (55 - 60deg C).	79	59
	15/09 Wednesday		
C	15sec prewash,30sec soak,15 sec main wash	40	30
T	15sec prewash,30sec soak, 1 min airjet + 20 sec wash clean hot water 60 deg C	**14**	**8**
	solid base		
T	15sec prewash,30sec soak + 1 min airjet, 20 sec wash clean hot water 55 deg C	**11**	**5**
	6/10 Wednesday		
C	15sec prewash,30sec soak,15 sec main wash	74	56
T	Stantard prewash, standard soak + 15 sec vibration & cold rinse	73	41
T	Standard prewash, standard soak + 15 sec vibration & hot rinse	65	46
T	Standard prewash, standard soak + 15 sec vibration + 60 secs air jet & hot rinse	38	29
	7/10 Thursday		
C	Standard prewash, standard soak	56	41
T	Standard prewash, standard soak + 15 sec vibration + 60 secs air jet & cold rinse	32	**28**
	solid base		
T	Standard prewash, standard soak + 15 sec vibration + 60 secs air jet & hot rinse 60 deg C	34	**22**

4.3.4 Trial series C

The visual assessment scores for this series of trials are set out in Table 4.9 below. Perhaps not surprisingly, treatments by ultrasonics achieve a good cleaning of the crates (27/10). The use of air-jets produced mixed results (26/10) with a good and a poor result. It is noted that in some cases, the crates were relatively clean at the start and thus the scope for improvement by washing was less. The especially clean crates on 28/10 are another anomaly that can not be easily

explained. More generally, the inside floor of the crate (both solid and grid design) were easier to clean that the sides.

Table 4.9: Summary of assessments for the experiments that make up trial **series C**

	Treatment	*Relative to unwashed* Improvement Total After" as a % of "before"	Improvement Internal base After" as a % of "before"
	25/10 Monday		
C	15sec prewash,30sec soak,15 sec main wash	38	**22**
T	Control wash then 120 secs steam	63	55
	26/10 Tuesday		
C	15sec prewash,30sec soak,15 sec main wash	58	57
T	Contol wash; rinse with hot clean water; 250ml of 0.5% Virkon	66	58
T	Contol wash; rinse with hot clean water; 60 secs air + 250ml of 0.5% Virkon	38	28
T	Contol wash; rinse with cold clean water; 60 secs air + 250ml of 0.5% Virkon	49	50
	27/10 Wednesday		
C	15sec prewash,30sec soak,15 sec main wash	47	**18**
T	Contol wash; rinse with hot clean water; 60 secs air + 60 secs under UV lamps	47	32
C	No US (soak only) - control	51	**24**
T	2 kW US	48	**21**
T	4 kW US	31	**25**
	28/10 Thursday		
C	15sec prewash,30sec soak,15 sec main wash	50	59
T	Contol wash; rinse with hot clean water + 120 secs steam	44	43
T	Contol wash + 250ml of 0.5% Virkon	59	61
T	Contol wash; rinse with hot clean water + 250ml of 0.5% Virkon	**14**	**7**

4.3.5 Trial series D

The visual assessment scores for this series of trials are set out in Table 4.10 below. Not surprisingly, some of the best results were with brushing but it should be noted that this only applied to the inside base of the crates. Improvements to the whole crate suggest that factors other than brushing played a part.

The commercial cleaner (24/11) was effective in cleaning the crates visually even if the level of microbes were hardly reduced. Good results were achieved with using detergents in a hot wash (25/11) which coincided with a reduction of 1-2 $\log_{10}$ in microbe numbers (section 4.2.5) but a poorer wash resulted with a cold wash with detergent, again as expected.

It is noted that the most effective use of the Virkon chemical disinfectant coincided with the production of cleaner crate - see the last three trials on 23/11 in Table 4.10 and compare with the microbiological results on Table 4.4. However, there is some ambiguity as to whether the brushing contributed directly to reducing the microbe numbers or indirectly by allowing a more effective use of chemical disinfectant (by removing some of the neutralising debris).

Table 4.10: Summary of assessments for the experiments that make up trial **series D**

	Treatment	*Relative to unwashed*	
		Improvement Total After" as a % of "before"	Improvement Internal base After" as a % of "before"
	22/11 Monday		
C	15sec,30sec clean soak (52deg.C) 15sec rinse hot (63deg.C)	31	**21**
T	Control + 300 secs brushing then 20 sec rinse with hot water	**10**	**2**
	23/11 Tuesday		
C	15sec,30sec cold dirty soak,15 sec, hot (63deg.C) rinse	47	33
T	Control + 500ml Virkon at 1%	44	41
T	Control + 500ml Virkon at 2%	51	38
T	Control + 120 sec scrub, hot rinse + 500ml Virkon at 2%	**23**	**6**
	24/11 Wednesday		
C	15sec, 30sec cold soak,15 sec hot (63deg.C) rinse	66	54
T	Control + 300 secs steam	39	26
T	Control + 300 secs blower		
T	Control + 300 secs blower + 250 ml Virkon at 0.5%	66	44
T	70 secs in commercial tray cleaner (steam) "Oliver-Douglas"	28	**23**
	25/11 Thursday		
C	15sec,30sec hot dirty watersoak, 15 sec hot (63deg.C) rinse	39	33
T	Control + 0.1% detergent in soak tank x1 wash	41	**25**
T	Control + 0.1% detergent in soak tank x2 wash	33	**21**
T	Control + 0.1% detergent in soak tank x3 wash	**15**	**3**
	26/12 Friday		
C	15sec,30sec cold dirty soak,15 sec hot (60deg.C) rinse	39	27
T	Control + 0.1% detergent in soak tank x1 wash	34	**21**

4.3.6 Trial series E

The visual assessment scores for this series of trials are set out in Table 4.11 below. The use of ultrasonic treatment consistently produced cleaner crates consistently (11/2) but the effect of brushing was this time more variable - little benefit evident on 7/2, reasonable cleaning on 8/2 and totally clean crates (floor only) on 9/2. The crates were generally cleaner on the latter two days but it was not clear why.

When comparing with the microbiological results (Table 4.5), the many inconsistencies make any

firm conclusions difficult to draw. However, the best results do seem to coincide with the cleanest crates as might be expected. Of course, it does not follow that achieving visually clean crates are necessarily and indication of a substantial reduction in microbe numbers.

Table 4.11: summary of assessments for the experiments that make up trial **series E**

	Treatment	Relative to unwashed: Improvement Total After" as a % of "before"	Relative to unwashed: Improvement Internal base After" as a % of "before"
	7/2 Monday		
C	Control - 15s prewash,30s soak and 15s rinse in cold clean water	57	45
T	Contol + 90s brushing	55	36
	8/2 Tuesday		
C	Control - 15s prewash,30s soak and 15s rinse in hot (55deg.C) clean water	44	35
T	Control + 90s rotary brushing + 0.1% detergent + 2 washes + 500 ml Virkon @ 2%	42	28
T	Control (Tues am) + 90s rotary brushing + 0.1% detergent + 500 ml Virkon @ 2%	44	**20**
T	Control (Tues am) + 0.1% detergent + 500 ml Virkon @ 2%	43	28
	9/2 Wednesday		
C	Control - 15s prewash,30s soak and 15s rinse in cold dirty water	45	27
T	Control + 500 ml Virkon @ 2%	42	30
T	Control (Wed am) + 90 secs hand brushing + 250 ml Virkon @ 1%	**25**	**0**
T	Control (Wed am) + 250 ml Virkon @ 1%	**25**	**0**
	10/2 Thursday		
C	Control - 15s prewash,30s soak in hot (55) dirty water; 15s rinse hot clean water	35	29
T	Control + 500 ml Virkon @ 2%	32	**17**
T	Control (Thurs am) + 500 ml Virkon @ 1%	42	27
C	Control (as Thurs am)	55	27
	11/2 Friday		
C	Control - 15s prewash,30s soak cold dirty water, 30s brush,15s rinse cold clean water	53	29
T	Control + 6mins US at 4kW at 65 deg.C (2% additive)	**15**	**0**
T	Control + 3mins US at 4kW at 65 deg.C (2% additive)	**23**	**17**
T	Control + 6mins US at 4kW at 65 deg.C (2% additive) but no brushing	28	**15**

4.3.7 Trial series F

The visual assessment scores for this final series of trials are set out in Table 4.12. These were much more variable than expected. Experiment A1 produced the expected improvement in cleanliness from ultrasonics but this was not repeated in the duplicate experiment A2. The benefit

of ultransonics (over the control of just immersing the crate in a hot tank of water) is evident in experiment B. Brushing did not improve a mediocre wash with a brief sonication in experiment C.

Of greatest interest from this final series of experiments (F1and F2) is that the cleaned crates on their return to the factory were visually cleaner prior to washing and very clean after the second wash in a hot sonication bath. This agrees with the microbiological benefits as set out in section 4.3.7. It can thus be concluded that there is evidence of the cumulative effect of an effective washing regime.

Table 4.12: Summary of assessments for the experiments that make up trial **series F**

	Treatment	**Relative to unwashed**	
		Improvement Total After" as a % of "before"	Improvement Internal base After" as a % of "before"
	Trial A1		
C	Control - commercial wash + 3mins at 60 deg.C		
T	Commercial wash + 3mins at 60 deg.C with US	31	**0**
	Trial A2		
C	Control - commercial wash + 3mins at 60 deg.C	64	54
T	Commercial wash + 3mins at 60 deg.C with US	42	44
	Trial B		
C	Commercial wash + 1mins at 60 deg.C	65	65
T	Commercial wash + 1mins at 60 deg.C with US	29	**13**
	Trial C		
C	Control - commercial wash + 30secs at 60 deg.C with US	51	33
T	Commercial wash + 60 s brushing + 30secs at 60 deg.C with US	51	29
	Trial F1 (Recycled crates from A1)		
C	Washed controls (commercial unt only)		
C	Control - commercial wash + 3mins at 60 deg.C	**23**	**0**
T	Commercial wash + 3mins at 60 deg.C with US	**0**	**0**
	Trial F2 (Recycled crates from A2)		
C	Washed controls (commercial unt only)		
C	Control - commercial wash + 3mins at 60 deg.C	42	**0**
T	Commercial wash + 3mins at 60 deg.C with US	42	**0**

4.4 Thermal treatment of wash water

4.4.1 Experimental programme

In parallel with the fifth series of crate-washing trials (series E), a small programme of work was carried out using a thermal treatment unit. This is illustrated schematically in Figure 4.1 below; the equipment is also described in detail in Appendix 1, section 7. In summary, it comprised a feed

effluent tank (V1) using soak tank water, a feed pump (P1) running at around 1 litre per minute, a heat exchanger (H.Ex), a retention vessel (RI) and a treated water tank (V2).

Soak tank dirty water passes through the first stage of the heat exchanger where it is warmed to 50-60 deg.C using the returning hot effluent. The last stage of heating is achieved from a separate loop with hot water (see Figure 4.1). This involves in-line water heaters (H) and a re-circulation pump, P2 and represents the main energy demand from the process. The heating water is around 80-90 deg.C thus enabling the final temperature of the dirty water to reach the target temperature of 70 deg.C. This is held in a mixed vessel for a minimum of 5 minutes before it flows back to the heat exchanger where it is cooled prior to discharge.

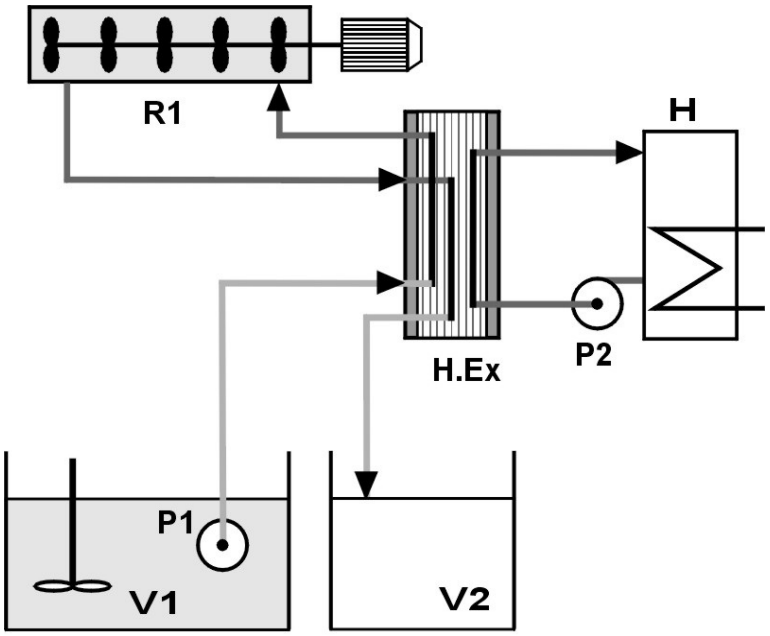


Figure 4.1: schematic layout of thermal treatment system for dirty water and similar effluents.

The implied heat recovery is crucial in the operation of any thermal treatment process which can otherwise be prohibitively expensive. A great deal depends on the flow rates and the type and size of heat exchanger: in this case a double pipe heat exchanger was used. Heat recovery was 76 to 83% (see Table 4.13). This implies that the heating cost was around 20% of the total heating duty. Greater heat recovery (up to 95%) is possible with larger heat exchangers especially the plate type.

COMMERCIAL - IN CONFIDENCE

Table 4.13: Presentation of results of water treatment trials carried out in parallel with the penultimate series of crate-washing trials (Trials E) and using soak tank water removed from the commercial line. Part 1: treatment conditions.

Time	**Temperatures** (deg C)			% heat recovery
	Feed	Treatment	Final	
Thursday 10th February 2005				
13:20	19	30	68	78
14:20	28	35	67	82
14:50	31	38	68	82
15:20	35	41	69	83
16:00	21	33	70	76
16:30	21	33	71	76
17:00	22	33	70	77
Friday 11th February 2005				
13:20	16	24	61	83
13:40	16	25	58	79
14:30	14	25	62	76
15:20	14	25	58	76

4.4.2 Results of water treatment trials

Samples of dirty water were taken before and after thermal treatment. These were enumerated for total aerobes, Enterobacteriaceae, and *Camplyobacter*. The microbial numbers per ml of water before and after treatment are set out in Table 4.14 below.

Table 4.14: Presentation of results of water treatment trials carried out in parallel with the penultimate series of crate-washing trials (Trials E) and using soak tank water removed from the commercial line. Part 2: microbiology results.

Time	**Feed sample** (log_{10} cfu/ml)			**Treated sample** (log_{10} cfu/ml)			**Comments**
	Log PCA	Log VRBG (F)	Log CCDA	Log PCA	Log VRBG (F)	Log CCDA	
Thursday 10th February 2005							
13:20	5.2	2.3	n/d	4.7	0.0*	n/d	A
14:20	7.2	3.3	n/d	2.7	0.0*	n/d	B
14:50	5.4	2.9	n/d	3.2	0.0*	n/d	
15:20	5.3	1.7	n/d	3.0	0.0*	n/d	
16:00	3.6	3.3	n/d	n/d	0.0*	n/d	C
16:30	4.3	3.3	n/d	3.2	0.0*	n/d	
17:00	3.5	3.3	n/d	2.4	0.0*	n/d	
Friday 11th February 2005							
13:20	5.4	2.0	2.8	5.4	1.6	2.4	D
13:40							
14:30	7.0	1.0	2.8	5.3	0.0*	1.9	
15:20	6.2	0.0	4.5	4.1	0.0*	0.0*	E

Table notes

A System warmed using hot clean water; switched to effluent at 13:19 - hence feed was effluent but treated was clean water. Effluent taken from production line soak tank (cold)

B Treated effluent recycled to feed tank

C Treated effluent switched to waste; feed tank topped up with dirty water from production line soak tank

D Plant heated up using recycled effluent; switched to waste at 13:20

E Blockage interrupted flow for several minutes; flowrate nominally 1 litre per minute.

* Zero indicates no colonies (below one log)

The effect of thermal treatment at 70°C is reduction in aerobes by 2 to 4 log_{10} units. In the case of the Enterobacteriaceae, reduction of numbers to below detection threshold was achieved in most cases. This implies at least a three log reduction but this may well have been greater had the initial concentration been higher. The effect on *Campylobacter* was less clear as analysis of the treated water was not done on the first trial (12/2). On the second day (11/2), the performance improved as the trial progressed with a reduction to below detection (over four log_{10} units) by the end of the experiment.

One might expect a more rigorous treatment if temperatures are raised higher -clearly this is readily possibly up to 80 or even 90°C Total sterilisation is theoretically possible but a higher thermal cost is inevitable; in addition, the problem of fouling in the heat exchanger can be expected leading to the need for more frequent cleaning. Nonetheless, thermal treatment of wastewaters to enable their subsequent use in cleaning duties has potential practical application as demonstrated in this brief study.

COMMERCIAL - IN CONFIDENCE

5. CONCLUSIONS

5.1 What are the best techniques

5.1.1 Introduction

To be effective, any revised or new technique needs to significantly improve microbiological levels compared to the current best available commercial standard at the current time. Studies and factory trials carried out suggest that whilst "visually clean" crates may be possible, the current state of the art technology rarely achieves even a one log reduction in microbe counts on the crate surface. Often even this is not achieved and in some poorly run systems and the count may actually increase following the washing process. To be significant, a valid crate-washing alternative needs to reduce the surface count by at least 2 $\log_{10}$ units relative to current practice, implying three logs relative to the unwashed crate. A four log reduction would, however, be more convincing.

Of the techniques studied, none by themselves achieved a reduction in the microbe count of four $\log_{10}$ units but several in combination could. In two cases, (Trial E) Enterobacteriaceae were reduced by 5.4 $\log_{10}$ units by a such a combination of methods. Five techniques were particularly effective:

1. The use of brushes;
2. Using hot water (60 deg.C +) in soaking and spraying;
3. The use of hot water with detergents;
4. Ultrasonics
5. The use of disinfecting chemicals.

5.1.2 Cost considerations

Clearly, as well as being effective, any new washing technique must be affordable within the context of the commercial operation. An indication of cost is thus appropriate which can enable some sort of value for each $\log_{10}$ unit of reduction achieved. Estimated figures are given below, and the basis of calculation are the assumptions that follow.

Entrained water As part of the final set of trials (F), measurements were made of the amount of water retained on a crate. 10 *grid* floor crates taken at random were weighed dry then immersed in a tank of soak-tank water before being weighed again. The crates were then jetted with a compressed air line before being weighed a third time. The mean dry weight was 9318 g (standard deviation of 3%). The amount of entrained water was 165 g (sd 17%) and after blowing with compressed air, this fell to just 22 g (sd 30%). It is expected that much larger amounts of water would be carried over with solid floor crates. For the purpose of calculation, water removed per crate is taken as 200 g per crate (reflecting the inclusion of some solid floor crates).

Chemical costs These can vary widely depending on the chemical. Disinfectants such as Virkon S (used in the trials reported here) costs around 1p per gram when bought in bulk. Thus a 1% solution works out at 10p per litre. There are cheaper chemicals such as quaternary compounds

which are around £1 per litre for the concentrate. Used at 2% strength, the price works out as 2p per litre. Detergents as a concentrate are taken as £5 per litre or 0.5p per litre used at the recommended 0.1% strength. The chemicals used in the ultrasonic bath are also costed as £5 per litre for the concentrate; this time diluted to 2% for use thus 10p per litre.

Energy costs. Electricity taken as 5p per kWh; steam taken as half this value, ie, 2.5p per kWh (thermal). Cold water at 20 deg.C heated to 60 deg.C requires 0.046 kWh per litre (kg) of water or 0.12p per litre of hot water (steam heating).

Brushes. Estimated cost as £1000 per set of brushes that need replacing after 100,000 crates. Thus cost per crate of 1 pence.

5.1.3 The use of brushes

Brushes achieved the following:

- 1 to 2 logs reduction in microbial counts, even from moderate brushing
- Contributes to producing a visually clean crate
- Not all areas reached
- Problem of lost bristles in system and of brush maintenance
- Medium investment – elaborate brushing frames
- Running cost (new brushes) ~1 pence per crate (basis of l set of brushes per 1-2 months or 100,000 crates at £1,000 per set - installed).
- Possible avoidance of chemical use.

There remains the central matter of how crate brushing could be achieved in a continuous production line; one option is sketched out in Figure 5.1 (above) but clearly further study is required.

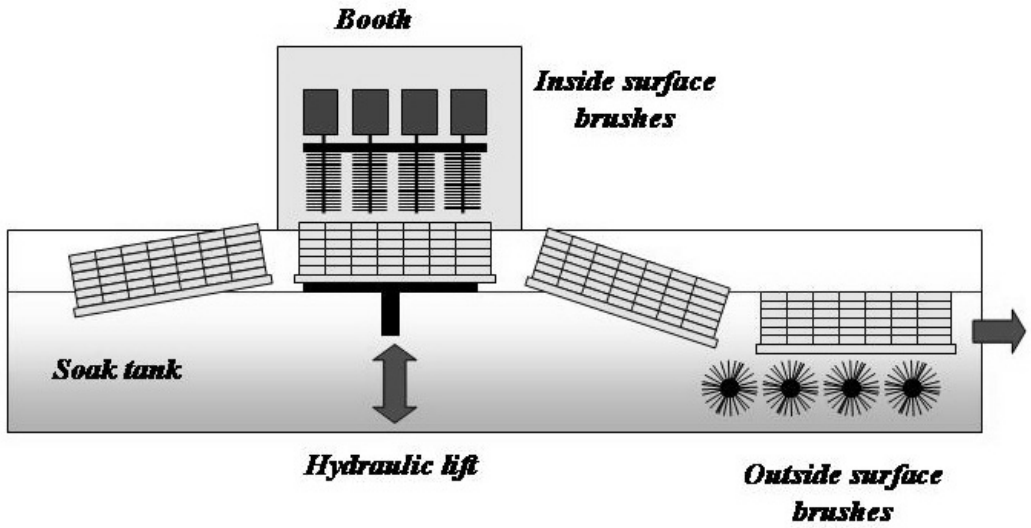


Figure 5.1: Possible deployment of a brush cleaning system in a modified soak tank of a commercial crate washer.

5.1.4 Using hot water (60°C +) in soaking and spraying

The following results are important to consider:

- 2 to 3 logs reduction when soaking – especially for faecal bacteria; less effective in reducing total aerobe count
- The temperature needs to be over 50°C (and preferably over 60°C)
- Hot water rinsing alone has little effect on microbe numbers
- Problem of fog in the lairage area
- Small investment (mostly containment of fog and heaters)
- Running cost (heating) ~0.12 pence per crate (on the basis of l litre of hot water lost per crate including evaporation).

5.1.5 The use of hot water with detergents

The following results are important to consider:

- 2 to 4 logs reduction from combined hot soak + detergent
- Detergent did not work in cold soak
- Multiple washing only useful if first stage ineffective
- Problem of fog in the lairage area
- Low investment – as for hot soak plus dosing system
- Possible running cost (heating) ~0.12 pence per crate (basis of l litre of hot water lost per crate including evaporation) plus ~0.1 pence for chemical used (basis of 0.1% concentration and 200 ml lost per crate).
- Much higher consumption of detergent when high levels of organic matter present owing to the neutralizing effect.
- Possible avoidance of subsequent disinfectant chemical use.

5.1.6 Ultrasonic treatments

The following results are important to consider:

- Up to 4 logs reduction from combined hot soak + ultrasonics
- Ultrasonics *not* effective in cold water
- Use associated chemicals at 2% concentration to enhance decontamination effect
- Problem of fog in the lairage area
- Medium investment – second soak tank after main wash: ultrasonics equipment and chemical dosing equipment.
- Importance of a water treatment loop to avoid the loss of performance from an increasing concentration of suspended matter in the water.
- Possible running cost (heating) ~0.3 pence per crate (basis of l litre of hot water lost per crate including evaporation) plus ~2 pence for chemical at 2% concentration (basis of 200ml water lost per crate).
- Electricity costs: 1 minute crate exposure at 4 kW = 0.06 kWh costing 0.3 p per crate.

5.1.7 The use of disinfecting chemicals

The following results are important to consider:

- 2 to 4 logs reduction from combined wash plus disinfectant application

- The product, Virkon S did not work (a) at low concentration (0.5%) or when crate was poorly washed
- High doses on clean crates very effective
- Low investment – only efficient spray system needed - booth would help cut losses and improve safety.
- Possible running ~5 pence for chemical at 500ml per crate at 1% concentration. Cheaper alternatives available but higher concentrations may then be needed.
- Environmental problems from high usage of chemical.
- Staff and bird health risks

5.1.8 Ineffective treatments

The following treatments had little or no impact on microbial contamination of transport crates:

- Increasing the soak or wash time
- Including a pre-wash
- Use of tepid or warm (40^{o}C) soak temperature
- Rinsing with clean, cold water
- Removing excess water using vibration or air jets
- Low level steam treatments
- Exposure to U.V. light

Some of these such as the use of air jets did contribute to the general cleaning process and thus (indirectly) to the overall reduction of the microbial load on the crate surface.

5.2 Development of an advisory code of good practice

A key part of this project has been the development of guidelines to enable the Food Business Operator (FBO) to identify and follow best practice. This has gone through several stages of discussion and a final version of the proposed code has been set out in Appendix 2. For the purpose of organisation, these guidelines have been divided into six parts corresponding to the main operations in the current process:

- Crate inversion
- Pre-washing
- Soaking stage
- Main wash
- Rinse stage
- Disinfection stage

The recommended practices are not all equally applicable and depend on the individual plant and the space available. Clearly, there is more scope with new installations to include revised methods of crate washing. There is even more scope to improve the process as part of product development with equipment manufacturers. For these reasons, recommendations are coded according to their

practicability:

Code A: applies to all crate washing systems
Code B: applies to existing crate washing systems where appropriate modifications can be made
Code C: applies to newly installed systems only
Code D: applies to future designs of crate washers.
Code O: optional - omission would not represent a contravention of the code if suggested alternatives are used.

Further discussion on future design options is given below.

5.3 Future developments in crate washing

The effectiveness of some alternative methods have been demonstrated in the project work reported here but there remains further development work to be done before such ideas can be implemented. Some indication of what this means is given below. In addition, comment is added on the cleaning process of modules (although not strictly part of this project).

5.3.1 Hot water problems

Several techniques for improved crate washing are based around the use of hot water. However in winter, the cool conditions around the lairage area will inevitably lead to the development of a fog in the immediate area. This represents a deterioration in the local working environment as well as a loss of water and heat. Extraction systems are clearly required but so too are methods of minimizing heat and water losses such as insulation around the related equipment and (as far as possible) closed washing systems. Such measures would also need to provide necessary operator protection in the event of using water over 50^{o}C.

5.3.2 Installation of brushes

Brushing as a technique may work but the development of low cost mechanical systems for continuous lines will be essential if this is to progress. Some indication of the possible approach required is given in section 5.1.3 above but a far more detailed study is needed. Brushing systems already exist in many industries and could certainly be adapted and developed for crate washing at a cost. What is needed here is a “clever” mechanism that disrupts the main operation as littel as possible and which fits into the existing crate washing operations.

5.3.3 Sonication

The value of ultra-sonics has been demonstrated in this study and one can easily envisage the technique being implemented in the soak tank stage. This could be done with minimal changes but provision for heating (see above) would be needed. The main limitation on using sonication is the need for a system to combat the build up of suspended solids in the water which would

progressively reduce the strength of the transmitted energy. For this, a water treatment loop is envisaged which would essentially be a clarification operation. The method would need to be inexpensive, reliable and sufficient (but no more) to ensure an efficient sonication process to continue. Such techniques as settling and plate and frame filtration as possibilities that would allow low costs. Membrane filtration would achieve a better clarification but such systems are more costly to run and maintain. A crucial question is the minimum clarification needed to enable the process to continue.

5.3.4 Dip tanks/air jets

As an alternative to spraying disinfectant with the associated problems of aerosols etc., there is the option of totally immersing the crate in a bath full of the disinfectant. Such techniques have already been tried in other European countries and the effectiveness of the method has been shown. However, there is inevitably a high loss of chemical with this process unless most can be recovered. The use of air jets in a booth may be able to achieve this - as little as 20 ml of solution per crate is implied by trials with air jets (section 5.1.2). Clearly further studies on such practical issues are needed before this approach can be recommended.

5.3.5 Water treatment

Although the benefit of using potable water over re-used water was not demonstrated in this project, water treatment remains a useful technology with applications that extend outside the cratewashing activity. Much of this relates to the general need to reduce water consumption in the industry but without compromising food safety. Using recovered water for crate washing and other cleaning duties is an option so long as the microbial quality of the water can be assured. The brief trials done with this project indicated the potential of the process but more studies are needed before a process can be confidently specified.

5.3.6 Crate modules

The steel frames that hold the crates are washed separately prior to being refilled with the cleaned crates. They differ to crates in five ways:

1. They are an open structure thus targeting of water jets is more important.
2. Being of steel construction, much higher cleaning temperatures can be used safely.
3. One module normally holds 12 crates which allows twelve times longer for the cleaning operation.
4. Modules have more “corners” especially around the base, where debris can be trapped.
5. Modules come into direct contact with the floor of poultry houses; as they are slid around, the risk of picking up (and depositing) debris is high.

In response to this, improvements are possible in three areas:

A. In the design of the frames to avoid (or minimise) the areas where debris can collect.
B. In the washing process - eg: the use of very hot water dip tanks as one possible option.

C. In the way the frames are used on the farms - eg: the use of a “shoe” kept at each farm on which the frame is set prior to movement around the poultry house.

It is likely that all three areas need to be developed to ensure that the risks of cross contamination presented by modules is adequately reduced; this is clearly the basis of a separate study.

References

Corry, J.E.L., Allen, V.M., Hudson, W.R., Breslin, M.F., Davies, R.H. (2001) Sources of Salmonella on broiler carcasses during transportation and processing: modes of contamination and methods of control. Journal of Applied Microbiology 91, 1-9

Line, J.E., Bailey, J.S., Cox, N.A., Stern, N.J. (1997). Yeast treatment to reduce Salmonella and Campylobacter populations associated with broiler chickens subjected to transport stress. Poultry Science. 76, 1227-1231

James, W.O; Prucha, J.C; Brewer, R.L; Williams, W.O; Christensen, W.A; Thaler, A.M; Hogue, A.T. (1992) Effects of countercurrent scalding and postscald spray on the bacteriological profile of raw chicken carcasses. Journal of the Amercian Veterinary Medical Association 1992 201 No.5 705-708

Mason, T.J., Lorimer, J.P. (2002) Applied Sonochemistry: The Uses of Power Ultrasound in Chemistry and Processing. Chapter 4 Sonochemistry in environmental protection and remediation. Wiley-VCH, Germany. ISBN 3527302050, 9783527302055. 303 pages

Mead, G.C., Hudson, W.R., Hinton, M.H. (1995). Effect of changes in processing to improve hygiene control on contamination of poultry carcasses with campylobacter. Epidemiol. Infect. 115, 495-500

Mulder, R.W.A.W. (1995). Impact of transport and related stresses on the incidence and extent of human pathogens in pig meat and poultry. Journal of Food Safety. 15, 239-246

Rajkowski, K.T; Rice, E.W; Huynh, B; Patsey, J. (1996) Growth of Salmonella spp and Vibrio-Cholerae in reconditioned waste-water. Journal of Food Protection 1996 59 No.6 577-581

Shane, S.M. (1992) The significance of Camplyobacter-Jeujuni infection in poultry - a review. Avian Pathology 1992 21 No.2 189-213

Stern, N.J., Clavero, M.R.S., Bailey, J.S., Cox, N.A,. Robach, M.C. (1995) Campylobacter spp. in broilers on the farm and after transport. Poultry Science. 74, 937-94

Turner , C., Williams, S.M., Burton, C.H.,Farrent, J.W.,and Wilkinson, P.J. (1998) Laboratory scale inactivation of pig viruses in pig slurry and pilot plant design, Presented at IAWQ Conference, Vancouver, June 1998. Paper accompanying talk accepted for publication in Water Science and Technology

COMMERCIAL - IN CONFIDENCE

COMMERCIAL - IN CONFIDENCE

APPENDICES

1. State of the art report on crate washing

1a. Use of Enterobacteriaceae counts for determining the likely efficiency of poultry transport crate decontamination

1b. Extracts from *Easyload Maintenance Manual* supplied by Anglia Autoflow Ltd

.

1c. Presentation on the current technology for crate washing: its strengths and weaknesses.

2. Proposed code of good practice for the design and operation of crate washing processes for poultry transport crates.

3. Design and construction of a mobile test rig for washing poultry transport crates.

3a. Details of programme of factory based trials using the pilot scale test rig for crate washing.

3a. Full results from the programme of factory trials

4. Micrographs from studies of crate surfaces using scanning electron microscopy.

5. Micro-biological procedures used in this project.

APPENDIX 1

Reducing microbial contamination of poultry transport crates by improved cleaning and disinfection systems based on better water use a state-of-the-art report on crate washing.

C H Burton and R T Whyte*
V M Allen and J E L Corry[o]
R Davies[+]

* Silsoe Research Institute
[o] University of Bristol (Langford)
[+] Veterinary Laboratories (Weybridge)

20th February 2004

SUMMARY

Published work has established beyond doubt that crates used to transport poultry from farm to abattoir (poultry transport crates) are a source of contamination and cross contamination with respect to zoonotic pathogens, especially *Salmonella* and *Campylobacter*. Swabs taken from washed crates yield high counts (*eg*, up to 10^7 cfu each of *Campylobacter*, coliforms and Enterobacteriaceae from the base of a single crate). Far from achieving a reduction in numbers, existing washing processes have been shown to result in crates with *higher* counts of *Camplyobacter* compared to unwashed crates even if they are *visibly clean*.

Crate washing systems in the UK are most commonly based around a system that involves a pre-wash booth, a soak tank, a main wash booth and a final disinfection/rinse stage. There is a large variation in crate washers in the UK reflecting (i) improvements achieved over recent years by the manufacturer, (ii) the importance given to the washing process and (iii) changes to the washing system implemented by the processing company itself. However, due to the limited use of water, it is unlikely, even with the best current crate washing systems, that a sufficient reduction in microbe counts (around 4 $\log_{10}$ units) will be achieved and the poorer systems fail to achieve even visually clean crates. Stipulating and enforcing the use of potable water is unlikely to achieve any significant reduction in microbe numbers on the crates with the current technology.

Potential improvements to the process fall into three categories: (i) those which can be easily implemented, (ii) changes to existing equipment requiring considerable investment and (iii) changes that are only possible with a new installation. The first group includes a range of items that come under the title "good housekeeping" as well as the general observation of best practice. This includes keeping the work area clean, avoiding spillages onto the floor, regular changing of water, more frequent cleaning of screens, inclusion of a rinse stage with clean water and effective disinfection. The use of a detergent in the soak tank and/or hot water are further options. It still remains likely that the sum of these changes may not be enough but a clear standard is lacking and what defines "enough" may be subjective. Any reduction in microbe numbers by more than 2 $\log_{10}$ units would be welcome but fully effective cleaning and disinfection should ideally reduce microbe numbers by 4-5 $\log_{10}$ units. The major improvements will require better water management with larger volumes passing through the system in a countercurrent system. Recycled water should not be used in the final washing stages unless thermally treated. Adequate quantities of clean (or even hot) water in a final rinse stage preceded and followed by good drainage is recommended. A range of other possible improvements requiring further research to validate their effectiveness include: drying technologies, brushes, UV light, sonication, and steam decontamination

CONTENTS

1. INTRODUCTION

1.1 Background to the problem - the need for clean crates

The FSA has set itself the target of reducing the numbers of cases of food-borne infection in the UK by 20% by 2006. As most food-borne infections are caused by *Campylobacter* spp., and poultry are considered to be a major source of *Campylobacter*s, reduction or elimination of such organisms from poultry on retail sale will contribute to achievement of this target. A limited number of studies have shown that poultry crates are a vector for spreading bacteria that can cause food poisoning (including *Salmonella* and *Campylobacter*) to poultry from other previously infected flocks. These studies have shown that poultry crates are poorly cleaned at most poultry processing plants, and in many cases the microbiological contamination can be exacerbated by the cleaning process.

Legislation The Transport of Animals (Cleaning and Disinfection) (England No.3) Order 2003. This order requires all animal transport vehicles and containers to be cleansed and disinfected after each use and within 24 hours of the journey being completed. The legislation covers transport of all mammals and birds, and includes the removal of all feed, bedding, excreta and other material of animal origin.

Industry quality organisations The Assured Chicken Production Scheme (APCS) is an industry-wide initiative that seeks to address important issues concerning chicken production and to assure consumers of high standards of food safety and animal welfare. The provision of cleaning and sanitation equipment for poultry crates and transporters, and that transporters and poultry crates must be washed after unloading, form part of the rules of membership.

1.2 Evidence for persistence of microbes on washed crates

Literature searches and questionnaires reveal that most crate washing systems, as installed and used in processing plants, are not capable of reliably removing *Salmonella* and *Campylobacter* (Humphrey and Allen, 2002). Literature searches also revealed that there are relatively few papers on transport crate hygiene. Two were published within the last year and are derived from the UK poultry industry (Corry *et al*.,2002; Slader *et al*.,2002) while the other two were published over 20 years ago and were based upon the Canadian poultry industry (Rigby *et al*.,1980a and 1980b). From the evidence presented in these papers Humphrey and Allen (2002) conclude that there has been little improvement to the hygiene of crates. The Canadian researchers were particularly interested in the infection of broiler flocks by *Salmonella* with transport forming part of the investigation. The recent UK research focussed more on the role of crates in reinfecting broiler flocks with *Campylobacter* and *Salmonella*.

Significantly, the Canadian results from 20 years ago and the recent UK results showed that more crates were *Salmonella* positive after being passed through a crate wash system than before. Crate

cleaning was thus increasing the likelihood of cross contamination rather than serving as a control point.

Lister (2001) cites work carried out by the Belgian Faculty of Veterinary Medicine and the EU Food and Veterinary Service in Dublin. This showed that *Salmonella* and *Campylobacter* could be isolated from poultry carried in contaminated transport crates whereas previously the flocks were free from contamination on the farm. Further work carried out by these researchers confirm the findings of Rigby *et al*., (1980a) that the number of contaminated crates increased after passing through a crate washing system. McKenna *et al*., (2001) examined the contamination of transport crates and modules by *Campylobacter* at three processing plants in December and January. They found that at one plant the number of crates contaminated with *Campylobacter* increased after passing through the crate washing process. At another plant the crate washing appeared to be very effective, the incidence of detection of *Campylobacter* falling to zero after the washing process. However, the results from the same plant in June showed that the crate contamination rate was as high after washing as before no explanation was given for this change.

Although post-wash chemical disinfection should offer an effective means of ensuring crate microbial cleanliness, Corry *et al*., (2002) found that the current methods used were ineffective, as disinfectant was generally applied at a concentration lower than recommended and faecal soiling was still evident after cleaning and disinfection. Even after thorough application of disinfectant by hand at the recommended concentrations (which are probably too low), crates were not reliably free of *Salmonella*.

1.3 The importance of the engineering dimension

The largest manufacturer of transport crate washing systems for the UK poultry industry is Anglia Autoflow with Stork b.v., the second largest, a long way behind. However, in both cases, crate washing systems form a relatively minor part of their businesses. The maintenance program of these systems is generally produced by the processing plant engineers. Few processing plants have maintenance contracts with the system suppliers. There is also limited guidance from the crate washer manufacturers regarding best practice use of the equipment. Recommendations by Humphrey and Allen (2002) include more targeted guidance from the manufacturers, but it appears that they are awaiting authoritative research-based information on crate hygiene before best practice operation of crate washing systems will be revised.

Crate washing systems are most conveniently sited near to the lairage area where the poultry are hung on to the shackles. The introduction of a crate washing system is generally limited by the space available, which in turn limits the time of the crate washing treatment. In most processing plants after the poultry have been removed, the crates immediately move on to the washing system. For most plants the process involves vigorously inverting the crates to remove loose debris, passing them through a pre-wash spray (at some plants), passing them through a soak tank at room temperature for between 30 and 60 seconds at a rate of approximately 400 per hour before they are finally pressure spray washed.

A great deal of the thinking behind the development of crate washing systems has been undertaken with the objective of producing "visually clean crates". However, even if acheiving crates free from visual contamination can be assured, there is now no also the need to meet hygiene criteria.. There is already evidence that suggests that crates can leave a washing process with a higher microbial load than the dirty crates entering the system. It is thus unlikely that "more of the same" will meet the increasingly tough requirements, and there is a clear risk of making matters worse. The strategies needed require a more fundamental study of the system with the broad recognition that water can contaminate as well as clean. On the positive side, this does not mean that enhanced cleaning will necessarily lead to either more costly or more elaborate systems; rather that some change of approach may be needed in some areas to achieve the desired results.

There is certainly scope for improvement simply by ensuring the correct operation of existing equipment; minor modification should be able to gain further advantage. However, some changes may require more substantial refurbishment of existing equipment and some will only be an option for future new installations.

2. THE CRATE WASHING PROCESS

2.1 Crate design and use

The Anglia Autoflow system
Poultry transport crates are part of a system for moving live birds from the farm to the slaughterhouse. Most systems are designed to convey broilers although units which are also available for turkeys and other species could be similarly handled. In the most common design developed by Anglia Autoflow, the complete system comprises 12 plastic crates fitted as drawers in an open steel frame (module). The module is strong enough for movement by a forklift truck with the forklift tines inserted in slots in the base; on the top and bottom of the frame are location lugs to allow for the safe stacking of several modules.

The poultry transport crate is manufactured from high density polypropylene and is approximately 120 x 75 x 25 cm in size. It is highly webbed on the external surfaces to give it strength and rigidity while the internal surfaces are smooth. There are two designs of crate base: those that have closed floors with a few perforations to allow water drainage, and those that are open, highly perforated

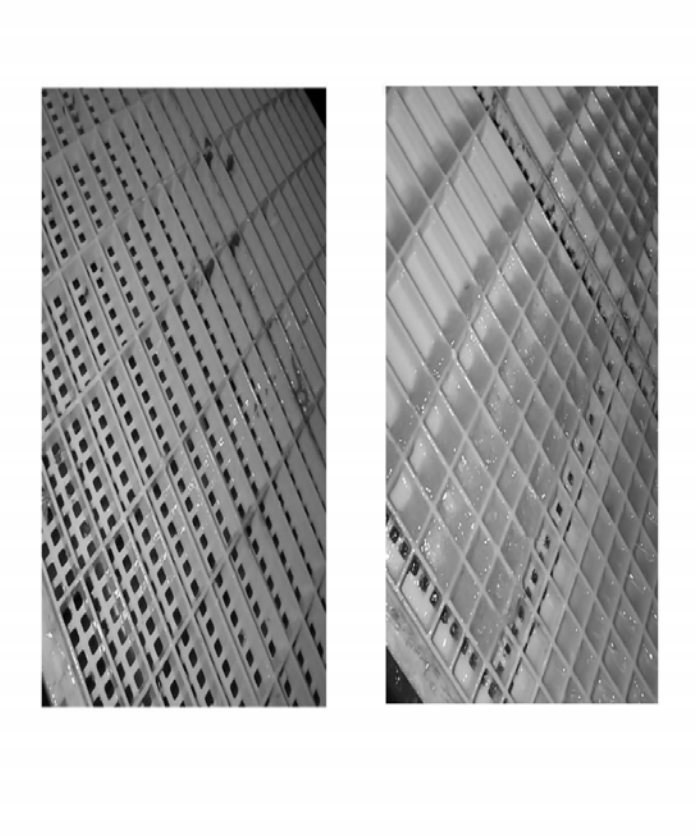


Figure 1: examples of open (grid floor) and solid floor crates (underside shown). Both types are in common use and arguments exist in favour for each. Higher HAS scores can be awarded for factories using exclusively the solid floor crates but they can be more difficult to wash especially due to drainage problems.

with holes approximately 1 cm square covering the whole surface of the crate floor (Figure 1). The sides are highly perforated to allow air movement to cool the birds while in transit. A single crate can hold 20-25 birds. It is open-topped, relying on being slid into the module to contain the birds (such that the base of the crate above forms the "ceiling" of the one below). Poultry crates are traditionally yellow in colour (chosen to minimise plastic deterioration by ultra violet light in sunlight), but blue crates are now becoming more common for animal welfare reasons (blue is thought to have a calming effect).

Some companies avoid open floor crates as they claim to be penalised in terms of the awarded HAS score by the visiting OVS (Official Veterinary Surgeon). Nonetheless, open floor crates are becoming preferred as, anecdotally, the birds are cleaner on arrival at the processing plant. Closed floors were thought to prevent excreta from birds on the upper tiers falling on those below. However the birds became more soiled in their own excreta, possibly being exacerbated by greater stress as the poultry are unable to grip the floor during vehicle movement. Open floors may also enable greater air movement (reducing heat stress) but this benefit may be small as the birds themselves represent the main obstruction to air circulation. Any increase in air movement may help to dry the excreta resulting in less bird soiling. A further possible advantage of open floors is that there is less likelihood of air entrapment beneath the inverted crates in the soaking tank during the washing process.

The webbing on the base and sides of the crate increases the overall surface area and provides numerous small surfaces at right angles to each other that have to be effectively cleaned. The orientation of these surfaces reduces the effectiveness of certain cleaning and disinfection processes such as medium pressure sprays and UV light as a result of "shadowing".

The surface of the transport crate when in use will become covered in a biofilm, a thin layer of biomaterial that supports and preserves microbes. It is notoriously difficult to remove by pressure sprays, even from smooth surfaces. The biofilm on crates will occupy the surface and attach particularly in the cracks, scratches and crevices that develop with use.

The modules in which 12 crates are housed during loading with poultry and transport are made from hot dip galvanised steel and therefore have holes in the tubing, providing a potential reservoir of organic matter and organisms. The structure of the modules is also conducive to retaining poultry house litter compacted into recesses in the base. During transport, large pieces of litter fall off the upper part of the modules on to the crates and modules underneath. The module itself has a skeletal structure which occupies a large volume and therefore requires a specialised washing process to adequately clean all its surfaces from low levels of visible contamination to highly compacted large quantities of material. Module washing systems comprise spray booths that are highly ineffective at removing visible contamination. Changes in module orientation, both laterally and vertically, together with specifically orientated jets that deliver a higher flow rate at greater pressure seem to be initial requirements necessary in such a system.

Modules, with their crates, are sent to the farm for collection of the birds. They are often pushed

along the floor of the broiler building and sited to act as a barrier to assist in bird catching; as such, the base can become fouled by the litter on the floor. Litter from previous flocks can be dislodged during this process to act as a source of infection for remaining birds in a house being thinned. Direct contamination is less of a problem with the crates themselves which rarely come in direct contact with the building. The modules plus crates are then transported to the lairage at the abattoir where they are loaded onto a conveying system when required. Crates are pulled out by hand to allow the removal of birds. Once emptied, they are placed onto a conveying line to be carried to the crate washer. The emptied modules are themselves moved on to their own washer, washed crates and modules being recombined after washing.

The Stork b.v. system

Rarely used in the UK (but common in other parts of Europe) is a system built by Stork b.v. and other suppliers, which consists of cages retained in a frame; birds are removed via flaps set on one side. In this approach, a steel frame is divided into sections (roughly the size of a typical poultry crate holding 20-30 broilers) by perforated plastic floors and sides. The number of compartments varies but eight to twelve arranged in two columns is common (giving a similar overall appearance to the Anglia Autoflow module with its drawers in place). The front section can be slid inwards to enable easier loading. At the back are hinged metal flaps for the purpose of unloading. The latter is achieved by gently tipping the whole assembly, discharging the birds onto a conveyor from where they are taken and hung on shackles. Because this system does not come apart in normal usage, the whole must be washed and disinfected as a single entity which is difficult to do effectively. This is often done near the lairage area, with the wet containers being transferred to cleaned lorries to make the return journey to the farm. In this, and many other respects, the two transport systems are used in a similar way. Beyond aspects relating to cleaning, the various merits of each handling system are beyond the scope of this report.

The situation in the UK

In the UK, most (over 90%) of poultry handling systems follow the Anglia Autoflow design using removable crates. For this reason, the details that follow in this and subsequent sections of this report relate solely to the Anglia Autoflow system with one main exception. For the sake of completeness, section 2.9 is given over to the fixed crate system marketed by Stork b.v. and the washing system used. Of particular interest are the differences between the two approaches and whether these bring benefits or disadvantages related to cleaning.

2.2 The overall purpose of the washing process

The general scheme for crate washing is summarised in Figure 2. The process is operated continuously over the production run which typically lasts 12-16 hours from early in the morning. Late evening or overnight, the whole washing plant is drained and thoroughly cleaned with water jet hoses. Tanks are refilled with clean water for the next day's operation. Water may also be changed during a production run (*e.g.* during the midday break) but this is not always the case and can depend on other factors in the running of the factory.

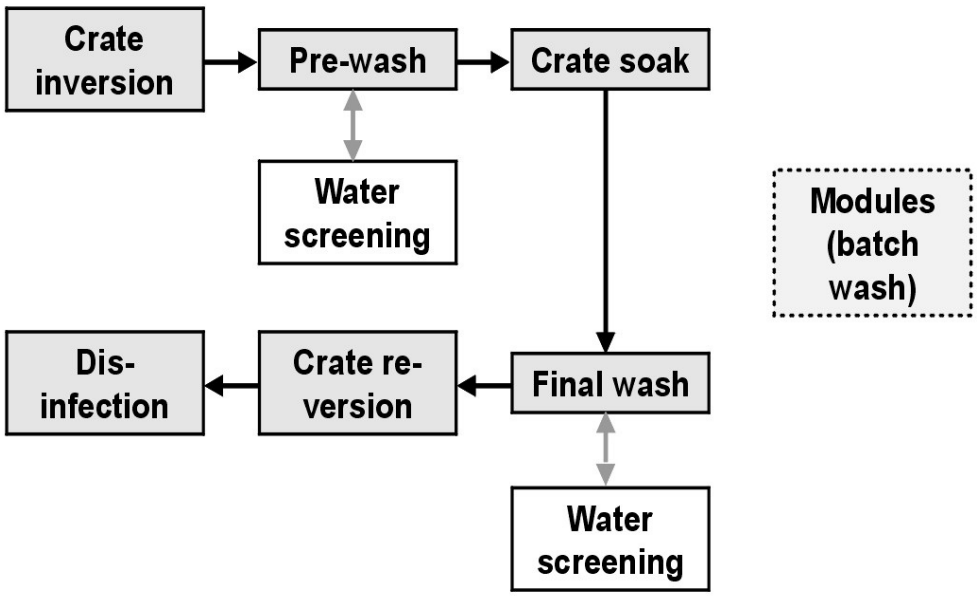


Figure 2: Schematic layout of a typical crate washing process.

At present, the generally accepted target of the washing operation is to remove debris to leave a visibly clean crate. This serves to promote general hygiene in the context of "good housekeeping". At the time of preparing this report, specific requirements based on microbiological objectives were not part of the washing operation.; this may change if clear benefits from practical measures can be demonstrated.

2.3 Crate inversion and pre-washing

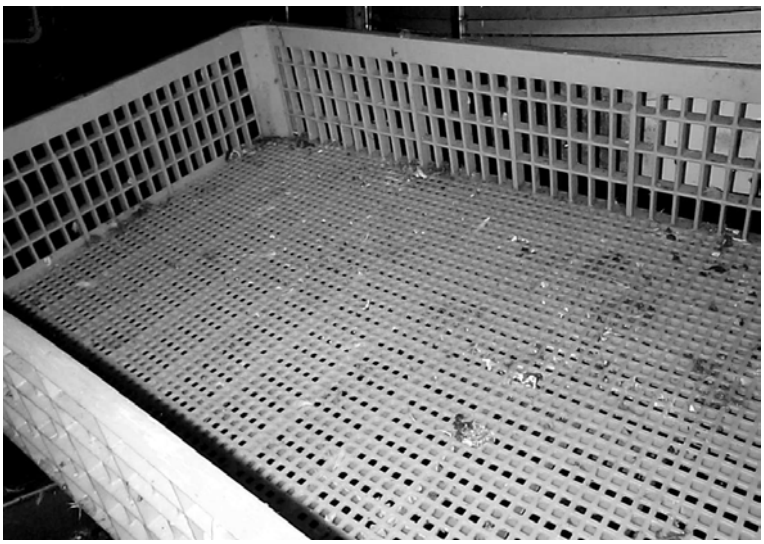


Figure 3: An example of an open floor crate prior to washing with a moderate amount of debris left by the poultry.

Crates are soiled by a mixture of faeces, uric acid and feathers from the birds. (Figure 3).

The first operation of the washing process is inversion (Figure 4) to place the crates face-down. The

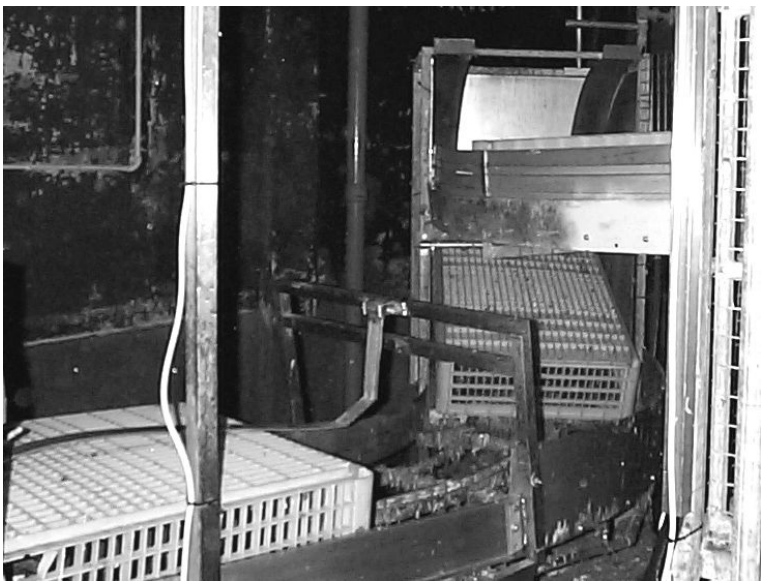


Figure 4: The inversion of the crate represents the first stage of the cleaning process. The turning process is intentionally done with some force to help knock out loose debris.

flipping process is done with some force to encourage the removal of loose debris which is collected in vessels located underneath the processing line.

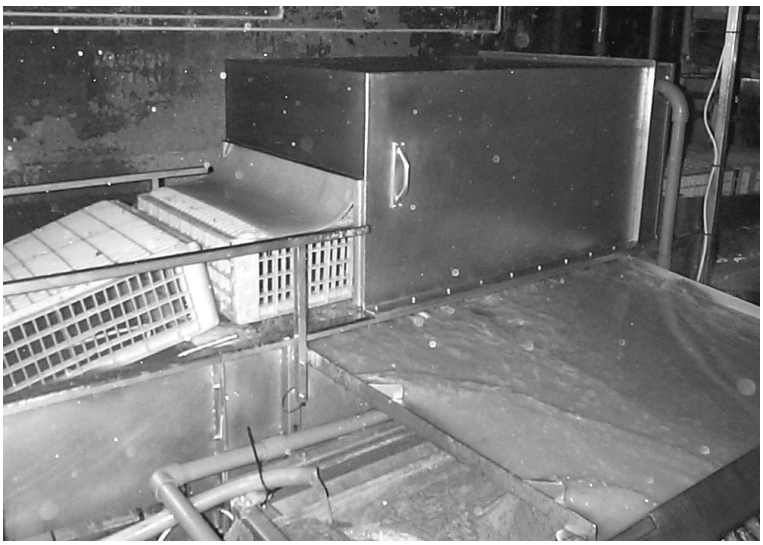


Figure 5: Pre wash booth and associated water recirculation equipment. The run down screen that filters the water is just out of view from the bottom right of the photograph. Screened water collects in the main tank from where it is recycled to the jets in the booth and to the soak tank. Make up water is added to this tank.

The pre-washing stage (Figure 5) consists of a booth where crates are sprayed with cold recycled water (often taken from the soak tank) via a series of pressure jets. It is anticipated that a large part of the remaining debris will be removed by this operation hence this water loop is often kept separate from the remainder of the washing process. Drainage water is cleaned by a run-down screen before collecting in a tank where make up water is added. There is the option of adding cleaning chemicals at this stage but they are rarely used. The operation is continuous for the duration of the shift at the end of which water is replaced and the equipment cleaned. However, the process is not "steady-state" and one might expect a fall in cleaning performance as the day progresses and debris in the wash water builds up.

2.4 The soak tank

A key part of the crate washing process is the soak tank (Figure 6) where there is the opportunity for attached debris to be softened. The actual soaking time will be determined by a combination of crate throughput (typically 5-10 per minute) and the length of the trough making up the tank (10-15 metres). Two minutes of soaking is typical but in some cases this can be longer to enhance the cleaning process. In one plant visited, there was no soak tank due to space constraints, a longer main wash booth being used instead.

There is little movement of water in the soak tank although the passage of crates does cause some circulation. In one plant, this had been addressed by the incorporation of air jets to encourage agitation and dislodge more material, but the extent of this improvement was not clear. Other plants use warm (25-50 deg.C) or even hot (60+ deg.C) water in the soak tank which can be expected to enhance the cleaning process. The use of cleaning and disinfectant chemicals added direct to the soak tank is fairly commonplace but the amounts used vary widely. Tanks are usually much deeper than necessary to submerge the crates; this feature allows for the collection of settled sludge in the tank bottom (for separate removal).

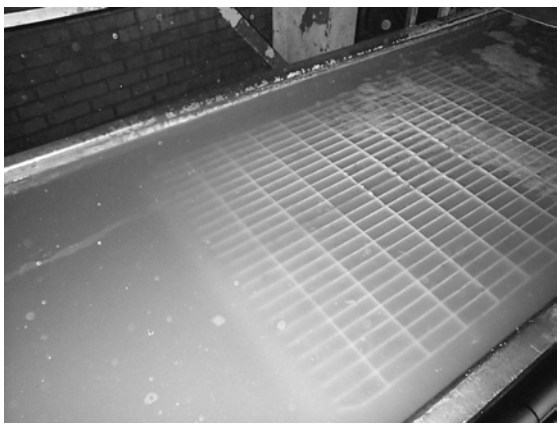


Figure 6: The main soak tank with crates passing through submerged. The length of these tanks can vary but typically they equate to 5 10 crate lengths. Plant speed will affect the actual residence time but around a minute would be usual.

Feed water is often added to the soak tank at the main wash end (where the crates leave the tank); the crates themselves carry over as much as a litre of water (or more for solid floor crates) into the main wash section. Some of this drains back into the soak tank along with additional water from the main wash; make up water is added direct to the soak tank along with chemicals. In the latter case, the feed point can be at the start of the soak tank generating a co-current flow but water velocities along the tank are very low.

2.5 Main wash and disinfectant application

The crates, having been subjected to a pre-wash and a soaking stage, receive the main wash in a second spray booth (Figure 7). This appears to be a large version of the pre-wash booth but it may also include rinse and disinfectant stages. The main wash unit is raised up (relative to the soak tank) by around a metre to (i) aid drainage and (ii) provide a drop space for the re-inversion of the crates after washing. The main water jets again deliver recycled water, at pressure, on to the various surfaces of the crates; water drains to a collection tank (via a run-down screen) which is underneath. This same vessel receives make-up water.

Figure 7: Crates entering main wash booth. The run down screen is visible in the lower part of the photograph; some fouling is evident.

In some plants, the final rinse is with hot clean water but this is not commonplace; indeed, rinsing even with clean cold water is surprisingly uncommon considering that fresh water is added to the main wash water tank. Disinfectant is applied via a series of spray jets at the end of the booth usually where the cleaned crates tumble as they revert to the 'right way up'. A wide range of disinfection systems and chemicals are in use but the process is not universal.

2.6 Re-use of water/screening

The vast majority of plants recycle wash water in order to minimise both effluent disposal costs and supply costs of fresh water. In many cases, the same water is re-used throughout a single daily shift with just make-up water being added to cover losses. The main treatment processes are (i) use of a run-down screen and (ii) the addition of chemicals. The latter is variable but screens are usually part of the plant. Aperture size is around 1mm leading to the effective removal of coarse matter but fine particles pass through, leading to cloudiness developing in the water from early in the day.

2.7 Re-version of crates and storage

The final stage of the washing process is the flipping over of the crates and their placement back into the separately cleaned modules (Figure 8). This operation can be done either by hand or automatically. There are no direct drying systems although the crates/modules may sometimes dry during transport back to the farm.

Figure 8: Washed crates stacked in steel transport frames (modules). These frames (modules) are washed separately in a batch wise fashion in large booths using sprays of recirculated water. Wash water can be seen dripping off the top surfaces of the module.

2.8 Module washers

The washing system for modules follows a semi-batch scheme with the whole operation taking place in a single booth. The module (still on a conveying system) is moved into the vacated booth. The broad principles follow that of the crate washers, with jets of cold water being directed at the frame as it is moved back and forth on the transfer system. Drained water is filtered as it flows over a run-down screen and collects in a tank where chemicals may be added along with make up water. The wash cycle is complete in 1-2 minutes and the module is ejected to receive its complement of cleaned crates. Although the cleaning cycle could incorporate both a rinse and disinfectant cycle, this is rarely the case.

2.9 Washing fixed drawer units

Fixed draw systems such as manufactured by Stork b.v. present some unique features in the washing operation. The Stork system uses a series of discreet stages: (i) pre-wash, (ii) soaking, (iii) main wash and (iv) disinfection. The pre-wash and main washes are carried out in booths in which water jets mounted on movable carriages are passed back and forth over the assembly which is tilted 5-10 degrees off horizontal to aid drainage. The pre-wash (and often the main wash as well) typically recycles water via a screen as is done in the Anglia Autoflow system. What is described as a "soaking stage" amounts to retention of the assembly for a period of time whilst queuing for the main wash; there is no immersion at any stage. Following the main wash there are the options of a rinse cycle (with clean water) and disinfection.

Many of the issues of the washing operation are similar to those of washing the empty modules in

the booths of the Anglia Autoflow system (*e.g.* use of hot water, chemicals and drainage/drying). However, the longer distances that the jets must reach and difficulty in accessing some of the internal surfaces will always put the fixed drawer system at a disadvantage with respect to cleaning. It is important to make clear that this does not mean that the fixed drawer system will necessarily result in a poorer wash; a great deal will depend on the individual washing plant and the discipline of the company that operates it. Rather, the overall message is that in the event of poor design or practice with fixed drawer systems, the consequences of a poor wash will be much more apparent.

3. THE EFFECTIVENESS OF THE PROCESS

3.1 Introductory note

This section highlights many of the difficulties and shortcomings of the crate washing process. It must be stated though that in some cases the problems were confined to only one or two crate washing plants out of a dozen or so seen. In other cases, there had been modifications by factory engineers which deviated from the original design of the manufacturer but which may still represent an improvement. Finally, the constraints of the plant operation in some cases led to some crate washing systems not being operated as per manufacturer's instructions. However, some issues relating to poor performance are more common throughout the industry and these may indicate areas where design and/or operation improvements could be implemented. Where the nature of the issues highlighted below is not common throughout the industry, this has been made clear.

3.2 Collection of debris from crate inversion and from the run-down screens

One difficulty in the lairage area (where crates are cleaned) is keeping the general area clean and, especially, keeping it dry. Inevitably, even the proper use of jet washers will (i) generate aerosols and (ii) maintain a wet environment. The latter can work against the cleaning process by spreading debris around and generating an environment which can allow microbes to multiply. Unless there is large scale use of disinfectant on floor areas, a minimum use of cleaning water is advisable. This can be achieved simply by the adequate collection of debris knocked out from the crates at the beginning of the washing process. In reality, most plants lack this facility and some (or all) of the debris falls onto the floor.

Floor contamination also commonly occurs from the run-down screens. In this case, it is often an operational problem with the screens being cleaned insufficiently resulting in a large volume of overflow (with the removed debris) cascading onto the floor. Cleaning the screens can add to the problem as this often involves the use of jet washers which are sometimes part of the process plant itself: the inevitable result is more water and debris driven onto the floor. Improvements would be made by better containment of wash water and the better (easier) removal of the screened debris. In a few cases, the level of water in the tanks seemed unnecessarily high leading to inevitable spillages.

3.3 The benefit of pre-wash

It is not contested that the pre-wash operation makes some contribution to the washing process; the pre-wash does remove some debris (which would otherwise add to the organic load in the soak tank.) Rather, it is suggested that the benefit is relatively small and it is not the best use of finite space and resources. The crates are normally dry prior to this operation and dwell time is only 10-20 seconds, thereby not allowing full benefit of the process to be achieved. However, relocation of this stage to a place just after the soak tank would mean that the jets would then be working on

softened debris. There may also be the option of combining the water loops of the main and pre-wash using a counter-current operation: *i.e.* the wash water from the main wash would be used in the pre-wash then into the soak tank. This last step could be an important consideration as a settling stage is necessary to deal with the build up of fine material in the water: this clarification is only provided in the large soak tank.

Clarification of the wash water (or limitation on the level of suspended solids that can be tolerated) is an important consideration as jet blockage is a common problem of all plants. The jets in the module wash were particularly vulnerable but cleaning of those in the pre-wash (and the main wash) was a common requirement. In some cases, jet cleaning could be required daily. The concern here is that prior to attention, the washing process would be compromised by the loss of water flow through one or more jets. As it is generally difficult to see if the water jets are blocked, a better strategy may be routine cleaning combined with measures to limit the level of debris in the wash water. As a general point on equipment design, the easier the jets are to be accessed and cleaned the more likely it is that this will be done.

3.4 The soaking stage

Soak tanks come as two types: standard and double-length. No soak tank was present at one plant where space was very limited. The value of soaking comes from both the opportunity to settle out fine material in the lower part of the sump and in providing some loosening of dirt in preparation for the main wash. One might expect such benefits but it is not clear how much extra cleaning increased soaking really brings and whether a double-length soak tank is much better than the more common 5-6 metre tank. However, if space is not a problem, then the running cost of any soak tank is relatively small and its inclusion would seem justified.

The use of chemicals in the crate soaking stage is variable, being a common practice in some factories and absent elsewhere. The effectiveness of such a measure is apparent from the observation that crates are noticeably much cleaner after washing at the beginning of the shift than at the end. This might be expected, as the water becomes progressively more turbid as it accumulates more crate debris, at the same time any added detergent (or disinfectant) would also be neutralised more quickly. Of course, this effect is also apparent if no chemicals are used - the cleaning will always be best at the beginning of a shift and deteriorate until the soak tank water is changed. In the absence of chemicals, one might expect the initial cleaning to be poorer and the rate of deterioration to be quicker. However, it remains a central point that any benefit from the use of cleaning and/or disinfectant chemicals in the soak stage will be progressively lost as the water becomes progressively contaminated.

The main problem with the soak tank is that it enables easy transfer of bacteria from one crate to the many that follow. The environment can also be very conducive to microbial growth (especially with temperatures in the range 20 to 40 deg.C) thus accentuating this effect. There is also the absence of any counter-current water flow which could potentially "sweep" contaminated water away from the washed crates. Producing strong water currents in the soak tank is not an attractive

option though as they would require either a large consumption of fresh water or recycling of the wash water via a sterilizing unit.

3.5 Hot, warm or cold water?

With very few exceptions, crates and modules are washed with cold water, which in the winter can be below 10°C. In a few cases, a final rinse (after the main wash) is done with warm (25-50°C) or hot (over 60°C) water. Warm water in the soak tank has also been tried. The problem with warm water is that it can serve to encourage the growth of bacterial cultures in a medium which is already loaded with degradable organic matter. Any application of heat can be expected to drive off any remaining oxygen favoring the survival of certain bacteria, such as *Camplylobacter* which prefer a low oxygen enviroment.

The real benefit of heat application only becomes apparent when temperatures exceed 60°C. At this temperature there is a large reduction of microbe activity; numbers rapidly diminish at 70°C and above. Higher temperatures do bring their own set of problems especially in winter with large amounts of fog being generated, equating to energy losses unless a large amount of containment and insulation is used. There are also issues of safety to personnel nearby. In addition it is noted that higher water temperatures, even as high as 90°C, are no guarantee of a sterile crate unless all surfaces are warmed to the same level - dead zones and various corners can remain at a lower temperature where microbes can survive to some extent depending on exposure time.

The main benefit of higher temperatures is better washing, which is likely to be true at each stage including soaking and main washing. Most detergents and disinfectants work better at increased temperatures although a few disinfectants may lose potency as the result of thermal degradation. Overall, it is by no means clear if higher temperatures and the implied costs are necessary if the washing system is adequate in other respects. However, if used, some containment and extraction system may be necessary to minimise the dispersion of fog, itself a possible mechanism of transferring bacteria around the lairage area and potentially onto the clean crates.

Figure 9: Crates leaving the soak tank to enter the main wash module (bottom corner only visible in top left of photograph). Water carried over by the crates and the drainage water from the wash booth falls back into the main water tank via a run down screen. The ball cock controlling the make up water is visible in the centre of the photograph.

3.6 The importance of rinsing and drainage

Rinsing with clean water is surprisingly uncommon in crate washing systems. The typical crate can carry a litre or more of

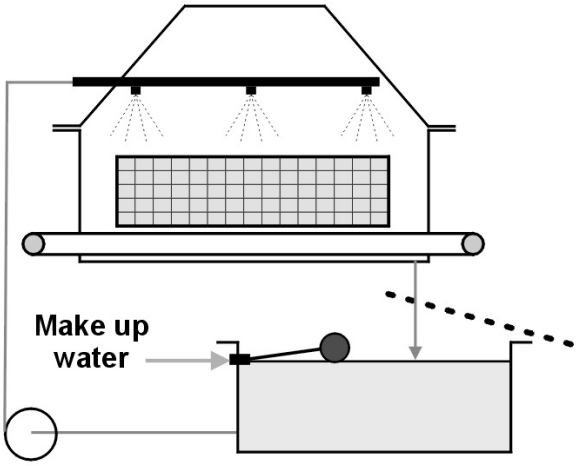


Figure 10: Scheme of operation of main washing booth. Recycled water is drawn from a holding tank and returns via a run-down screen. Make up water may be potable but this brings no benefit as it is added directly to the used water.

contaminated wash water out with it; rinsing with fresh water would be a simple way of reducing this effect. This is especially important as there seems to be no clear mechanism for encouraging drainage other than what may occur in the module on its way back to the farm. Once a crate has been replaced into the module, there is then the possibility of drained water dripping on the lower crates. The absence of rinsing and drainage has a further negative effect on any disinfection stage that may follow, as the chemical is more likely to be diluted and neutralised by the large amounts of contaminated wash water still on the crate.

Make up water is added directly to the main holding tank (Figures 9 and 10). Because of this, it matters little whether it is potable or not as any benefit from clean water is immediately negated due to the high level of solids in the holding tank. Although the flow of make up water is low (relative to the recirculation rate) the opportunity is still present to introduce it to the system via a final row of jets in the main wash and so make best use of it to rinse off washwater.

3.7 Disinfectant application

As with the use of disinfectant in the soak tank, there is a wide range of practice followed for the final application of disinfectant to the cleaned crate. There is the general assumption within the industry that the mere application of a chemical implies that disinfection has been done, although there may be no reduction in the numbers of microbes (see section 4). Such ineffective treatment may be the result of:

1. Poor washing of the crate, leaving a high organic load in the form of debris specks and biofilm layers;
2. Poor drainage of the crate, leaving large amounts of entrained water that effectively dilutes the chemical;
3. Inadequate volumes of chemical used;
4. Inappropriate chemical used or of the incorrect strength;
5. Poor application of the spray; resulting in limited covering of the crate surface;
6. Poor location of the spray resulting in intermittent application.

The last two issues are illustrated in Figure 11. This shows a poor spray (large droplets) located in a poor position at the re-inversion point for the crates.

The effect of this is (i) the fast moving (falling) crates are in contact with the spray for the briefest of moments and (ii) most of the spray is wasted, falling onto the floor. In addition, it is difficult to see how all of the crate surface could be coated by the system even if the crates were moving more slowly. Relocation of the spray bar is required in this case along with the choice of better nozzles.

Figure 11: The continuous application of disinfectant in this example is clearly wasteful as well as being inadequate from a very poor jet. However, this is typical of many units but it still adds to the awarded HAS score. Some crate wash facilities have no disinfection system.

4. THE USE OF CHEMICALS

4.1 Introduction

Heat and chemical disinfection are the two main methods used for decontamination of contaminated surfaces. Properly applied heat, (*i.e.* a sufficiently high temperature applied for a suitable contact time) will always be the more robust of these methods as heat penetration can be reliably predicted, assured and monitored. However, heat application may not be cost-effective for large surfaces such as buildings or high throughput operations such as slaughter lines and current poultry transport crate wash systems. Chemical disinfectants might provide some residual protection against re-contamination by settled organisms which have been aerosolised during the prior cleaning processes. It is important to consider the purpose of the decontamination treatment. In some circumstances, *e.g.* surgical implants, absolute sterility must be guaranteed. In other situations the requirement is simply to either eliminate defined target organisms or to reduce them to a level at which they would not be considered a significant risk. This is the situation in poultry houses (and on transport crates) where a level of disinfection capable of eliminating 10^5 *Salmonella* or *Campylobacter* per cm^2 surface area will still leave total viable counts of other organisms of 10^4-10^6 cm^2 (Davies *et al*. 1998). This might be a desirable situation since these other organisms tend to be better environmental survivors than pathogens so would provide some competitive antibacterial effect which contributes to the final reduction of pathogens and provides a partial barrier against establishment of pathogens involved in recontamination.

4.2 Definitions relating to 'Disinfection'

A great deal of misunderstanding with the use of disinfectants stems from different interpretation of seemingly common words. The following scientific terms reported by Block (1983) are commonly accepted and are used in this report:

Disinfectant	an agent that frees inanimate objects from infection; usually a chemical agent that destroys disease germs or other harmful micro-organisms or inactivates viruses.
Antiseptic	as above but refers to an agent intended to be applied to living tissue.
Germicide	an agent that destroys micro-organisms, especially pathogens and including fungi, viruses and non-bacterial micro-organisms.
Bactericide	an agent that kills bacteria.
Fungicide	an agent that kills fungi.
Virucide	an agent that destroys or inactivates viruses.
Sporicide	an agent which is capable of inactivating microbial spores, which are more resistant than vegetative cells. A sporicide would be capable of full chemical sterilisation.
Biocide	a substance that kills all living organisms, both pathogenic and non-pathogenic.

Bacteriostat an agent that prevents growth of bacteria but does not necessarily kill them.
Antibiotic an organic chemical substance produced by micro-organisms that has the capacity, in dilute solutions, to destroy or inhibit the growth of bacteria. (Antimicrobial agent: same but also includes synthetic agents).
Sterilization the act or process, physical or chemical, that destroys or eliminates all forms of life, especially micro-organisms.
Sanitiser an agent that reduces the number of bacterial contaminates to safe levels as judged by public health requirements.

4.3 The main types of disinfectant products

Types of Disinfectant; their pro's and con's
Most disinfectants are commercial blends of various disinfectant groups, acids, detergents, etc. The main types are summarised in Table 4.1 (below).

How Disinfectants Work
The various disinfectants act on bacterial cells in different ways: these are summarised in Table 4.2 (below). Organisms must maintain a positive osmotic pressure by the activity of the cell wall and cytoplasmic membrane. Many products interact with elements of the cell wall and membranes to degrade their integrity or functionality. Other disinfectants may interfere with the activity of the cytoplasmic or nuclear mechanisms involved in DNA or protein synthesis or energy metabolism. Often disinfectants will have more than one action but the primary action of major disinfectants is listed below (OIE 1995):

Acidic and Alkaline Compounds: H+ ions destroy bonds in nucleic acids, disrupt cytoplasmic pH and coagulate proteins. OH- ions saponify cell membrane lipids. pH > 10.0 disrupts peptidoglycan cell wall.

Chlorine based Products: electronegativity leads to oxidation of peptide links and protein denaturation. Dissolution in water produces hypochloric acid which decomposes into free chlorine and oxygen, thereby oxidising thiol groups.

Table 4.1: common disinfectants and the main features.

Disinfectant Group	Advantages	Disadvantages
Iodine based (Iodophor)	low toxicity and pH, acids reduce limescale so good for disinfecting water lines, broad spectrum.	corrosive, staining, food taint by vapour, poor organic soil tolerance, lack of residual activity.
Chlorine based	fast action, broad spectrum, low toxicity, low cost.	corrosive, bleaching, pungent, pH dependant, poor organic soil tolerance, volatile.

Amphoteric Compounds	pleasant to use.	poor efficiency, poor organic load tolerance.
Peracetic acid/hydrogen peroxide compounds	non fuming, no odour, non staining, fast action, biodegradable.	poor-moderate organic soil tolerance, corrosive at effective concentrations.
Phenolics	broad spectrum, good soil tolerance.	smell, taint, pH dependent, poor biodegradability, poor solubility.
Quaternary Ammonium Compounds (QAC)	broad spectrum, odourless, colourless, good residual activity, non toxic, non corrosive, biodegradable.	poor-moderate organic soil tolerance, formulation dependent.
Chlorhexidine	pleasant to use.	poor organic soil tolerance.
Cresylic Acids	good organic soil tolerance.	narrow spectrum, odour.
Cresylic Acid/Phenolic Combinations	proper formulations, good organic soil tolerance.	toxic, odour.
Glutaraldehyde	less volatile than formaldehyde.	only active at high concentrations, very toxic, respiratory sensitiser.
Glutaraldehyde/QAC combinations	highly effective, more user-friendly than formaldehyde.	very toxic
Formaldehyde	excellent organic soil penetration, sporicidal, cheap, readily biodegradable	slow action, very toxic
Formaldehyde/Glutaraldehyde/ QAC combinations	highly effective, more user-friendly than formaldehyde	very toxic
Sodium Hydroxide	very effective at high concentrations, cheap.	very corrosive.

Quaternary Ammonium Compounds: these bind to membrane phospholipids and proteins and so impair permeability.

Amphoteric Compounds: these integrate with and puncture the cell wall.

Phenolic Compounds: cell membrane enzyme inactivation.

Peracetic acid/Hydrogen Peroxide Combinations: these denature proteins and lipids leading to membrane damage.

Iodine Compounds: interference with electron transfer mechanisms of respiratory chain and binding of some cytoplasmic membrane proteins.

Ozone: oxidation damage to proteins.

Table 4.2: Disinfection Mechanisms: numbers indicating activity, which appear in several columns for a given compound, demonstrate the multiple actions for the compound concerned. This activity is nearly always concentration dependent, and the number indicates the order of concentration at which the effect is elicited, *i.e*. 1, elicited at low concentrations: 3, elicited at high concentrations. When a number appears in only one target column, this is the only known site of action of the agent. (W.B. Hugo; 1992).

Cellular target for non-antibiotic antibacterial agents	**Non-antibiotic antimicrobial agents**																		
	Acridine dyes	Alcohols	Anilides (TCS, TCC)	Bronopol	Chlorhexidine	Copper (II) salts	Ethylene oxide	Formaldehyde	Glutaraldehyde	Hexachlorophane	Hydrogen peroxide	Hypochlorites, chlorine releasers	Iodine	Mercury (II) Salts, organic mercurials	Phenole	â-Propiolactone	Quaternary ammonium compounds	Silver (I) salts	Sulphur dioxide, sulphites
Cell wall								1				1		1	1				
Cytoplasmic membrane																			
Action on membrane potentials			1							1					1				
Action on membrane enzymes																			
Electron transport chain										1									
Adenosine triphosphatase					1	1	1												
Enzymes with thiol groups				1			1		1		1	1	1	1		1		1	1
Action on general membrane permeability		1	1		1										2		1		
Cytoplasm																			
General coagulation					3	3			2	3				3	3		3	3	
Ribosomes											1			1					
Nucleic acids	1																		
Thiol groups				1		1	1		1		1	1	1	1		1		1	
Amino groups							1	2	1			1				1			1
Highly reactive compounds							1		1			1				1			

Formaldehyde: denaturation of proteins and nucleic acids by alkylation.

Glutaraldehyde: similar to formaldehyde but activity more dependent on ideal physio-chemical conditions.

Biguanides: cytoplasmic membrane damage.

The disinfection mechanisms are summarized in Table 4.2. In practice most commercial disinfectant products are blends of various different varieties of active chemical group combined with surfactants, stabilisers, pH regulators, etc. It is therefore not normally possible to accurately assess the likely activity of a disinfectant product just by looking at its declared chemical composition. Even when a full breakdown of the exact ingredients is provided it is still difficult to critically evaluate the product, although products based on certain chemical groups are more likely than others to be adversely affected by organic matter or bioflims (Carpentier & Cerf 1993).

4.4 Approval of Disinfectants

In the UK disinfectants for agricultural use are normally submitted for Defra approval. The test for action against general bacteria involves exposure of a suspension of *Salmonella choleraesuis* together with a yeast carrier to disinfectant at 4°C for 30 minutes. Products which produce a 99.99% reduction in *Salmonella* at the concentration nominated by the manufacturer are deemed to have passed the test and that concentration then becomes the *Defra General Orders approved* rate for use in cases of general bacterial contamination. Some products are also approved for use against *Mycobacterium bovis* at a higher concentration or contagious viruses such as Foot and Mouth Disease and Newcastle Disease at lower concentrations. Although these tests are useful the results are sometimes misleading and many products which perform poorly in the presence of proteinacious or fatty organic residues pass the test at relatively low concentrations (Bloomfield *et al.* 1991). Conversely highly effective disinfectants such as formaldehyde do not perform well in the test because of their slow initial activity.

There is currently no test which adequately simulates the difficulties of disinfection of damaged and porous livestock containment surfaces, with the inevitable biofilms and residual faecal matter, although reasonable simulations have been developed at VLA (Defra Projects OZ0122 *Salmonella* contamination: cleansing and disinfection studies; OZ0134 Epidemiological studies of multiresistant *Salmonella typhimurium* in pigs). In these models only formaldehyde based products and phenolic products, particularly synthetic biphenyls, worked adequately at Defra recommended rates and this has been confirmed by numerous field studies carried out by VLA and laboratory studies by others (Davies & Wray 1995, Berchieri & Barrow 1996, Davison *et al.* 1996). Peroxygen products, chlorines, iodine based products and quaternary ammonium compounds (QAC) were frequently ineffective. Although such models are much more realistic tests of disinfectants they do involve biological variability so cannot easily be standardised between laboratories. It is therefore necessary to carry out comparisons of the products at the same time and using the same material, which is not suitable for a disinfectant approval procedure. More work is needed to design, evaluate

and validate a robust and reproducible surface disinfection model. In such models, still provide less rigorous conditions for disinfection than the field situation (Taylor *et al.,* 1999), it would also be preferable to aim for total elimination of a realistic level of the target bacteria rather than a 99.99% reduction. This would also simplify the test and allow more replicate tests for the same cost.

4.5 Disinfection of poultry transport crates

Problems of disinfection of crates, crate modules and transport vehicles are universal (Rigby *et al.,* 1980, Carr *et al.* 1999). Experimental simulations have suggested that certain disinfectants or combinations of moderate heat and disinfectants could be effective (El-Assaad *et al.,* 1993, Ramesh *et al.,* 2002) but these studies are highly artificial and cannot be substantiated in real high throughput commercial operations (Carr *et al.,* 1999). It is therefore necessary to develop a new approach, which cannot be done without spending additional money on the crate washing process. It will be necessary to improve the thermal efficiency of any heating stages using modern heat recovery techniques. It is also essential to accurately monitor and maintain suitable effective disinfectant levels throughout the working day and to design systems for efficient removal of organic matter from recycled soak and wash water.

Given that a disinfectant formulation is suitable for the target organism in its expected location, then the next requirement is to ensure that the application rate, concentration, contact time and temperature are appropriate. It is desirable that all surfaces are saturated with disinfectant, this is most effectively done by the total immersion in disinfectant (dipping) this system is in place for crates in some poultry processing plants in Denmark. Disinfectant can also be effectively applied to crates by high pressure spray. This system is used for hatcher and chick delivery baskets in hatcheries and works well when the right products are used (Davies *et al.* 2001). These crates are smaller and more simple than broiler transport crates, either with no lids or totally removable lids. Cleaning and disinfection of the interior of these crates is easier than with the larger broiler crates, where some of the surfaces are not readily accessed by the spray. This may be overcome by using high concentration disinfectant at high pressure in a spray booth. This would facilitate distribution of spray by rebound and aerosolisation, but would still be inferior to dipping. The use of adequate volumes of disinfectant would inevitably create a large amount of run-off which should ideally be re-used, after filtration of any entrained organic matter. The same applies to drippage of excess disinfectant from crates which have been immersed or dipped. This could be achieved by including a drippage channel which slopes back towards the dip tank or spray disinfectant reservoir.

The most important aspect of successful disinfection is to apply sufficient concentration for long enough to achieve the desired result (Assanta & Roy 2001). This depends on circumstances: for example, very low concentrations of chlorine (or oxygenating agents) can control bacterial contamination of clean water in mains supplies or swimming pools, but much higher concentrations would be needed to restrict the growth of bacteria in biofilms (Hood & Zottola 1995) and higher levels still to attempt elimination of bacterial biofilms. When organic matter is present even higher concentrations are needed (Ruano *et al.* 2001) and this requirement increases still further if there are large populations of organisms in the water which is being used to dilute the disinfectants

(Davison *et al*. 1996). All these constraints apply to poultry transport crate washing as currently carried out. Increasing the concentrations of disinfectants often becomes problematic because of corrosiveness, noxious vapours and problems of disposal.

In the FSA Project ZB0033 (Humphrey and Allen, 2002) it was suggested that 10 of 15 companies with soak tanks used either QAC or caustic detergents and two companies without such tanks incorporated a caustic detergent or peroxygen disinfectant in wash water. 13 companies had disinfectant spray bars for application of disinfectant to washed crates but these were not always used. Disinfectants used were QACs at a relatively low concentration or peroxygens at variable concentrations. These regimes would not be expected to provide realistic disinfection so would need to be upgraded. Application of disinfectants or detergents in soak water may enhance the removal of adherent faecal matter but would not significantly improve removal of biofilms (Gibson *et al.* 1999). Addition of disinfectants such as hypochlorites may however limit the attachment of newly deposited cells (Rossoni & Gaylarde 2000) and reduce multiplication of organisms in the wash water and cross-contamination. Disinfection of crates is considerably complicated by the fact that the most effective disinfectants such as phenols or formaldehyde cannot be used in a food processing situation.

Current work at the VLA, which is studying the effectiveness of disinfection of cages in laying flocks, suggests that washing is counterproductive unless there is adequate subsequent drying and disinfection is carried out to a very high standard. Better results have been achieved by dry cleaning followed by disinfection and this approach should also be considered for poultry transport crates. This approach should be particularly effective for *Camplyobacter* which is intolerant of dry conditions. If this was done, then crates may be more thoroughly cleaned on a rolling basis (rather than after every batch), and more time could be directed at cleaning and thoroughly disinfecting modules - which could use more effective chemicals if carried out in a separate area to the crates.

Use of disinfectants on transport crates

In the short term at least, the only measure that is sure to reduce pathogen numbers on crates that is both practical and effective is likely to be by the application of an oxidizing disinfectant (*e.g*. hydrogen peroxide - perchloric acid blend). Crates need to have been cleaned and partially dried before immersion into a dunk tank containing the disinfectant at a concentration of three to five times that given by the Defra General Order. The negative effect of the presence of excess water (causing dilution of the disinfectant) is so pronounced, that one might even consider dry cleaning to enable higher efficiencies.

5. MICROBIOLOGICAL ASPECTS

The following information is based on previous studies and preliminary investigations undertaken as part of the present project.

5.1 Assessment of visual crate cleanliness

There appeared to be no significant difference between visually clean crates and those with biofilms (old firmly adhering visible faecal matter) in the numbers of APC (plate aerobic counts) and Enterobacteriaceae recovered when swabbing areas free from built up debris. Therefore crates were scored in terms of grams of debris estimated visually. Three scores were given per crate: these were the total amount of debris in grams on three sites, 1) total surfaces inside the crate, 2) the outside walls of the crate and 3) the outside base. As expected, there was a wide variation in the scores of unwashed crates although this diminished after washing especially on the outside of the crates (Table 5.1). In Company C, due to the high degree of automation, it was not possible to examine the crates from the line after the birds were removed but only after the pre-wash stage.

Table 5.1: Range of scores on transport crates with median value in parenthesis (n=12)

Company	Processing stage	Site 1	Site 2	Site 3
A	Untreated	5-10 [7]	3-16 [6]	1-5 [2]
B	Untreated	2-20 [4]	0-10 [3.5]	0-10 [2]
C	Pre-washed	2-10 [4]	1-4 [2]	1-3 [1]
A	Final wash	0-2 [0.5]	0-2 [1]	0-2 [0]
B	Final wash	0-8 [4.5]	0-2 [0]	0-1 [0]
C	Final wash	0-2 [1]	0-3 [0]	0-2 [0]

5.2 Assessment of crate microbiological load

The microbiological load on the inside base of the crates was assessed since this surface posed the greatest risk of contamination onto the birds. The base was sampled using four large dry cotton wool swabs (code MW104J, Medical Wire, Corsham), each wiping a quarter of the base. These were placed together into 10ml Maximum Recovery Diluent (MRD Oxoid CM733), transported at 1°C, and examined in the laboratory within 4 hours. Decimal dilutions were made in MRD from the samples following vortexing. These were plated onto Plate Count agar (PCA Oxoid CM325) incubated aerobically at 30°C/48h, Violet Red Bile Glucose Agar (VRBG Oxoid CM485) incubated at 37°C/24h and mCCDA (Modified Charcoal Cefoperazone Deoxycholate Agar, Oxoid CM739+SR155) incubated at 37°C/ 48h in a microaerobic atmosphere.

Table 5.2: Microbial numbers (mean $\log_{10}$ cfu/ base + StDev) recovered from the inside base of crates (n=12)

Company	Processing stage	APC	Enterobacteriaceae	*Campylobacter*
A	Untreated	7.80 0.37	6.87 1.02	6.91 0.85
B	Untreated	7.90 0.73	7.56 0.72	5.60*
C	Pre-wash (post)	8.06 0.36	7.07 0.72	4.11 ± 0.16
A	Final wash (post)	7.57 0.37	6.06 0.34	5.66 0.01
B	Final wash (post)	7.93 ± 0.52	7.35 ± 0.62	2.93 ± 0.86
C	Final wash (post)	7.73 ± 0.33	5.96 ± 0.22	5.34 0.06

* 1 crate sampled only

In Company B, 12 crates were examined after being held in the lairage for 12h after the final wash. The numbers of APC, Enterobacteriaceae and *Campylobacter* appeared to be very consistent as shown in Table 5.3 below.

Table 5.3: Microbial numbers (mean $\log_{10}$ cfu/ base + StDev) recovered from the inside base of crates following 12h storage (n=8)

Company	Processing stage	APC	Enterobacteriaceae	*Campylobacter*
B	Stored after wash	7.82 ± 0.64	6.79 0.92	3.97 0.51

In Company B low foaming alkaline detergent was added to the soak tank. This concentration was increased approximately 10-fold to monitor the effect. The numbers of Enterobacteriaceae were reduced from several logs to below detectable numbers (<$\log_{10}$ cfu/base 2.70); the numbers of APC on the crates were reduced from $\log_{10}$ cfu/base 8.58 0.5 to 5.96 0.3 (close to a 4 $\log_{10}$ reduction). However, using this level of detergent would have cost and staff safety issues that need to be resolved. However, hot alkaline washes have been used for cleaning eggs - one might thus expect that such technology could be transferred to crates if desired.

The use of *Enterobacteriaceae* counts as an indication of the effectiveness in cleaning poultry transport crates is discussed further in a brief paper given in Appendix 2.

5.3 Numbers and types of microbes in recycled water and soak tank water

Some abattoirs use 'white' water, from offal flumes for the prewash and crate-soak stages. There is little information about the numbers and types of microbes present in this water prior to its use on the crates, but unpublished results during MAFF project FS3301 showed *Salmonella* sometimes present, and numbers of coliforms per ml in the region 10^4 to 10^5. The microbiological quality of the water taken from the soak tank was similar - whether white water or good quality bore-hole water was used. This is not surprising, since most of the dirt rinsed off the crates is faecal matter. However, the results of *Salmonella* examination during project FS3301 did show that crates soaked in white water tended to be contaminated more frequently with *Salmonella* than crates soaked in

bore hole water.

In the present study the microbial numbers recovered from soak tank water in Company B for APC, ranged from $\log_{10}$cfu/ml 6.58 to 4.00 depending on the concentration of detergent while the numbers of Enterobacteriaceae were below detectable numbers (<$\log_{10}$ cfu/ml 1.70). On the other hand, for Companies A and C, these levels were considerably higher as set out in Table 5.4 below:

Table 5.4: Microbial numbers (mean $\log_{10}$ cfu/ base + StDev) recovered from soak tank water.

Company	Processing stage	APC	Enterobacteriaceae	*Campylobacter*
A	Soak tank	7.47 0.1	8.64 0.5	5.64 0.2
B	Soak tank	4.0 to 6.6*	< 1.70	-
C	Soak tank	6.72 0.1	6.96 0.8	5.34 0.1

* depending on the level of detergent used.

However, this difference in numbers appeared to have little impact on the microbial load of the washed crates. Slader *et al*. (2002) also found that although increased detergent (QAC) level reduced *Campylobacter* numbers in crate wash water below detectable levels, it did not eliminate the organisms from the crates. The authors found that immersion of crates in 100ppm hypochlorite or 10% QAC was more effective in eliminating *Campylobacter* than *spraying* with 10% QAC or immersion in 0.25% peracetic acid. It is noted though that with respect to peracetic acid, the concentration used may be too low to be effective.

5.4 Crate to crate cross-contamination

Soaking large numbers of crates in the same bath of water without subsequent proper disinfection, or including a disinfectant in the soak tank water, is likely to result in microbes being washed off some crates and distributed onto others. Evidence of this happening can be found in several published papers, which found increased proportions of crates contaminated with *Salmonella* or *Campylobacter* after cleaning (Rigby *et al*., 1980a; McKenna *et al*., 2001; Corry *et al*., 2002; Slader *et al*., 2002).

5.5 Crate to farm cross-contamination

There is circumstantial evidence for this. Unpublished data gathered during FSA project no. FS3306, showed that crates were contaminated with *Campylobacter* when birds were put into them on the farm. In addition, on many occasions, when flocks were harvested in stages ('thinned') the flocks were negative for *Campylobacter* prior to thinning, but the remainder of the flock became positive a few days after thinning. Similar results have been reported from Denmark (Hald *et al*., 2001). Other possible sources of *Campylobacter* contamination during thinning include the human catchers, the crate modules and the vehicles that move the modules full of crates.

5.6 Do contaminated crates infect birds during transportation to the abattoir?

Information on this is limited. A study by Slader *et al*., (2002) found that birds from uninfected flocks were contaminated with *Campylobacter*s on their feathers during catching on the farm, and sometimes from the transport crates. They found no evidence for colonisation of the caeca, which is not surprising since the birds were in the crates for only 2 hours. An investigation by Jacobs-Reitsma and Bolder (1998) of the presence/absence of *Campylobacter* in various parts of the intestines of uninfected birds left for 4 hours in crates heavily contaminated with *Campylobacter*, found positive results in 8/10 (oesophagus), 1/10 (duodenum), 2/10 (ileum), 1/20 (caecum). It seems likely, therefore that the longer the transport plus lairage time, the more likely that the intestines of the birds will be colonised by *Campylobacter* from contaminated crates, and the further down the tract they will be found. Numbers per gram of intestinal contents are also likely to increase with time.

5.7 Targets of acceptable cleanliness

The first aim should be to achieve visibly clean crates, since microbes in solid faecal matter would be extremely difficult to inactivate. Preliminary results of microbiological examination of parts of crates free of visible contamination show that they often carry total colony-forming units exceeding 10^7 per cm^2, while numbers of Enterobacteriaceae are often about one order of magnitude lower, and numbers of *Campylobacter* at least two log cycles lower than Enterobacteriaceae. It is not practical to expect to produce sterile crates, but treatment that reduces numbers of Enterobacteriaceae by 4-5 log cycles (i.e. down to 10^2 per cm^2) should result in extremely low levels of *Campylobacter* and *Salmonella*, the two pathogens of most concern.

6. IMPROVEMENT OPTIONS TO EXISTING EQUIPMENT

6.1 Improved housekeeping

A series of general improvements that can be classified "good housekeeping" is readily achievable to some degree by all processors. Adequate available space is a pre-requisite for measures based on better separation of operations, but even in relatively cramped conditions there is the opportunity to tidy up the operation to reduce the microbial hazard. These measures include:

- *Avoidance of spillages*: the floor should be kept as clean as practical and also as dry as possible. This is because water is a transfer medium and in the use of jet washers any debris will have the chance of being caught up in aerosols that can spread throughout the lairage area and potentially contaminate clean crates. The less spillage, the less the need for cleaning and the less water on the floor. All overflow wash water should be contained as far as possible and channelled direct to drain.
- *Collection of loose debris*: solid debris and effluent should not be allowed to fall on the floor on the basis that it can be washed away to drain; better to collect it at the point of source in a suitable container.
- *Good access*: it is easier to keep equipment clean if there is good access at least in places where debris can accumulate. Good separation of the various operations in the general area will reduce the risk of contamination; clean crates especially should be kept well away from washing and lairage activities.

6.2 Relocation of pre-washer to add an extra rinsing stage after the main wash

Space is often at a premium at the crate-washing end of poultry processing hence solutions that require more space are unlikely to be attractive. The relatively limited value of the pre-wash stage thus leads to the question, "would it be better relocated to the main wash end"? Hence, the water jets would be aimed at debris already softened by the soaking action. No extra space and limited extra equipment is implied although this change is more readily implemented in new installations. An extension to the main wash unit in any way then opens the possibility of two or more stages of cleaning with dirty water being used for the early stages and cleaner water for the later stages. A final rinse using the clean water (originally intended for make up) is a further possibility. (See also 6.9 below).

6.3 Changes to the design and operation of the soak tank

In some ways, the soak tank is the heart of the cleaning process but the benefit of submerging a crate for 1-2 minutes in contaminated water can be small without changes. Possible changes (some of which have been explored by some companies) include:

- *Agitation by use of air jets*: although not vigorous, the circulation this causes can be expected to make some improvements to cleaning and the aeration may remove some of the organic load by stimulating aerobic microbial activity. If aeration is carried out in a controlled way (*e.g.* regulated to sustain an oxygen level as monitored by a dissolved oxygen probe) it will be possible to maintain an aerobic environment which will not favour the survival of bacteria such as *Campylobacter* which prefer a low oxygen environment However, the amount of aeration implied can be substantial (to overcome the effect of organic matter present) and the growth of other undesirable organisms may be stimulated.
- *Agitation by use of water jets and/or mixers*: again some benefit is expected so long as the action produces good movement of the water at crates surfaces. However, any agitation may also be counterproductive if it stirs up sludge (or prevents such settlement).
- *Inducing counter-current water flow*: soak tanks are generally static with respect to water movement. In the common set up, a circulation pattern can be induced if water for the pre-wash stage is taken from the last stage of washing, but this is only circulating equally contaminated water. A better alternative would be the addition of clean water at the point where crates leave the tank, inducing a flow running down the tank - the dirtiest crates would then be cleaned with the dirtiest water in the classic countercurrent system. This concept could of course be extended over the whole crate washing plant with water for the soak tank coming from the final washing stages. A key limitation is the amount of fresh water available, leading to the need for at least some recycling - optimal amounts may be decided on the basis of cost-benefit studies.
- *Multistage soak tank* splitting the soak tank into two (or more) stages opens the way to keep the dirty wash water separate from the cleaned crates in much the same way as the previous counter-current water flow concept. The advantage of distinct stages is (i) settled sludges are kept separate and (ii) lower flow rates of water are necessary. The crate being washed is effectively submerged in cleaner and cleaner water. The final stage could be with hot water.
- *Frequent removal of settled sludge*: a small but significant improvement may be achieved by the periodic pumping out of accumulated sludge in the bottom of the soak tank - this going to effluent waste. This is particularly important if any degree of additional agitation is to be used as well. The alternative could be a re-circulation loop with filtration (see below).

Air-jet agitation, counter-current water flow and multistage tanks are already well understood in poultry plants having been incorporated into most scalding tank systems over recent years.

6.4 Use of hotter water in washing

The benefits of hot water may be best utilised by using it in conjunction with a counter-current flow as previously described. Thus the hottest water (well over 70 deg.C) would be used in the final rinse with the drainage water contributing to warming the earlier stages. The draw back of increased microbial growth in warm water would be largely offset by the final wash which would effectively act as a disinfectant stage. Arrangements would still be necessary to contain the fogs generated by hot water (especially in cold weather) and extraction equipment may be needed.

Energy considerations based on warming a 10 kg plastic crate by 50 deg.C implies a heat load of one M.Joule per crate (specific heat capacity taken as 2000 J/kg.K). This is a third of a kilowatt hour or around 2p in terms of electrically supplied heat (or half this amount for steam from oil or gas).

6.5 Use of chemicals in the soak stage

Chemicals in this case mean detergents for cleaning although there may be some benefit in terms of disinfectant as well. The key issue is one of avoiding the need for large amounts by restricting the use to the final wash stages: the implication here is again a multistage soak tank with the bulk of the debris removed in the first stage. The amount of cleaning agent will relate directly to the amount of debris to be removed and enough must be added if it is not to be totally neutralised.

6.6 Reduction in water carry-over; better drainage

Crates, especially those with solid floors, have the capacity to carry dirty wash water to the next stage of the process. This can be as much as a litre of water representing a significant contamination of the next stage of the process. Thus drainage is an important consideration. This is especially the case prior to any final disinfection stage where residual water will dilute the concentration of the applied disinfectant and organic debris may neutralise it. The removal of surplus water can be achieved by several means:

- Natural drainage: assisted by tipping the crate and even inverting it (in the case of the solid floor type). Sufficient time needs to be allowed to enable a large amount of residual water to drain away.
- Use of vibration: to enhance the water removal akin to "shaking dry". The attraction of this approach is that it is simple and involves only relatively minor changes. Drainage time is reduced as a result.
- Use of air jets: are more effective than vibration and they can impart some drying action. However, they can lead to the generation of aerosols and the process is only effective on surfaces reached by the air jet.
- Drying: only an option for the last stage of the process and one that normally requires at least some previous use of heat to enhance the process. Dry crates represent a greatly reduced risk of cross contamination; any disinfectant subsequently applied is also likely to be much more effective.

6.7 More [potable] water use in the final rinse stage

There is little value in specifying the use of potable water unless:

(i) it is applied direct to a cleaned crate and
(ii) the subsequent handling process also operates to a high standard of cleanliness.

Therefore the most likely place of application is as the final rinse. Even if this is the case, the full benefit requires adequate quantities of water to ensure that contaminated water is washed away along with entrained debris leaving a crate wetted only by clean water. It remains likely though that the washed crate will still contain residual levels of bacteria that leave the benefit of specifying potable water as in the current regulations (rather than just clean water) in question.

6.8 Use of increased [targeted] amounts of disinfectant

Several improvements to the application of disinfectant could be achieved by minor changes.

- *Spray jet evenness*: The use of spray heads that generate a fine even spray to evenly wet a larger area. The risk of blockage should be minimal so long as mixtures are made up with clean water but periodic checking will still be required. Many chemical manufacturers (along with the suppliers of spray equipment) provide extensive guidelines for such application.
- *Quantities of disinfectant needed*: A good spray does not in itself guarantee enough chemical or the total coverage of the crate. Quantities will depend on the residual microbial and other organic matter on the washed crate. Guidelines are available from chemical suppliers but these can be too general leaving the operator unclear of the correct amounts needed for crates. Furthermore, there is always the suspicion that recommended disinfectant quantities err on the side of excess to promote sales (the reverse may actually be the case with suppliers underestimating effective quantities to avoid appearing too expensive). Separate tests using swabs to establish a reduction of microbe numbers to an acceptable level will be necessary to identify the appropriate application for a given crate wash operation: this should be carried out as part of the plant's procedures following HACCP principles. It follows that better cleaning of the crates will have the benefit of a lower quantity of disinfectant being required to meet any set standard.
- *Deployment of spray jets*: Total coverage of the crate by disinfectant requires the deployment of sprays in a way that all surfaces are covered. In addition disinfectant wastage needs to be minimised and this is unlikely to be achieved with a single row of jets. A constraint to laying out jets is the need for the crates to move throughout the system without snagging on the nozzles. A moving nozzle system, although more complex, could enable an even application with minimal wastage.
- *Spraying time*: As well as laying out the spray nozzles in an optimal way, it is important to ensure that there is sufficient time for application; the re-version section (following the main washer) is inappropriate in this respect owing to a very brief exposure of the fast moving crate to the spray. Relocation of the spray bars to a position before (or after) the tumbling action would allow more time for application and less wastage. Some savings in chemical could be made by controlling the spray jets to only operate when crates are passing the application zone.
- *Use of a spray booth*: If disinfection by spraying is to be used, the best application would require the use of a dedicated spray booth. Clearly this implies changes to existing equipment however it would be a relatively easy item to add to new installations. As well as ensuring an improved application of disinfectant in a safe environment, containment would also enable

the recovery of surplus chemical by means of a collection sump located under the booth.

- *Disinfection by immersion in a dip tank*: The immersing of crates (and separately, modules) in a tank of disinfectant (used in Denmark) seems excessive and wasteful in the use of the chemical, on the basis of a large amount of liquid carryover. Nonetheless, it does represent a well-contained system and it might be expected to be more effective than sprays, furthermore, good engineering may improve drainage and thus reduce losses. The level of disinfection achieved is generally better than that achieved by sprays: improved reliability can also be expected by avoiding such problems such as spray blockage.

Recent studies in the USA (Ramesh *et al*. 2003) have demonstrated the removal of coliforms (to below detectable levels) from fixed drawer poultry transport containers by immersion in a tank of water with 1000ppm sodium hypochlorite at 70 ^{o}C for 2 minutes. The implication is at least a six log10 reduction in coliform numbers. In separate experiments with (a) clean water at 70 deg.C and (b) cold water with 1000 ppm sodium hypochlorite, coliforms numbers were reduced by 2.4 and 4.2 $\log_{10}$ units respectively. In all cases, the transport containers had previously been washed using very high pressure water jets (60 Bar). Although an effective method, the study lacked an economic assessment. One might expect the benefit to be greater with the fixed drawer systems as they are more difficult to disinfect in the first place but the costs are likely to be significant.

6.9 Improved module washer design and operation

Many of the potential improvements for the module washer follow those already set out for the crate washing system. In particular these include:

- Use of hot water;
- Improved quality of recycled water;
- Use of a distinct rinse cycle with clean water;
- Drainage and drying options;
- Better disinfectant application.

There are also other issues relating to the design and deployment of modules which are discussed in the next section as they represent a substantial change to the current system.

Although many of the above issues are common to crate washing, the process for modules is very different in two key respects. The first of these is the use of a semi-batch operation rather than the continuous operation used for crates. The main consequence of this is that all operations are carried out in the same booth. This both makes improvements easier and harder. It is easier in that the process is well defined and can be well controlled rather like a domestic washing machine but it is harder in that the separate stages are less distinct and the risk of cross contamination is greater.

The second key difference with modules is, unlike crates, they are mostly empty space. Water jets on crates are easily targeted at sides and bottom, whereas with modules a large amount of the water is almost bound to miss. This is the case even allowing for the movement of the frame backwards and forwards within the booth. The problem applies equally to washing, rinsing and disinfectant

application. The concept of submersion of the whole frame in a series of large tanks has some attraction in this respect but has its own set of problems. An alternative may be the use of moving jets that follow the outline of the framework but this is not a modification that can be done easily to existing systems.

6.10 The best operation of existing systems

Systems not operating as specified
There are certainly crate washing systems that are not being operated in accordance with the manufacturer's guidelines. This includes poor maintenance and operation such as;

(a) infrequent checking/cleaning of water nozzles,
(b) infrequent removal of debris from the run-down screens,
(c) poor maintenance of the disinfection system,
(d) insufficient (or no chemical application),
(e) inadequate cleaning of equipment
(f) overfilling of tanks,
(g) excessive build up of debris in the wash water.
(h) modifications eg removal of a soak tank

However, it is important to make clear that the rectification of all of these points would be no guarantee of satisfactory cleaning in terms of microbiological criteria: In some cases, it is uncertain whether following good practice would even achieve visibly clean crates although this is certainly possible if the water is not allowed to get too loaded with debris. The picture is confused by the variations of washers that exist representing development over recent years. The correct operation of equipment is a logical starting point and the use of the most modern equipment can be expected to give the best results, but even here, it remains unlikely that any consistent and significant benefit will be gained in terms of reducing microbial numbers.

Operation manuals
In keeping with good practice, manufacturers of crate washing systems supply full documentation with any equipment. As an example, extracts from a manual supplied with crate washers from Anglia Autoflow are included in Appendix 1B. Such documentation concentrates primarily on (i) safe operation and (ii) mechanical maintenance such as lubrication to ensure long service. The best operation of the supplied washer in terms of gaining the cleanest crates is less clear. Information such as frequency of changing wash water and type & quantity of disinfectant is not evident: one might add that this sort of advice is more subjective depending on the level of cleanliness desired. Nonetheless, there remains scope to better inform the operators by means of advised best operation even if the standard of cleanliness is not specified.

Even if detailed information is supplied on the best (or most appropriate) washing regime, there remains two more considerations to ensure good information transfer. On the part of the supplier this is to ensure that instruction/advice is clear, accessible and readily understood; *e.g.* by the

inclusion of a set of laminated summary cards. On the part of the user (operator), it is important that any supplied information is passed onto key staff involved. The operation of the crate washer should be a procedure based on HACCP principles with documentation and provision for training to ensure that all personnel are aware of the required operation practices.

Plants with improvement modifications
Several plants have had features added at some point since delivery/commissioning by the manufacturer. These have been largely carried out under the instruction of factory management with the intention of gaining better cleaning. Three changes in particular have been explored:

- Use of agitation in the soak tank especially from air bubblers;
- Use of a heater in the soak tank;
- Use of a rinse stage with clean hot water.

All of these might be expected to improve the cleaning process but again, it can not be stated that the level of cleanliness in terms of microbial numbers will meet any particular standard. Rinsing with hot water ought to reduce the microbe count on the crate surface, if enough water is used, on the basis of displacing any unattached bacteria. Other studies looking at the effect of spraying poultry carcasses has demonstrated this to be the case even with cold water (Allen *et al*., 2000).

Key improvements with current equipment
Existing plant ought to be run in accordance with manufacturer's directions; it follows that these ought to be clear and readily available and periodically updated. In addition to this, good housekeeping ought to be encouraged with the operational area and equipment kept clean and spillages on to the floor minimised. Beyond such self evident advice other measures that would be expected to produce improvements include the following:

- Setting a minimum soaking time;
- Specifying a minimum amount of water to be used per crate;
- Using detergent and in the correct amount
- Specifying the maximum number of crates that can be washed before the water is changed;
- Setting a minimum time for drainage after the soaking stage and especially after the main wash stage;
- Rinsing with clean water. The requirement of potable water may be excessive and offer little real advantage;
- Criteria for the disinfection process; either in terms of quantity of chemical used or in terms of the reduction in microbe counts achieved.

Work needs to be undertaken to provide quantitative data to inform application of the measures listed.

Alternatively to such a series of process criteria, it may be easier to specify a maximum tolerable level of one or more bacteria types on the cleaned crate surface with a specified sampling and testing

method. This would leave it to the individual company to decide how this is to be achieved (although guidelines should be made available). Any such standard ought to be realistic, reflecting what can reasonably be achieved with the currently available technology.

7. NEW AND ALTERNATIVE TECHNOLOGIES

7.1 Improved treatment of recycled water

A major weakness in current crate washer designs is the inadequate treatment of water that is recycled around the process. The build up of suspended matter is inevitable along with the increase in bacteria numbers. The run-down screens used are cheap and simple in operation but they can only remove coarse particles (over 1mm). If kept clean, they remain a useful first step in treatment but further stages should be considered to improve water quality. This implies pulling water out of the system and passing it through a distinct treatment loop that meets a specific objective:

- *Sedimentation*: a simple concept requiring a stagnant tank with minimal disturbance. Flocculants may be mixed in prior to feeding the wash water into the vessel. Natural gravity separation leads to the production of a sludge concentrate at the bottom and a clarified supernatant at the top (Figure 12).

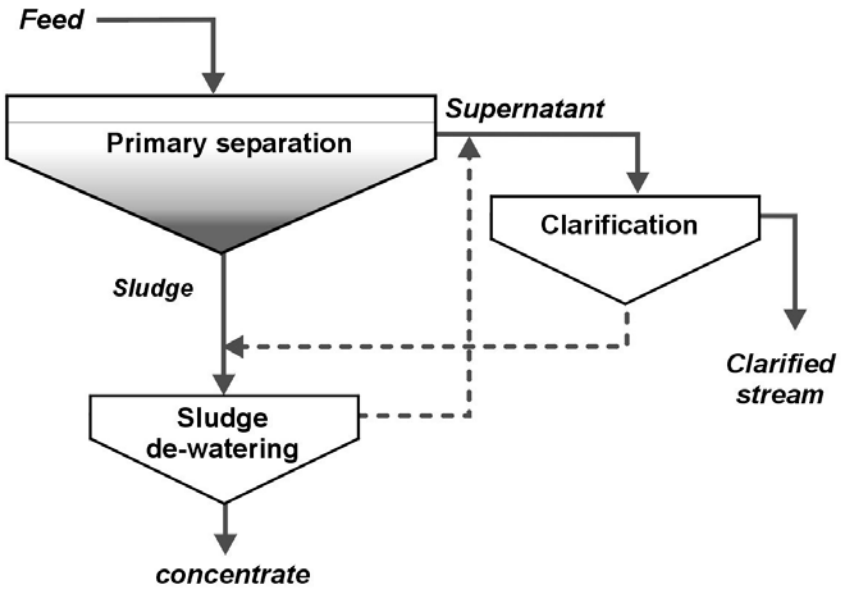
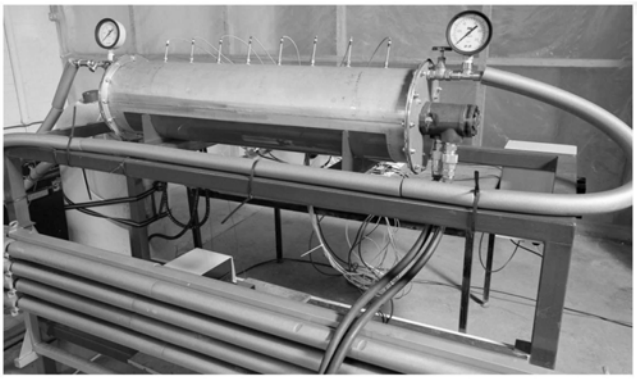


Figure 12: Options for clarification of effluent streams loaded with suspended matter. After a primary separation, further filtration of the supernatant and/or concentration of the sludge may be required.

Typically, the process takes up to 24 hours but the time can be halved if the water is warm and/or flocculants added. Residence time governs the volume of the vessel. If the sludge is low in concentration, it can represent an excessive proportion of the original volume and further concentration (*e.g.* by centrifuge or hydrocyclones) may be necessary. The supernatant should contain only a low level of suspended matter making the option of

subsequent filtration by plate and frame filter a realistic choice.

- *Membrane processes*: clarification can only affect the microbe concentration if very tight filters are used such as ultrafiltration membranes. This is a valid option so long as the wash water has been already clarified, as such membranes are susceptible to blockage. They also generate a large volume of waste concentrate as clean water is progressively "squeezed out". It is doubtful that the benefit of such an elaborate treatment justifies the cost in the case of recycling wash water for poultry crates.
- *Thermal treatment*: although recognised as effective, this technology is often overlooked owing to the perceived high cost. However, this is not the case if heat recovery is used which can bring running costs down to 30 to 40 p per tonne of water (Figure 13). Pre-clarification may be included to remove particulate matter but it is not critical for thermal treatment which essentially returns sterile water for further washing duties.



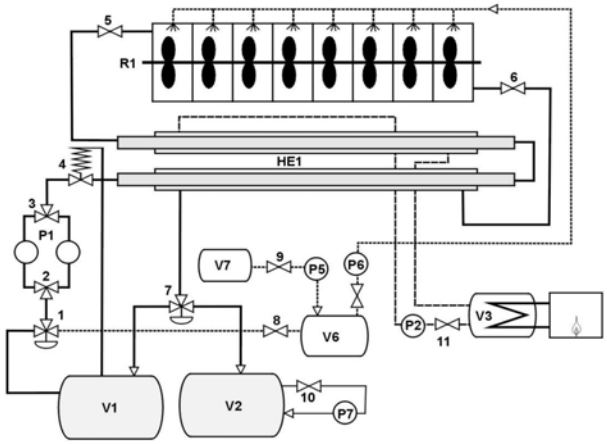


Figure 13: Continuous pilot plant for the thermal treatment of water (100 litres per hour) successfully demonstrated for the total removal of a range of viruses from effluents (Turner *et al.* 1999). Heat recovery was included in the design and successfully brought running costs down to below 40 pence per tonne.

7.2 Use of sonication in the soak stage

The expected benefit from sonication is one of loosening up attached debris (including microbes) which suggests that it may be especially useful in the soak tank. Limitations with the technology include the tendency of plastics to absorb the energy, thereby negating its effect, and the importance of a gas-free water medium for good transmission. Published work does also suggest that some destruction of bacteria can still be expected even in less than ideal conditions.

7.3 Brushes

The use of brushes for poultry transport crates is limited because of crate design which includes many surfaces that are difficult to access. Nonetheless, brushes have been tried in at least one plant with limited success. The attraction lies with the mechanical removal of biofilm layers and where surfaces are readily accessible, this seems a reasonable expectation. Even if brushes can only reach some of the crate surfaces, their use may still be worthwhile as part of a system. Brushes used in a submerged location such as in the soak tank, might also be expected to achieve further benefit by causing liquid agitation. The extent of cleaning by brushing will increase with the

number used but this will also require a much more sophisticated machine. The simplest system would be a single rotary brush that could only clean the base of the crate; further brushes could include the sides but reaching the inside of the crate would require a more elaborate system. In addition to the investment costs implied would be the periodic replacement cost of the brushes A further consideration is the cleaning of the brushes themselves so as not to be a source of contamination.

An alternative use of brushes is in a dry-cleaning operation in which the disadvantages of using water are avoided. The level of cleaning can be expected to be poorer but subsequent disinfection of a dry surface ought to be better so long as residual dirt can be kept to a few flecks.

7.4 Steam drying and disinfection options

The use of steam in food production always appears to be a relatively costly process in relation to many other operations and its use would also require containment of the fogs produced. However, it does allow for a chemical free disinfection such as might be achieved in a steam tunnel. The likelihood of total disinfection of all surfaces is greatly increased over chemical sprays in that all surfaces will be in contact with the vapour and thus potentially heated by the condensate formed. It will be important that the temperature of the surface of the crate reaches 70 deg.C or more and is held at this level for at least a minute to ensure a large reduction in microbe numbers. This implies that the crate should be in the steam tunnel for more than this period of time implying a length similar to the soak tank unless the crates can be turned on their side to save space. An alternative approach might be to steam treat the crates once reinserted in to their module.

The poor conductivity of the plastic should minimise steam consumption - only the surface would be heated possibly equating to no more than 10-20% of the crate volume. Assuming this implies a running cost of less than 0.5 pence per crate as explained earlier (section 6.4) depending on efficiency.

Larger amounts of steam could enable some drying of the crates on the basis that it (i) enhances drainage and (ii) enables subsequent evaporation on entering a cooler, dryer, environment outside the steam tunnel. The latter is not unlike the drying of domestic crockery on a draining-board after washing in hot water - the local air around the hot object is warmed with a rapid fall in relative humidity (RH) which increases evaporation. The extent of this benefit remains to be evaluated as does the potential shortening of the crate life as a result of including thermal cycles in the cleaning process.

7.5 Use of UV in the final stages

The use of UV light for microbe destruction is already well established in other industries such as potable water treatment. Illumination of the crate surface at the appropriate level could be expected to similarly reduce microbe numbers. The method does have the attraction of easy deployment implying little more than the fitting of a row of lamps around the final stages of crate washing with

some shielding for operator protection. However, there are four possible drawbacks to such an approach that point to the need for further research:

- UV light will only penetrate transparent surfaces; it will not effect microbes "protected" by debris remaining on the crate surfaces after cleaning. It is essential that the crate is visually clean and well drained prior to UV treatment;
- the light application would need to be maintained for a period of time implying a series of lamps possibly set up in a treatment tunnel;
- any surface in shadow will be untreated; for poultry crates, this may mean a high proportion of the surfaces unless many lamps are used to illuminate from many directions;
- UV light will shorten the life of the plastic the crate is made from.

7.6 Drying rooms

In dry conditions, bacteria will not multiply and indeed, numbers can be expected to fall as many die off. Hence getting the crates dry prior to returning them to the lorry is an effective way of reducing microbe numbers (including *Campylobacter*). Dry crates can also be expected to be less of a cross contamination risk to poultry grower units. The benefit is extended if the crates are held in a dry state for a period of time running into hours or even days. Low cost drying could be achieved in drying rooms with cold air ventilation. The cost of this approach would be for (a) the additional space required and more importantly (b) the capital tied up in the extra crates required. At around £50 per crate and 5 per minute passing through, a drying room holding crates for 4 hours implies £60,000 of extra crates required.

7.7 Modules: design and use issues

The issues of module washing have already been set out in section 6.9. In addition to this are factors concerning the way in which a module is dirtied. For the transport crates, the soiling remains an uncontrollable part of the process but this is not the case for modules: some control may be possible depending on how they are used at the farm. For example, the problem of debris compacting on the base may be reduced if the module is fitted onto a "shoe" on arrival at the farm; which is removed and retained at the farm once the module is full. There is also the design of the module which includes many locations that can trap debris as it is pushed around the floor of a poultry house. The use of plates on the top (to retain the birds in the top row of drawers) makes the washing process more difficult as it inhibits drainage and removal of collected debris. In such ways, there may be scope to improve module washing by minimising the extent of dirt it receives in the first place. However, such ideas demand a more rigorous study that takes into account the other important design criteria which is outside the subject area of this report.

7.8 Research options

The ideas set out in this section (and indeed many other more straightforward improvements detailed in earlier sections) need to be evaluated in a systematic way against set standards. Are they

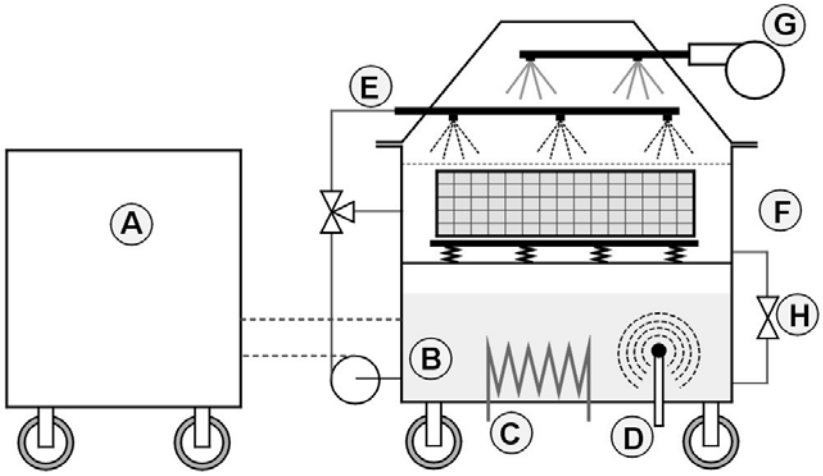


Figure 14: Scheme of test rig to evaluate the effectiveness of alternative crate washing methods. Possible features include: A water treatment; B soaking water; C heater; D sonication; E spraying jets; F vibration tray; G air blower; H drainage. Other possible features (not shown) include uUV disinfection and steam.

effective? What do they cost per crate? Ultimately research requires the use of a test rig to be located at a participating company where there is a ready source of dirty crates (such as Figure 14). Various options could be tested out in a batch concept with parameters including soak time, water temperature, drainage, water jet pressure, use of air curtains, air and/or steam drying, disinfection system etc. The best option(s) will need to meet the cleaning criteria at a lowest cost with a low investment - *relative* to the currently used technologies.

8. CONCLUSIONS: POTENTIAL IMPROVEMENTS FOR CRATE WASHING

General

- Keep the general area clean; undertake good housekeeping practices.
- Avoid spillages onto the floor.
- Collect loose debris from inversion into a container to reduce fouling of the floor.
- Improve the use of space/resources by replacing pre-wash with a longer main wash.

Soak tank

- Have a counter current flow; use larger volumes of water
- Split soak tank into two or more stages; use detergent and larger amounts of in the final stages
- Remove settled sludge frequently
- Use hot water

The main spray booth operation

- Improve filtration of recycled water.
- Have a distinct rinse stage (using clean make up water).
- Use hot water.
- Improve water removal (drainage) *e.g.* by use of air jets or vibrating platform.

Water recycling and treatment

- Increase water use (using recycling)
- Implement counter current flow strategy with the dirtiest water used for pre washing.
- Improve water filtration, consider thermal treatment
- Inspect jets, filters and other equipment regularly

Potable water

- Only use *potable* water as a final rinse stage on crates already cleaned to a high microbiological standard
- Use clean water for the rest of the process as a cost effective measure.
- Use cost saving benefits elsewhere in the process.

The effectiveness of disinfection

- Correct disinfectant choice + correct concentration + recovery of unused chemical (with filtration) and reuse.
- Consider relocation of jets

- Install better jets achieving higher flow rates and more effective chemical usage
- Synchronise intermittent spray with passing of crates, or add chemical to the rinse water
- Improve drainage prior to applying disinfectant
- Consider disinfection by dip tank

Module washing

- Improve water filtration
- Use rinse water in latter stages of cleaning
- Target spray jets more effectively
- Disinfection issues as for crates
- Consider design and farm deployment issues

Other possible technologies

- Use of a final steam disinfection stage
- Use of infra-red heaters
- Use of sonication in the soak stage
- Use of UV in the final stages
- Brushes
- Thermal decontamination of recirculated water
- Air jets to remove water
- Vibration to remove water
- Steam drying options
- Drying rooms
- Dry cleaning and improved disinfection.

Best plant operation?

- Some plants do not run the equipment as specified by the equipment supplier; others lack (or have compromised) certain features, BUT there is no evidence that even if this were not the case, an adequate wash would be achieved.
- Some plants have "improvements" added by the factory engineer; AGAIN there is no evidence that these significantly improve the washing process or (if it does) that this provides adequate washing.

References

ALLEN, V.M.; CORRY, J.E.L.; BURTON, C.H.; WHYTE, R.T.; MEAD, G.C. (2000) Hygiene aspects of modern poultry chilling. International Journal of Food Microbiology, **58** (2000) 39-48

ANON (2000) Chemical disinfectants and antiseptics - quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field - Test method and requirements. BS EN 1656:2000. The Stationery Office, London.

ASSANTA, M.A.; ROY, D. (2001) Disinfecting and sterilising agents used in food industry. In: Guide to Foodborne Pathogens. Ed. R.G. Labbé & S. Garca. John Wiley & Son, Inc., Chichester, UK.

BERCHIERI, A.; BARROW, P.A. (1996) The antibacterial effects for *Salmonella* Enteritidis phage type 4 of different chemical disinfectants and cleaning agents tested under different conditions. Avian Pathology **25**, 663-673.

BLOCK, S.S. (1983) Disinfection, Sterilization and Preservation. Lea & Febiger, Philadelphia, 877-881.

BLOOMFIELD, S. F., ARTHUR, M., LOONEY, E., BEGUN, K.; PATEL, H. (1991) Comparative testing of disinfectant and antiseptic products using proposed European suspension testing methods. Letters in Applied Microbiology **13**, 233-237.

CARPENTIER, B.; CERF, O. (1993) Biofilms and their consequences, with particular reference to hygiene in the food industry. Journal of Applied Bacteriology **75**, 499-511.

CARR, L., RIGAKOS, L., CARPENTER, G., BERNEY, G.; JOSEPH, S. (1999) An assessment of livehaul poultry transport container decontamination. Dairy, Food & Environmental Sanitation **19**, 753-759.

CORRY, J.E.L.; ALLEN, V.M.; HUDSON, W.R.; BRESLIN M.F.; DAVIES, R.H. (2002). Sources of *Salmonella* on broiler carcasses during transportation and processing: modes of contamination and methods of control. Journal of Applied Microbiology, **92**, 424-432.

DAVIES, R.H.;WRAY, C. (1995) Observations on disinfection regimens used on *Salmonella* enteritidis infected poultry units. Poultry Science **74**, 638-647.

DAVIES, R.H.; BEDFORD, S.; WRAY, C. (1998) A semi-quantitative study of the effectiveness of disinfection of broiler-breeder houses contaminated with *Salmonella*. Proceedings of the 4th World Conference on Foodborne Infections and Intoxications, 7-12 June 1998, Berlin, 414-424.

DAVIES, R.; BRESLIN, M.; CORRY, J.E.L.;HUDSON, W.; ALLEN, V. (2001) Observations on the distribution and control of *Salmonella* species in two integrated broiler companies. Veterinary Record **149**, 227-232.

DAVISON, S., BENSON, C.E.; ECKROADE, R.J. (1996) Evaluation of disinfectants against Salmonella Enteritidis. Avian Diseases **40**, 272-277.

EL-ASSAAD, F.; STEWART, L.E.; MALLINSON, E.T.;CARR, L.E.; JOSEPH, S.W.; BERNEY, G. (1993) Decontamination of poultry transport cages. Proceedings of the Annual Meeting of the American Society of Agricultural Engineers, ASAE Paper No.93-3010, p.1-26.

GIBSON, H.; TAYLOR, J.H.; HALL, K.E.; HOLAH, J.T. (1999) Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. Journal of Applied Microbiology **87**, 41-48.

HALD, B.; RATTENBORG, E.; MADSEN, M. (2001) Role of batch depletion of broiler houses on the occurrence of *Campylobacter* spp. in chicken flocks. Letters in Applied Microbiology, **32**, 253-256.

HOOD, S.K.; ZOTTOLA, E.A. (1995) Biofilms in food processing. Food Control 6, 9-18.

Hugo, W.B. (1992) Disinfection Mechanisms in: Principles and Practice of Disinfection, Preservation and Sterilisation. Second Edition. Eds. A.D. Russell, W.B. Hugo & G.A.J. Ayliffe. Blackwell Scientific Publications, Oxford, UK, 187-210.

HUMPHREY, T.J.; ALLEN,V.M. (2002) Poultry crate hygiene: Final Report. FSA Project ZB00033

LISTER, S. (2001) The importance of vehicle disinfection in the poultry industry. Antec International Ltd. http://www.antecint.co.uk/main/livhaua3.htm

JACOBS-REITSMA, W.F.; BOLDER, N. (1998) The role of transport crates in *Campylobacter* contamination of broilers. In "*Campylobacter*, Helicobacter and Related Organisms" Proceedings of the 9th International Workshop, Capetown. (ed) Lastovica, A.J., Newell, D.G. and Lastovica, E.E. pp379-380. South Africa, Capetown University.

MCKENNA, J.P.; OZA, A.N.;MCDOWELL, S.W.J. (2001). The role of transport vehicles, modules and transport crates as potential sources of *Campylobacter* infection for broilers. Poster presented at the International meeting on *Campylobacter*, Heliobacter and related organisms. Freiberg, Germany in September 2001.

OIE (1995) Revue Scientifique et Technique 14 : Disinfectants: actions and applications.
RAMESH, N.; JOSEPH, S.W.; CARR, L.E.; DOUGLAS, L.W.; WHEATON, F.W. (2002) Evaluation of chemical disinfectants for the elimination of *Salmonella* biofilms from poultry transport containers. Poultry Science **81**, 904-910.
RAMESH, N.; JOSEPH, S.W.; CARR, L.E.; DOUGLASS, L.W.; WHEATON, F.W. (2003) Serial disinfection with heat and chlorine to reduce microorganism populations on poultry transport containers. Journal of Food Protection **66**, 5, 793-797.
RIGBY, C.E.; PETIT, J.R.; BAKER, M.F.; BENTLEY, A.H.; SALOMENS, M.O.; LIOR, H. (1980a) Sources of *Salmonella* in an uninfected commercially processed broiler flock. Canadian Journal of Comparative Bacteriology **44**, 267-274.
RIGBY, C.E.; PETTIT, J.R.; BAKER, M.F.; BENTLEY, A.H.; SALOMONS, M.O.; LIOR, H. (1980b) Flock infection and transport as sources of *Salmonella*e in broiler chickens and carcasses. Canadian Journal of Comparative Medicine, **44**, 323-337.
ROSSONI, E.M.M.; GAYLARDE, C.C. (2000) Comparison of sodium hypochlorite and peracetic acid as sanitising agents for stainless steel food processing surfaces using epifluorescence microscopy. International Journal of Food Microbiology, **61**, 81-85.
RUANO, M.; EL-ATTRACHE, J.; VILLEGAS, P. (2001) Efficiency comparisons of disinfectants used by the commercial poultry industry. Avian Diseases **45**, 972-977.
SLADER, J.; DOMINGUE, G.; JØRGENSEN, F.; MCALPINE, K.; OWEN, R.J.; BOLTON, F.J. HUMPHREY; T.J. (2002). Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. Applied and Environmental Microbiology. **68**, 713-719
TAYLOR, J.H.; ROGERS, S.J.; HOLAH, J.T. (1999) A comparison of the bactericidal efficiency of 18 disinfectants used in the food industry against Escherichia coli O157:H7 and Pseudomonas aeruginosa at 10 and 20^{o}C. Journal of Applied Microbiology **87**, 718-725.
TURNER C.; WILLIAMS S.M.; BURTON, C.H.; CUMBY, T.R.; WILKINSON, P.J.; FARRENT, J.W. (1999) Pilot scale thermal treatment of pig slurry for the inactivation of animal virus pathogens. Journal of Environmental Science and Health Vol B34 (**6**), 989 -1007 1999

APPENDIX 1a

Use of Enterobacteriaceae Counts for Determining the Likely Efficiency of Poultry Transport Crate Decontamination

---

Dr R Davies

Although there is some debate as to whether more robust organisms such as Enterococci should be used as indicator organisms, Enterobacteriaceae or coliform counts are most commonly used as an indicator of surface hygiene (Danilenko 1971). This is especially valid when the need is related to correlations with activity of chemical or physical agents against enteric gram negative organisms such as *Salmonella* and *Campylobacter*. Studies of naturally occurring contamination of poultry carcases have shown a poor correlation between levels of Enterobacteriaceae and *Salmonella* (Jimenez *et al.,* 2002) or *Campylobacter* (Cason and Berrang, 2002), although the correlation is good between Enterobacteriaceae counts and total aerobic counts or *Escherichia coli*.

The current question is what could be used as a suitable indicator of the success of decontamination of broiler transport crates. Limited studies have shown a good correlation between the response of *Campylobacter* to chemical or heat decontamination and that of Enterobacteriaceae or total coliforms (Davies, 1995; Berrang *et a.,l* 2001). More studies have confirmed a strong association between reduction of indicator organisms and *Salmonella* (Cover *et al.,* 1985; Castillo *et al.,* 1998a,b; Davies *et al.,* 1998; Hill *et al.,* 2000; Stivarius *et al.,* 2000).

Since this association exists it should be possible to define either a log reduction in Enterobacteriaceae counts or a post-treatment level which could act as an indicator of the effectiveness of any process used for decontamination of poultry transport crates. The main problem is the lack of data on the levels of *Salmonella* and *Campylobacter* which are surviving on effectively washed crates when birds from highly infected flocks have been transported. Carcass rinses of birds taken before defeathering typically show levels of *Campylobacter* of 2-3 logs (Dickens *et al*., 2000; Berrang *et al.,* 2001; with increases occurring after defeathering. It is reasonable to assume that similar or higher levels may be present on poultry transport crates from which gross-faecal contamination has been removed. *Salmonella* levels are always lower than total Enterobacteriaceae (Cason *et al.,* 2002), but may occasionally approach similar numbers in faecal contamination from highly infected flocks (Davies *et al.,* 1998).

Procedures such as spraying of beef carcases with hot water at 95^{o}C are capable of producing a 3log reduction in coliforms and *Salmonella* (Castillo *et al*., 1998b). Pressure washing at 6094kPa followed by immersion in chlorinated water (1 g/l sodium hypochlorite) at 70^{o}C for two minutes has been shown to be capable of total elimination of very high levels of coliforms on poultry transport crates (Niraja-Ramesh *et al*., 1999). This procedure could probably be speeded up if a high temperature soak tank, primary hot wash stage and final hot wash containing higher levels of disinfectant were used. The biggest challenge is likely to be with solid-based crates, which do lead to increased contamination of birds, at least up to the defeathering stage (Buhr *et al.,* 2000). It is also easier to sample solid-floored crates to obtain representative samples so these could be used for comparative work.

Work carried out in this project so far suggests that levels of *Campylobacter* of up to log 6 per 10 cm^2 of poorly washed crate surface may be found. It would therefore be necessary to achieve a >4 log reduction in Enterobacteriaceae, or a maximum residual Enterobacteriaceae count of log 2, to achieve a minimal risk of survival of significant levels of *Campylobacter* on crates by the time they are re-used on nearby farms. For practical purposes this would equate to negative Violet Red Bile Glucose Agar sample dilution spread plates so it would be more useful to develop a direct plating, contact plate or dipstick approach to be used in poultry company laboratories. Since no real data exists on the effect of various improved decontamination measures on *Campylobacter* in transport crates it is recommended that the current project includes further comparative work to correlate the response of Enterobacteriaceae and *Campylobacter* to the washing, heat and chemical treatments to be evaluated. If crates which have been used for transporting birds which are highly infected with *Salmonella* become available it would also be useful to make comparisons using a simple *Salmonella* dilution-enrichment technique. A publication describing this work would contribute significantly to the literature and future quantitative risk assessments.

References

BERRANG, M.E., LADELY, S.R., & BUHR, R.J. (2001) Presence and level of *Campylobacter*, coliforms, Escherichia coli, and total aerobic bacteria recovered from broiler parts with and without skin. Journal of Food Protection **64**: 184-188.

BUHR, R.J., CASON, J.A., DICKENS, J.A., HINTON, A. Jr. & INGRAM, K.D. (2000) Influence of flooring type during transport and holding on bacteria recovery from broiler carcass rinses before and after defeathering. Poultry Science **79**: 436-441.

CASON, J.A. & BERRANG, M.E. (2002) Variation in numbers of bacteria on paired chicken carcass halves. Poultry Science **81**: 126-133.

CASTILLO, A., DICKSON, J.S., CLAYTON, R.P., LUCIA, L.M. & ACUFF, G.R. (1998a) Chemical dehairing of bovine skin to reduce pathogenic bacteria and bacteria of fecal origin. Journal of Food Protection **61**: 623-625.

CASTILLO, A., LUCIA, L.M., GOODSON, K.J., SAVELL, J.W. & ACUFF, G.R. (1998b) Use of hot water for beef carcass decontamination. Journal of Food Protection **61**: 19-25.

COVER, M.S., GARY, J.T. Jr., BINDER, S.F. & SNOEYENBOS, G.H. (1985) Reduction of standard plate counts, total coliform counts and *Salmonella* by pelletizing animal feed. Proc. International Symposium on *Salmonella*, New Orleans, 19-20 July 1984. 221-231.

DANILENKO, I.P. (1971) Enterococci titre as indicator of faecal contamination of milking equipment and of effectiveness of disinfection. Trudy,-Vsesoyuznyi-Nauchno-issledovatel'skii-Institut-Veterinarnoi-Sanitarii **39**: 98-101.

DAVIES, R.H. (1995) Final Report. MAFF Funded Project OZ0122: *Salmonella* contamination; cleansing and disinfection studies.

DAVIES, R.H., BEDFORD, S. & WRAY, C. (1998) A semi-quantitative study of the effectiveness of disinfection of broiler breeder houses contaminated with *Salmonella*. Proc. 4th World Congress on Foodborne Infections and Intoxications, 7-12 June 1998, Berlin, 414-424.

DICKENS, J.A., BERRANG, M.E. & COX, N.A. (2000) Efficacy of an herbal extract on the microbiological quality of broiler carcasses during a simulated chill. Poultry Science 7: 1200-1203.

HILL, V.R., SOBSEY, M.D., REDDY, K.R.(ed.) & KADLEC, R.H. (2000) Removal of *Salmonella* and microbial indicators in constructed wetlands treating swine wastewater. Selected Proc. 7th International Conference on Wetland Systems for Water Pollution Control 2000, Florida, USA, 11-16 November. Water Science and

Technology **44**: 215-222.

JIMENEZ, S.M., SALSI, M.S., TIBURZI, M.C. & PIROVANI, M.E. (2002) A comparison between broiler chicken carcasses with and without visible faecal contamination during the slaughtering process on hazard identification of *Salmonella* spp. Journal of Applied Microbiology **93**: 593-598.

NIRAJA-RAMESH, JOSEPH, S.W., CARR, L.E., WHEATON, F.W. & RAMESH, N. (1999) Cleaning transport containers as an approach toward reducing food contamination in poultry products. ASAE CSAE SCGR Annual International Meeting, Toronto, Ontario, Canada, 18-21 July, ASAE Paper No.6092.

STIVARIUS, M.R., POHLMAN, F.W., MCELYEA, K.S., JOHNSON, Z.B., APPLE, J.K., JOHNSON, M.G. & WALDROUP, A.L. (2000) Reduction of microbial pathogens in ground beef utilizing hurdle technology and a novel ozone generator. Research Series Arkansas Agricultural Experiment Station **478**: 37-39.

APPENDIX 1b

Extracts from *Easyload Maintenance Manual* supplied by Anglia Autoflow Ltd

---

Copies of these instructions are provided to all customers buying Anglia Autoflow crate-washers for Anglia Autoflow poultry transport crates. The extracts reproduced here, with permission, relate to the correct operation and maintenance of the equipment.

CLEANING

DRAWER WASHER SET UP
AND RUNNING MAINTENANCE.

Drawer Washer

1. Turn on the fast fill taps for both pre wash and the soak tank and fill to a point approximately 50mm below the lip, level with the feed to the pre wash filter system.

2. Turn on the supply to the ball cock valve on the pre-wash re-circulating tank, and fill to the pre set level of 25mm below the lip. Insure that the overflow/scum drain is adjusted to the correct level just above the waterline.

3. Repeat the above procedure with the main drawer washer re-circulating tank.

4. Check that the supply to all ball cock valves are turned on, and that they are not adding to the pre set levels of the tanks.

   Note: The ball cock valve fitted to each tank is there to maintain water levels during production. In some instances the supply from the top up system may not be sufficient to cope with excessive water loss, in these cases the system should be toped up using the fast fill.

5. Ensure that all parabolic screens are clean and free from grit, feathers and fatty deposits.

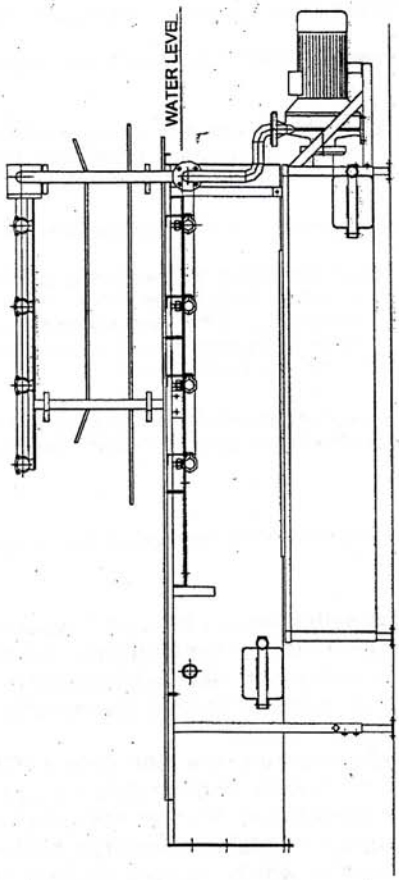
   Note: The water screening system is designed to filter out all deposits over 1mm in diameter, and collect them in a linear deposit on the bottom of the screen profile. As the volume of deposits increase they should be pushed further down the screen, until the eventually drop off in to the collection trough. If there is an excessive build up of debris up the screen, water will tend to flow over rather than trough the screen, resulting in water loss down the collection trough.

6. To insure that the washer performance in maintained at the highest level, all tanks screens and pipe work should be cleaned and flushed through at the end of production.

WARNING

Overfilling washer tanks and poor screen maintenance will result in continuous water loss throughout production.

DRAWER PRE-WASHER.



MODULE WASHER RUNNING MAINTENANCE

1. Turn on the fast fill tap for the re-circulating tank, and fill to a point where the water flows in to the module washer through the return weir. Continue to fill the module washer via the weir, until the water reaches the under side of the jet banks across the base of the module washer.

2. Check that the supply to all ball-cock valves are turned on, and that they are not adding to the pre set levels of the tanks.

   Note: The ball cock valve fitted to each tank is there to maintain water levels during production. In some instances the supply from the top up system may not be sufficient to cope with excessive water loss, in which cases the system should be toped up using the fast fill.

3. Ensure that all parabolic screens are clean and free from grit, feathers and fatty deposits.

   Note: The water screening system is designed to filter out all solids over 1mm in diameter, and collect them in a linear deposit on the bottom of the screen profile. As the volume of deposits increase they should be pushed further down the screen, until the eventually drop off in to the collection trough. If there is an excessive build up of debris up the screen, water will tend to flow over rather than trough the screen, resulting in water loss down the collection trough.

4. To insure that the washer performance in maintained at the highest level, all tanks screens and pipe work should be cleaned and flushed through at the end of production.

WARNING

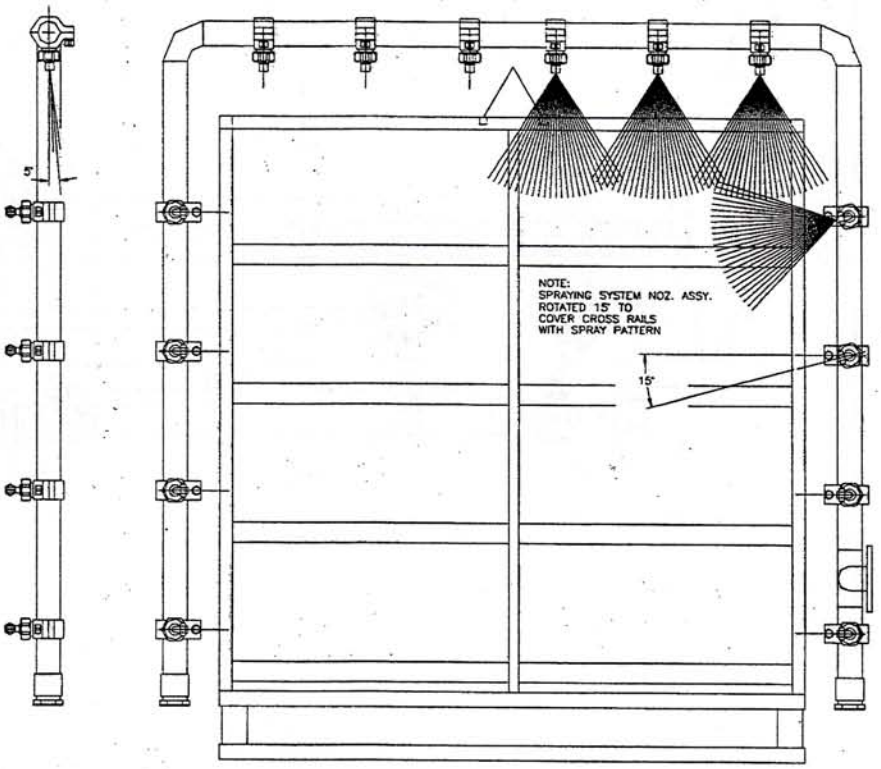
Overfilling washer tanks and poor screen maintenance will result in continuous water loss throughout production.

Washer jet-banks

To maintain the best possible results from your washing system, attention should be paid to the orientation and condition of washing jets. As indicated on the following drawings each jet is designed to sweep and clean a particular part of the module, providing complete coverage of the areas collecting the majority of the litter.

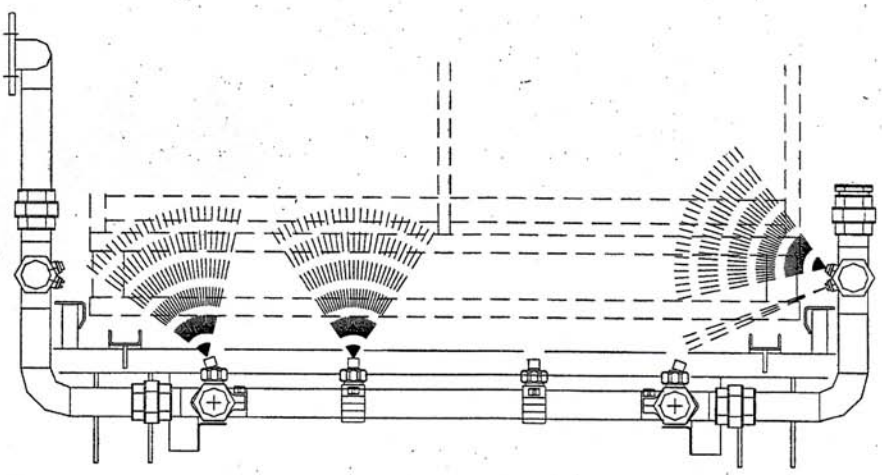
The two jet banks mounted around the module in a "Goal Post" arrangement are designed to sweep the roof and cross rails, and the jet bank mounted in the bottom of the washer is designed to cover the pallet base. In most cases individual jets are adjustable in direction by releasing and resetting the retaining cap. The design of the eyelet jet holder provides a quick-change nozzle facility, which involves no tools or readjustment due to a twist lock fit. Care should be taken to insure the correct direction and orientation of the nozzle should the complete holder be removed from the jet bank, and the following drawings should provide a guide to their general set up.

JET BANKS 1 & 2



M:\S40-S45\S4048\DOCS\CAMAN.DOC

JET BANK 3



MAINTENANCE REQUIREMENTS

Daily

1) Check condition of parabolic filter screen, and remove any blockages by cleaning with a high-pressure hose.

2) Check the air supply is clean & dry and at required pressure.

3) Check oil level in filter/regulator/lubricator units feeding pneumatic equipment, top up as necessary using a recommended grade of oil.

4) Check, and if necessary, clean filter elements in each of the above mentioned units.

5) Check efficiency of jets in drawer washing machine, remove and clean if blocked, replace if damaged. To achieve correct angle of jet when re-fitting, use.

6) Check efficiency of jets in module washer, remove and clean jets if blocked and replace if damaged

7) Check efficiency of jets in drawer pre-wash section, remove and clean jets if blocked and replace if damaged.

8) Check drawer washer pump inlet and outlet pipe work for obstructions.
NB Do not under any circumstances start the pumps in a dry condition.

9) Check the condition of all acetal wheels and lubricate if necessary, including height sensor trolley wheels.

10) Check low level battery warning light for PLC is not illuminated. Replace if necessary with Lithium type.

11) Check ULS indicator is not flashing. If indicator is flashing, check proximity switch settings on lift and height sensor.

12) Check that the underneath of the 'Platform' guard, at the Infeed & Outfeed, is clear of debris and able to pivot.

BEFORE UNDERTAKING ANY MAINTENANCE CHECKS ENSURE THAT MACHINERY IS IN A SAFE CONDITION (i.e. TURNED OFF & ISOLATOR LOCKED TO PREVENT UNAUTHORISED START UP).

IF GUARDS OR COVERS ARE REMOVED FOR MAINTENANCE, ENSURE THAT THEY ARE REFITTED CORRECTLY BEFORE RE-STARTING THE MACHINERY.

AL

At End of Production

(1) Flush out dosing pump thoroughly after use with clean water only for approximately 10 to 15 minutes. Detergents left to soak overnight will be detrimental to the seals and will considerably reduce the expected life of the pump.

(2) Check, and replace if necessary, the watertight seals on the dosing pump.

Note

In regions where the water is hard (high calcium content) the dispenser should be de-scaled periodically. If this precaution is not taken, the seals are likely to harden and cause leaks.

A7

MAINTENANCE REQUIREMENTS

Weekly

1) Check pneumatics for leaks and damage to pipe work etc.

2) Check pumps on the drawer washing and module washing machines for excessive leaking.

NB: Do not allow the pumps to run dry.

3) Check condition of all other pneumatic valves and condition of exhaust filter. Clean if blocked, replace if damaged.

) Check if any one of the drawer washer jet banks has become blocked and remove drain plugs and flush out if required.

5) Check if any one of the module washer jet banks has become blocked and remove drain plugs and flush out if required.

6) Check condition of all plastic chain for damage and wear. Lubricate and adjust tension if required.

7) Inspect and test safety ledge system, on hydraulic lift table, to ensure satisfactory operation.

8) Inspect hydraulic lift table for any fluid leaks etc.

9) Inspect all hoses and pipes on hydraulic lift table.

10) Check the condition of plastic chain stock wheels for build up of dirt. Clean if necessary.

NB If an accumulation of dirt is allowed to build up it will cause the chains to jump.

11) Check tension and condition of Coventry Mk 5 roller chain at shuttle conveyor. Adjust tension if required.

12) Check that both the Torque limiters, fitted on the module shuttle conveyor and drawer reloader, are set correctly to ensure correct operation.

13) Check tension and condition of Coventry Mk 5 roller chain at re-loader. Adjust tension if required.

14) Check condition of 'V' wheels on shuttle conveyor, lubricate if necessary. Check condition of runners for accumulation of dirt, clean as necessary.

AR

15) Check 'Energy chain' cable management system at rear of shuttle conveyor is free to extend and no links are broken. If links are broken replace the chain.

MAINTENANCE REQUIREMENTS

Weekly (cont..)

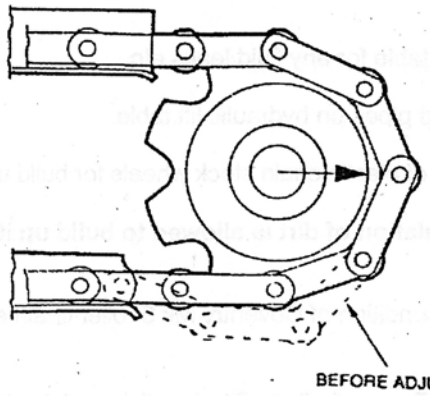
17) Check condition of all electrical cable, conduit and electrical connections within main control panels. Replace if damaged.

BEFORE UNDERTAKING ANY MAINTENANCE CHECKS ENSURE THAT MACHINERY IS IN A SAFE CONDITION (i.e. TURNED OFF & ISOLATOR LOCKED TO PREVENT UNAUTHORISED START UP).

IF ANY GUARDS OR COVERS ARE REMOVED, ENSURE THAT THEY ARE REFITTED CORRECTLY. THE MACHINERY SHOULD NOT BE OPERATED WITHOUT GUARDS.

18) Check tension and condition of heavy duty roller chain. Lubricate and adjust if required. The following is a general guide to adjustment procedure.

a) Check the shaft is in line, i.e. the bearings are an equal distance back in the slides.
b) Correct the adjuster bolts a few turns at a time, equally each side, until the chain no longer falls away from the bottom of the stock wheel.



c) Run the conveyor for a least one complete circuit to allow the chain to settle and re-check the chain adjustment.
d) Re-adjust if necessary.

If replacing the following is a guide.

a) Secure the chain on both sides of the assembly point.

A9

b) Lubricate the connecting pins with grease or oil and insert the pins into the two ends of the chain.

MAINTENANCE REQUIREMENTS

Weekly (cont..)

c) Place the loose plate over the ends of the connecting pins and support the fixed plate side of the chain against the reaction force.
d) Progressively force the loose plate onto the connecting pins necks equally and alternately, using a hollow punch and hammer or hydraulic press.
e) When the plate is fully seated, apply the connector e.g. nut, split pin or circlip.
f) Check that the assembled joint flexes freely. It is tight, a light blow on the opposite end of the connecting pins should free the joint.

For normal conditions a good quality mineral oil with medium viscosity is recommended. Mineral oils are suitable for chains operating in normal atmospheric conditions. For wet conditions water repellent grease should be used, but this will not penetrate into the chain bearing surfaces and therefore additional application of oil is recommended.

A dry film lubricant such as Colloidal Graphite suspended in a white spirit carrier can lubricate Chains operating in abrasive conditions. For exceptional abrasive conditions, grease gun lubricated chain may be used.

BEFORE UNDERTAKING ANY MAINTENANCE CHECKS ENSURE THAT MACHINERY IS IN A SAFE CONDITION (i.e. TURNED OFF & ISOLATOR LOCKED TO PREVENT UNAUTHORISED START UP).

IF ANY GUARDS OR COVERS ARE REMOVED ENSURE THAT THEY ARE REFITTED CORRECTLY. THE MACHINERY SHOULD NOT BE OPERATED WITHOUT GUARDS.

19) Inspect hydraulic lift table platform to ensure it is flat and without obstructions.

20) Check oil level in hydraulic lift table pump reservoir and top up if necessary with a recommended grade.

Note Refilling should be carried out when the lift is in the closed position. Fill the reservoir with sufficient. **CLEAN** oil to maintain the level above the filter. Use a clean funnel fitted with a fine mesh wire screen and not a cloth strainer.

Note UNDER NO CIRCUMSTANCES MUST THE HYDRAULIC PUMP BE RUN WITHOUT OIL.

21) Check hydraulic lift table solenoid valve and adjust as necessary. (This adjusts the lowering speed).

22) Check hydraulic lift table for tightness and location of all fixing down bolts.

23) Check hydraulic lift table hinge blocks and pivot pins/wheels and lubricate. If badly worn replace.

MAINTENANCE REQUIREMENTS

Weekly (cont..)

24) Inspect hydraulic lift table cylinders and check seals, replace if necessary.

25) Inspect hydraulic lift table cylinder mountings for wear.

26) Check the operation of the hydraulic lift table safetychocks.

27) Oil (or grease where nipples are fitted) all pivot bush locations on the hydraulic lift table.

28) Check and lubricate transmission chain on all drive units.

Recommended Lubricants

Ambient Temperature °C	°F (approx)	Lubricant Viscosity SAE	Rating BS4231
-5 to +5	20 to 40	20	46 to 68
5 to 40	40 to 100	30	100
40 to 50	100 to 120	40	150 to 220
50 to 60	120 to 140	50	320

For the majority of applications in the above temperature range a Multigrade SAE 20/50 oil would be suitable.

BEFORE UNDERTAKING ANY MAINTENANCE CHECKS ENSURE THAT MACHINERY IS IN A SAFE CONDITION (i.e. TURNED OFF & ISOLATOR LOCKED TO PREVENT UNAUTHORISED START UP).

IF ANY GUARDS OR COVERS ARE REMOVED ENSURE THAT THEY ARE REFITTED CORRECTLY. THE MACHINERY SHOULD NOT BE OPERATED WITHOUT GUARDS.

WARNING IF UNDERTAKING ANY WORK ON THE HYDRAULIC LIFT TABLE THE SAFETY CHOCKS SHOULD ALWAYS BE USED.

29) Check efficiency of jets on chain wash, remove and clean if blocked.

30) Check the pump motor bearings for temperature, if high check lubrication or alternatively replace them.

31) Check the pump motor bearings for vibration, if excessive replace as necessary.

A11

Use any good grease, which has a Lithium base and avoid overfilling the bearing housing.

A12

MAINTENANCE REQUIREMENTS

Monthly

1) Check condition of heavy-duty roller chain stock wheels for wear, if necessary replace.

2) Check condition of all pillow block bearings for wear. Grease all bearings.

Temp Range	*Grease*	*Supplier*
-20 to +90°C	*Alvania R3*	*Shell UK Oil*
	Alvania RA	
	Energrease LS3	*BP*
	Beacon 3	*Esso*
	Multi Special 3	*Total*
	Lupus A3	*Century Oils*
	Mobilux 3	*Mobil*

3) Check oil level in all gearboxes. Top up as necessary with Shell Macoma R460, or equivalent.

NB **It may be necessary to check all of the above on a daily basis depending upon the environment within the reception bay.**

BEFORE UNDERTAKING ANY MAINTENANCE CHECKS ENSURE THAT MACHINERY IS IN A SAFE CONDITION (i.e. TURNED OFF & ISOLATOR LOCKED TO PREVENT UNAUTHORISED START UP).

IF ANY GUARDS OR COVERS ARE REMOVED ENSURE THAT THEY ARE REFITTED CORRECTLY. THE MACHINERY SHOULD NOT BE OPERATED WITHOUT GUARDS.

WARNING IF UNDERTAKING ANY WORK ON THE HYDRAULIC LIFT TABLE THE SAFETY CHOCKS SHOULD ALWAYS BE USED.

APPENDIX 1c

Presentation on the current technology for crate washing

FSA Project MO 1023

Reducing microbial contamination of poultry crates by improved cleaning and disinfection systems based on better water use

Colin H Burton
(Silsoe Research Institute)

Viv M Allen
(Bristol University)

Presentation to the Food Standards Agency - Wednesday 9th April 2003

- **Introduction to the current FSA-funded project**
- **Quick summary of the crate washing process**
- **Key issues of crate washing**
- **Current short-comings of the equipment**
- **Longer term improvements under study**

Introduction to the current FSA-funded project

Project partners

Silsoe Research Institute (Coordinating and Engineering)
Mr Colin Burton, Mr Robin Whyte, Mr Dave Wilkinson
Bristol University (Microbiology)
Dr Viv Allen, Dr Janet Corry
Consultants
Dr Rob Davis (Veterinary Laboratories) - use of disinfectants
Prof Geoff Mead - hygiene in poultry processing

Introduction to the current FSA-funded project

Industrial partners

- ***Anglia Autoflow - supplier of crates and washing systems***
- ***Faccenda Chicken - poultry processor - broilers***
- ***Lloyd Maunders - poultry processor - broilers***
- ***Sun Valley Foods - poultry processor - broilers***
- ***Banham Poultry Produce - poultry processor - broilers***
- ***Grampian Prepared Meals - poultry processor - broilers***
- ***Bernard Matthews - poultry processor - turkeys***
- ***PLC Ltd - ultrasonic cleaning systems***
- ***British Poultry Council - consultancy***

Introduction to the current FSA-funded project

Project objectives

To review the existing methods of washing poultry crates and crate modules with the intention of understanding the underlying cleaning processes

Then to develop:

- **best operating practices for existing systems**
- **components of a revised system to achieve a rapid, effective and efficient physical and microbial decontamination of poultry transport crates.**

Introduction to the current FSA-funded project

Research approaches

- **Optimisation of water application (soaking, sprays, scrubbing, sonication etc) to maximise the removal of debris and to minimise contamination problems caused by back mixing.**
- **Improving crate cleaning by using larger volumes of water per crate in counter-current flow while minimising extra costs by recycling spent water via a decontamination unit using e.g. thermal (with 80%+ heat recovery), and/or ultrasonic treatments.**
- **A final drying stage - with efficiency improved by enhanced drainage (e.g.; removal of water droplets by air jets, air knives, vibration etc.) and use of residual heat in the crates**
- **Better use of disinfectants**

Project time-frame

Effective project start date:	**Oct 02**
Initial project planning meeting:	**Dec 02**
First project meeting:	**Mar 03**
Second project meeting:	**Sept 03**
Third project meeting:	**Mar 04**
Final meeting:	**Oct 04**
Final report:	**Nov 04**

6 months into a 24 month project


COMMERCIAL–IN–CONFIDENCE

CONTRACT REPORT

REDUCING MICROBIAL CONTAMINATION OF POULTRY TRANSPORT CRATES BY IMPROVED CLEANING & DISINFECTION SYSTEMS BASED ON BETTER WATER USE

A STATE-OF-THE-ART REPORT ON CRATE WASHING

April 2003

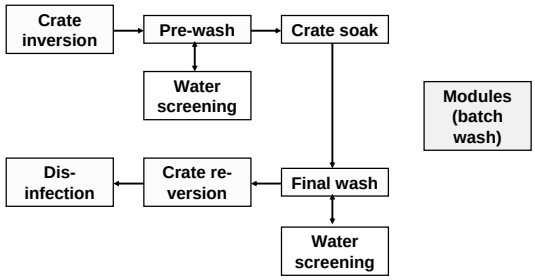
Copy No:

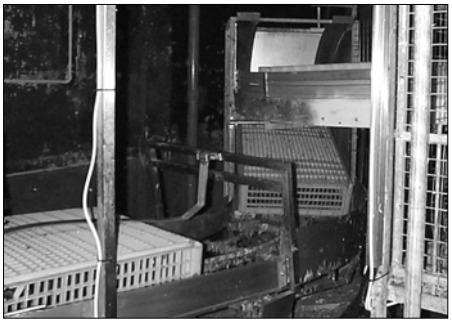
State-of-the-Art Report on Cratewashing

Draft copy being circulated to all partners: final version of report due out May 2003

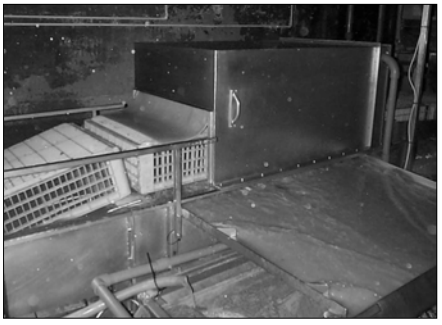
- **Introduction to the current FSA-funded project**
- **Quick summary of the crate washing process**
- **Key issues of crate washing**
- **Current short-comings of the equipment**
- **Longer term improvements under study**

The main process operation steps



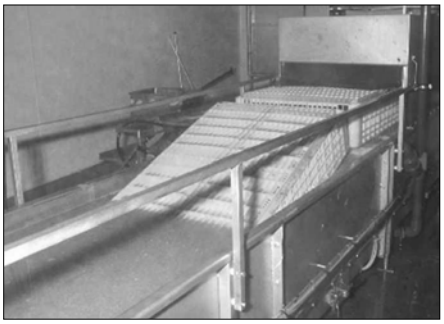


Crate inversion



Pre-wash and water recycling via screens

Quick summary of the crate washing process



Crates entering the soak tank

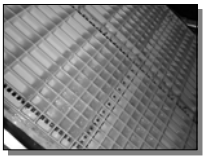
Quick summary of the crate washing process

Crates moving into main wash booth; water recycling via a run down screen and make up tank

Quick summary of the crate washing process

Washed crates stacked in separately washed modules

- **Introduction to the current FSA-funded project**
- **Quick summary of the crate washing process**
- **Key issues of crate washing**
- **Current short-comings of the equipment**
- **Longer term improvements under study**



Key issues of crate washing

- **Cross contamination via poultry transport crates; evidence includes salmonellas or campylobacters . Key process is the crate cleaning operation; crates may appear clean but can carry microbial loads**

- **Simply using larger amounts of water will increase supply and effluent disposal costs: recycling is implied**

Key issues of crate washing

- **Proportion of crates contaminated with salmonella and campylobacter is often unaffected by the washing process.**
- **Cleaning can even make things worse**

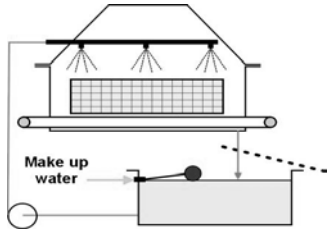
  the proportion of salmonella positive crates sometimes increases after cleaning, which could be due to cross contamination during cleaning

Key issues of crate washing

- **Preliminary comparison of cleaned and dirty crates has shown that they differ little microbiologically.**
- ***Cleaned*** **crates carry 10^5 to 10^6 cfu cm^{-2} of total counts and Enterobacteriaceae, and 10^3 to 10^4 cfu cm^{-2} of *Campylobacter* spp.**
- **Visually clean and obviously dirty areas have similar levels of microbial contamination**

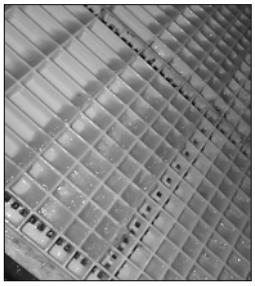
Key issues of crate washing

- **Potable water: such water is used in some plants but typically, the "fresh" water is fed direct into the soak tank or recycle water tank. Hence the quality of the make-up will have no effect on the washing process as the re-circulated water is certainly loaded with contaminants.**



Key issues of crate washing

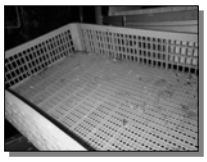
- **Solid or open floor crates? Drainage is easier with open floor crates**



Key issues of crate washing

- **Use of disinfectants are no guarantee of control as they can be neutralized by the large organic load present**
- **Other technologies possible (u/v, ozone, sonication, etc) but *avoidance of water is not practical***
- **Improvements must be straightforward but effective; elaborate changes are unlikely to be widely embraced in the short term**

- **Introduction to the current FSA-funded project**
- **Quick summary of the crate washing process**
- **Key issues of crate washing**
- **Current short-comings of the equipment**
- **Longer term improvements under study**



Current short-comings of the equipment

The contribution from the pre-washing stage

- **Collection of debris falling out from the crate on inversion**
- **Limited benefit of pre-wash as soak tank will wet crates**
- **Soak tank water used**
- **Removed debris flows into the soak tank**

<u>Possible improvements</u>

1. **Collection of loose debris in box to reduce fouling of floor**
2. **Better us of space/resources by replacing pre-wash with a longer main wash at the other end**

Current short-comings of the equipment

Soak tank considerations

- Hot water - improved cleaning but problems with fogs and microbe growth; costs
- Use of detergent: often inadequate quantities
- Collection of debris in sump
- Longer residence time needed?
- Use of other treatments such as sonication?

Possible improvements

1. Counter-current flow; larger volumes of water
2. Split soak tank into two; use (larger amounts of) detergent but only in second stage
3. Frequent removal of settled sludges

Current short-comings of the equipment

The main booth operation

- Blockage of jets
- Poor separation of wash and rinse stages
- Drainage problems (especially with solid floor crates)
- Use of effluent (recycled water)

Possible improvements

1. Better filtration of recycled water
2. Use of a distinct rinse stages (using clean make-up water)
3. Improved water removal (drainage) both before and after rinse stage *eg;* by use of air jets or vibrating platform

Current short-comings of the equipment

Water recycle and treatment

- Treatment by screening (and occasionally by chemicals)
- Screening can only remove coarse solids; no effect on dissolved and fine solids
- Problems with screen blockage and water overflow
- Water management important issue
- Use of recycled water best kept to early stages of washing

Possible improvements

1. Counter-current flow strategy with the dirtiest water used for pre-washing.
2. Improved water filtration; possible thermal treatment
3. Regular inspection of jets, filters and other equipment
4. Avoidance of spillage of water/effluent on to floor

Current short-comings of the equipment

The effectiveness of disinfection

The continuous application of disinfectant is clearly wasteful as well as being inadequate from a poor jet. This example is very typical of many units but it still adds to the awarded HAS score

Current short-comings of the equipment

The effectiveness of disinfection

- Definition of the term “disinfection”
- Inadequate spraying time
- Inadequate spray volume
- Poor coverage
- Neutralising effect of residual effluent

Possible improvements

1. Relocation of jets
2. Better jets; higher flowrate and more chemical usuage

3a. Intermittent spray synchronised with passing of crate

3b. Or chemical added to rinse water

4. Improved drainage prior to applying disinfectant

Current short-comings of the equipment

Module washing

- Design and use (at farm) contributory factors
- Semi-batch operation (cf: continuous for crates)
- Unlike crates, most water in washer “misses”
- Importance of targeted sprays
- Re-cycled effluent; blockage problems
- No separate rinse cycle with clean water

Possible improvements

1. Improved water filtration
2. Use of rinse water in latter stages of cleaning
3. Targeted sprays
4. Disinfection issues as for crates
5. *Design and farm deployment issues*

Current short-comings of the equipment

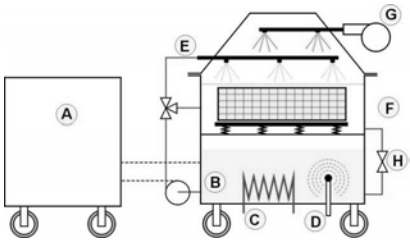
Best plant operation?

- Some plants do not run the equipment as specified by the equipment supplier; others lack (or have compromised certain features, BUT there is not evidence that even if this were not the case, an adequate wash would be achieved.
- Some plants have "improvements" added by the factory engineer; AGAIN there is no evidence that these significantly improve the washing process or (if it does) that this provides adequate washing

- Introduction to the current FSA-funded project
- Quick summary of the crate washing process
- Key issues of crate washing
- Current short-comings of the equipment
- Longer term improvements under study

Longer term improvements under study

Prototype cleaning system



Possible included features: A-water treatment; B-soaking water; C-heater; D-sonication; E-spraying jets; F-vibration tray; G-air blower; H-drainage. Other possible features (not shown) include U/V, disinfection and steam.

Longer term improvements under study

Other technologies being studied

- Use of increased [targeted] amounts of disinfectant
- More [potable] water use in the final rinse stage
- Use of a final steam disinfection stage
- Use of hotter water in the soak stage
- Use of detergents in the soak stage
- Use of sonication in the soak stage
- Use of u/v in the final stages
- Brushes
- Counter-flow water circulation
- Thermal decontamination of re-circulated water
- Increased water use (with re-cycle)
- Air jets to remove water
- Vibration to remove water
- Steam drying options
- Drying rooms

Longer term improvements under study

Draft (preliminary) code of good practice for effective crate washing

- It will set out the best operation procedure of *current* equipment including minor modifications
- It will include improved design concepts for inclusion in new installations
- It will be based on both the state-of-the-art report (currently available as draft), other published work and on the findings from the first 12 months of this project
- It will be sent out for limited circulation/consultation in October 2003
- The final version planned for release November 2004

Any questions?

APPENDIX 2

Proposed code of good practice for the design and operation of crate washing systems for live poultry transport crates

---

The guidelines below are intended to enable poultry producers achieve a defined standard of crate cleanliness following the washing process that takes place in the lairage area. The proposed defined standard is yet to be set out in precise terms and so far only includes the broad requirement that the crates are visually clean following the washing process. However, almost certainly there will be a microbiological criterion added at some point and the guidelines below are aimed to cover this as well. As an indication of adequate improvement in microbiological terms, a reduction in count of at least 4 log10 units has been suggested. This may be too difficult to achieve consistently but a minimum of 2 log10 units is quite realistic. Following the guidelines set out in the code below properly should reliably achieve this.

Notes

Visually clean is defined as *no visible deposits of faecal material on the surface of the crate.*

Microbiologically clean can be defined as a crate with a level of floor surface contamination that does not exceed a specific limit, as determined by a set swabbing and plate count procedure. Alternatively, a reduction by **X** log10 units relative to the unwashed crate may be required.

Code A: applies to all crate washing systems
Code B: applies to existing crate washing systems where appropriate modifications can be made
Code C: applies to newly installed systems only
Code D: applies to future designs of crate washers.
Code O: optional - omission would not represent a contravention of the code if suggested alternatives are used

No.	***Operation***	**Requirement**	**Explanation**	**Code**
0	General layout	Crate washing systems essentially need four main components that will achieve a crate that is both visually and microbiologically clean: (a) initial removal of large amounts of solid debris, (b) an effective soaking stage to loosen adherent dirt, (c) a washing stage to actually remove the dirt and (d) a disinfection stage.	General design criteria for good washing	A
0a	General layout	Layout is important: screen off the crate washing operation and especially the clean crates from the hanging area and crates with live birds	The unwashed crates and the birds both represent potential sources of recontamination of the cleaned crates; good separation is desirable.	B

0b	General layout	Access to all parts of plant needing regular maintenance must be both easy and safe for operators	Regular servicing is more likely if the equipment is easily accessible.	A
1	Crate inversion	All debris removed by flipping action must be collected in adequate bins; any floor spillage is unacceptable.	Floors represent a potential source of re-contamination for cleaned crates	A
2	Pre-washing	This is an optional step necessary only for heavily soiled crates. Its omission is justified if additional cleaning time is given to the subsequent soaking and main wash stages.	The main advantage of pre-wash is to reduce the amount of suspended matter entering the water loop in the soak and main wash cycles; therefore, the pre-wash water loop must be kept separate.	O
3	Pre-washing	Avoidance of spillage of wash water onto the floor - either by leakage or by splashing/spraying. Use of guards, shields; overflow from separator screen directed to a drain.	The water in the pre-wash represents a major source of contamination of the rest of the area including that used to store cleaned crates. Aerosols, in particular, should be minimised.	A
4	Pre-washing	Frequent cleaning of run down screen; set a definite check routine assigned to a specific operator - suggested frequency, once an hour	As well as ensuring efficient separation of debris (and thus reducing jet blockage), this also minimises the overflow of water to the drains/floor.	A
4a	Pre-washing	As an alternative to item 4, there is the option for the development of self-cleaning systems.		D
5	Pre-washing	Frequent changing of wash water - at every plant stoppage (eg: lunch and tea-breaks).	A great deal depends on the level of crate soiling more frequent water changes being needed for dirtier crates. Neglect of this matter will lead to poorer cleaning and the greater likelihood of jet blockage.	A
6	Pre-washing	Frequent inspection of water jet nozzles; set a definite check routine assigned to a definite operator - suggested frequency, once an hour. Any defect must be corrected as soon as possible.	Blockage of nozzles is a common problem which often goes unnoticed for long periods of time. The loss of one or more nozzles greatly reduces the efficiency of cleaning.	A
6a	Pre-washing	As an alternative to item 6, there is the option of the development of better nozzle design and/or specification		D
7	Soaking stage	Minimum residence time of 30 seconds	Use of shorter periods of immersion can result in failure to achieve a visibly clean wash If hot soaking is used (see below) then there is increased benefit with longer periods than the 30 sec indicated.	B
8	Soaking stage	Minimum soak temperature of 50 deg.C Intermediate temperatures of 25-40 can improve the *cleaning* process but the effect may also be to enhance the growth of bacteria in the large volumes of retained water leading to microbiological problems	There is very strong evidence that soaking in water at 50 deg C both enhances the physical cleaning process and reduces microbial contamination of surfaces by 2 log10 units or more. Raising the temperature to 62 deg.C substantially increases this benefit but brings the need for operator protection.	B

9	Soaking stage	Use of a recommended detergent - ammonia or alkaline addition would have a detergent and antibacterial effect. Manufacturer's instructions to be followed.	Detergent usage is necessary to produce a visibly clean crate. To gain full benefit, additional quantities should be added throughout the shift reflecting the volumes of make up water added.	A
10	Soaking stage	Take make up water from the main washing stage.	The use of mains water for making up lost volumes is wasteful and serves no benefit. The clean water would be better used in a final rinse stage (see below).	B
11	Soaking stage	Recirculation of soak tank water at rates in excess of 10 tank volumes per hour in countercurrent route - ie: the water is removed from the point at which crates enter and upgraded water (see below) returned to the end where crates are lifted out.	Setting up a strong flow along the length of the soak tank will assist in conveying debris away from the crates as they move along. In addition, there will be some cleaning benefit from the physical effect of the water flow	C
12	Soaking stage	Clarification and recirculation of soak tank water - use of sedimentation or filtration methods to treat the water. Frequent removal of the sludges produced to separate closed vessels.	Any large build up of debris in the recirculated water is likely to reduce the cleaning efficiency of the water. The associated organic content will have a further detrimental effect in nullifying the effect of the detergent added.	C
13	Main wash	Avoidance of spillage of wash water onto the floor - either by leakage or by splashing/spraying. Use of guards, shields; overflow from separator screen directed to a drain.	The water in the main wash still represents a potential source of contamination for the rest of the area, including the cleaned crates (often stored nearby). Aerosols are of particular concern and should be minimised.	A
14	Main wash	Frequent cleaning of run down screen; set a definite check routine assigned to a specific operator - suggested frequency, once an hour	As well as ensuring an efficient debris separation (and thus reducing jet blockage), this also minimises the overflow of water to the drains/floor.	A
15	Main wash	Frequent inspection of water jet nozzles; set a definite check routine assigned to a specific operator - suggested frequency, once an hour Any defect must be corrected as soon as possible.	Blockage of nozzles is a common problem that often goes unnoticed for long periods of time. The loss of one or more nozzles greatly reduces the efficiency of cleaning.	A
16	Main wash	Removal of entrained water on crates emerging from the soak tank - eg: by use of air jets or vibration mechanism located on the lifting section of the track	Entrained water will lead to a faster build up of contamination in the water of the main wash loop. If a hot wash is used, the same entrained water will lead to faster cooling of the wash water and a higher energy cost.	B
17	Main wash	Use of hot-water sprays (30 to 60 deg.C): this is an option that can be omitted if adequate cleaning is achieved in the other stages of the process and if a hot rinse step follows.	Spraying crates with hot water has been shown to enhance both visual cleaning and the reduction in microbial contamination. Even water at 30 - 40 deg.C is likely to be better than cold water.	O

18	Main wash	Minimum residence time of 15 seconds - implying 5-10 crates held within the main wash cabinet.	There is a clear benefit in extending the wash time although this must have a practical limit owing to space limitations. Doubling the current residence time of 5 - 10 seconds to 15 - 20 seconds may be achieved by foregoing the pre-wash section with a minimal loss of performance.	C
19	Main wash	Use of detergent/disinfectant in the main wash - option that can be omitted if satisfactory cleaning is achieved using other methods	The effectiveness of cleaning chemicals has been well demonstrated with domestic dishwashers but they may represent an unnecessary step in the main crate wash. It is more important to ensure adequate volumes of chemicals for the soak stage and for subsequent disinfection.	O
20	Rinse stage	Removal of residual water on crates emerging from main wash section by use of air jets or a vibrating section of the track.	In order to gain the maximum benefit from the final rinse stage, as much residual water as possible should be removed from crates leaving the main wash.	C
21	Rinse stage	Provision of an adequate rinsing using clean (potable) water - minimum volume of 2 litres per crate	With several thousand crates passing through a typical cratewasher each day, this may seem a large usage of fresh water However this would represent the total feed of clean water to the whole crate washing plant. The overflow from this process would act as make up for all previous stages in a countercurrent route. The volume of 2 litres represents approximately twice that retained on a solid floor crate.	B/C
22	Rinse stage	Use of hot clean water (40-60 deg.C) for the purpose of final rinsing. This can be omitted if preceded by a hot main wash but the energy saving would be small. The benefits would be increased if hotter sprays (up to 80 deg.C) could be accommodated. Above 60 deg.C, operator protection will be required.	The purpose of a rinse stage is to displace any residual water on the crate from the main wash; use of hot water would slightly improve this process as well as enabling better drainage ahead of disinfection. On the basis that all clean water enters the system via the final rinse, using cold water would not greatly reduce the energy bill if a hot main wash is to follow.	O
22a	Rinse stage	The hot water effluent would efficiently be used as a top up for the soak tank or recycled back to a heater/treatment unit	Any thermal process must include efficient energy management and control of evaporation (and fog production in winter)	C
23	Rinse stage	Use of plastic flaps to separate the main wash section from the rinse section	To gain full benefit from the rinse stage, some protection of crates from the sprays generated at the main wash section is appropriate. The alternative of using separate booths for each operation is likely to be an unnecessarily complication. **Note**: if a hot rinse is main antibacterial action there must be protection from recontamination by separate booth	B

24	Disinfection stage	Removal of residual water on crates emerging from main wash section by use of air jets or a vibrating section of the track. This can be omitted but the penalty would be a larger volume of chemical disinfectant per crate.	Residual water will reduce the effectiveness of disinfectant by dilution. A solid floor crate can retain over a litre of water - applied volumes of disinfectant would normally be much less than this amount. If the residual water contains organic matter, the effectiveness of the disinfectant is further reduced.	O
25	Disinfection stage	Separation of disinfectant stage from the main wash and rinse stages - eg: use of plastic flaps if sharing the same booth.	Avoid unnecessary contamination of the cleaned and disinfected crates.	B
26	Disinfection stage	Disinfection to be carried out in its own booth with the collection and re-use of drained chemical	A high proportion of disinfectant will not land on the crate surface or will drain off rapidly. The use of a booth will allow collection and re-application of this material ensuring both a better coverage and less waste. A booth will have the additional and important advantage of protecting nearby staff.	C
27	Disinfection stage	Applied dose of disinfectant to reflect recommended amount eg: for Virkon S, 250 ml at 1% strength per crate. Other disinfectants may be considered including peroxygens such as "Sorgene 5" - some are less sensitive to organic debris.	There is good evidence that a reduction of 2 or more $\log_{10}$ units is achieved when the recommended dose of disinfectant is administered. Lower doses may be suitable if crates are sprayed in a booth but only if very clean and use of high pressure rebound	A
28	Disinfection stage	Disinfectant jets located to spray all surfaces of the passing crate	Both the outside and inside of the crate need to be wetted by the spray although the inside is more important. Outside possibly more important in terms of contamination of workers hands and poultry houses at thinning – any untreated pooled water which gets into the house will be important for solid floor crate interiors It will be difficult to achieve an efficient application without using a booth.	B
28a	Disinfection stage	As an alternative to item 28, dip tanks may be considered although this may result in a higher consumption of disinfectant. Efficient removal of liquid would be a minimal requirement with this alternative. total immersion is always the best option for application of disinfectant – suitable arrangements to reduce and correct for dilution by wash water, passage of inverted crates or an inversion stage and drainage of disinfectant back to the tank could easily be arranged.		
29	Disinfection stage	Frequent inspection of disinfectant jet nozzles; set a definite check routine assigned to a specific operator - suggested frequency, once an hour – and correct problems as soon as possible.	Blockage of nozzles is a common problem even for disinfectant jets. Even a partially blocked nozzle will lead to a poor application.	A
30	Disinfection stage	A minimum of 3 seconds spray contact time – but this may not be enough unless pressure is good and rebound chamber	This is to ensure good wetting of all surfaces of the crate	

APPENDIX 3

Design and construction of a mobile test rig for washing poultry transport crates

---

1. Design concept

1.1 Why use a test rig?

The requirement of a test rig was to enable investigations into crate cleaning to be done at the poultry processing factory with crates removed from a production line. During the project, a limited number of crates were removed from the production line and transported to test facilities at other sites. However, this was both limited and there would always be the question of the effect of drying on of debris. The alternative of re-soiling a set of cleaned crates with "standard dirt" was explored. However, the mere application of debris by soaking (or spraying) was not considered to be representative of the dirtying process that occurred from the cycle of collecting and transporting live birds. Artificially dirtied crates tended to be easier to clean and disinfect as there was little development of biofilms or of other attachment mechanisms.

Several advantages lie with the cleaning of crates at the factory:
(i) there is a very large supply of crates to be studied
(ii) crates taken are representative of those being washed by the commercial system
(iii) logistic problems of large batches (collection, transport and return) are avoided
(iv) a greater appreciation of the prevailing factory conditions.

However, the frenetic activity around the lairage area where the transport crates are handled makes any activity that interferes with the commercial washing equipment impractical. There would also be a limit to the number and extent of modifications that could be made to the commercial crate washer. Thus the use of a separate washing rig was agreed; this would take a small sample of crates which were washed away from the main activity. There would be the option of exploring a wide range of parameters. This could include some combinations that clearly would not provide an adequate wash but which would provide necessary information fo the project; such crates would then be separately cleaned by jet washer prior to returning to the production line.

1.2 Operation mode

Although initial thoughts were of a *continuous* washing test rig (with crates passing through as with the full size units) the engineering required would have been excessive for the few dozen intended crates to be washed. The implied sophistication would have added little to the project and the rig would have been larger than necessary with a chain drive and several separate cleaning stages that copied the commercial unit.

The alternative strategy was to reproduce the various cleaning stages in a sequence but batchwise with the crates being presented to the rig one at a time. Thus, in a process not unlike a domestic washing machine, the crate would be sequentially sprayed, soaked, drained etc in any number of combinations. The rig produced was thus simpler in design but far more flexible. Most importantly, the process model could remain close to that used in the commercial plant. Thus

if the soak time in the continuous process was deemed to be 15 seconds, the crate could be soaked in the rig for the same amount of time. But the rig offered a range of treatments that wold not be possible on the commercial line thus enabling a very wide range of parameters to be explored.

2. The design and operation of the crate washing rig.

2.1 Rig description

A general illustration of the built test rig is given in Figure 1 below. The whole process including control and electrics was built onto the back of a trailer that could be towed by van to the operation area. The trailer itself was rated to support the load of a rig full of water which was effectively an extra 700-800 kg but for transport it was always drained first.

The rig comprised five parts as illustrated in Figures 2 and 3: (i) the trailer, (ii) the washing unit itself, (iii) external tanks, (iv) circulating pumps and (v) electrics and control. A thermal treatment unit used to treat water during the reported trials shared the trailer but was not part of the crate washing operation.

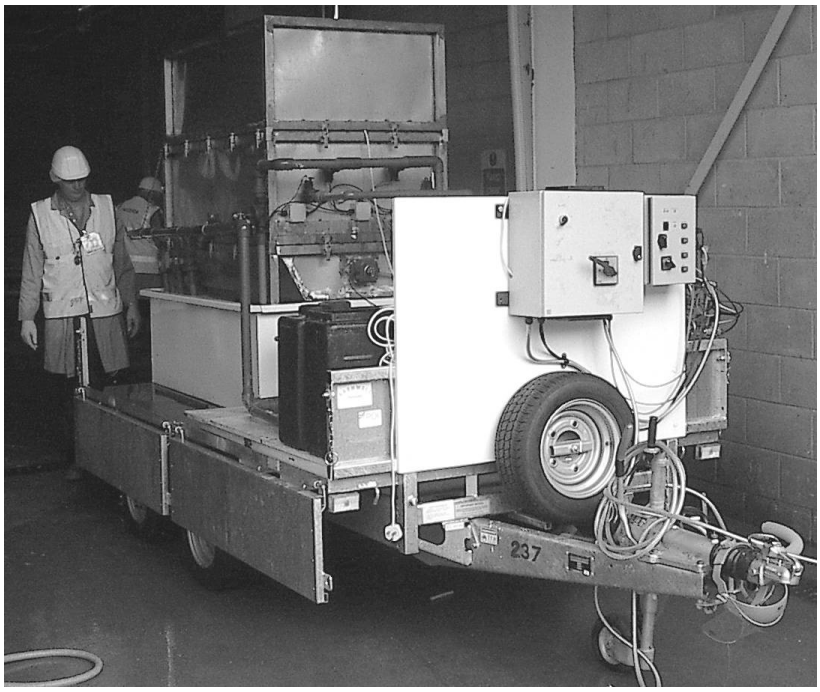
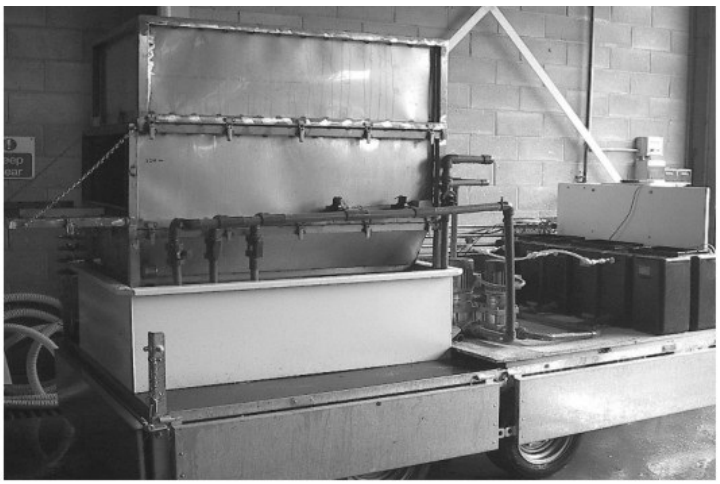


Figure 1: mobile crate washing rig used in factory based trials.

The washing unit (Figure 2) consists of two tanks; an outer one and an inner one. It was convenient (and compact) to site one within the other but this was not necessary for the process. The inner tank comprised three parts: a sump, a central section where the crate was placed and a top hood. The crate itself sat on a pair of runners below which is mounted two rows (each of 6) 1mm spray nozzles. Above the crate was mounted a second set of 12 nozzles. The lower section (the sump) would remain full of water for the whole period of operation. It contained 6kW heating elements for occasions when hot water for soaking was needed. With the front "door" closed (having placed the crate within the washer), water from the outer tank could be pumped in so flooding the middle section and immersing the crate in a "soaking stage". The level of water never exceeded the top of the middle section or entered the top section or hood. The latter served both to contain water during spraying and to mount additional equipment such as u/v lamps as required in some of the trials. Once a soak stage had been completed, water would be pumped back to the outer tank.

External tanks were used as the source of water for the various spraying options. These could



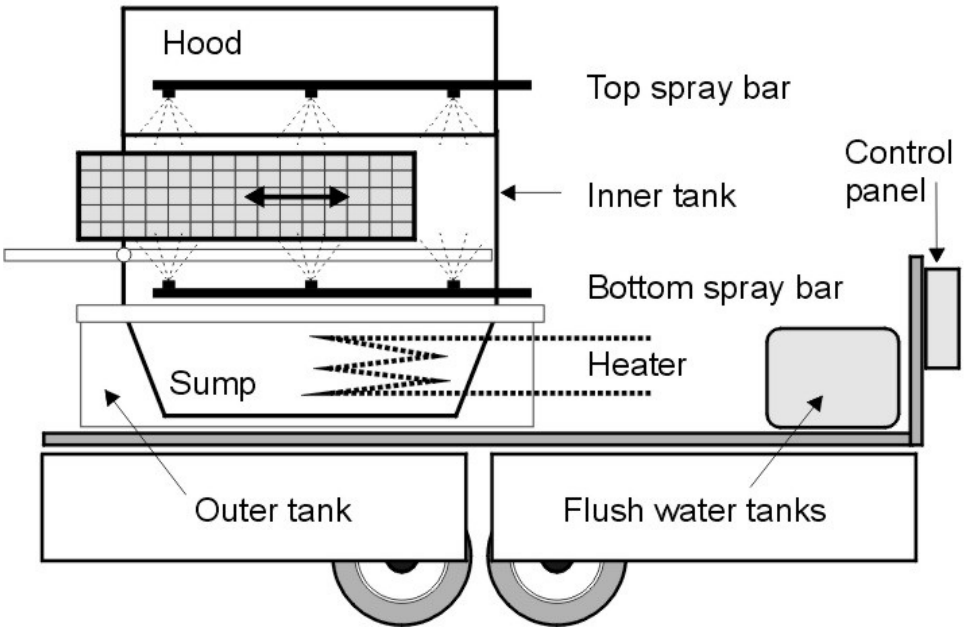


Figure 2: main components of the crate washing rig.

be filled with the same water as the crate washer but this could equally be taken direct form the outer or inner tank (unless it was to be separately heated). A more important use of the external tanks was for the supply of clean water for spraying (hot or cold), the water in the washer itself more likely to be dirty water taken from the commercial plant.

Figure 3: circulation pumps.

In the original design, the plant was to operate automatically making use of solenoid valves. This proved an unnecessary level of sophistication which also limited the operation versatility of the plant. Thus, manual valves were substituted for the automatic devices and operation sequences were entirely controlled manually by the operator. In the final version, only temperature control (for the hot water) was left to run automatically.

2.2 Rig operation

2.2.1 General set up

For all the trials in this project, the test rig was set up in a generous space on level concrete under cover and near to the transport crate handling in the lairage area. Good access all around the plant was important both to enable an efficient operation and to ensure a high level of safety in carrying out the work. Prior to operating the plant, the trailer was secured on its jacks and a three phase power supply (via a fly lead) was run to the plant. Preparations at the factory site included the provision of hot (50°C) and cold mains water via hose and compressed air. A 2-inch pipe had also been run from the soak tank on the commercial plant to the rig running clear of any moving equipment or passageways; this enabled the safe transfer by pump of dirty water from the soak tank to the rig as required. The selected area included adequate drains for water and effluent discarded from the plant during and at the end of a trial.

Preparation of the plant for a trial would depend on the specific conditions: the lower tank and the sump (in the upper tank) would be filled with clean or dirty water as required. The flush water tanks would be filled with the water to be used for spraying (if not to be that taken from the washer itself). Chemicals would be added as required. Heating if needed for hot water would need to be set up several hours before the commencement of the trial; a raft of balls on the outer tank would be used to cut heat losses by evaporation.

2.2.2 The spray circuit

The pipework in the test rig is arranged into two circuits, each with a pump: (a) the spray circuit and (b) the transfer circuit described further on. The feed and discharge for both pumps are linked via a valve to enable pumps to be switched if necessary (*e.g.* in the event of breakdown).

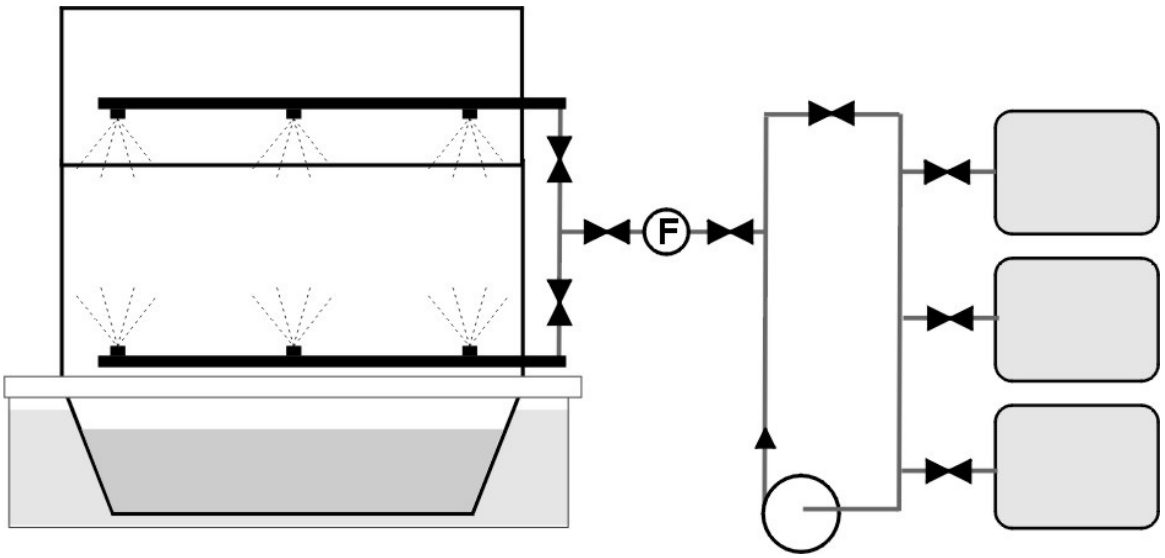


Figure 4: the spray circuit

The pumps (illustrated in Figure 3) are otherwise similar, both being high pressure vane (Grunfoss CR4-30) were run continuously; flow was controlled by opening/closing valves. Pipework is one and a half inch plastic throughout.

The spray circuit is shown diagrammatically in Figure 4 above and some of the pipework is illustrated in Figure 6 below. The layout of pipework is inevitably more complicated in a three-

dimensional reality than a two-dimension diagram but the route and location of the valves are the same.

When spraying is not required, the pump in the spray circuit merely circulates the water around the immediate loop. From here, valves can be opened to send the water to one or other or both sets of sprays via a filter, F. One of the flush tanks is selected and the related valve opened. Flow to the nozzles is started once the valve at the top of the loop is closed (Figure 4).

2.2.3 Transfer circuit

The second circuit serves to move water from the lower to the upper tanks in the washer (and vice versa). This is illustrated schematically in Figure 5 below.

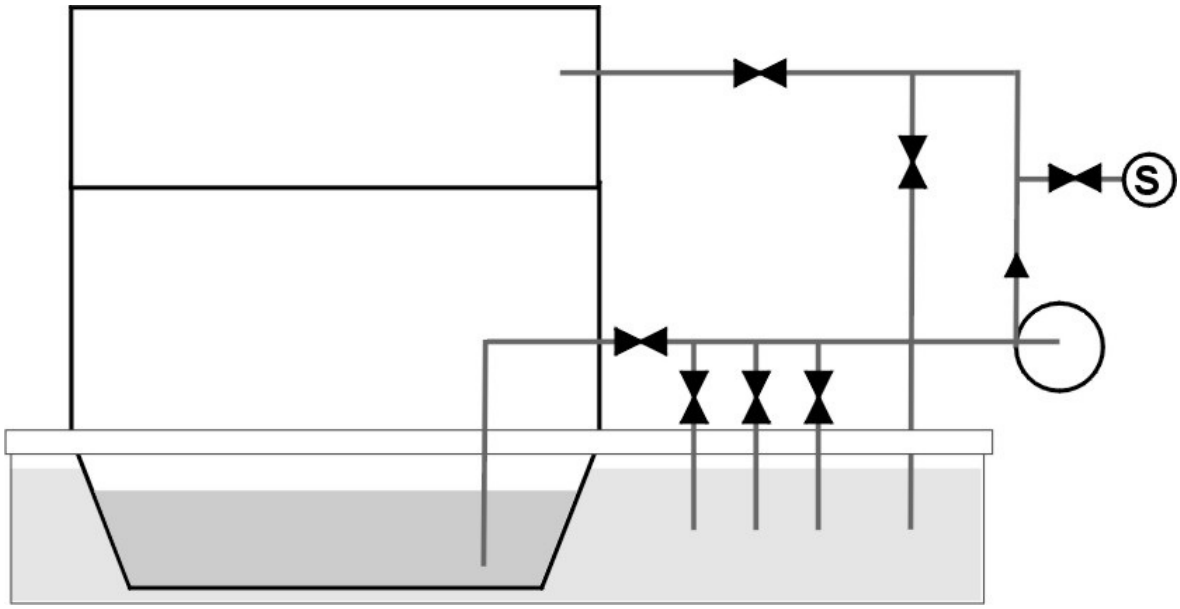


Figure 5: transfer circuit

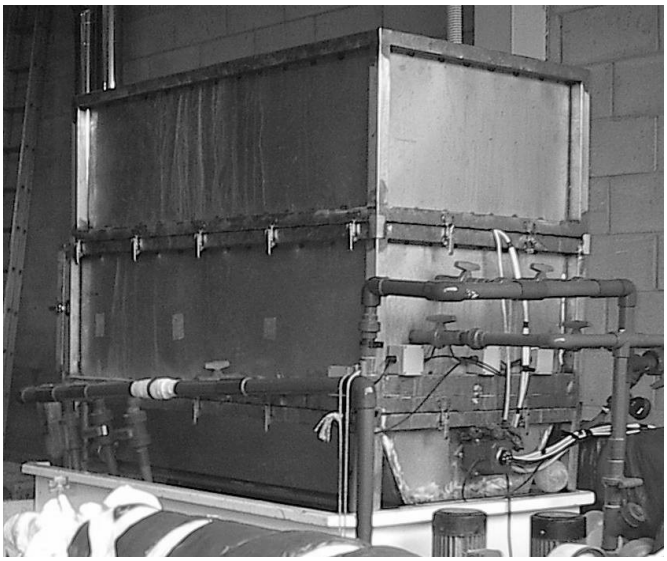


Figure 6: details of pipe runs to and from the crate washer.

In this case, the pump (when not transferring) takes water from the outer tank and returns it to the same place thus providing some mixing of the contents. By opening/closing the appropriate valves there is the option to take water from either tank an to return it to either tank.

It is noted that the fill operation can take several minutes, clearly more than the soaking time would be. However, only the bottom of the crate is assessed micro-biologically and this (the crate being inverted) is only immersed at the end of the fill cycle.

APPENDIX 3a

Details of programme of factory based trials using the pilot scale test rig for crate washing.

---

1. Experimental protocol

1.1 General

All trials were carried out in the lairage area of the Faccenda Poultry Processing plant at Buckingham Road, Brackley. Crates were removed from the nearby line just after they had been emptied but before the first stage of washing. Each trial lasted approximately one week. The equipment was set up on the Monday morning and a maximum of eight experiments took place: Monday (pm) to Friday (am); one experiment per half day. The equipment was removed Friday afternoon and returned to Silsoe for modifications ahead of the next trial.

1.2 Preparation

The mobile experimental rig was delivered to factory site prior to trials and located in a previously agreed area of yard. The equipment was near a 3ph/32Amp power supply and under cover. Other services included a local clean water supply, a drainage point and compressed air. At the beginning of each trial the rig would be set-up during the morning of a Monday by installing and running service, filled with clean water and a check made that all equipment was functioning correctly and safely. The rig would then be drained and filled with representative water from the factory crate washing system.

1.3 Experimental trials

Each experiment used 12-15 poultry transport crates removed from the lairage area prior to the washing stage. The selection was random but within certain set constraints (*e.g.* solid floor crates or old crates may be specified). All crates were removed at the same time and stacked next to the test rig away from direct sunlight or wetting/drying conditions. 10 crates were normally used in the experiment: five replicates for the test and five for the washed control: the others were left unwashed controls.

"Standard Control" (statistical sense) conditions are given with the schedule, these represent the typical cleaning conditions for crates. As such this represents a benchmark against which improvements achieved in the "test" are measured. In a few stated cases, control conditions are modified where the effects of specific parameters are being studied. As far as possible, only one parameter is varied in each experiment.

The crates were fed to the test rig, once ready, one at a time. Where possible, the wash conditions alternated, control-test-control-test etc. If this was not practical, *e.g.* where there are large temperature differences, the 5 controls were followed by the five test crates.

After washing crates were (i) visually inspected and photographed (using a digital camera) and scored following previous procedures (State of the Art Report on Crate Washing, Burton *et al.*,

2004) and (ii), swabbed over the crate floor to determine the presence of indicator organisms in contact with the birds. Procedures were followed as previously used by Bristol University. **(Appendix 5).** Wash water was sampled in some trials and assessed micro-biologically (as for the crate swabs).

1.4 Conclusion of trial work

On completion of the week's trials, the equipment was drained and packed up ready for safe transport back to Silsoe Research where it was thoroughly checked (and modified as necessary) prior to despatch to the next trial site. All removed transport crates were left in a clean state (including jet washing if necessary).

2. Definition of control conditions used

Two controls were used; one represents a ***standard wash***. It is noted that the test rig took between 2 and 4 minutes to fill and the same time to empty. Thus the soaking stage was longer than the 30 secs indicated. However, only the floor of the crate was swabbed and this would be unaffected by the fill time (being only immersed when the vessel was filled). For the purpose of the experimental program outlined below, soak times are defined as that for the period of total immersion; *i.e.* it excludes fill/empty time.

Selection of crates was random over a time period of 10-20 minutes but very damaged (or otherwise untypical) crates were avoided. Standard control wash conditions (unless stated otherwise):

Pre-wash - 15 secs spray (recycled water)
Soak - 30 seconds - cold (15°C) water
Main wash - 15 secs spray (recycled water)
No detergent
No disinfectant
No fresh rinse water
No active water removal from crates
Open floor (grid) crates

The second control is an **unwashed crate** (at least two crates and as many as 5). These were swabbed to provide a typical level of surface contamination of the unwashed crate thus enabling the effect of washing to be established.

3. Trial A - 12th to 16th July 2004

3.1 Purpose of trial

The main theme of this first study was an evaluation of the current crate washing system used. Attention was given to establishing which (if any) of the normal parameters are critical for effective washing (both in terms of debris removal and microbial numbers). The trials also set out to evaluate the operation and performance of the test rig especially in relation to the factory crate washer located nearby.

3.2 Monday 12th July

- Plant filled with clean mains water - 16°C
- 12 grid floor crates removed from plant around 3pm (numbered 1/1 to 1/12)
- Crate 1/1 to 1/5 washed as control (above)
  i.e.: 15secs prewash, 30secs soak; 15sec main wash
- Crate 1/1 rejected due to irregular wash cycle.
- Crates 1/6 to 1/10 washed as follows (expt **A1**)
  *i.e. 15secs prewash, **5mins** soak; 15sec main wash*
- Crate 1/10 not used due to lack of time.
- Crates 1/11 and 1/12 unwashed controls.
- All crates swabbed (crate floor only).
- All crates visually inspected and scored for debris.

NB: Problems of crates floating during soak stage (held down by weights!).

3.3 Tuesday 13th July

- Plant filled with water from soak tank - 16°C
- 12 grid floor crates removed from plant around 10am (numbered 2/1 to 2/12)
- Crate 1/1 to 1/5 washed as control (above)
- Crates 1/6 to 1/10 intended to be washed as follows (expt **A2**)
  (*i.e. 15secs prewash, 30sec soak; 15sec main wash **40°C***)
  Trial abandoned due to continuing power supply problems
- Crates 2/11 and 2/12 unwashed controls.
- Only crates 2/1 to 2/5 and 2/11 to 2/12 swabbed (floor only).
- Crates visually inspected and scored for debris.

NB: Experimental trials frustrated due to frequent power trips during heating phase. Electrical supply repaired during the evening with new (32A) cable being run to socket.

3.4 Wednesday 14th July

- Plant left with same water from soak tank taken on Tuesday.
- Soak tank water temperature 22°C
- 12 grid floor crates removed from plant in morning (numbered 3/1 to 3/12)
- Crate 3/1 to 3/5 washed as control (above)
- Crates 3/6 to 3/10 washed as follows (expt **A3**)
  *i.e. **no** prewash, 30secs soak; **60sec** main wash*
- Crates 3/11 and 3/12 unwashed controls.

- 12 grid floor crates removed from plant in afternoon (numbered 4/1 to 4/12)
- Crate 4/1 to 4/5 washed as follows (expt **A4**)
  *i.e. **no** prewash, 30secs soak; **5mins** main wash*
- Crates 4/6 to 4/10 washed as follows (expt **A5**)
  *i.e. **5mins** prewash, 30secs soak; **5mins** main wash*
- Crates 4/11 and 4/12 unwashed controls.
- All crates swabbed (floor only).
- All crates visually inspected and scored for debris.

3.5 Thursday 15th July

- Plant filled with "fresh" water from soak tank taken during the morning.
- Soak tank water temperature 20°C
- 12 grid floor crates removed from plant in morning (numbered 5/1 to 5/12)
- Crate 5/1 to 5/5 washed as control (above)
- Crates 5/6 to 5/10 washed as follows (expt **A6**)
  i.e. 15secs prewash, ***5mins soak****; 15sec main wash*
  Soak water temperature 40°C
- Crates 5/11 and 5/12 unwashed controls.

- 12 grid floor crates removed from plant in afternoon (numbered 6/1 to 6/12)
- Crate 6/1 to 6/5 washed as follows (expt **A7**)
  i.e. 15secs prewash, 30secs soak; 15secs main wash
  Soak water temperature 40°C
- Crates 6/6 to 6/10 washed as follows (expt **A8**)
  i.e. 15secs prewash, ***5mins soak****; 15sec main wash*
  Soak water temperature 60°C*
  ***NB**: The actual temperature drifted down to 55°C during the experiment as the heaters struggled against an intermittent power supply to sustain temperature. The temperature of the spray water was much cooler than 50/60°C possibly due to evaporative cooling. The crates from this trial alone were visibly cleaner than the control.

- Crates 6/11 and 6/12 unwashed controls.
- All crates swabbed (floor only).
- All crates visually inspected and scored for debris.

3.6 Friday 16th July

- Plant filled with "fresh" water from soak tank taken during the morning.
- Soak tank water temperature 20°C
- 12 grid floor crates removed from plant in morning (numbered 7/1 to 7/12)
- Crates 7/1 to 7/5 washed as follows (expt **A9**)
  i.e. ***no*** *prewash,* ***1 mins soak*; no*** *main wash*
  ***NB**: In this trial alone, the soak tank remained full for the whole period. The top spray bar had been removed and crates were dunked precisely for the 1 minute as indicated.
- Crate 7/6 to 7/10 washed as control (above)
- Crates 7/11 and 7/12 unwashed controls.

- 12 grid floor crates removed from plant in late morning (numbered 8/1 to 8/12)
- Crate 6/1 to 6/5 washed as follows (expt **A10**)
  i.e. ***no*** *prewash,* ***no soak; 5mins*** *main wash*
  Soak water temperature cold, 20°C
- Crates 8/6 swabbed as an unwashed control.
- Crates 8/7 to 8/12 **not used**.
- All crates swabbed (floor only).
- All crates visually inspected and scored for debris.
- All crates photographed before and after washing.

4. Trial B - 13th to 17th September 2004

4.1 Purpose of trial

The main theme of this second study was to evaluate physical water removal systems, in particular use of air knives and vibration. The opportunity was also taken to repeat experiments from Trial A where clarification was required.

Owing to persistent problems with the vibration rig, the programme was ended early after three days. The remaining work will be carried out on a second visit covering 1-2 days provisionally set as during week 4- 8th October.

4.2 Monday 13th September

- Plant filled with cold soak tank water - noted as very contaminated with suspended debris
- 12 grid floor crates removed from plant around 2pm (numbered 1/1 to 1/12)
- Crate 1/1 to 1/5 washed as control (above)
  i.e.: 15secs prewash, 30secs soak; 15sec main wash
- Problems with nozzle jets blocking (frequent cleaning needed)
- Crates 1/6 to 1/10 washed as follows (expt **B1**; a **repeat** of expt **A10** -previous trial)
  i.e. ***no*** *prewash,* ***no*** *soak;* ***5 mins*** *main wash*
- Blockage problems on second set less following cleaning of filter
- Crates 1/11 and 1/12 unwashed controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

4.3 Tuesday 14th September

- Plant re-filled with "cleaner" dirty water taken from the soak tank around 9:30am.
- Soak tank water temperature around 18°C
- 12 grid floor crates removed from plant around 9 am (numbered 2/1 to 2/12)
- Crate 2/1 to 2/5 washed as control (above)
- Crates 2/6 to 2/10 washed as follows (expt **B2)**
  (*i.e. 15secs prewash, 30sec soak; 15sec main wash/rinse with* ***clean cold water***)
- Crates 2/11 and 2/12 unwashed controls.
- Crates visually inspected photographed and scored for debris.
- All crates swabbed (floor only).

- 12 grid floor crates removed from plant around 2 pm (numbered 3/1 to 3/12)
- Crate 3/1 to 3/5 washed as follows (expt **B3)**
  (*i.e. 15secs prewash, 30sec soak;* ***1 min wiping with compressed air jet*;*** *15sec main wash* ***with clean cold water***)
  * **NB**: The crate was removed from the rig and placed on the floor. An compressed air jet (fitted with a 6mm outlet) was passed over the inside and outside of the crate taking around 1 minute. The effect was to both remove water droplets (very efficiently) *and* also to remove debris. The latter raises the option of greater use of compressed air for a cleaning operation. "Dry cleaning" of the crate by compressed air may yet be an option. Greater benefit is achieved here by cleaning by compressed air after a soaking operation.

- Crates 3/6 to 3/10 washed as follows (expt **B4)**
  (*i.e. 15secs prewash, 30sec soak; 15sec main wash with* ***clean hot water 55 to 60°C***)
- Crates 3/11 and 3/12 unwashed controls.
- Crates visually inspected photographed and scored for debris.
- All crates swabbed (floor only).

4.4 Wednesday 15th September

- Plant left with same dirty water as used on Tuesday.
- 12 grid floor crates removed from plant in morning (numbered 4/1 to 4/12)
- Crate 4/1 to 4/5 washed as control (above)
- Crates 4/6 to 4/10 washed as follows (expt **B5)**
  (*i.e. 15secs prewash, 30sec soak;* ***1 min wiping with compressed air jet*; 20****sec main wash* ***with clean hot water***)
- Temperatures of wash water:
  4/6 65°C
  4/7 60°C
  4/8 60°C
  4/9 58°C
  4/10 55°C
- Crates 4/11 and 4/12 unwashed controls.
- Crates visually inspected photographed and scored for debris.
- All crates swabbed (floor only).

- 7 **solid** floor crates removed from plant in afternoon (numbered 4/13 to 4/19)
- Crate 4/13 to 4/17 washed as follows (expt **B6)** (repeat of expt B5 but with solid floor crates) **NB**: The solid floor crates were less easily cleaned with the air jet. As the jet could not blow through the holes, the tendency was to spread the water and debris around.
  (*i.e. 15secs prewash, 30sec soak;* ***1 min wiping with compressed air jet*; 20****sec main wash* ***with clean hot water***)
- Temperatures of wash water:
  4/13 55°C
  4/14 55°C
  4/15 55°C
  4/16 55°C
  4/10 55°C
- Crates 4/18 and 4/19 unwashed controls.
- Crates visually inspected photographed and scored for debris.
- All crates swabbed (floor only).

4.5. Trial B (part 2) - background

This could not be completed in September due to equipment problems: in particular, the arrangement for vibration needed revision. In preparation for the return to the factory to finish the trials, a stand-alone vibration platform was borrowed. Crates removed from the test rig were placed on this to enable removal of surplus water before returning to the rig for a final rinse stage. It was noted that the effect of vibration was also to dislodge some of the remaining solid debris. This same effect was also seen with the use of air jets (also intended primarily to remove

surplus water). Thus both air knives and vibration systems are noted as having a *direct* cleaning effect on the crates. More generally the strategy of water removal is to enhance the final rinse and/or disinfection stages of the process.

4.6 Wednesday 6th October

- Plant filled with cold soak tank water
- 12 grid floor crates removed from plant around 10am (numbered 5/1 to 5/12)
- Crate 5/1 to 5/5 washed as **control** (as above)
  i.e. 15secs prewash, 30secs soak; 15sec main wash
- Crates 5/6 to 5/10 washed as follows (expt **B7)**
  15secs prewash, 30secs soak, ***15 secs vibration****,*
  *15 secs cold rinse (****clean water****)*
- Crates 5/11 and 5/12 unwashed controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

- 12 grid floor crates removed from plant around 2 pm (numbered 6/1 to 6/12)
- Crates 6/1 to 6/5 washed as follows (expt **B8)**
  15secs prewash, 30secs soak, ***15 secs vibration****,*
  then 15 secs ***hot*** *(60°C) rinse (****clean water****)*
- Crates 6/6 to 6/10 washed as follows (expt **B9)**
  15secs prewash, 30secs soak, ***15 secs vibration****,*
  then ***60 secs wiping with a compressed air jet***
  then 15 secs ***hot*** *(60°C) rinse (****clean water****)*
- Crates 6/11 and 6/12 unwashed controls.
- All crates visually inspected, photographed, scored for debris and swabbed

4.7 Thursday 7th October

- Plant left with cold soak tank water from previous day
- 12 grid floor crates removed from plant around 10am (numbered 7/1 to 7/12)
- Crate 7/1 to 7/5 washed as control
- *i.e.: 15secs prewash, 30secs soak; 15sec main wash*
- Crates 7/6 to 7/10 washed as follows (expt **B10)**
  15secs prewash, 30secs soak, ***15 secs vibration****,*
  then ***60 secs wiping with a compressed air jet***
  then 15 secs ***cold*** *rinse (****clean water****)*
- Crates 7/11 and 7/12 unwashed controls.
- All crates visually inspected, photographed, scored for debris and swabbed

- 7 **solid** floor crates removed from plant around 2pm (numbered 8/1 to 8/7)
- Crate 8/1 to 8/5 washed as follows (expt **B11)**
  *15secs prewash, 30secs soak,****15 secs vibration****,*
  then ***60 secs wiping with a compressed air jet***
  then 15 secs ***hot*** *(60°C) rinse (****clean water****)*
- Crates 8/6 and 8/7 unwashed controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

5. Trials C - 25th to 28th October

5.1 Purpose of trial

The central theme for the third set of trials was disinfection systems. In all cases, it is assumed that an adequate washing has taken place before hand and some direct comparison of effectiveness of the various options is made. However, it is noted that the effect of a poor wash will inhibit some disinfection systems more than others. Four disinfection processes were considered: (a) chemical, (b) steam, (c) ultra-sonics and (d) ultraviolet irradiation.

5.1.1 Chemical disinfection

The chemical used was Virkon S at which is one of four suggested by Rob Davies (VLA) as examples of a popular and effective choice for poultry plants. The concentration used (0.5% in cold water) is half of the concentration set out in the Defra *General Orders*. The volume applied (250 ml) is also half the volume recommended on a per square metre basis. Thus the applied chemical per crate was 25% of that advised for dirty surfaces. The low value was chosen (a) because of concerns of over-treating and thus losing the chance to distinguish trends in the various options and (b) because of concerns of excessive use of chemical and the safety consequences of this. Application was by an "up market" 5 litre garden sprayer; 250ml was dispensed in approximately 20 seconds; spraying of the crate (inside surfaces only) was done from a height of approximately one metre. Swabbing done 5 minutes after application.

5.1.2 Steam disinfection

The steam application was done by a small domestic unit involving a 1.5 kW boiler and an application pipe and hood (100mm x 75 mm). This was slowly swept over the inside surface of the crate taking 2 minutes. In this time, 90 grams of steam were applied equating to a cost of 0.3 pence. Ideally, steam application would be within a steam chamber and probably with more steam per crate being consumed than 90g.

5.1.3 Disinfection by the use of ultrasonics

The ultra-sonics treatment was done on equipment supplied and operated by Phil Slapp of PLC ltd. This consisted of a tank filled with water (45-50ºC) with 3% chemical to enhance the ultrasonic effect. The tank was large enough to submerge one crate set on its side; the u/s generator was likewise located on its side thus directing the energy at the crate in a horizontal path. The crate floor was about 0.5 m from the source. Treatment was for one minute. Power levels were either high (4 kW), low (2kW) or off. The last was done to discount any effect from the soaking in water.

5.1.4 Disinfection by the use of ultra-violet radiation

The u/v was applied via a set of 4 strip lights located in the hood of the crate-washing rig. Thus this disinfection option alone was demonstrated within the rig itself. However, the crate had to be re-verted after washing to present the floor to the u/v radiation. The light power was 4 x 20

watts; the strips were located approx. 0.5 metres above the crate floor.

5.2 Monday 25th October 2004

- Plant filled with cold soak tank water
- 12 grid floor crates removed from plant around 10am (numbered 1/1 to 1/12)
- Crate 1/1 to 1/5 washed as **control** (as described above)
  i.e.: 15secs prewash, 30secs soak; 15sec main wash
- Crates 1/6 to 1/10 washed as follows (expt **C1)**
  15secs prewash, 30secs soak, 15 secs main wash,
  120 secs steam treatment
- Crates 1/11 and 1/12 unwashed and un-disinfected controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

5.3 Tuesday 26th October 2004

- Plant left with soak tank water from previous day
- 12 grid floor crates removed from plant around 10am (numbered 2/1 to 2/12)
- Crate 2/1 to 2/5 washed as **control**
  i.e.: 15secs prewash, 30secs soak; 15sec main wash
- Crates 2/6 to 2/10 washed as follows (expt **C2)**
  15secs prewash, 30secs soak, 15 secs rinse with ***hot clean*** *water*
  Spray with Virkon S
- Crates 2/11 and 2/12 unwashed and un-disinfected controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

- 12 grid floor crates removed from plant around 2pm (numbered 3/1 to 3/12)
- Crates 3/1 to 3/5 washed as follows (expt **C3)**
  15secs prewash, 30secs soak, 15 secs rinse with ***hot clean*** *water*
  wipe with compressed ***air jet for 60 secs***
  Spray with Virkon S
- Crates 3/6 to 3/10 washed as follows (expt **C4)**
  15secs prewash, 30secs soak, 15 secs rinse with ***cold clean*** *water*
  wipe with compressed ***air jet for 60 secs***
  Spray with Virkon S
- Crates 3/11 and 3/12 unwashed and un-disinfected controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

5.4 Wednesday 27th October 2004

- Plant left with soak tank water from previous day
- 12 grid floor crates removed from plant around 10am (numbered 4/1 to 4/12)
- Crate 4/1 to 4/5 washed as control
  i.e. 15secs prewash, 30secs soak; 15sec main wash
- Crates 4/6 to 4/10 washed as follows (expt **C5)**
  15secs prewash, 30secs soak, 15 secs rinse with ***hot clean*** *water*

then ***expose crates to u/v light for 60 secs***

- Crates 4/11 and 4/12 unwashed and un-disinfected controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

- 12 grid floor crates removed from plant around 2 pm (numbered 5/1 to 5/12)
- **All** crates washed as follows (expt **C6)**
  *15secs prewash, 30secs soak, 15 secs rinse with **hot clean** water* then .....
  ..... 4 crates then treated by 120s ultrasonics at NO power (5/1, 5/4, 5/7, 5/10)
  ..... 4 crates then treated by 120s ultrasonics at low power (2kW) (5/2, 5/5, 5/8, 5/11)
  ..... 4 crates then treated by 120s ultrasonics at high power (4kW) (5/3, 5/6, 5/9, 5/12)
- During the trial, the ultrasonic tank water fell from 47 to 44.5°C and picked up a slight cloudiness. Final tank water sampled (RAW3).
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

5.5 Thursday 28th October 2004

- Plant left with soak tank water from previous day
- 12 grid floor crates removed from plant around 10am (numbered 6/1 to 6/12)
- Crate 6/1 to 6/5 washed as control
  i.e. 15secs prewash, 30secs soak; 15sec main wash
- Crates 4/6 to 4/10 washed as follows (expt **C7)**
  *15secs prewash, 30secs soak, 15 secs rinse with **hot clean** water*
  then ***120 secs steam treatment***
- Crates 6/11 and 6/12 unwashed controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

- 12 grid floor crates removed from plant around 10am (numbered 7/1 to 7/12)
- Crate 7/1 to 7/5 washed as follows (expt **C8)**
  i.e. 15secs prewash, 30secs soak; 15sec main wash
  then ***Spray with Virkon S***
- Crates 4/6 to 4/10 washed as follows (expt **C9)**
- *15secs prewash, 30secs soak, 15 secs rinse with **hot clean** water*
  then ***Spray with Virkon S***
- Crates 7/11 and 7/12 unwashed **controls** but ***spray with Virkon S***
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

5.6. Initial water treatment trials

5.6.1 Wednesday 27th October

In parallel with the main crate wash work described above, some of the surplus wash water effluent was taken from the rig and passed through a thermal treatment unit. Flowrate - 100 litres per hour; residence time of water treatment rig ~ nominal 30 minutes (volume 50 litres). After a couple of hours, steady conditions reached (exit temperature 69°C) and monitoring started (time = 0 mins). Samples taken: untreated feed (RAW 1 and 2) at 0 and 20 mins; treated water (TRT

1, 2 and 3) at 20, 40 and 60 mins. Some problems with persisting "cold" zones in treatment retention vessel due to poor mixing. Sample RAW 3 was actually the final water from the sonication trials (see section 5.3).

5.6.2 Thursday 28th October

Repeat of Wednesday trial with further problems of poor mixing leading to cold spots. Problems again with circulation pump; flowrate varied between 60 and 100 litres per hour. After 2 hours, exit temperature still at 50°C; trial started (time = 0). Samples taken: untreated feed (RAW 4 to 9) and treated water (TRT 4, to 9) as pairs at 0, 20, 45, 65, 90, 120 mins. The corresponding exit temperatures were: 53, 54, 54, 54, 58, 60°C.

NB: at the end of the trial, feed other temperatures were noted as: feed 24, final water, 31°C and maximum temperature 61°C. Hence, heat loss is 7°C in a heating load of 37 representing 19% or a heat recovery of 81%.

6. Trial D - 22 to 26th November 2004

6.1. Purpose of trial

The broad theme for the fourth series of trials is "advanced washing systems" but some repeat work from the earlier trials was also included. Three concepts are of special interest are (a) repeated washing using detergents, (b) drying and (c) physical scrubbing. The first is intended to explore possible cumulative benefits of repeated washing cycles. The second endeavors to assess the benefit of drying in reducing microbe numbers noting that this may inevitably occur in summer weather anyway; if a clear benefit, the process option may be considered for use during cool damp weather. Drying may be expected to increase the efficacity of any applied disinfectant on the basis that dilution effects are removed. The purpose of physical scrubbing is both to ascertain whether there is merit in brushing systems and to establish the possible limit to microbe reduction for the most vigorous cleaning systems. In the same way, larger doses of disinfectant (Virkon S) are used to establish the maximum (realistic) effect of the chemical.

6.1.1 Chemical disinfection

The disinfectant used was Virkon S (as for previous trial C). Three concentrations were made up, the recommended level of 1%, double this (2%) and half this (0.5%). Either a full treatment of 500ml of the solution was sprayed onto the inside of the crate over 30 secs the spray covering inside sides and floor; a reduced treatment was done with 250ml. The crate was left lying in a horizontal position for 10 minutes to allow time for the disinfectant to work. Swabbing was carried out on the **un**-rinsed surface. It is noted that disinfectant entrapped and remaining on the swab may enhance the chemical effect. The alternative of rinsing first would have also affected the residual microbe population, hence the decision was made as representing more closely the likely industrial situation.

6.1.2 Steam disinfection

A very simple rig was prepared for the purpose of evaluating the concept. The crate was placed upside-down on a table, the steam injected via the underside through a hole in the floor. The

crate was covered with plastic sheet to contain the steam. Supply rate of steam was 50g per minute (from a 1.5kW kettle). Five minutes operation ensured that the crate was warm to the touch on removal but there was uncertainty if the peak temperature was enough to significantly affect the bacteria numbers. Cost for this amount of steam would be around 1 pence per crate.

6.1.3 Drying

The drying rig was essentially a Flymo Garden Vac blower (as used in gardens for leave collection etc) - model EV650. This had a 650 watt motor providing a strong air current enough to remove free water from the crate. Some drying (enhanced by the hot rinse leaving a warm crate) is expected and after 5 mins application the surface *appeared* dry. A further 10 minutes was allowed before swabbing for residual microbes.

6.1.4 Scrubbing options

Two types of scrubbing were used, a more rigorous approach lasting 5 minutes (experiment 1) which attempted to leave a very clean crate with minimal residual microbes and a shorter 2 minute scrub (experiment 3) to remove visible dirt for the purpose of reducing any neutralizing effect on the applied disinfectant. In both cases, scrubbing was done manually using conventional scrubbing brushes and long bristle brushes to work into the corners. It is noted that the effect was most pronounced on the exposed and accessible upper surfaces; swabbing would likewise concentrate on these same surfaces.

6.1.5 Use of tray washer

A more vigorous steam application was carried out using a commercial tray washer as a separate experiment. Dwell time was around 70 seconds with the main cleaning process coming from the large applications of steam.

6.1.6 Use of detergents

Cleaning detergents were added to the soak tank for one experiments 8-10. Hot clean water (available on tap) was used because of the difficulty of heating up cold wastewater from the soak tank. This however also ensured that the maximum benefit of the detergent was achieved. The chemical used was Johnson Diversey Spectak G low foam caustic (Sodium Hydroxide) at 0.1%v/v. As the holding tank on the rig has a volume of 750 litres, 750 ml of chemical was added. It is noted that with the passage of the experiment this would be progressively diluted with the addition of 15 litres of water per crate washed. With 5 controls and 12 experiments, 255 litres of rinse water were added implying a final concentration of 0.08%. There would also be a small accumulation of debris removed from the crates - the final crate would thus be washed in slightly soiled water but the rinse in each case was clean (no detergent) hot water.

To overcome the changes in the washing system, the set of related experiments was done as a single trial with a rotation of crates from each experiment. Thus for experiment 8, the related set of crates were washed in time slots 1st, 4th, 7th and 10th The washing slots for experiment 9 were 2nd, 5th, 8th, 11th and so on.

6.2 Monday 22nd November

- Plant filled with ***hot clean water*** which is warmed to 52ºC
- 15 grid floor crates removed from plant around 9 am (numbered 1/1 to 1/15)
- Crate 1/1 to 1/5 washed as **control but** with hot clean water
  i.e.: 15secs prewash, 30secs soak; 15sec main wash/rinse (60-66ºC)
- Crates 1/6 to 1/10 washed as follows (expt **D1)**
  *15secs prewash, 30secs soak, **300 secs hand brushing**,*
  *return to rig for **20sec** main wash/rinse* (60-66ºC)
- Crates 1/11 to 1/15 unwashed controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

6.3 Tuesday 23rd November

- Plant filled with ***cold*** (18ºC) soak tank water taken at 9:30am
- 15 grid floor crates removed from plant around 9 am (numbered 2/1 to 2/15)
- Crate 2/1 to 2/5 washed as control but with hot clean water rinse
  *i.e.: 15secs prewash, 30secs cold soak; 15sec **hot** **(60-66ºC)** main wash/rinse*
- Crates 2/6 to 2/10 washed as follows (expt **D2)**
  *15secs prewash, 30secs cold soak; 15sec **hot** **(60-66ºC)** main wash/rinse*
  *then spray with Virkon (**1%**, 500 mls) - leave for 10 mins before swabbing*
- Crates 2/11 to 2/15 unwashed and untreated controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed after being left for 10 mins (floor only).

- Plant left with ***cold (18ºC)*** soak tank water taken in the morning.
- 12 grid floor crates removed from plant around 2 pm (numbered 3/1 to 3/12)
- Crates 3/1 to 3/5 washed as follows (repeat of experiment **D3 but** with different Virkon concentration).
  *15secs prewash, 30secs cold soak; 15sec **hot** **(60-66ºC)** main wash/rinse*
  *then spray with Virkon (**2%**, 500 mls) - leave for 10 mins before swabbing*
- Crates 3/6 to 3/10 washed as follows (expt **D4)**
  15secs prewash, 30secs soak; scrub for 120 secs to remove visible debris from floor
  *return to rig for 15sec **hot** **(60-66ºC)** main wash/rinse*
  *then spray with Virkon (**2%**, 500 mls) - leave for 10 mins before swabbing*
- Crates 3/11 and 3/12 unwashed and untreated controls.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed after being left for 10 mins with disinfectant (floor only).

6.4 Wednesday 24th November

- Plant left with cold (20ºC) soak tank water from previous day.
- 15 grid floor crates removed from plant around 9 am (numbered 2/1 to 2/15)
- Crate 4/6 to 4/10 washed as control but with hot clean water rinse
  *i.e.: 15secs prewash, 30secs cold soak; 15sec **hot (60-66ºC)** main wash/rinse*
- Crates 4/11 to 4/15 washed as follows (expt **D5)**
  *15secs prewash, 30secs cold soak; 15sec **hot (60-66 ºC)** main wash/rinse*
  then 300 secs in a steam cabinet

- Crates 4/1 to 4/5 unwashed and untreated **controls.**
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

- Plant left with same soak tank water.
- 15 grid floor crates removed from plant around 2 pm (numbered 5/1 to 5/15)
- Crates 5/1 to 5/5 washed as follows (expt **D6)**
  15secs prewash, 30secs cold soak; 15sec ***hot (60-66°C)*** *main wash/rinse then 300 secs drying using a blower*
- Leave 10 minutes before swabbing
- Crates 5/6 to 5/10 washed as follows (expt **D7)**
  15secs prewash, 30secs cold soak; 15sec ***hot (60-66°C)*** *main wash/rinse then 300 secs drying using a blower then spray with Virkon (**0.5%**, 250 mls) - leave for 10 mins before swabbing*
- Crates 5/11 to 5/15 washed as follows (expt **D8)**
  70 secs in a commercial (Oliver Douglas) tray washer - steam application
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

6.5 Thursday 25th November

- The residual soak tank water from the previous day was heated overnight to **50-55°C.**
- 25 grid floor crates removed from plant around 9 am (numbered 6/1 to 6/25)
- Crates 6/1 to 6/5 unwashed and ***untreated controls*.**
- Crate 6/6 to 6/10 washed as control but with hot soak (dirty water) and a hot clean water rinse
  i.e.: 15secs prewash, 30secs ***hot*** **(55-60°C)** *soak in dirty water; 15sec* ***hot*** *main wash/rinse using clean water*
- Crates 6/11 to 6/25 washed as follows (expt **D9, D10 & D11)**
  Wash conditions: *15secs prewash, 30secs* ***hot*** **(55-60°C)** *soak in clean water; soak tank water with the addition of 0.1% detergent; 15sec* ***hot*** *main wash/rinse* using *clean* water
- Experiment **D9** - one wash only; experiment **D10**, two wash cycles; experiment **D11**, three wash cycles.
- Experiments done in sequence: 9-10-11, 9-10-11 etc. Only four sets completed. Thus crates 6/15, 6/20 and 6/25 not used.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

6.6 Friday 26th November

- Plant completely drained and refilled with cold (16-19ºC) soak tank water taken from the commercial line around 9:30 am.
- 15 grid floor crates removed from plant around 9 am (numbered 7/1 to 7/15)
- Crates 7/1 to 7/5 unwashed and untreated **controls**.
- Crate 7/6 to 7/10 washed as control but with hot clean water rinse
  i.e.: 15secs prewash, 30secs cold soak; 15sec ***hot*** **(60ºC)** *main wash/rinse*
- Crates 7/11 to 7/15 washed as follows (expt **D12) -** ***0.1% detergent*** added to soak tank

water; *15secs prewash, 30secs cold soak; 15sec* ***hot*** **(60ºC)** *main wash/rinse*
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

7. Trial E - 7th to 11th February 2005

7.1 Purpose of trial

The broad theme for the fifth set of trials is "using the best options". Five themes have been identified from the previous four series of trials that achieve a large reduction in microbe counts on the surface of the crates:

1 use of large amounts of disinfectant,
2 brushing,
3 hot (50+ ºC) washing,
4 repeated washes (with detergent)
5 ultra-sonics. (us)

The best results arose from combinations of two or more of these factors. The general strategy of the final series of trials was to include all of these factors in some trials but then to remove them one at a time from subsequent trials. The purpose was to (a) confirm the earlier results and (b) to rank the methods in order of effectiveness.

7.1.1 Chemical disinfection

The disinfectant used was Virkon S (as used for all previous trials). Two concentrations were made up, the recommended level of 1%, and double this (2%). Either a full treatment of 500ml of the solution was sprayed onto the inside of the crate over 30 secs (the spray covering inside floor); or a reduced treatment was done with 250ml. The crate was left lying in a horizontal position for 5-10 minutes to allow time for the disinfectant to work before swabbing. It is noted that entrained disinfectant on the swab may enhance the bactericidal effect. The alternative of rinsing off the residual disinfectant would have also affected the residual microbe population. It was considered that this represented more closely the likely industrial situation.

7.1.2 Scrubbing options

Two types of scrubbing were used, one using a rotary attachment to a drill (effectively a scrubbing brush fitted to a 400 rpm battery powered drill) the other manual brushing. In both cases, scrubbing was confined to the base and carried out for 90 seconds with the base covered in an even way.

It is anticipated that in any commercial process, brushing would be both much quicker but much more vigorous as well with multiple brush heads. The purpose of the experiments carried out was to verify that the mere action of brushing could achieve some direct reduction of microbe numbers. The enhanced cleaning (removal of visual debris) may also be expected to enable more effective disinfection as well.

7.1.3 Use of detergents

Cleaning detergents were added to the soak tank for some experiments - this was necessarily hot as the detergents were found to have little benefit in cold water. Hot water was achieved by leaving the tank on heat overnight. The chemical used was Johnson Diversey Spectak G low foam caustic (Sodium Hydroxide) at 0.1%v/v. As the holding tank on the rig has a volume of 750 litres, 750 ml of chemical was added. It is noted that with the passage of the experiment, this would be progressively diluted with the addition of 10-20 litres of water per crate washed. With 5 controls and 10 experiments, up to 300 litres of rinse water we added implying a final concentration of around 0.07%. There would also be a small accumulation of debris removed from the crates - the final crate would thus be washed in slightly soiled water but the rinse in each case was clean (no detergent) hot water.

7.1.4 Repeated washing

This was included to demonstrate (as appropriate) the scope of the washing process -ie: if significant improvement could be achieved with a second successive wash, then the first wash would be deemed incomplete. This was only carried out for the hot wash (with detergent) to explore the limits of the washing process.

7.1.5 Use of ultrasonics

The ultrasonics treatment (ust)was done with equipment supplied and operated by Phil Slapp of PLC ltd. This consisted of a tank filled with water (60-65^{o}C) with 2% chemical to enhance the ultrasonic effect. The tank was large enough to submerge one crate set on its side; the ust generator was likewise located on its side thus directing the energy at the crate in a horizontal path. The crate floor was about 0.5 m from the source. Power levels were set at high (4 kW) and residence times were 3 and 6 minutes. The crates were firstly washed using a standard wash (but including 30 seconds brushing) to protect the ust systems from excessive contamination from fine particles.

7.2 ***Monday 7th February***

- Plant filled with ***cold dirty water from the commercial soak tank***
- 15 grid floor crates removed from plant around 9 am (numbered 1/1 to 1/15)
- Crates 1/1 to 1/5 unwashed (control).
- Crates 1/6 to 1/10 washed as control:
  15 secs prewash, 30secs soak 15 secs rinse with ***clean*** *cold water,*
- Crates 1/11 to 1/15 washed as folows (expt **E1)**
  Control wash (above) ***plus*** *90 seconds rotary brushing before final rinse*.
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).
- Plant drained and filled with clean water; heated up overnight.

7.3 Tuesday 8th February

- Plant filled with ***hot clean water*** *at nominally 55^{o}C*
- 15 grid floor crates removed from plant around 9 am (numbered 2/1 to 2/15)
- Crates 2/1 to 2/5 unwashed and untreated controls
- Crates 2/6 to 2/10.washed as **contro**l
  i.e. 15secs prewash, 30secs ***hot*** *(55^{o}C) clean water soak; 15sec* ***hot*** *(60^{o}C) main*

wash/rinse with clean water (not from soak tank).

- Crates 2/11 to 2/15 washed as follows (expt **E2)**
  Control wash (above) but with 0.1% detergent added to the soak tank water
  *15secs prewash, 30secs hot soak; 15sec **hot** main wash/rinse with clean water*
  90 seconds rotary brushing before final rinse
  second wash (but skipping the pre-wash): soak - brush - rinse
  then spray with Virkon (2%, 500 mls) - leave for 10 mins before swabbing
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed after being left for 10 mins (floor only).

- Plant left with ***hot*** soak tank water used in the morning.
- 15 grid floor crates removed from plant around 2 pm (numbered 3/1 to 3/15)
- Crates 3/1 to 3/5 unwashed and untreated controls
- Crates 3/6 to 3/10 washed as follows (expt **E3)**
  Control wash (above) but with 0.1% detergent added to the soak tank water
  *15secs prewash, 30secs hot soak; 15sec **hot** main wash/rinse with clean water*
  90 seconds rotary brushing before final rinse
  ***no** second wash*
  then spray with Virkon (2%, 500 mls) - leave for 10 mins before swabbing
- Crates 3/11 to 3/15 washed as follows (expt **E4)**
  Control wash (above) but with 0.1% detergent added to the soak tank water
  *15secs prewash, 30secs hot soak; 15sec **hot** main wash/rinse with clean water*
  ***no** brushing*
  ***no** second wash*
  then spray with Virkon (2%, 500 mls) - leave for 10 mins before swabbing
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed after being left for 10 mins with disinfectant (floor only).
- Tank water discharged at the end of the day.

7.4 Wednesday 9th February

- Plant filled with fresh ***cold dirty water*** *from the commercial soak tank*
- 15 grid floor crates removed from plant around 9 am (numbered 4/1 to 4/15)
- Crates 4/1 to 4/5 unwashed (control).
- Crate 4/6 to 4/10 washed as **control**
  *i.e. 15secs prewash, 30secs cold soak; 15sec **cold dirty soak water for** main wash/rinse*
- Crates 4/11 to 4/15 washed as follows (expt **E5)**
  Control wash (above)
  *then spray with **Virkon** (**2%**, 500 ml) - leave for 10 mins before swabbing*
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

- Plant left with same soak tank water.
- 15 grid floor crates removed from plant around 2 pm (numbered 5/1 to 5/15)
- Crates 5/1 to 5/5 unwashed (control).
- Crates 5/6 to 5/10 washed as follows (expt **E6)**
  Control wash (above)
  *then spray with **Virkon** (**1%**, 250 ml) - leave for 10 mins before swabbing*
- Crates 5/11 to 5/15 washed as follows (expt **E7)**

Control wash (above)
*but 90 secs **brushing** (manual) between the soak and rinse stages*
*then spray with **Virkon** (**1%**, 250 ml) - leave for 10 mins before swabbing*

- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).
- Rig water retained and left heating overnight.

7.5 Thursday 10th February

- The residual soak tank water from the previous day was heated overnight to **55-60°C.**
- 15 grid floor crates removed from plant around 2 pm (numbered 6/1 to 6/15)
- Crates 6/1 to 6/5 unwashed (control).
- Crate 6/6 to 6/10 washed as control but with hot soak (dirty water) and a hot clean water rinse
  *i.e. 15secs prewash, 30secs **hot** **(55-60°C)** soak in dirty water; 15sec **hot** main wash/rinse using clean water*
- Crates 6/11 to 6/15 washed as follows (expt **E8)**
  Control wash (above)
  *then spray with **Virkon** (**2%**, 500 ml) - leave for 10 mins before swabbing*

- 15 grid floor crates removed from plant around 2 pm (numbered 7/1 to 7/15)
- Crates 7/1 to 7/5 unwashed (control).
- Crates 7/6 to 7/10 washed as follows
  Same as the control wash (above)
- Crates 7/11 to 7/15 washed as follows (expt **E9)**
  Control wash (above)
  *then spray with **Virkon** (**1%**, 500 ml) - leave for 10 mins before swabbing*
- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

7.6 Friday 11th February

- Plant completely drained and refilled with cold (16-19°C) soak tank water taken from the commercial line around 9:30 am.
- 15 grid floor crates removed from plant around 9 am (numbered 8/1 to 8/15)
- Crates 8/1 to 8/5 unwashed and untreated controls.
- Crate 8/6 to 8/10 washed as control
  *i.e. 15secs prewash, 30secs cold soak; 15sec **cold** main wash/rinse plus 30 secs manual brushing*
- Crates 8/11 to 8/15 washed as follows (expt **E10)**
  *Control wash (above) plus **ultrasonic** treatment for **6 mins** at 65°C and 4kW*

- 15 grid floor crates removed from plant around 2 pm (numbered 9/1 to 9/15)
- Crates 9/1 to 9/5 unwashed and untreated controls.
- Crates 9/6 to 9/10 washed as follows (expt **E11)**
  *Control wash (above) plus **ultrasonic** treatment for **3 mins** at 65°C and 4kW*
- Crates 9/11 to 9/15 washed as follows (expt **E12)**
  Control wash (above) but no brushing;

*plus **ultrasonic** treatment for **6 mins** at 65°C and 4kW*

- All crates visually inspected, photographed and scored for debris.
- All crates swabbed (floor only).

7.7 Water treatment trials

7.7.1 Thursday 10th February

The thermal treatment plant was warmed with hot clean water during the morning. Around 1:30 pm, the water in the feed tank was drained and replaced with dirty water taken direct from the commercial soak tank. This was circulated for 2 hours and three pairs of samples taken (feed and treated effluent). Circulation rate was nominally 1 litre per minute and treatment temperature was 70°C.

At 3:30pm, the return flow was directed to drain and the feed tank topped up with more dirty water from the commercial plant. The treatment unit was allowed to run for another one and half hours and three further pairs of samples were taken. Operation temperature and flowrates were unchanged.

7.7.2 Friday 11th February

The feed tank was filled with dirty water effluent from the soak tank and the thermal treatment plant was pre-warmed by circulating effluent during the morning. Around 1pm, the flow was directed to waste and the feed tank topped up with "fresh" effluent. Three pairs of samples were taken over the next two hours (feed and treated). The operation temperature was nominally 60°C and the flowrate 1 litre per minute.

8. Trials F - 7th to 8th April 2004

8.1 Purpose of work and definition of control conditions used

This final study was carried out in response to requests made at the final project meeting held on March 9th 2005. There were three outstanding pieces of work to be completed:

1. Running trials with ultrasonics alongside controls carried out in the same tank but without the sonication.
2. Repeat trials on crates that were cleaned, sent to the farm and re-cleaned on their return.
3. Establishment of the weight of water retained on crates on removal from a water tank; in addition, recalculate once free water is blown off using a compressed air line.

The crate washing rig was not used for these trials which were carried out over a two day visit. The ultrasonic kit provided by Phil Slapp of PLC was set up on the previous day being left overnight to heat up.

Controls for this work (unless otherwise stated) were the final washed crates as removed from the existing commercial crate washing plant. To assess the washing procedure, crates were also removed from the discharge end of the soak tank and a further batch of unwashed crates were also taken.

Crates were inspected by (a), swabbing for Total Count (on PCA), Enterobacteriaceae (on VRBG), and *Campylobacter* (on CCDA) and (b), visual inspection for estimating the total weight of entrained solid debris. Photo's of each crate were also taken in respect to the latter.

8.2 Programme of work - Thursday 7th April 2004

Grid floor crates were used throughout.

FA1 *Remove 12 crates from the end of the washing line (label 1/1 to 1/12)*

All crates numbered and tags/labels added.

1/1 to 1/4 pre-washed controls - commercial wash only
1/5 to 1/8 washed controls - dip for 3 minutes in US bath but ultrasonics swtched OFF
1/9 to 1/12 dip for 3 minutes in US bath at 60ºC with ultrasonics switched on at 4 kW.

All washed crates put into one module and module tagged with special instructions for despatch. The module plus original crates was tracked (with thanks to Faccenda staff) and retrieved on the following morning.

FA2 *Duplicate of **FA1**; remove 12 crates from the end of the washing line (label 1/13 to 1/24)*

All crates numbered and tags/labels added.

1/13 to 1/16 pre-washed controls - commercial wash only
1/17 to 1/20 washed controls - dip for 3 minutes in US bath but ultrasonics switched OFF
1/21 to 1/24 dip for 3 minutes in US bath at 60ºC with ultrasonics switched on at 4 kW.

Put all crates into one module and tag module; special instructions for despatch as A1.

FB *10 crates removed from the end of the soak tank (label 3/1 to 3/10)*

2/1 to 2/5 washed controls - dip for 1 minute in US bath but ultrasonics switched OFF
2/6 to 2/10 dip for 1 minute in US bath with ultrasonics switched on at 4 kW.

Crates 2/6 to 2/10 left to dry over lunch.
Dried crates 2/6 to 2/10 weighed.
Dipped into now dirty water in US tank, minimal drainage and weighed again.
Compressed air jets applied for 1 minute and weigh crate again.
Amount of retained water determined in each case.

FC *15 crates removed from the end of the soak tank (label 3/1 to 3/15)*

3/1 to 3/5 washed controls - dip for 30 seconds in US bath but ultrasonics switched OFF
3/6 to 3/10 dip for 30 seconds in US bath with ultrasonics switched on at 4 kW.
3/11 to 3/15 ***brushed*** for 60s then 30s in US bath with ultrasonics switched on at 4 kW.

FE *5 unwashed crates removed from line (label 6/1 to 6/5)*

4/1 to 4/5 swabbed as a second set of unwashed dry controls

8.3 Programme of work - Friday 7th April 2004

FF1 *Tagged module/crates identified and removed from the end of the washing line (same label 1/1 to 1/12)*

1/1 to 1/4 pre-washed controls - commercial pre-wash and soak tank only
1/5 to 1/8 washed controls - dip for 3 minutes in US bath but ultrasonics swtched OFF
1/9 to 1/12 dip for 3 minutes in US bath at 60°C with ultrasonics switched on at 4 kW.

FF2 *Tagged module/crates identified and removed from the end of the washing line (same label 1/13 to 1/24)*

1/13 to 1/16 pre-washed controls - commercial pre-wash and soak tank only
1/17 to 1/20 washed controls - dip for 3 minutes in US bath but ultrasonics swtched OFF
1/21 to 1/24 dip for 3 minutes in US bath at 60°C with ultrasonics switched on at 4 kW.

Drain and pack away ultrasound equipment and depart.

APPENDIX 3b

Full results from the programme of factory Trials A to F

Results are presented in tabulated form, one set of tables for each trial. Analysis of the data is given in the main text.

Notes:

1) The data for the unwashed treatments (UT), also called unwashed controls elsewhere, are combined for the complete day, rather than morning and afternoon;- as such they were more representative and based on a larger sample.

2) Data in the spreadsheets behind Appendix 3b were generally calculated to 6 decimal places so rounding errors occur when reducing the results to 1 decimal place as included here and in the tables in Section 4 of the report.

3) Experiment codes are in line with those given in Appendix 3a.

All temperatures are ambient unless shown otherwise. "Hot" wash was a nominal 60^{o}C.

Abbreviations used in the following tables:

C:- Control Washed Trial

T:- Experimental trial

UT:- Un-Treated; AKA UnWashed Controls

PCA:- Total Aerobes

VRBG:- Enterobacteriaceae

CCDA:- *Campylobacter*

Trial Series A - 12-16 July 2004

Microbiological results

		Trial A1 (A)- 12/7 Mon pm						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1								
2	C	15sec prewash,30sec soak,15 sec main wash	7.5	**7.7**	0.2	5.7	**6.0**	0.2
3	C	15sec prewash,30sec soak,15 sec main wash	7.8			5.9		
4	C	15sec prewash,30sec soak,15 sec main wash	7.9			6.0		
5	C	15sec prewash,30sec soak,15 sec main wash	7.6			6.2		
6	T A1	15sec prewash,5 mins soak,15 sec main wash Ambient temp	7.8	**7.8**	0.1	6.2	**5.6**	0.2
7	T A1	15sec prewash,5 mins soak,15 sec main wash Ambient temp	7.9			5.8		
8	T A1	15sec prewash,5 mins soak,15 sec main wash Ambient temp	7.7			5.4		
9	T A1	15sec prewash,5 mins soak,15 sec main wash Ambient temp	7.7			5.6		
10								
11	UT	Unwashed control	8.0	8.0	0.0	6.0	5.9	0.2
12	UT	Unwashed control	8.0			5.8		
		Tank Water (counts per ml)	5.3			3.8		

		Trial A2 (B) - 13/7 Tues am						
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	C	15sec prewash,30sec soak,15 sec main wash	7.8	**9.4**	0.9	8.1	**8.0**	0.8
2	C	15sec prewash,30sec soak,15 sec main wash	9.5			9.4		
3	C	15sec prewash,30sec soak,15 sec main wash	9.7			7.2		
4	C	15sec prewash,30sec soak,15 sec main wash	10.2			7.5		
5	C	15sec prewash,30sec soak,15 sec main wash	9.6			7.7		
6	A2							
7	A2							
8	A2							
9	A2							
10	A2							
11	UT	Unwashed control	8.5	8.5	0.0	8.1	7.7	0.6
12	UT	Unwashed control	8.5			7.3		
		Tank Water (counts per ml)	7.0					

		Trial A3 (C)- 14/7 Wed am						
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	C	15sec prewash,30sec soak,15 sec main wash	8.7	**8.6**	0.1	8.3	**7.8**	0.5
2	C	15sec prewash,30sec soak,15 sec main wash	8.7			8.0		
3	C	15sec prewash,30sec soak,15 sec main wash	8.5			7.8		
4	C	15sec prewash,30sec soak,15 sec main wash	8.5			6.9		
5	C	15sec prewash,30sec soak,15 sec main wash	8.6			8.1		
6	TA3	No prewash, 30sec soak, 60sec main wash Ambient temp	8.5	**8.7**	1.1	7.8	**9.2**	1.6
7	TA3	No prewash, 30sec soak, 60sec main wash Ambient temp	10.5			10.2		
8	TA3	No prewash, 30sec soak, 60sec main wash Ambient temp	7.8			10.6		
9	TA3	No prewash, 30sec soak, 60sec main wash Ambient temp	7.8			10.4		
10	TA3	No prewash, 30sec soak, 60sec main wash Ambient temp	8.6			7.3		
11	UT	Unwashed control	9.1	9.0	0.2	8.7	8.4	0.4
12	UT	Unwashed control	8.8			8.1		
		Tank Water (counts per ml)	3.7					

		Trial A4 & A5 (D)- 14/7 Wed pm						
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	TA4	No prewash, 30sec soak, 5min main wash	8.1	**8.2**	0.3	6.6	**6.4**	0.7
2	TA4	No prewash, 30sec soak, 5min main wash	8.4			7.2		
3	TA4	No prewash, 30sec soak, 5min main wash	8.3			6.8		
4	TA4	No prewash, 30sec soak, 5min main wash	7.8			5.6		
5	TA4	No prewash, 30sec soak, 5min main wash	8.5			5.7		
6	TA5	5min prewash, 30sec soak, 5min main wash	8.0	**8.4**	0.3	6.5	**6.4**	1.3
7	TA5	5min prewash, 30sec soak, 5min main wash	8.5			6.6		
8	TA5	5min prewash, 30sec soak, 5min main wash	8.1			7.9		
9	TA5	5min prewash, 30sec soak, 5min main wash	8.8			4.2		
10	TA5	5min prewash, 30sec soak, 5min main wash	8.6			6.5		
11	UT	Unwashed control	9.8	9.5	0.4	8.8	8.1	0.9

12	UT	Unwashed control	9.3			7.5		
		Tank Water (counts per ml)	5.1					

Trial A6 (E) - 15/7 Thurs am								
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	C	15sec prewash,30sec soak,15 sec main wash	8.8	**8.7**	0.3	6.2	**6.6**	0.4
2	C	15sec prewash,30sec soak,15 sec main wash	9.1			6.1		
3	C	15sec prewash,30sec soak,15 sec main wash	8.3			6.7		
4	C	15sec prewash,30sec soak,15 sec main wash	8.5			7.2		
5	C	15sec prewash,30sec soak,15 sec main wash	8.7			6.7		
6	TA6	15sec prewash, 5min soak (40C), 15sec main wash	9.7	**8.3**	0.8	6.0	**6.0**	0.5
7	TA6	15sec prewash, 5min soak (40C), 15sec main wash	8.3			6.6		
8	TA6	15sec prewash, 5min soak (40C), 15sec main wash	8.3			6.3		
9	TA6	15sec prewash, 5min soak (40C), 15sec main wash	8.2			5.3		
10	TA6	15sec prewash, 5min soak (40C), 15sec main wash	7.3			6.1		
11	UT	Unwashed control	8.2	8.3	0.1	7.1	7.1	
12	UT	Unwashed control	8.4					
		Tank Water (counts per ml)						

Trial A7 & A8 (F) - 15/7 Thurs pm								
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	TA7	15sec prewash, 30sec soak (40C), 15 sec main wash	8.0	**8.0**	0.3	8.0	**7.2**	1.2
2	TA7	15sec prewash, 30sec soak (40C), 15 sec main wash	8.3			7.9		
3	TA7	15sec prewash, 30sec soak (40C), 15 sec main wash	7.9			5.9		
4	TA7	15sec prewash, 30sec soak (40C), 15 sec main wash	7.5			6.0		
5	TA7	15sec prewash, 30sec soak (40C), 15 sec main wash	8.1			8.4		
6	TA8	15sec prewash, 5min soak (60C nom), 15sec main wash		**7.3**	0.4	6.0	**5.6**	1.1
7	TA8	15sec prewash, 5min soak (60C nom), 15sec main wash	7.6			6.3		
8	TA8	15sec prewash, 5min soak (60C nom), 15sec main wash				5.9		
9	TA8	15sec prewash, 5min soak (60C nom), 15sec main wash				6.3		
10	TA8	15sec prewash, 5min soak (60C nom), 15sec main wash	7.0			3.6		
11	UT	Unwashed control	9.7	9.6	0.2	6.0	7.1	1.5
12	UT	Unwashed control	9.5			8.1		
		Tank Water (counts per ml)						

Trial A9 (G) - 16/7 Fri am								
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	TA9	No prewash, 1min soak*, no main wash	8.9	**8.7**	0.3	7.2	**7.3**	0.6
2	TA9	No prewash, 1min soak*, no main wash	8.5			8.0		
3	TA9	No prewash, 1min soak*, no main wash	8.4			7.4		
4	TA9	No prewash, 1min soak*, no main wash	9.1			7.6		
5	TA9	No prewash, 1min soak*, no main wash	8.6			6.4		
6	C	15sec prewash,30sec soak,15 sec main wash	9.5	**8.3**	0.7	5.6	**6.3**	1.0
7	C	15sec prewash,30sec soak,15 sec main wash	7.8			6.0		
8	C	15sec prewash,30sec soak,15 sec main wash	7.8			5.5		
9	C	15sec prewash,30sec soak,15 sec main wash	8.5			6.7		
10	C	15sec prewash,30sec soak,15 sec main wash	7.8			7.9		
11	UT	Unwashed control	8.2	9.1	1.3	7.9	8.8	1.2
12	UT	Unwashed control	10.0			9.6		
		Tank Water (counts per ml)						

Trial A10 (H) - 16/7 Fri pm								
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	TA10	No prewash, no soak, 5min main wash	9.9	**10.0**	0.2	5.7	**6.9**	1.9
2	TA10	No prewash, no soak, 5min main wash	9.8			7.1		
3	TA10	No prewash, no soak, 5min main wash	9.9			5.6		
4	TA10	No prewash, no soak, 5min main wash	10.0			5.7		
5	TA10	No prewash, no soak, 5min main wash	10.4			10.2		
6	UT	Unwashed control	12.4			12.2		
		Tank Water (counts per ml)	4.4					

Trial Series A - 12-16 July 2004

Visual assessment

Mon 12 July

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
2	3	0.5	1	4.5	0.5	0.5	1	2.0		
3	2	0.5	4	6.5	1.5	0.5	2	4.0		
4	1	1	3	5.0	1	0.5	1.5	3.0		
5	4	2	4	10.0	3	1.5	1	5.5		
AVE	**2.5**	**1.0**	**3.0**	**6.5**	**1.5**	**0.8**	**1.4**	**3.6**	**56**	**60**
6	5	1	4	10.0	4	1	2	7.0		
7	2	0.5	5	7.5	1.5	0	4	5.5		
8	2	0.5	5	7.5	2	0.5	3	5.5		
9	4	1	6	11.0	3	1	3	7.0		
AVE	**3.3**	**0.8**	**5.0**	**9.0**	**2.6**	**0.6**	**3.0**	**6.3**	**69**	**81**

Tues 8 July am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	4	2	4	10.0	2	1	2	5.0		
2	6	2	6	14.0	2	1	2	5.0		
3	3	1	4	8.0	3	1	3	7.0		
4	5	1	4	10.0	3	1	2	6.0		
5	6	2	3	11.0	4	1	2	7.0		
AVE	**4.8**	**1.6**	**4.2**	**10.6**	**2.8**	**1.0**	**2.2**	**6.0**	**57**	**58**

Wed 14 July am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	4	2	3	9.0	2.5	2	3	7.5		
2	4	2	4	10.0	2	1.5	2	5.5		
3	4	2	4	10.0	2	2	2	6.0		
4	3	2	4	9.0	2	1.5	3	6.5		
5	4	1	4	9.0	1.5	1.5	2	5.0		
AVE	**3.8**	**1.8**	**3.8**	**9.4**	**2.0**	**1.7**	**2.4**	**6.1**	**65**	**53**
6	3	1	4	8.0	1	1	2	4.0		
7	3	0.5	4	7.5	3	0.5	2.5	6.0		
8	3.5	2	3	8.5	1	2	1	4.0		
9	3.5	1.5	3	8.0	1	1	2	4.0		
10	2.5	1	3	6.5	0.5	0.5	1	2.0		
AVE	**3.1**	**1.2**	**3.4**	**7.7**	**1.3**	**1.0**	**1.7**	**4.0**	**52**	**42**

Wed 14 July pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	2	5	10.0	2	1	2	5.0		
2	5	2	6	13.0	2	1	3	6.0		
3	5	0.5	6	11.5	0.5	0.5	1	2.0		
4	2	1	5	8.0	0.5	1	2	3.5		
5	2	0.5	4	6.5	0.5	1.5	1	3.0		
AVE	**3.4**	**1.2**	**5.2**	**9.8**	**1.1**	**1.0**	**1.8**	**3.9**	**40**	**32**
6	2	1	5	8.0	1.5	1	1.5	4.0		
7	3	3	4	10.0	2	2	1.5	5.5		
8	3.5	0.5	5	9.0	2.5	1	1.5	5.0		
9	1.5	1	4	6.5	0.5	1	1	2.5		
10	3	1	4	8.0	1	1	1	3.0		
AVE	**2.6**	**1.3**	**4.4**	**8.3**	**1.5**	**1.2**	**1.3**	**4.0**	**48**	**58**

Thurs 15 July am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	10	4	5	19.0	3	1	3	7.0		
2	10	4	7	21.0	1	0.5	1.5	3.0		
3	10	2	5	17.0	3	1	2	6.0		
4	7	1	5	13.0	0.5	0.5	1	2.0		

5	6	1	4	11.0	1	0.5	1.5	3.0		
AVE	**8.6**	**2.4**	**5.2**	**16.2**	**1.7**	**0.7**	**1.8**	**4.2**	**26**	**20**
6	5	3	6	14.0	0.5	0.5	2	3.0		
7	6	3	6	15.0	1	0.5	1	2.5		
8	4	0.5	5	9.5	0.5	0.5	2	3.0		
9	4.5	2	4.5	11.0	0.5	1	1	2.5		
10	5.5	1	4	10.5	0.5	1	1	2.5		
AVE	**5.0**	**1.9**	**5.1**	**12.0**	**0.6**	**0.7**	**1.4**	**2.7**	**23**	**12**

Thurs 15 July pm

	Before (g)				**After (g)**				**Improvement - total**	**Improvement - I Base**
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	8	2	4.5	14.5	3	1.5	4	8.5		
2	5	2	6	13.0	3	1	4.5	8.5		
3	3	1	3	7.0	0.5	0.5	1	2.0		
4	3	2	4	9.0	0.5	0.5	1.5	2.5		
5	4.5	2	5	11.5	1	1	2.5	4.5		
AVE	**4.7**	**1.8**	**4.5**	**11.0**	**1.6**	**0.9**	**2.7**	**5.2**	**47**	**34**
6	4	1	5	10.0	0.5	0.5	1.5	2.5		
7	5	2	6	13.0	1	1	2	4.0		
8	3	2	7	12.0	2	1	4	7.0		
9	3	2	4	9.0	1	1	2	4.0		
10	5.5	3	4	12.5	2	2	2	6.0		
AVE	**4.1**	**2.0**	**5.2**	**11.3**	**1.3**	**1.1**	**2.3**	**4.7**	**42**	**32**

Fri 16 July am

	Before (g)				**After (g)**				**Improvement - total**	**Improvement - I Base**
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	4	2	4	10.0	3	1	3	7.0		
2	5	2	6	13.0	4	2	5	11.0		
3	3	1	3	7.0	3	1	3	7.0		
4	3	2	4	9.0	2	1	3	6.0		
5	3	1	3	7.0	2	1	3	6.0		
AVE	**3.6**	**1.6**	**4.0**	**9.2**	**2.8**	**1.2**	**3.4**	**7.4**	**80**	**78**
6	4	2	4	10.0	1.5	1	2	4.5		
7	2.5	1	2	5.5	1	0.5	1	2.5		
8	2	1	3.5	6.5	0.5	0.5	1	2.0		
9	3	2	4	9.0	1	1	1.5	3.5		
10	3	1	4	8.0	1	0.5	1	2.5		
AVE	**2.9**	**1.4**	**3.5**	**7.8**	**1.0**	**0.7**	**1.3**	**3.0**	**38**	**34**

Fri 16 July pm

	Before (g)				**After (g)**				**Improvement - total**	**Improvement - I Base**
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	3	7.0	0.5	1	1.5	3.0		
2	4	2	6	12.0	1	0.5	2	3.5		
3	2.5	1	4	7.5	1	0.5	1	2.5		
4	3	1	3	7.0	0.5	1	1	2.5		
5	3	2	4	9.0	0.5	0.5	1	2.0		
AVE	**3.1**	**1.4**	**4.0**	**8.5**	**0.7**	**0.7**	**1.3**	**2.7**	**32**	**23**

Trial Series B: 13-17th September 2004 and 6-7th October 2004

Microbiological results

		Trial B1 (A) - Mon 13/09 pm						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	8.1	**8.1**	0.3	7.2	**7.3**	0.2
2	C	15sec prewash,30sec soak,15 sec main wash	7.8			7.2		
3	C	15sec prewash,30sec soak,15 sec main wash	7.9			7.3		
4	C	15sec prewash,30sec soak,15 sec main wash	7.9			7.5		
5	C	15sec prewash,30sec soak,15 sec main wash	8.6			7.6		
6	B1	5 minutes main wash	7.9	**8.3**	0.5	7.5	**7.4**	0.7
7	B1	5 minutes main wash	9.4			8.1		
8	B1	5 minutes main wash	7.8			6.3		
9	B1	5 minutes main wash	8.1			7.5		
10	B1	5 minutes main wash	8.1			7.3		
11	UN	Unwashed controls	8.6	8.2	0.6	7.4	7.5	0.1
12	UN	Unwashed controls	7.8			7.5		
		Tank Water (counts per ml)	*7.1*			*5.2*		

		Trial B2 (B) - Tues 14/09 am						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	7.9	7.9	0.2	7.1	7.1	0.4
2	C	15sec prewash,30sec soak,15 sec main wash	7.7			6.6		
3	C	15sec prewash,30sec soak,15 sec main wash	7.7			7.3		
4	C	15sec prewash,30sec soak,15 sec main wash	8.1			7.0		
5	C	15sec prewash,30sec soak,15 sec main wash	8.0			7.6		
6	B2	15 sec prewash, 30 sec soak +	7.8	7.6	0.1	6.9	7.0	0.1
7	B2	15 sec main wash/rinse with clean cold water	7.5			7.0		
8	B2	" "	7.6			6.8		
9	B2	" "	7.5			7.1		
10	B2	" "	7.4			7.0		
11	UN	Unwashed controls	9.9	8.4	2.1	7.6	7.6	0.0
12	UN	Unwashed controls	7.0			7.5		
		Tank Water (counts per ml)	*7.2*			*6.9*		

		Trial B3 & B4(C) - Tues 14/09 pm						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
1	B3	15sec prewash,30sec soak, I min air jet +	7.7	7.7	0.1	6.6	6.9	0.2
2	B3	15 sec wash with clean cold water	7.8			6.8		
3	B3	" "	7.6			7.0		
4	B3	" "	7.6			7.3		
5	B3	" "	7.6			6.8		
6	B4	15sec prewash,30sec soak +	7.8	7.8	0.1	6.8	6.8	0.1
7	B4	15 sec rinse with clean hot water (55 - 60deg C).	7.9			6.7		
8	B4	" "	7.5			6.8		
9	B4	" "	7.8			6.8		
10	B4	" "	7.8			7.1		
11	UN	Unwashed controls	7.9	7.9	0.1	8.8	8.9	0.2
12	UN	Unwashed controls	7.8			9.0		
		Tank Water (counts per ml)	*7.7*			*7.4*		

		Trial B5 (D) - Wed 15/09 am						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	7.5	7.4	0.2	7.3	6.9	0.4
2	C	15sec prewash,30sec soak,15 sec main wash	7.6			6.4		
3	C	15sec prewash,30sec soak,15 sec main wash	7.5			6.9		
4	C	15sec prewash,30sec soak,15 sec main wash	7.1			7.2		
5	C	15sec prewash,30sec soak,15 sec main wash	7.3			6.6		
6	B5	15sec prewash,30sec soak, 1 min airjet +	9.6	8.8	0.9	6.3	6.5	0.2
7	B5	20 sec wash clean hot water nominal 60 deg C	8.1			6.4		
8	B5	" "	9.4			6.7		
9	B5	" "	9.5			6.9		
10	B5	" "	7.5			6.4		
11	UN	Unwashed controls	7.6	8.1	0.7	7.2	6.3	1.3
12	UN	Unwashed controls	8.6			5.4		

		Tank Water (counts per ml)						

		Trial B6 (D) - Wed 15/09 pm						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
		solid base						
13	B6	15sec prewash,30sec soak +	8.1	9.1	0.6	7.4	7.4	1.0
14	B6	1 min airjet, 20 sec wash clean hot water 55 deg C	9.2			6.7		
15	B6	" "	9.7			9.1		
16	B6	" "	9.2			7.0		
17	B6	" "	9.4			6.6		
18	UN	Unwashed controls	8.1	9.0	1.3	7.4	7.5	0.1
19	UN	Unwashed controls	10.0			7.6		
		Tank Water (counts per ml)	*5.3*			*4.7*		

		Trial B7 (E) - Wed 06/10 am						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	9.3	9.4	0.4	6.7	7.0	0.1
2	C	15sec prewash,30sec soak,15 sec main wash	9.3			7.0		
3	C	15sec prewash,30sec soak,15 sec main wash	9.0			7.0		
4	C	15sec prewash,30sec soak,15 sec main wash	9.3			7.1		
5	C	15sec prewash,30sec soak,15 sec main wash	10.1			7.0		
7	B7	Standard prewash, standard soak +	9.2	9.0	0.2	7.1	6.9	0.4
8	B7	15 sec vibration & cold rinse	9.2			6.9		
9	B7	" "	8.9			6.8		
10	B7	" "	8.9			6.3		
11	B7	" "	8.9			7.2		
6	UN	Unwashed controls	11.3	10.1	1.7	6.8	7.6	1.1
12	UN	Unwashed controls	8.9			8.3		
		Tank Water (counts per ml)						

		Trial B8 & B9 (F) - Wed 06/10 pm						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
1	B8	Standard prewash, standard soak +	8.9	8.9	0.2	6.3	6.5	0.4
2	B8	15 sec vibration & hot (60 deg C)rinse	9.1			6.0		
3	B8	" "	8.8			6.8		
4	B8	" "	9.1			7.0		
5	B8	" "	8.5			6.6		
7	B9	Standard prewash, standard soak +	9.7	9.6	0.6	6.4	6.7	0.4
8	B9	15 sec vibration + 60 secs air jet & hot (60 deg C) rinse	10.3			6.4		
9	B9	" "	10.1			6.9		
10	B9	" "	8.9			6.3		
11	B9	" "	9.0			7.2		
6	UN	Unwashed controls	9.9			7.2		
12	UN	Unwashed controls	10.1	10.0	0.2	6.3	6.7	0.6
		Tank Water (counts per ml)	*7.2*					

		Trial B10 (G) - Thurs 07/10 am						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
1	C	Standard prewash, standard soak	9.3	9.0	0.3	9.3	9.0	0.3
2	C	Standard prewash, standard soak	8.4			8.4		
3	C	Standard prewash, standard soak	9.2			9.2		
4	C	Standard prewash, standard soak	9.0			9.0		
5	C	Standard prewash, standard soak	8.9			8.9		
7	B10	Standard prewash, standard soak +	8.9	8.8	0.4	8.9	8.8	0.4
8	B10	15 sec vibration + 60 secs air jet & cold rinse	9.3			9.3		
9	B10	" "	9.0			9.0		
10	B10	" "	8.6			8.6		
11	B10	" "	8.4			8.4		
6	UN	Unwashed controls	9.1			9.1		
12	UN	Unwashed controls	11.0	10.0	1.4	11.0	10.0	1.4
		Tank Water (counts per ml)	*7.4*			*7.4*		

		Trial B11 (H) - 07/10 pm						
		Treatment	Log - PCA	***average***	*STDEV*	Log - VRBG	***average***	*STDEV*
		solid base						
1	B11	Standard prewash, standard soak +	9.8	9.7	0.2	8.3	8.3	0.1

2	B11	15 sec vibration + 60 secs air jet & 15 secs hot rinse 60 deg C	9.8			8.2		
3	B11	" "	9.8			8.3		
4	B11	" "	9.4			8.1		
5	B11	" "	9.9			8.3		
6	UN	Unwashed controls	11.6	11.6	0.1	9.2	9.4	0.3
7	UN	Unwashed controls	11.5			9.7		
		Tank Water (counts per ml)	*7.5*			*7.0*		

Trial Series B: 13-17th September 2004 and 6-7th October 2004

Visual assessment

Mon 13 Sept pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	4	2	2	8.0	1.5	2	2	5.5		
2	5	1	1.5	7.5	1	0.5	2	3.5		
3	3.5	2	2.5	8.0	1.5	1	0.5	3.0		
4	2.5	1.5	2	6.0	1	1.5	2	4.5		
5	4	2	2	8.0	1.5	1	2	4.5		
	3.8	**1.7**	**2.0**	**7.5**	**1.3**	**1.2**	**1.7**	**4.2**	**56**	**34**
6	4.5	1	1.5	7.0	0.5	0.5	0.5	1.5		
7	2	1	2	5.0	0.5	0.5	1	2.0		
8	4.5	1	1.5	7.0	0.5	0.5	1.5	2.5		
9	3	2	2	7.0	2.5	1	1.5	5.0		
10	1.5	1	1	3.5	2	0.5	0.5	3.0		
AVE	**3.1**	**1.2**	**1.6**	**5.9**	**1.2**	**0.6**	**1.0**	**2.8**	**47**	**39**

Tues 14 Sept am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	4	1.5	1	6.5	1.5	0.5	1.5	3.5		
2	3	2	1.5	6.5	1	0.5	0.5	2.0		
3	5	2	2	9.0	0.5	2	2	4.5		
4	2	1	1.5	4.5	1	1	1	3.0		
5	3	1.5	1	5.5	1	1	0.5	2.5		
AVE	**3.4**	**1.6**	**1.4**	**6.4**	**1.0**	**1.0**	**1.1**	**3.1**	**48**	**29**
6	2	1.5	1	4.5	1	0.5	1	2.5		
7	3	2	2	7.0	0.5	2	1.5	4.0		
8	2	1.5	1.5	5.0	0.5	0.5	1	2.0		
9	3	1.5	1.5	6.0	0.5	0.5	1	2.0		
10	2.5	2.5	2	7.0	0.5	1.5	1	3.0		
AVE	**2.5**	**1.8**	**1.6**	**5.9**	**0.6**	**1.0**	**1.1**	**2.7**	**46**	**24**

Tues 14 Sept pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	1.5	0.5	1	3.0	0.5	0	0.5	1.0		
2	4	1	1.5	6.5	0	0	0.5	0.5		
3	2	0.5	1	3.5	0	0	0.5	0.5		
4	4	1.5	1	6.5	0	0	0.5	0.5		
5	3	1.5	1	5.5	0	0	1	1.0		
AVE	**3.0**	**2.9**	**1.0**	**1.1**	**5.0**	**0.1**	**0.0**	**0.6**	**55**	**167**
6	2	1	1	4.0	1.5	0.5	1	3.0		
7	3	1	3	7.0	1	0.5	1.5	3.0		
8	2	1.5	1	4.5	1.5	1	1	3.5		
9	1	1	1	3.0	1	0.5	0.5	2.0		
10	2	2	1	5.0	1	0.5	1.5	3.0		
AVE	**8.0**	**2.0**	**1.3**	**1.4**	**4.7**	**1.2**	**0.6**	**1.1**	**79**	**59**

Wed 15 Sept am/pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	5	1.5	2	8.5	1.5	1	1	3.5		
2	4	1.5	2	7.5	2	0.5	2	4.5		
3	4	1	2	7.0	0.5	0	0.5	1.0		
4	3	1.5	2	6.5	2	1	2	5.0		
5	6	2	3	11.0	0.5	0.5	1	2.0		
AVE	**4.4**	**1.5**	**2.2**	**8.1**	**1.3**	**0.6**	**1.3**	**3.2**	**40**	**30**
6	4.5	2	3	9.5	0.5	0	0.5	1.0		
7	6	2.5	2	10.5	0.5	0	0.5	1.0		
8	2	1.5	2	5.5	0	0.5	1	1.5		
9	2	1.5	4	7.5	0	0	0.5	0.5		
10	4	1	2.5	7.5	0.5	0.5	0.5	1.5		
AVE	**3.7**	**1.7**	**2.7**	**8.1**	**0.3**	**0.2**	**0.6**	**1.1**	**14**	**8**
11	4	2	0.5	6.5	0	1	0	1.0		

12	3.5	2	0.5	6.0	0	0	0	0.0		
13	4	3	0.5	7.5	0	0.5	0	0.5		
14	4.5	2	0	6.5	1	0.5	0.5	2.0		
15	6	3	0.5	9.5	0	0.5	0	0.5		
AVE	**4.4**	**2.4**	**0.4**	**7.2**	**0.2**	**0.5**	**0.1**	**0.8**	**11**	**5**

Wed 6 Oct am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	1.5	5.5	1	1	1.5	3.5		
2	4	1	2	7.0	2	1	2	5.0		
3	3	1	4	8.0	2	2	2	6.0		
4	3	1	4	8.0	2	1	3	6.0		
5	3	2	3	8.0	2	2	2.5	6.5		
AVE	**3.2**	**1.2**	**2.9**	**7.3**	**1.8**	**1.4**	**2.2**	**5.4**	**74**	**56**
6	3	1	1.5	5.5	2	2	1.5	5.5		
7	2	1	3	6.0	1	1	3	5.0		
8	4	1	3	8.0	1.5	1.5	3	6.0		
9	1.5	1	2	4.5	0.5	1	1.5	3.0		
10	3	2	2	7.0	0.5	1	1.5	3.0		
AVE	**2.7**	**1.2**	**2.3**	**6.2**	**1.1**	**1.3**	**2.1**	**4.5**	**73**	**41**

Wed 6 Oct pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	0.5	2	5.5	1	0	1.5	2.5		
2	2	0.5	3	5.5	0.5	0.5	2	3.0		
3	2	0.5	1	3.5	1.5	0.5	1	3.0		
4	3	0.5	2	5.5	1.5	1	1.5	4.0		
5	2	0.5	2	4.5	1	0.5	2	3.5		
AVE	**2.4**	**0.5**	**2.0**	**4.9**	**1.1**	**0.5**	**1.6**	**3.2**	**65**	**46**
6	2	0.5	2	4.5	0	0	0	0.0		
7	3	0.5	2	5.5	1	0.5	1.5	3.0		
8	2.5	1	2	5.5	0.5	0	1	1.5		
9	2	1	2.5	5.5	1	0	2	3.0		
10	2.5	1	3	6.5	1	0	2	3.0		
AVE	**2.4**	**0.8**	**2.3**	**5.5**	**0.7**	**0.1**	**1.3**	**2.1**	**38**	**29**

Thurs 7 Oct am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	4	8.0	1	1	2	4.0		
2	4	2	4	10.0	1	1	2	4.0		
3	4	1	5	10.0	2	0.5	4	6.5		
4	2.5	1	2	5.5	1.5	1	1	3.5		
5	2.5	1	2	5.5	1	1	2	4.0		
AVE	**3.2**	**1.2**	**3.4**	**7.8**	**1.3**	**0.9**	**2.2**	**4.4**	**56**	**41**
6	3	1	3	7.0	1	1	1	3.0		
7	4	2	4	10.0	2	1	2	5.0		
8	5	3	4	12.0	1	1	1	3.0		
9	2	2	3	7.0	0.5	0.5	1	2.0		
10	2	1	2	5.0	0	0	0	0.0		
AVE	**3.2**	**1.8**	**3.2**	**8.2**	**0.9**	**0.7**	**1.0**	**2.6**	**32**	**28**

Thurs 7 Oct pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	1	5.0	0.5	0.5	0.5	1.5		
2	3.5	1	2	6.5	1	0.5	1	2.5		
3	4	2	2	8.0	1	0.5	1	2.5		
4	5	3	3	11.0	1	1	2	4.0		
5	5	2	2	9.0	1	1	1	3.0		
AVE	**4.1**	**1.8**	**2.0**	**7.9**	**0.9**	**0.7**	**1.1**	**2.7**	**34**	**22**

Trial Series C - 25 to 28th October 2004

Microbiological results

		Trial C1 (A) - 25/10 Mon pm						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	8.6			7.5		
2	C	15sec prewash,30sec soak,15 sec main wash	8.9			8.3		
3	C	15sec prewash,30sec soak,15 sec main wash	8.8			8.0		
4	C	15sec prewash,30sec soak,15 sec main wash	8.7			7.7		
5	C	15sec prewash,30sec soak,15 sec main wash	8.9	**8.8**	0.1	8.0	**7.9**	0.3
6	C1	Control wash then 120 secs steam	9.2			7.8		
7	C1	Control wash then 120 secs steam	8.8			7.8		
8	C1	Control wash then 120 secs steam	8.8			6.8		
9	C1	Control wash then 120 secs steam	9.0			8.7		
10	C1	Control wash then 120 secs steam	8.7	**8.9**	0.2	7.4	**7.7**	0.6
11	UT	Unwashed control	10.3			9.3		
12	UT	Unwashed control	10.2	**10.3**		7.7	**8.5**	
		Tank Water (counts per ml)	*6.6*			*6.4*		

		Trial C2 (B) - 26/10 Tues am						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	9.03			8.30		
2	C	15sec prewash,30sec soak,15 sec main wash	9.18			8.28		
3	C	15sec prewash,30sec soak,15 sec main wash	8.48			7.36		
4	C	15sec prewash,30sec soak,15 sec main wash	8.40			8.52		
5	C	15sec prewash,30sec soak,15 sec main wash	9.70	**9.0**	0.5	8.53	**8.2**	0.4
6	C2	Contol wash; rinse with hot clean water; 250ml of 0.5% Virkon	8.38			6.45		
7	C2	Contol wash; rinse with hot clean water; 250ml of 0.5% Virkon	8.40			6.60		
8	C2	Contol wash; rinse with hot clean water; 250ml of 0.5% Virkon	8.63			6.89		
9	C2	Contol wash; rinse with hot clean water; 250ml of 0.5% Virkon	7.36			5.59		
10	C2	Contol wash; rinse with hot clean water; 250ml of 0.5% Virkon	8.51	**8.3**	0.5	6.51	**6.4**	0.4
11	UT	Unwashed control	9.94			7.85		
12	UT	Unwashed control	10.01	**10.0**		7.00	**7.4**	
		Tank Water (counts per ml)	*7.79*			*6.69*		

		Trial C3 & C4 (C) - 26/10 Tues pm						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C3	Contol wash; rinse with hot clean water; 60 secs air +	8.27			6.00		
2	C3	250ml of 0.5% Virkon	8.71			6.67		
3	C3	" " "	8.06			5.60		
4	C3	" " "	8.53			7.94		
5	C3	" " "	8.69	**8.5**	0.3	5.97	**6.4**	0.8
6	C4	Contol wash; rinse with cold clean water; 60 secs air +	8.18			7.60		
7	C4	250ml of 0.5% Virkon	8.79			6.53		
8	C4	" " "	7.12			7.33		
9	C4	" " "	8.25			5.90		
10	C4	" " "	8.39	**8.1**	0.6	5.26	**6.5**	0.9
11	UT	Unwashed control	10.00			9.54		
12	UT	Unwashed control	9.67	**9.8**		8.00	**8.8**	
		Tank Water (counts per ml)	*7.68*			*6.70*		

		Trial C5 (D) - 27/10 Wed am						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	8.76			7.30		
2	C	15sec prewash,30sec soak,15 sec main wash	8.65			6.86		
3	C	15sec prewash,30sec soak,15 sec main wash	8.09			6.41		
4	C	15sec prewash,30sec soak,15 sec main wash	9.78			6.84		
5	C	15sec prewash,30sec soak,15 sec main wash	8.84	**8.8**	0.5	7.94	**7.1**	0.5
6	C5	Contol wash; rinse with hot clean water; 60 secs air +	9.02			8.02		
7	C5	60 secs under u/v lamps	5.32			8.42		
8	C5	" " "	8.64			8.09		
9	C5	" " "	8.96			8.09		
10	C5	" " "	9.01	**8.2**	1.4	8.17	**8.2**	0.1

11	UT	Unwashed control	9.06			8.50		
12	UT	Unwashed control	8.08	**8.6**			**8.5**	
		Tank Water (counts per ml)						

		Trial C6 (E) - 27/10 Wed pm						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C(6)	15sec prewash,30sec soak,15 sec main wash; 1 min us soak	8.04			6.54		
2	C6L	15sec prewash,30sec soak,15 sec main wash; 1 min us 2 kW	9.07			7.15		
3	C6H	15sec prewash,30sec soak,15 sec main wash; 1 min us 4 kW	7.54			5.81		
4	C(6)	15sec prewash,30sec soak,15 sec main wash; 1 min us soak	9.18			6.64		
5	C6L	15sec prewash,30sec soak,15 sec main wash; 1 min us 2 kW	9.05			5.70		
6	C6H	15sec prewash,30sec soak,15 sec main wash; 1 min us 4 kW	7.98			6.15		
7	C(6)	15sec prewash,30sec soak,15 sec main wash; 1 min us soak	9.01			6.98		
8	C6L	15sec prewash,30sec soak,15 sec main wash; 1 min us 2 kW	9.06			6.49		
9	C6H	15sec prewash,30sec soak,15 sec main wash; 1 min us 4 kW	9.17			6.56		
10	C(6)	15sec prewash,30sec soak,15 sec main wash; 1 min us soak	8.69			6.28		
11	C6L	15sec prewash,30sec soak,15 sec main wash; 1 min us 2 kW	8.11			6.74		
12	C6H	15sec prewash,30sec soak,15 sec main wash; 1 min us 4 kW						
	C(6)	No us (soak only)		**8.7**			**6.6**	
	C6L	2 kW us		**8.8**			**6.5**	
	C6H	4 kW us		**8.2**			**6.2**	
		Tank Water (counts per ml)						

		Trial C7 (F) - 28/10 Thurs am						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C	15sec prewash,30sec soak,15 sec main wash	8.99			7.33		
2	C	15sec prewash,30sec soak,15 sec main wash	8.69			5.43		
3	C	15sec prewash,30sec soak,15 sec main wash	5.14			4.72		
4	C	15sec prewash,30sec soak,15 sec main wash	9.05			8.48		
5	C	15sec prewash,30sec soak,15 sec main wash	9.34	**8.2**	1.6	8.68	**6.9**	1.6
6	C7	Contol wash; rinse with hot clean water + 120 secs steam	8.64			5.32		
7	C7	Contol wash; rinse with hot clean water + 120 secs steam	8.61			7.37		
8	C7	Contol wash; rinse with hot clean water + 120 secs steam	9.23			7.41		
9	C7	Contol wash; rinse with hot clean water + 120 secs steam	8.81			7.06		
10	C7	Contol wash; rinse with hot clean water + 120 secs steam	8.90	**8.8**	0.2	7.50	**6.9**	0.8
11	UT	Unwashed control	10.39			8.68		
12	UT	Unwashed control	8.41	**9.4**		7.60	**8.1**	
		Tank Water (counts per ml)	*6.97*			*6.09*		

		Trial C8 & C9 (G) - 28/10 Thurs pm						
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C8	Contol wash + 250ml of 0.5% Virkon	8.6			8.7		
2	C8	Contol wash + 250ml of 0.5% Virkon	8.9			7.4		
3	C8	Contol wash + 250ml of 0.5% Virkon	9.0			6.9		
4	C8	Contol wash + 250ml of 0.5% Virkon	9.3			7.2		
5	C8	Contol wash + 250ml of 0.5% Virkon	8.0	**8.8**	0.4	7.3	**7.5**	0.6
6	C9	Contol wash; rinse with hot clean water + 250ml of 0.5% Virkon	8.0			7.3		
7	C9	Contol wash; rinse with hot clean water + 250ml of 0.5% Virkon	7.6			7.0		
8	C9	Contol wash; rinse with hot clean water + 250ml of 0.5% Virkon	8.0			7.2		
9	C9	Contol wash; rinse with hot clean water + 250ml of 0.5% Virkon	8.0			7.1		
10	C9	Contol wash; rinse with hot clean water + 250ml of 0.5% Virkon	7.8	**7.9**	0.2	6.8	**7.1**	0.2
11	UT	Unwashed control + 250ml of 0.5% Virkon	9.7			7.5		
12	UT	Unwashed control + 250ml of 0.5% Virkon	7.9	**8.8**		7.1	**7.3**	
		Tank Water (counts per ml)	*7.7*			*6.1*		

Trial Series C - 25 to 28th October 2004

Visual assessment

Mon 25 Oct pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	3	7.0	1	0.5	1.5	3.0		
2	3	2	2	7.0	0.5	1	1.5	3.0		
3	3	2	3	8.0	1	1	1	3.0		
4	3	1	2	6.0	0.5	0.5	1.5	2.5		
5	4	2	4	10.0	0.5	0.5	2	3.0		
AVE	**3.2**	**1.6**	**2.8**	**7.6**	**0.7**	**0.7**	**1.5**	**2.9**	**38**	**22**
6	2	1	2	5.0	2	1	2	5.0		
7	3	1	2	6.0	2	1	2	5.0		
8	2	1	2	5.0	0.5	0.5	1	2.0		
9	2	1	3	6.0	0.5	0.5	1.5	2.5		
10	2	1	3	6.0	1	0.5	1.5	3.0		
AVE	**2.2**	**1.0**	**2.4**	**5.6**	**1.2**	**0.7**	**1.6**	**3.5**	**63**	**55**

Tues 26 Oct am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	6	3	6	15.0	2	1	2	5.0		
2	5	2	6	13.0	3	2	3	8.0		
3	4.5	2	4	10.5	3	1	3.5	7.5		
4	4	2	4	10.0	3	1	3	7.0		
5	3.5	1	3	7.5	2	1	2	5.0		
AVE	**4.6**	**2.0**	**4.6**	**11.2**	**2.6**	**1.2**	**2.7**	**6.5**	**58**	**57**
6	4	1	3	8.0	1.5	0.5	2	4.0		
7	3	1	4	8.0	2	1	3	6.0		
8	4	2	4	10.0	3	2	2	7.0		
9	3.5	2	3	8.5	1.5	1	2.5	5.0		
10	4.5	1	4	9.5	3	1	3	7.0		
AVE	**3.8**	**1.4**	**3.6**	**8.8**	**2.2**	**1.1**	**2.5**	**5.8**	**66**	**58**

Tues 26 Oct pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	6	1	2	9.0	2	1	2	5.0		
2	10	2	8	20.0	4	1	3.5	8.5		
3	10	1	6	17.0	3	1	4	8.0		
4	4	2	4	10.0	0.5	1	1.5	3.0		
5	11	3	8	22.0	2	1	2	5.0		
AVE	**8.2**	**1.8**	**5.6**	**15.6**	**2.3**	**1.0**	**2.6**	**5.9**	**38**	**28**
6	8	5	5	18.0	3	1	2	6.0		
7	7	1	3	11.0	2	1	1.5	4.5		
8	7	1	3	11.0	2	1	2.5	5.5		
9	6.5	2	4	12.5	1	1	2	4.0		
10	5	2	5	12.0	1	0.5	1.5	3.0		
AVE	**6.7**	**2.2**	**4.0**	**12.9**	**1.8**	**0.9**	**1.9**	**4.6**	**36**	**27**

Wed 27 Oct am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	2	5.0	0.5	0.5	2	3.0		
2	3	1	2	6.0	0.5	1	1	2.5		
3	3	1	2	6.0	0.5	1	2	3.5		
4	3	1	3	7.0	0.5	0.5	1.5	2.5		
5	3	1	2	6.0	0.5	0.5	1.5	2.5		
AVE	**2.8**	**1.0**	**2.2**	**6.0**	**0.5**	**0.7**	**1.6**	**2.8**	**47**	**18**
6	2	1	2	5.0	1.5	0.5	1.5	3.5		
7	3	1	2	6.0	0.5	0.5	2	3.0		
8	3	1	2	6.0	0.5	0.5	1	2.0		
9	3	1	3	7.0	1.5	1	1.5	4.0		
10	3	1	3	7.0	0.5	0	1.5	2.0		
AVE	**2.8**	**1.0**	**2.4**	**6.2**	**0.9**	**0.5**	**1.5**	**2.9**	**47**	**32**

Wed 27 Oct pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2.5	1	2	5.5	0	1	2	3.0		
4	3	1	2	6.0	0.5	0	0.5	1.0		
7	1.5	1	3	5.5	0.5	0.5	1.5	2.5		
10	1.5	0.5	1.5	3.5	1	1	2	4.0		
AVE	**2.1**	**0.9**	**2.1**	**5.1**	**0.5**	**0.6**	**1.5**	**2.6**	**51**	**24**
2	3	1	2	6.0	0	0.5	1.5	2.0		
5	2	1	2	5.0	0.5	0	1	1.5		
8	2.5	0.5	2	5.0	1	0	2	3.0		
11	2	0	2	4.0	0.5	0.5	2	3.0		
AVE	**2.4**	**0.6**	**2.0**	**5.0**	**0.5**	**0.3**	**1.6**	**2.4**	**48**	**21**
3	2.5	1	2	5.5	0	0	0.5	0.5		
6	2	1	2.5	5.5	0.5	0	0.5	1.0		
9	1	0	2	3.0	1	0	1.5	2.5		
12	2.5	1	2	5.5	0.5	0.5	1	2.0		
AVE	**2.0**	**0.8**	**2.1**	**4.9**	**0.5**	**0.1**	**0.9**	**1.5**	**31**	**25**

Thurs 28 Oct am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	3	6.0	1	0	0.5	1.5		
2	1	1	1	3.0	0	0	1	1.0		
3	2.5	1.5	1	5.0	1.5	1	0.5	3.0		
4	2	1.5	1.5	5.0	1.5	1	1.5	4.0		
5	3	1	1	**4.8**	2	0.5	1	**2.4**		
AVE	**2.1**	**1.3**	**1.1**	**4.8**	**1.3**	**0.6**	**1.0**	**2.4**	**50**	**59**
6	2.5	1	1.5	5.0	1	0.5	1	2.5		
7	2	2	2.5	6.5	0	0.5	0.5	1.0		
8	2.5	1	2	5.5	1.5	1	1.5	4.0		
9	1.5	1	1	3.5	1	0	0.5	1.5		
10	1	0.5	0.5	**5.1**	0.5	0	0.5	**2.3**		
AVE	**1.8**	**1.1**	**1.5**	**5.1**	**0.8**	**0.4**	**0.8**	**2.3**	**44**	**43**

Thurs 28 Oct pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	1.5	4.5	1.5	0.5	0.5	2.5		
2	2	2	2	6.0	1.5	1	1.5	4.0		
3	2.5	1	2	5.5	2	1	1	4.0		
4	3	1	2	6.0	1	1	1	3.0		
5	2	1	2	5.0	1	1	0.5	2.5		
AVE	**2.3**	**1.2**	**1.9**	**5.4**	**1.4**	**0.9**	**0.9**	**3.2**	**59**	**61**
6	4	1	3	8.0	0	0	0	0.0		
7	3	1	3	7.0	1	0.5	1	2.5		
8	2.5	0.5	2.5	5.5	0	0	0.5	0.5		
9	2.5	1.5	3	7.0	0	0	1	1.0		
10	2.5	1	2	5.5	0	0	0.5	0.5		
AVE	**2.9**	**1.0**	**2.7**	**6.6**	**0.2**	**0.1**	**0.6**	**0.9**	**14**	**7**

Trial Series D - 22 - 26th November

Microbiological results

Trial D1 (A) - 22/11 Mon pm								
		Treatment	*Log - PCA*	***average***	*STDEV*	*Log - VRBG*	***average***	*STDEV*
1	C	Control - 15sec prewash,30sec soak in hot (52deg.C)	7.8			7.2		
2	C	clean water; 15sec rinse with hot (63deg.C) clean water.	7.8			6.8		
3	C	" "	8.6			8.1		
4	C	" "	7.7			7.3		
5	C	" "	8.4	**8.1**	0.4	6.8	**7.2**	0.5
6	D1	15sec prewash,30sec soak in hot (52deg.C) clean water	6.6			5.9		
7	D1	300 secs brushing then 20 sec rinse with hot (63deg.C)	7.0			5.1		
8	D1	water	6.9			4.8		
9	D1	" "	6.7			4.3		
10	D1	" "	6.2	**6.7**	0.3	3.8	**4.8**	0.7
11	UT	Unwashed control	9.2			8.4		
12	UT	" "	9.4			8.5		
13	UT	" "	9.5			8.6		
14	UT	" "	8.1			8.7		
15	UT	" "	9.2	**9.1**	0.5	8.8	**8.6**	0.1
		Tank Water (counts per ml) at the end						

Trial D2 (B) - 23/11 Tues am								
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	C	Control - 15sec prewash,30sec cold soak,15 sec	8.0			7.0		
2	C	hot (63deg.C) rinse	8.1			7.3		
3	C	" "	9.6			7.3		
4	C	" "	10.1			7.7		
5	C	" "	9.9	**9.1**	0.9	8.4	**7.5**	0.5
6	D2	15sec prewash,30sec cold soak,15 sec	8.3			8.0		
7	D2	hot (63deg.C) rinse + 500ml Virkon at 1%	8.8			8.2		
8	D2	" "	8.6			6.7		
9	D2	" "	7.1			6.6		
10	D2	" "	8.5	**8.3**	0.6	6.4	**7.2**	0.8
11	UT	Unwashed control	8.9			8.6		
12	UT	Unwashed control	9.2			8.3		
13	UT	Unwashed control	9.1			8.4		
14	UT	Unwashed control	9.1			8.8		
15	UT	Unwashed control	9.2	**9.1**	0.1	8.7	**8.6**	0.2
		Tank Water (counts per ml)						

Trial D3 & D4 (C) - 23/11 Tues pm								
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	D3	15sec prewash,30sec cold soak,15 sec	4.3					
2	D3	hot (63deg.C) rinse + 500ml Virkon at 2%	7.8			6.6		
3	D3	" "	7.8			5.8		
4	D3	" "	9.3			5.0		
5	D3	" "	8.0	**7.4**	1.7	3.3	**5.2**	1.2
6	D4	15sec prewash,30sec cold soak,15 sec, 120 sec scrub	6.3			2.7		
7	D4	hot (63deg.C) rinse + 500ml Virkon at 2%	7.2			3.5		
8	D4	" "	6.7			2.7		
9	D4	" "	6.5			5.0		
10	D4	" "	7.1	**6.8**	0.4	3.0	**3.4**	0.8
11	UT	Unwashed control	9.4			8.8		
12	UT	Unwashed control	9.7	**9.6**	0.2	8.7	**8.8**	0.0
		Tank Water (counts per ml)						

Trial D5 (D) - 24/11 Wed am								
		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	UT	Unwashed control	9.4			8.4		
2	UT	Unwashed control	8.9			8.3		
3	UT	Unwashed control	9.5			7.1		
4	UT	Unwashed control	9.1			8.7		
5	UT	Unwashed control	9.4	**9.3**	0.3	8.5	**8.2**	0.6

6	C	Control - 15sec prewash,30sec cold soak,15 sec	8.4			6.7		
7	C	hot (63deg.C) rinse	8.9			8.4		
8	C	" "	8.7			6.8		
9	C	" "	8.7			7.0		
10	C	" "	9.7	**8.9**	0.4	8.5	**7.5**	0.8
11	D5	15sec prewash,30sec cold soak,15 sec	8.7			7.0		
12	D5	hot (63deg.C) rinse + 300 secs steam	8.1			6.7		
13	D5	" "	8.5			6.9		
14	D5	" "	8.7			6.8		
15	D5	" "	8.9	**8.6**	0.3	7.1	**6.9**	0.1
		Tank Water (counts per ml)						

Trial D6, D7 & D8 (E) - 24/11 Wed pm

		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	D6	15sec prewash,30sec cold soak,15 sec	8.5			6.9		
2	D6	hot (63deg.C) rinse + 300 secs blower	8.9			6.6		
3	D6	" "	8.6			5.9		
4	D6	" "	8.0			6.4		
5	D6	" "	8.7	**8.5**	0.3	6.8	**6.5**	0.3
6	D7	15sec prewash,30sec cold soak,15 sec hot (63deg.C)	8.9			6.7		
7	D7	rinse + 300 secs blower + 250 ml Virkon at 0.5%	8.3			7.3		
8	D7	" "	8.7			6.9		
9	D7	" "	8.6			7.0		
10	D7	" "	8.6	**8.6**	0.2	6.7	**6.9**	0.2
11	D8	70 secs in commercial tray cleaner (steam)	8.8			7.0		
12	D8	Oliver-Douglas"	9.4			6.6		
13	D8	" "	8.9			6.7		
14	D8	" "	6.6			6.8		
15	D8	" "	7.9	**8.3**	1.0	6.4	**6.7**	0.2
		Tank Water (counts per ml)						

Trial D9, D10 7 D11 (F) - 25/11 Thurs am/pm

		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	UT	Unwashed control	9.9			9.6		
2	UT	" "	10.3			7.5		
3	UT	" "	9.8			9.4		
4	UT	" "	10.3			9.3		
5	UT	" "	10.1	**10.1**	0.2	8.8	**8.9**	0.8
6	C	Control - 15sec prewash,30sec hot dirty watersoak,	9.0			6.9		
7	C	15 sec hot (63deg.C) rinse	9.0			7.3		
8	C	" "	8.9			5.2		
9	C	" "	8.4			8.5		
10	C	" "	8.7	**8.8**	0.2	6.5	**6.9**	1.1
11	D9	15sec prewash,30sec hot dirty watersoak	8.8			5.9		
12	D9	+ 0.1% detergent,15 sec hot (63deg.C) rinse x 1	7.3			6.8		
13	D9	" "	6.9			4.8		
14	D9	" "	6.5	**7.4**	0.9	5.5	**5.7**	0.7
16	D10	15sec prewash,30sec hot dirty watersoak	6.2			6.6		
17	D10	+ 0.1% detergent,15 sec hot (63deg.C) rinse x 2	7.0			4.4		
18	D10	" "	6.7			3.3		
19	D10	" "	6.3	**6.5**	0.3	4.7	**4.8**	1.2
21	D11	15sec prewash,30sec hot dirty watersoak	7.5			6.4		
22	D11	+ 0.1% detergent,15 sec hot (63deg.C) rinse x 3	7.6			4.4		
23	D11	" "	8.2			4.4		
24	D11	" "	6.6	**7.5**	0.6	3.5	**4.7**	1.1
		Tank Water (counts per ml) at the end	5.4					

Trial D12 (G) - 26/12 Fri am

		Treatment	Log - PCA	**average**	STDEV	Log - VRBG	**average**	STDEV
1	UT	Unwashed control	9.3			8.1		
2	UT	" "	9.5			5.0		
3	UT	" "	9.5			8.1		
4	UT	" "	9.8			8.3		
5	UT	" "	9.7	**9.5**	0.2	8.1	**7.5**	1.2
6	C	Control - 15sec prewash,30sec cold dirty soak,15 sec	9.9			8.1		

7	C	hot (60deg.C) rinse	9.6			5.2		
8	C	" "	8.9			8.4		
9	C	" "	8.8			7.9		
10	C	" "	9.8	**9.4**	0.4	7.6	**7.4**	1.1
11	D12	15sec prewash,30sec cold dirty soak + 0.1% detergent,	10.2			9.6		
12	D12	15 sec hot (60deg.C) rinse	10.2			8.6		
13	D12	" "	10.2			9.6		
14	D12	" "	10.1			9.5		
15	D12	" "	10.1	**10.1**	0.0	9.5	**9.3**	0.4
		Tank Water (counts per ml)	6.7					

Trial Series D - 22 - 26th November

Mon 22 Nov pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	3	6.0	0.5	0.5	1	2.0		
2	1.5	1	2	4.5	0.5	0.5	1	2.0		
3	4	2	4	10.0	0.5	0.5	1	2.0		
4	5	1.5	5	11.5	1	0.5	1.5	3.0		
5	4	3	3	10.0	1	1	2	4.0		
AVE	**3.3**	**1.7**	**3.4**	**8.4**	**0.7**	**0.6**	**1.3**	**2.6**	**31**	**21**
6	3	1	2	6.0	0	0	0	0.0		
7	6	1	4	11.0	0	0	1	1.0		
8	4	2	3	9.0	0	0	0	0.0		
9	3.5	2	2	7.5	0.5	0.5	1	2.0		
10	5	3	3	11.0	0	0.5	1	1.5		
AVE	**4.3**	**1.8**	**2.8**	**8.9**	**0.1**	**0.2**	**0.6**	**0.9**	**10**	**2**

Tues 23 Nov am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	2	5.0	1	1	1.5	3.5		
2	5	2	5.5	12.5	1	1	3	5.0		
3	5	2	4	11.0	2	1	3	6.0		
4	6	2	5	13.0	1.5	1	2	4.5		
5	5	2	4	11.0	2	1	2.5	5.5		
AVE	**4.6**	**1.8**	**4.1**	**10.5**	**1.5**	**1.0**	**2.4**	**4.9**	**47**	**33**
6	5	2	4	11.0	2	1	1.5	4.5		
7	4	1	3	8.0	1	0.5	1	2.5		
8	4	2	4	10.0	2	1	2	5.0		
9	4	2	4	10.0	2	1	2	5.0		
10	6	3	4	13.0	2.5	1.5	2	6.0		
AVE	**4.6**	**2.0**	**3.8**	**10.4**	**1.9**	**1.0**	**1.7**	**4.6**	**44**	**41**

Tues 23 Nov pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	3	7.0	1	1	2	4.0		
2	4	2	3	9.0	0.5	0.5	1.5	2.5		
3	3	1	3.5	7.5	1.5	1	2	4.5		
4	3	1	2	6.0	2	1.5	1	4.5		
5	3	2	4	9.0	1	1	2	4.0		
AVE	**3.2**	**1.4**	**3.1**	**7.7**	**1.2**	**1.0**	**1.7**	**3.9**	**51**	**38**
6	3	1	3	7.0	0	0.5	1	1.5		
7	4	1	4	9.0	0.5	0.5	2	3.0		
8	3	1	3	7.0	0	0.5	1	1.5		
9	3	1	3	7.0	0.5	0.5	1	2.0		
10	4	2	3	9.0	0	0.5	0.5	1.0		
AVE	**3.4**	**1.2**	**3.2**	**7.8**	**0.2**	**0.5**	**1.1**	**1.8**	**23**	**6**

Wed 24 Nov am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	3	7.0	2	1	2	5.0		
2	3	1	3	7.0	2	1	2	5.0		
3	3	1	2.5	6.5	1	1	2	4.0		
4	2	0.5	3	5.5	1	0.5	2	3.5		
5	2	1	3	6.0	1	0.5	2	3.5		
AVE	**2.6**	**0.9**	**2.9**	**6.4**	**1.4**	**0.8**	**2.0**	**4.2**	**66**	**54**
6	4	0.5	3	7.5	2	0.5	3	5.5		
7	3	0.5	4	7.5	2	0.5	3	5.5		
8	3	0.5	3	6.5	1	0.5	2	3.5		
9	4	0.5	3	7.5	0	0	0	0.0		
10	5	0.5	2.5	8.0	0	0	0	0.0		
AVE	**3.8**	**0.5**	**3.1**	**7.4**	**1.0**	**0.3**	**1.6**	**2.9**	**39**	**26**

Wed 24 Nov pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	0.5	4	7.5						
2	3	1	3	7.0						
3	2	1	4	7.0						
4	3	0.5	4	7.5						
5	3	1	3	7.0						
AVE	**2.8**	**0.8**	**3.6**	**7.2**						
6	3	1	4	8.0	3	1	3	7.0		
7	4	2	4	10.0	1	2	4	7.0		
8	3	2	4	9.0	1	1.5	2	4.5		
9	4	2	4	10.0	1	2	3	6.0		
10	4	2	4	10.0	2	0.5	4	6.5		
AVE	**3.6**	**1.8**	**4.0**	**9.4**	**1.6**	**1.4**	**3.2**	**6.2**	**66**	**44**
11	4	2	5	11.0	1	1	1.5	3.5		
12	4	1	5	10.0	0.5	0.5	1	2.0		
13	5	2	5	12.0	1	0.5	1	2.5		
14	5	2	5	12.0	1.5	0.5	1.5	3.5		
15	4	1	4	9.0	1	0.5	2	3.5		
AVE	**4.4**	**1.6**	**4.8**	**10.8**	**1.0**	**0.6**	**1.4**	**3.0**	**28**	**23**

hurs 25 Nov am/pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
6	3	1	3	7.0	0.5	0.5	0.5	1.5		
7	2	1	4	7.0	1	1	1	3.0		
8	4	2	4	10.0	2	1	2	5.0		
9	2.5	1	3	6.5	0.5	0.5	1.5	2.5		
10	2	0.5	2	4.5	0.5	0.5	0.5	1.5		
AVE	**2.7**	**1.1**	**3.2**	**7.0**	**0.9**	**0.7**	**1.1**	**2.7**	**39**	**33**
11	3	1	3	7.0	1	0.5	1	2.5		
12	3.5	1	3	7.5	0.5	1	1.5	3.0		
13	4	2	4	10.0						
14	3	2	3	8.0	1	1	2.5	4.5		
AVE	**3.4**	**1.5**	**3.3**	**8.1**	**0.8**	**0.8**	**1.7**	**3.3**	**41**	**25**
16	2	2	2	6.0	0.5	0.5	1	2.0		
17	2	2	3	7.0	0.5	0.5	1	2.0		
18	1.5	1	2	4.5	0.5	1	1	2.5		
19	4	1	2	7.0	0.5	0.5	0.5	1.5		
AVE	**2.4**	**1.5**	**2.3**	**6.1**	**0.5**	**0.6**	**0.9**	**2.0**	**33**	**21**
21	4	1	4	9.0	0.5	0.5	1	2.0		
22	4	1	3	8.0	0	0.5	0.5	1.0		
23	3.5	1	3	7.5	0	0.5	0.5	1.0		
24	3	1	4	8.0	0	0	1	1.0		
AVE	**3.6**	**1.0**	**3.5**	**8.1**	**0.1**	**0.4**	**0.8**	**1.3**	**15**	**3**

Fri 26 Nov pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	2	3	8.0	1	1	1	3.0		
2	2	1	3	6.0	0.5	0.5	1	2.0		
3	3	1	3	7.0	1	1	1.5	3.5		
4	4	1	3	8.0	1	0.5	1.5	3.0		
5	3	1	3	7.0	0.5	0.5	1.5	2.5		
AVE	**3.0**	**1.2**	**3.0**	**7.2**	**0.8**	**0.7**	**1.3**	**2.8**	**39**	**27**
6	4	1.5	3	8.5	1	1	2	4.0		
7	3	2	3.5	8.5	0.5	0.5	1	2.0		
8	2.5	1	2.5	6.0	0.5	0.5	1	2.0		
9	3	1	2	6.0	0.5	0.5	0.5	1.5		
10	2	1	2	5.0	0.5	0.5	1	2.0		
AVE	**2.9**	**1.3**	**2.6**	**6.8**	**0.6**	**0.6**	**1.1**	**2.3**	**34**	**21**

Trial Series E: 7 - 11th February

Microbiological results

		Trial E1 (A) - 7/2 Mon pm									
		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	9.3			8.3			7.7		
2	UT	" "	9.2			8.3			7.9		
3	UT	" "	9.0			8.5			7.6		
4	UT	" "	9.5			8.3			7.5		
5	UT	" "	8.9	**9.2**	0.2	8.2	**8.3**	0.1	7.7	**7.7**	0.1
6	C	Control - 15sec prewash,30sec soak in cold	8.8			8.3			6.6		
7	C	clean water; 15sec rinse with cold clean water.	8.7			7.7			6.0		
8	C	" "	8.9			8.2			6.2		
9	C	" "	8.8			6.8			6.4		
10	C	" "	9.0	**8.8**	0.1	8.4	**7.9**	0.6	8.0	**6.6**	0.7
11	E1	Contol + 90 secs rotary brushing	8.1			6.6			6.5		
12	E1	" "	6.8			6.2			6.4		
13	E1	Contol + 90 secs manual brushing	8.8			6.3			5.3		
14	E1	" "	8.8			5.5			6.0		
15	E1	" "	8.8	**8.3**	0.8	6.5	**6.2**	0.4	4.7	**5.8**	0.7
		Tank Water (counts per ml) at the end	9.4								

		Trial E2 (B) - 8/2 Tues am									
		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	9.0			7.7			7.3		
2	UT	" "	9.7			9.2			6.0		
3	UT	" "	8.8			8.1					
4	UT	" "	9.2			7.6					
5	UT	" "	9.6	**9.3**	0.3	9.3	**8.4**	0.7		**6.6**	0.7
6	C	Control - 15sec prewash,30sec soak in hot (55deg.C)	7.1			5.9					
7	C	clean water; 15sec rinse with hot clean water.	6.8			5.6			5.5		
8	C		7.1			6.1					
9	C		9.0			8.2			5.7		
10	C		9.1	**7.8**	1.0	7.7	**6.7**	1.0	5.9	**5.7**	0.2
11	E2	Control + 90 secs rotary brushing (after soak) + 0.1% det-	5.3			3.0			3.0		
12	E2	-gent + 2 washes + 500 ml Virkon @ 2%	5.3			3.0			3.0		
13	E2	" "	4.9			3.0			3.0		
14	E2	" "	5.1			3.0			3.0		
15	E2	" "	5.0	**5.1**	0.2	3.0	**3.0**	0.0	3.0	**3.0**	0.0
		Tank Water (counts per ml)	2.3								

		Trial E3 & E4 (C) - 8/2 Tues pm									
		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	9.4			8.4			7.9		
2	UT	" "	9.4			7.7			6.2		
3	UT	" "	9.7			8.5					
4	UT	" "	9.6			8.9					
5	UT	" "	7.7	**9.1**	0.7	8.4	**8.4**	0.4	6.0	**6.7**	0.8
6	E3	Control (Tues am) + 90 secs rotary brushing (after soak)	4.3			3.0					
7	E3	+ 0.1% detergent + 500 ml Virkon @ 2%	3.9			3.0					
8	E3	" "	4.7			3.0			3.0		
9	E3	" "	5.6			3.3					
10	E3	" "	4.2	**4.5**	0.6	3.0	**3.1**	0.1		**3.0**	0.0
11	E4	Control (Tues am) +	4.5			4.5			6.0		
12	E4	+ 0.1% detergent + 500 ml Virkon @ 2%	6.5			5.0			3.0		
13	E4	" "	3.9			3.0			3.0		
14	E4	" "	7.0			5.7			6.0		
15	E4	" "	4.0	**5.2**	1.3	3.0	**4.2**	1.1	3.0	**4.2**	1.5
		Tank Water (counts per ml)	2.6								

Trial E5 (D) - 9/2 Wed am

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	9.4			8.4					
2	UT	" "	9.2			8.3			8.0		
3	UT	" "	9.3			8.2					
4	UT	" "	9.8			8.0			7.7		
5	UT	" "	9.4	**9.4**	0.2	8.5	**8.3**	0.2		**7.8**	0.2
6	C	Control - 15sec prewash,30sec soak in cold	8.1			7.0					
7	C	dirty water; 15sec rinse with cold dirty water.	8.1			6.4			8.2		
8	C	" "	9.2			7.8					
9	C	" "	8.4			7.5			7.0		
10	C	" "	9.1	**8.6**	0.5	7.2	**7.2**	0.5		**7.6**	0.6
11	E5	Control + 500 ml Virkon @ 2%	7.1			6.9			7.5		
12	E5	" "	7.5			7.0			6.3		
13	E5	" "	7.7			7.0			5.5		
14	E5	" "	6.4			7.0			7.0		
15	E5	" "	7.8	**7.3**	0.5	6.4	**6.9**	0.2	7.3	**6.7**	0.7
		Tank Water (counts per ml)	6.9								

Trial E6 & E7 (E) - 9/2 Wed pm

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	9.6			6.9					
2	UT	" "	9.4			7.9					
3	UT	" "	9.3			8.4					
4	UT	" "	9.2			7.8					
5	UT	" "	6.8	**8.9**	1.0	8.9	**8.0**	0.7			
6	E6	Control (Wed am) + 250 ml Virkon @ 1%	7.8			6.4					
7	E6	" "	7.5			5.7					
8	E6	" "	7.5			6.7					
9	E6	" "	7.6			6.3					
10	E6	" "	7.5	**7.6**	0.1	6.4	**6.3**	0.3			
11	E7	Control (Wed am) + 90 secs hand brushing (after soak)	7.2			5.8					
12	E7	+ 250 ml Virkon @ 1%	6.9			4.6					
13	E7	" "	7.4			3.9					
14	E7	" "	7.1			4.3					
15	E7	" "	6.9	**7.1**	0.2	3.6	**4.4**	0.8			
		Tank Water (counts per ml)									

Trial E8 (F) - 10/2 Thurs am

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	8.7			6.2					
2	UT	" "	8.9			8.1					
3	UT	" "	9.4			8.2					
4	UT	" "	9.3			8.3					
5	UT	" "	9.1	**9.1**	0.2	8.6	**7.9**	0.8			
6	C	Control - 15sec prewash,30sec soak in hot (55deg.C)	8.9			5.0					
7	C	dirty water; 15sec rinse with hot clean water.	8.9			8.0					
8	C	" "	9.4			6.5					
9	C	" "	9.1			5.8					
10	C	" "	8.9	**9.0**	0.2	8.3	**6.7**	1.2			
11	E8	Control + 500 ml Virkon @ 2%	7.3			3.0					
12	E8	" "	6.0			3.8					
13	E8	" "	7.2			4.0					
14	E8	" "	5.9			6.0					
15	E8	" "	6.6	**6.6**	0.6	3.8	**4.1**	1.0			
		Tank Water (counts per ml) at the end	6.7								

Trial E9 (G) - 10/2 Thurs pm

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	9.4			7.6					
2	UT	" "	9.0			8.2					

3	UT	" "	9.3			7.8					
4	UT	" "	9.1			7.9					
5	UT	" "	9.5	**9.3**	0.2	8.2	**7.9**	0.2			
6	C	Control (Thurs am)	7.5								
7	C		7.7			7.9					
8	C		9.3			5.7					
9	C		7.7			7.0					
10	C		9.1	**8.3**	0.8	6.2	**6.7**	0.9			
11	E9	Control (Thurs am) + 500 ml Virkon @ 1%	8.7			3.0					
12	E9		7.1			4.3					
13	E9		6.3			3.5					
14	E9		9.0			6.0					
15	E9		7.6	**7.7**	1.0	3.0	**3.9**	1.1			
		Tank Water (counts per ml)	1.8								

Trial E10 (H) - 11/2 Fri am

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	9.3			6.7					
2	UT	" "	9.4			7.9					
3	UT	" "	9.4			8.3					
4	UT	" "	9.5			8.0					
5	UT	" "	9.3	**9.4**	0.1	6.6	**7.5**	0.7	5.5	**5.5**	0.0
6	C	Control - 15sec prewash,30sec soak in cold dirty water	9.5			5.7					
7	C	30 second brush then15sec rinse with cold clean water.	8.9			6.2					
8	C	" "	9.3			8.0					
9	C	" "	9.0			6.3					
10	C	" "	9.6	**9.3**	0.3	6.3	**6.5**	0.8			
11	E10	Control + 6mins us at 4kW at 65 deg.C (2% additive)	5.7			3.0			3.0		
12	E10	" "	7.3			3.0			3.0		
13	E10	" "	6.8			3.0			3.0		
14	E10	" "	7.0			3.0			3.0		
15	E10	" "	6.4	**6.6**	0.6	3.5	**3.1**	0.2	3.0	**3.0**	0.0
		Tank Water (counts per ml)	7.4								

Trial E11 & E12 (J) - 11/2 Fri pm

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Unwashed control	8.9			8.0					
2	UT	" "	8.6			8.3			4.7		
3	UT	" "	8.8			8.3					
4	UT	" "	8.5			7.7					
5	UT	" "	8.9	**8.7**	0.2	8.1	**8.1**	0.2	3.0	**3.9**	0.9
6	E11	Control + 3mins us at 4kW at 65 deg.C (2% additive)	6.1			3.9			3.0		
7	E11	" "	4.9			4.6			3.0		
8	E11	" "	5.3			3.6			3.3		
9	E11	" "	6.2			4.2			3.0		
10	E11	" "	6.6	**5.8**	0.6	4.6	**4.2**	0.4	3.0	**3.1**	0.1
11	E12	Control + 6mins us at 4kW at 65 deg.C (2% additive)	6.8			4.1			3.0		
12	E12	but no brushing	4.7			3.0			3.0		
13	E12	" "	7.3			4.2			3.0		
14	E12	" "	5.1			3.5			3.0		
15	E12	" "	7.1	**6.2**	1.1	3.0	**3.5**	0.5	3.0	**3.0**	0.0
		Tank Water (counts per ml)	8.1								

Trial Series E - 7-11th February

Visual assessment

Mon 7 Feb pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	4	2	5	11.0						
2	3	1	3	7.0						
3	4	2	3	9.0						
4	4	1.5	2	7.5						
5	4	2	4	10.0						
AVE	**3.8**	**1.7**	**3.4**	**8.9**						
6	3	2	4	9.0	1.5	1	2	4.5		
7	2.5	1	3	6.5	1	1	1.5	3.5		
8	4	1	3	8.0	1.5	1	2	4.5		
9	3.5	1	2	6.5	2	1	1.5	4.5		
10	3.5	1	3	7.5	1.5	1	2	4.5		
AVE	**3.3**	**1.2**	**3.0**	**7.5**	**1.5**	**1.0**	**1.8**	**4.3**	**57**	**45**
11	2	1	3	6.0	1	1	1.5	3.5		
12	2	2	3	7.0	1	1	2	4.0		
13	2	1	2	5.0	1	1	1.5	3.5		
14	2	1	2.5	5.5	0.5	1	1.5	3.0		
15	3	1.5	3	7.5	0.5	1	1.5	3.0		
AVE	**2.2**	**1.3**	**2.7**	**6.2**	**0.8**	**1.0**	**1.6**	**3.4**	**55**	**36**

Tues 8 Feb am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	3	6.0						
2	2	1	3	6.0						
3	3	1	3.5	7.5						
4	2	1	3	6.0						
5	2.5	1	2	5.5						
AVE	**2.3**	**1.0**	**2.9**	**6.2**						
6	2.5	1	2	5.5	0.5	0.5	1	2.0		
7	1.5	1	2	4.5	0.5	0.5	1	2.0		
8	1.5	0.5	1.5	3.5	0.5	0.5	0.5	1.5		
9	2.5	1	2	5.5	0.5	0.5	1	2.0		
10	2	1	2	5.0	1.5	0.5	1	3.0		
AVE	**2.0**	**0.9**	**1.9**	**4.8**	**0.7**	**0.5**	**0.9**	**2.1**	**44**	**35**
11	3	1	3	7.0	0.5	0.5	0.5	1.5		
12	1	0.5	2	3.5	0.5	0.5	1	2.0		
13	1.5	0.5	1.5	3.5	0.5	0.5	1	2.0		
14	1.5	0.5	1.5	3.5	0.5	0.5	1	2.0		
15	2	1	2	5.0	0.5	0.5	1	2.0		
AVE	**1.8**	**0.7**	**2.0**	**4.5**	**0.5**	**0.5**	**0.9**	**1.9**	**42**	**28**

Tues 8 Feb pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	4	8.0						
2	2.5	1	4	7.5						
3	3	1	4	8.0						
4	2.5	1	3	6.5						
5	3.5	1	4	8.5						
AVE	**2.9**	**1.0**	**3.8**	**7.7**						
6	3	1	3	7.0	0.5	0.5	1	2.0		
7	3	1	3	7.0	0.5	0.5	1	2.0		
8	2.5	0.5	3	6.0	0.5	1	2	3.5		
9	2	1	3	6.0	0.5	0.5	2	3.0		
10	2	1	2.5	5.5	0.5	1	2	3.5		
AVE	**2.5**	**0.9**	**2.9**	**6.3**	**0.5**	**0.7**	**1.6**	**2.8**	**44**	**20**
11	3	1	3	7.0	1	0.5	2	3.5		
12	3	1.5	3	7.5	1	1	2	4.0		
13	3	1	3	7.0	0.5	0.5	1	2.0		
14	3	1	2	6.0	1	1	1	3.0		
15	2.5	1	3	6.5	0.5	0.5	1	2.0		
AVE	**2.9**	**1.1**	**2.8**	**6.8**	**0.8**	**0.7**	**1.4**	**2.9**	**43**	**28**

Wed 9 Feb am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	3	7.0						
2	2.5	1	3.5	7.0						
3	2.5	1	3	6.5						
4	2	2	3	7.0						
5	3	1	3	7.0						
AVE	**2.6**	**1.2**	**3.1**	**6.9**						
6	3	1	3	7.0	1	1	2	4.0		
7	3	1	2	6.0	0.5	1	1.5	3.0		
8	3	1	3	7.0	1	0.5	2	3.5		
9	4	1	4	9.0	1	1	1	3.0		
10	3.5	1	3	7.5	1	1	1	3.0		
AVE	**3.3**	**1.0**	**3.0**	**7.3**	**0.9**	**0.9**	**1.5**	**3.3**	**45**	**27**
11	3	2	4	9.0	0	1	1.5	2.5		
12	4	2	4	10.0	2	0	2	4.0		
13	4	1	4	9.0	0	1	3	4.0		
14	2.5	1	3	6.5	1	1	1	3.0		
15	3	2	3	8.0	2	1.5	1	4.5		
AVE	**3.3**	**1.6**	**3.6**	**8.5**	**1.0**	**0.9**	**1.7**	**3.6**	**42**	**30**

Wed 9 Feb pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	1	4.0						
2	2	1	3	6.0						
3	5	4	2	11.0						
4	3	1	1.5	5.5						
5	2	2	2	6.0						
AVE	**2.8**	**1.8**	**1.9**	**6.5**						
6	2	1	1	4.0	0	0	1.5	1.5		
7	2	1	3	6.0	0	0	2	2.0		
8	3	1	2.5	6.5	0	0	1.5	1.5		
9	2	1	2	5.0	0	0	0	0.0		
10	2	1	2	5.0	0	0	1.5	1.5		
AVE	**2.2**	**1.0**	**2.1**	**5.3**	**0.0**	**0.0**	**1.3**	**1.3**	**25**	**0**
11	2	1	2	5.0	0	0	1.5	1.5		
12	2	1	2	5.0	0	0	1.5	1.5		
13	2	1	2	5.0	0	0	1.5	1.5		
14	3	2	3	8.0	0	0	1	1.0		
15	2	2	1.5	5.5	0	0.5	1	1.5		
AVE	**2.2**	**1.4**	**2.1**	**5.7**	**0.0**	**0.1**	**1.3**	**1.4**	**25**	**0**

Thurs 10 Feb am

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	1	2	2	5.0						
2	2.5	1	1.5	5.0						
3	2	2	2	6.0						
4	2.5	2	2	6.5						
5	2	2	2	6.0						
AVE	**2.0**	**1.8**	**1.9**	**5.7**						
6	1.5	1.5	1.5	4.5	0.5	0	1	1.5		
7	3	1	1	5.0	0.5	0.5	0.5	1.5		
8	2.5	1.5	1	5.0	0.5	0	0.5	1.0		
9	3	0	1	4.0	0.5	0	1	1.5		
10	4	1	2	7.0	2	1	0.5	3.5		
AVE	**2.8**	**1.0**	**1.3**	**5.1**	**0.8**	**0.3**	**0.7**	**1.8**	**35**	**29**
11	2	2	2	6.0	0.5	0	0.5	1.0		
12	2	1.5	2	5.5	1	0.5	1.5	3.0		
13	1.5	1	2	4.5	0	0.5	1	1.5		
14	2	2	1	5.0	0.5	0.5	1	2.0		
15	4	2.5	2	8.5	0	1	1	2.0		
AVE	**2.3**	**1.8**	**1.8**	**5.9**	**0.4**	**0.5**	**1.0**	**1.9**	**32**	**17**

Thurs 10 Feb pm

	Before (g)				After (g)				Improvement - total	Improvement - I Base

	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	2	3.5	8.5						
2	5	3	2.5	10.5						
3	4	2	3	9.0						
4	4.5	2.5	4	11.0						
5	3	1.5	2	6.5						
AVE	**3.9**	**2.2**	**3.0**	**9.1**						
6	2	2	2	6.0	0.5	0.5	1	2.0		
7	4.5	3	2	9.5	2	1	2	5.0		
8	3.5	2	2.5	8.0	1.5	1	2	4.5		
9	4.5	2	2	8.5	2	0.5	2	4.5		
10	4	3	3	10.0	3	2	2	7.0		
AVE	**3.7**	**2.4**	**2.3**	**8.4**	**1.8**	**1.0**	**1.8**	**4.6**	**55**	**49**
11	5	3	2	10.0	0	0	2	2.0		
12	3	2	2.5	7.5	0.5	0.5	2	3.0		
13	4	2	3	9.0	0.5	0.5	2	3.0		
14	7	4	3	14.0	3	1	2	6.0		
15	3	2	2	7.0	2	2	2	6.0		
AVE	**4.4**	**2.6**	**2.5**	**9.5**	**1.2**	**0.8**	**2.0**	**4.0**	**42**	**27**

Fri 11 Feb am

	Before (g)				**After (g)**				**Improvement - total**	**Improvement - I Base**
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	1	2.5	5.5						
2	3	1	2	6.0						
3	1.5	1.5	2	5.0						
4	2	1	3	6.0						
5	1.5	1	2	4.5						
AVE	**2.0**	**1.1**	**2.3**	**5.4**						
6	2	1	2	5.0	0.5	0	1.5	2.0		
7	3	1	2	6.0	0	0.5	0.5	1.0		
8	2	1	2.5	5.5	1	1	2	4.0		
9	1.5	1	2	4.5	0.5	0.5	2	3.0		
10	2	1	1.5	4.5	1	1	1.5	3.5		
AVE	**2.1**	**1.0**	**2.0**	**5.1**	**0.6**	**0.6**	**1.5**	**2.7**	**53**	**29**
11	1.5	0	2	3.5	0	0.5	0.5	1.0		
12	2	0.5	2	4.5	0	0	0	0.0		
13	3	2	1.5	6.5	0	0	0.5	0.5		
14	2	0.5	1.5	4.0	0	0.5	1	1.5		
15	2	2	1.5	5.5	0	0	0.5	0.5		
AVE	**2.1**	**1.0**	**1.7**	**4.8**	**0.0**	**0.2**	**0.5**	**0.7**	**15**	**0**

Fri 11 Feb pm

	Before (g)				**After (g)**				**Improvement - total**	**Improvement - I Base**
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2.5	1	3	6.5						
2	3	1	4	8.0						
3	4	2	3	9.0						
4	3	1	3	7.0						
5	3	1	4	8.0						
AVE	**3.1**	**1.2**	**3.4**	**7.7**						
6	2.5	1	2	5.5	0.5	0	0.5	1.0		
7	2	2	4	8.0	0.5	0.5	1	2.0		
8	5	2	3	10.0	0.5	0.5	1	2.0		
9	2	1	3	6.0	0.5	0.5	1	2.0		
10	3	2	4	9.0	0.5	0.5	1	2.0		
AVE	**2.9**	**1.6**	**3.2**	**7.7**	**0.5**	**0.4**	**0.9**	**1.8**	**23**	**17**
11	4	2	4	10.0	0.5	0.5	1.5	2.5		
12	2	1	3	6.0	0.5	0.5	1	2.0		
13	3	1	3	7.0	0.5	0.5	1	2.0		
14	1.5	1	2	4.5	0.5	0	1	1.5		
15	3	1	4	8.0	0	0.5	1.5	2.0		
AVE	**2.7**	**1.2**	**3.2**	**7.1**	**0.4**	**0.4**	**1.2**	**2.0**	**28**	**15**

Trial Series F: 7 - 8th April

Microbiological results

Trial FA1											
		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	C	Washed controls (commercial unt only)	8.2			6.3			5.3		
2	C	" "	7.4			5.0			5.0		
3	C	" "	7.7			5.3			5.0		
4	C	" "	6.1	**7.4**	0.8	5.3	**5.5**	0.5	5.0	**5.1**	0.1
5	C	Control - commercial wash + 3mins at 60 deg.C	5.6			3.6			5.0		
6	C	" "	8.2			3.0			5.0		
7	C	" "	8.1			3.9			5.0		
8	C	" "	7.1	**7.2**	1.1	4.1	**3.7**	0.4	5.0	**5.0**	0.0
9	T	Commercial wash + 3mins at 60 deg.C **with US**	5.7			2.3			2.3		
10	T	" "	4.6			2.3			2.3		
11	T	" "	4.4			2.3			2.3		
12	T	" "	5.6	**5.1**	0.6	2.3	**2.3**	0.0	2.3	**2.3**	0.0

Trial FA2											
		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
13	C	Washed controls (commercial unt only)	7.7			6.0			5		
14	C	" "	7.6			5.7			5		
15	C	" "	7.4			5.5			5		
16	C	" "	7.2	**7.4**	0.2	5.0	**5.5**	0.4	5	**5.0**	0.0
17	C	Control - commercial wash + 3mins at 60 deg.C	5.5			3.0			5		
18	C	" "	5.6			3.0			5		
19	C	" "	6.0			3.3			5		
20	C	" "	5.0	**5.5**	0.3	3.0	**3.1**	0.1	5	**5.0**	0.0
21	T	Commercial wash + 3mins at 60 deg. **C with US**	4.9			2.3			2.3		
22	T	" "	4.9			2.3			2.3		
23	T	" "	4.6			2.3			2.3		
24	T	" "	5.0	**4.9**	0.1	2.3	**2.3**	0.0	2.3	**2.3**	0.0

Trial FB											
		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	C	Washed controls (commercial unt only)	5.2			3.0			3.0		
2	C	" "	5.3			3.0			3.0		
3	C	" "	5.4			3.0			3.0		
4	C	" "	5.4			3.0			3.0		
5	C	" "	5.6	**5.4**	0.1	4.3	**3.3**	0.5	3.0	**3.0**	0.0
6	T	Commercial wash + 1mins at 60 deg. **C with US**	5.3			3.0			2.3		
7	T	" "	4.8			3.0			2.3		
8	T	" "	5.2			3.0			2.3		
9	T	" "	5.1			3.0			2.3		
10	T	" "	4.6	**5.0**	0.2	3.0	**3.0**	0.0	2.3	**2.3**	0.0

Trial FC											
		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	C	Washed controls (commercial unt only)	6.9			6.0			3.0		
2	C	" "	8.0			6.7			3.0		
3	C	" "	6.7			5.2			3.0		
4	C	" "	8.1			4.9			5.3		
5	C	" "	7.8	**7.5**	0.6	5.9	**5.7**	0.6	3.8	**3.6**	0.9
6	C	Control - commercial wash + 30secs at 60 deg. **C with US**	5.4			5.3			2.3		
7	C	" "	6.1			3.9			2.3		
8	C	" "	5.4			4.1			2.3		
9	C	" "	5.9			3.8			5.0		
10	C	" "	7.4	**6.0**	0.7	6.7	**4.8**	1.1	2.3	**2.8**	1.1

11	T	Commercial wash + 30secs at 60 deg. **C with US**	6.0			2.8			2.3			
12	T	after 60 secs manual brushing	6.0			3.7			2.3			
13	T	" "	5.6			3.0			2.3			
14	T	" "	5.7			4.4			2.3			
15	T	" "	5.8	**5.8**	0.1	4.1	**3.6**	0.6	2.3	**2.3**	0.0	

Trial FE

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	UT	Un-washed controls	9.8			8.2			5.0		
2	UT	" "	8.9			8.6			5.0		
3	UT	" "	8.5			8.3			5.0		
4	UT	" "	9.2			9.1			5.0		
5	UT	" "	8.7	**9.0**	0.5	8.4	**8.5**	0.3	5.3	**5.1**	0.1

Trial FF1

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
1	C	Washed controls (commercial unt only)	7.5			6.9			3.0		
2	C	(Recycled crates from A1)	7.9			6.6			3.0		
3	C	" "	8.4			6.7			3.0		
4	C	" "	7.7	**7.9**	0.3	6.9	**6.8**	0.1	3.0	**3.0**	0.0
5	C	Control - commercial wash + 3mins at 60 deg.C	5.9			2.3			2.3		
6	C	(Recycled crates from A1)	5.2			2.3			2.3		
7	C	" "	5.5			2.3			2.3		
8	C	" "	5.4	**5.5**	0.3	2.3	**2.3**	0.0	2.3	**2.3**	0.0
9	T	Commercial wash + 3mins at 60 deg. **C with US**	5.3			2.3			2.3		
10	T	(Recycled crates from A1)	5.4			2.3			2.3		
11	T	" "	5.3			2.3			2.3		
12	T	" "	5.3	**5.3**	0.1	2.3	**2.3**	0.0	2.3	**2.3**	0.0

Trial FF2

		Treatment	*Log - PCA*	***ave***	*sd*	*Log - VRBG*	***ave***	*sd*	*Log-CCDA*	***ave***	*sd*
13	C	Washed controls (commercial unt only)	7.7			6.5			3.0		
14	C	(Recycled crates from A1)	7.7			7.0			3.0		
15	C	" "	8.2			4.3			3.0		
16	C	" "	8.4	**8.0**	0.3	3.0	**5.2**	1.6	3.0	**3.0**	0.0
17	C	Control - commercial wash + 3mins at 60 deg.C	5.4			2.3			2.3		
18	C	(Recycled crates from A1)	5.7			2.3			2.3		
19	C	" "	5.6			2.3			2.3		
20	C	" "	5.3	**5.5**	0.2	2.3	**2.3**	0.0	2.3	**2.3**	0.0
21	T	Commercial wash + 3mins at 60 deg. **C with US**	5.2			2.3			2.3		
22	T	(Recycled crates from A1)	5.3			2.3			2.3		
23	T	" "	5.6			2.3			2.3		
24	T	" "	5.2	**5.3**	0.2	2.3	**2.3**	0.0	2.3	**2.3**	0.0

Trial Series F - 7-8th April 2005

Visual assessment

Trial A1

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	1	1	1.5	3.5						
2	1	2	1	4.0						
3	0.5	0.5	1	2.0						
4	0.5	0.5	0.5	1.5						
AVE	**0.8**	**1.0**	**1.0**	**2.8**						
5					0.5	0.5	0.5	1.5		
6					0.5	0.5	0.5	1.5		
7					0	0	0.5	0.5		
8					0	0	0.5	0.5		
AVE					**0.3**	**0.3**	**0.5**	**1.0**		
9					0	0	0.5	0.5		
10	1	1	1.5	3.5	0	0	0	0.0		
11	0.5	1	1	2.5	0	0.5	1	1.5		
12	1	1	0.5	2.5	0	0.5	1	1.5		
AVE	**0.8**	**1.0**	**1.0**	**2.8**	**0.0**	**0.3**	**0.6**	**0.9**	**31**	**0**

Trial A2

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
13	1	1	2	4.0						
14	1	1	1.5	3.5						
15	1	1	2	4.0						
16	0.5	1	1	2.5						
AVE	**0.9**	**1.0**	**1.6**	**3.5**						
17	2	2	1.5	5.5	1	0.5	1.5	3.0		
18	1.5	1	2	4.5	1	1	1.5	3.5		
19	1	1	1.5	3.5	0.5	0.5	1	2.0		
20	2	1	1.5	4.5	1	1	1	3.0		
AVE	**1.6**	**1.3**	**1.6**	**4.5**	**0.9**	**0.8**	**1.3**	**2.9**	**64**	**54**
21	1	1	1.5	3.5	0	0	0.5	0.5		
22	1.5	1	2	4.5	0.5	0.5	1	2.0		
23	1.5	1	2	4.5	1	1	1	3.0		
24	2	1	1.5	4.5	0.5	1	0.5	2.0		
AVE	**1.5**	**1.0**	**1.8**	**4.5**	**0.5**	**0.6**	**0.8**	**1.9**	**42**	**33**

Trial B

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	1	1	2.5	4.5	1	0.5	1	2.5		
2	1	1	2	4.0	0.5	0.5	1	2.0		
3	2	2	1	5.0	1.5	2	1.5	5.0		
4	2	0.5	2.5	5.0	0.5	0.5	1.5	2.5		
5	2.5	2	3	7.5	2	1	2	5.0		
AVE	**1.7**	**1.3**	**2.2**	**5.2**	**1.1**	**0.9**	**1.4**	**3.4**	**65**	**65**
6	1	0.5	1.5	3.0	0	0	1	1.0		
7	1	1	2	4.0	0	0.5	0.5	1.0		
8	2	2	2.5	6.5	0	1	1.5	2.5		
9	2	2	1	5.0	1	0.5	0.5	2.0		
10	2	2	2	6.0	0	0.5	0	0.5		
AVE	**1.6**	**1.5**	**1.8**	**4.9**	**0.2**	**0.5**	**0.7**	**1.4**	**29**	**13**

Trial C

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	2	0.5	1	3.5	2	1.5	1	4.5		
2	1.5	0.5	1	3.0	1.5	0.5	1.5	3.5		
3	2	0.5	1.5	4.0	1.5	0.5	1.5	3.5		
4	2	0.5	1.5	4.0	1.5	0.5	1.5	3.5		
5	2.5	0.5	1	4.0	2.5	1.5	1	5.0		
AVE	**2.0**	**0.5**	**1.2**	**3.7**	**1.8**	**0.9**	**1.3**	**4.0**	**108**	**90**
6	1.5	1	1.5	4.0	0.5	0.5	1	2.0		

7	2	1	1.5	4.5	1	0.5	1	2.5		
8	1	0.5	1.5	3.0	0.5	0	1	1.5		
9	2	0.5	1.5	4.0	0.5	0	1.5	2.0		
10	1	1	2	4.0	0	0.5	1.5	2.0		
AVE	**1.5**	**0.8**	**1.6**	**3.9**	**0.5**	**0.3**	**1.2**	**2.0**	**51**	**33**
11	1	0	1	2.0	0.5	0	0.5	1.0		
12	1.5	0.5	2	4.0	0.5	0	1.5	2.0		
13	2	0.5	2	4.5	0.5	0.5	1.5	2.5		
14	1	0.5	2	3.5	0	0	1.5	1.5		
15	1.5	0.5	1.5	3.5	0.5	0	1.5	2.0		
AVE	**1.4**	**0.4**	**1.7**	**3.5**	**0.4**	**0.1**	**1.3**	**1.8**	**51**	**29**

Trial E

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	3	1	2	6.0						
2	4	2	3	9.0						
3	5	1	3	9.0						
4	4	1	2	7.0						
5	5	1	3	9.0						
AVE	**4.2**	**1.2**	**2.6**	**8.0**						

Trial F1

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
1	0.5	0	0.5	1.0						
2	0.5	0	0.5	1.0						
3	0.5	0.5	0.5	1.5						
4	1	0.5	2	3.5						
AVE	**0.6**	**0.3**	**0.9**	**1.8**						
5	0.5	0	1	1.5	0	0	0	0.0		
6	0.5	0.5	1.5	2.5	0	0	1	1.0		
7	0	0	0	0.0	0	0	0	0.0		
8	1	0.5	1	2.5	0	0	0.5	0.5		
AVE	**0.5**	**0.3**	**0.9**	**1.6**	**0.0**	**0.0**	**0.4**	**0.4**	**23**	**0**
9	0	0	0.5	0.5	0	0	0	0.0		
10	0.5	0	0.5	1.0	0	0	0	0.0		
11	0	0.5	1	1.5	0	0	0	0.0		
12	1	0.5	0.5	2.0	0	0	0	0.0		
AVE	**0.4**	**0.3**	**0.6**	**1.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0**	**0**

Trial F2

	Before (g)				After (g)				Improvement - total	Improvement - I Base
	I Base	Sides	O Base	Total	I Base	Sides	O Base	Total	After" as a % of "before"	After" as a % of "before"
13	0	0.5	1	1.5						
14	0.5	0	1	1.5						
15	0.5	0.5	0.5	1.5						
16	1	0.5	1	2.5						
AVE	**0.5**	**0.4**	**0.9**	**1.8**						
17	0.5	0	0.5	1.0	0	0	0	0.0		
18	0	0	1	1.0	0	0	0.5	0.5		
19	0	0	0.5	0.5	0	0.5	0	0.5		
20	0.5	2	1	3.5	0	1	0.5	1.5		
AVE	**0.3**	**0.5**	**0.8**	**1.5**	**0.0**	**0.4**	**0.3**	**0.6**	**42**	**0**
21	0	0.5	0.5	1.0	0	0	0.5	0.5		
22	0.5	0.5	1	2.0	0	0	0.5	0.5		
23	1	0.5	0.5	2.0	0	0	0.5	0.5		
24	0	0	0.5	0.5	0	0.5	0.5	1.0		
AVE	**0.4**	**0.4**	**0.6**	**1.5**	**0.0**	**0.1**	**0.5**	**0.6**	**42**	**0**

APPENDIX 4

Micrographs from studies of crate surfaces using scanning (SEM) and transmission (TEM) electron microscopy

---

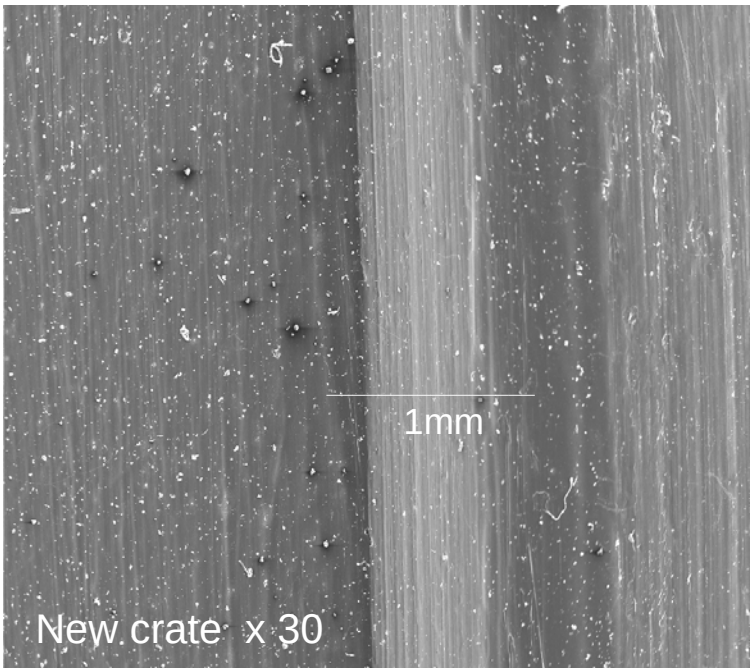


Figure 1. Appearance of unused crate section at low magnification by SEM. The granules on the surface are swarf from the cutting of the section.

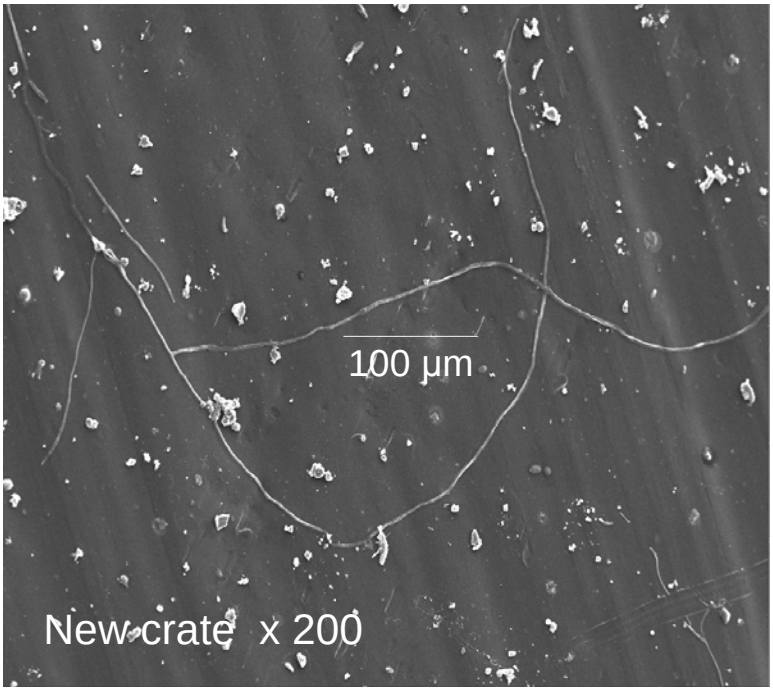


Figure 2. Higher magnification by SEM of the surface of unused crate showing fungal hyphae

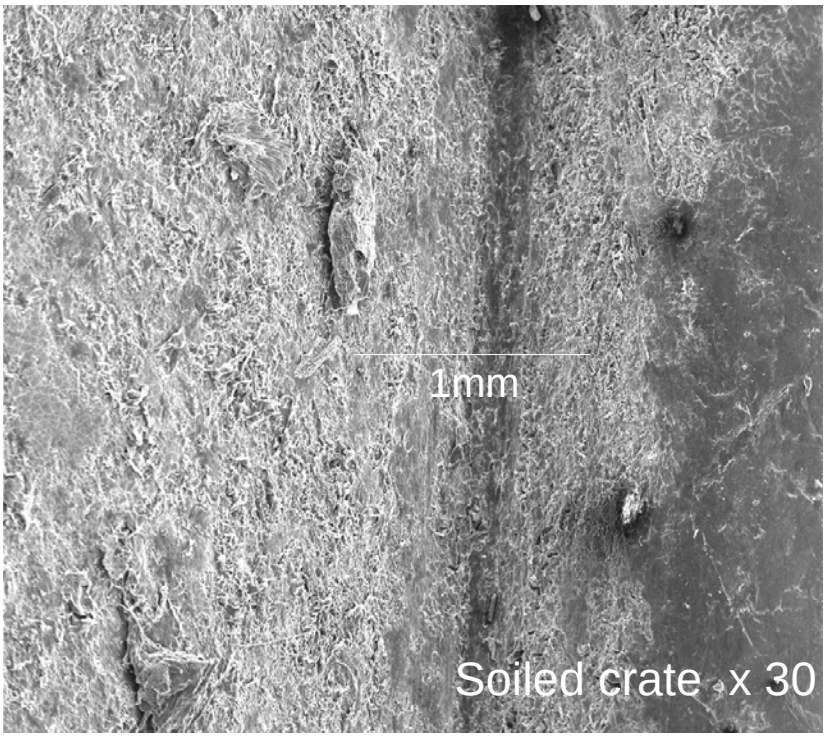


Figure 3 Appearance of used crate section at low magnification by SEM, in a similar area as Figure 1, showing a build up of a bio-film matrix.

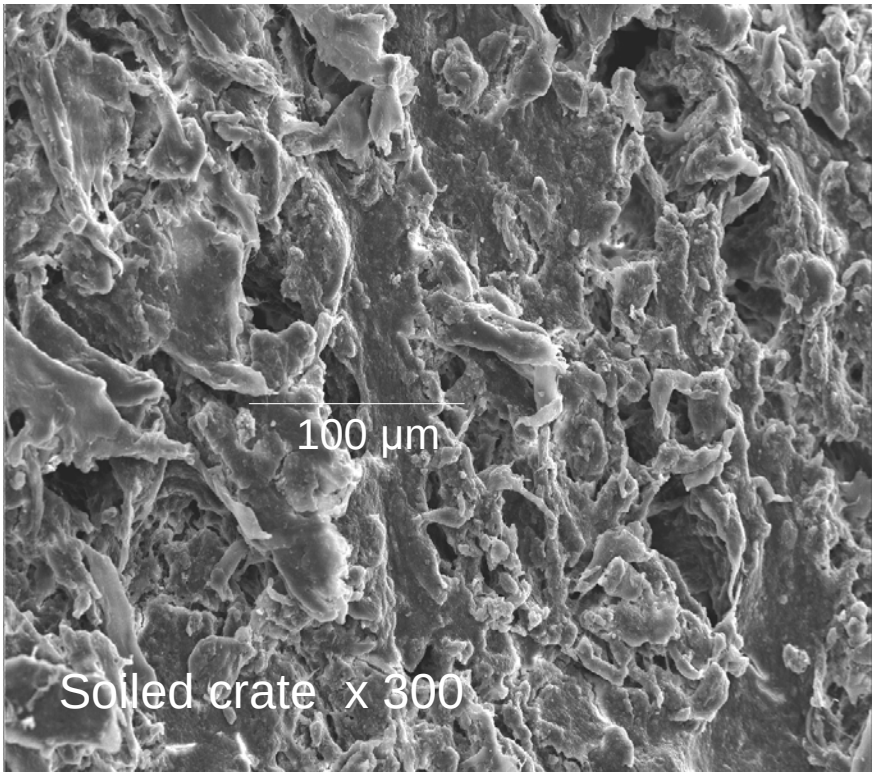


Figure 4. Higher magnification by SEM of the bio-film matrix

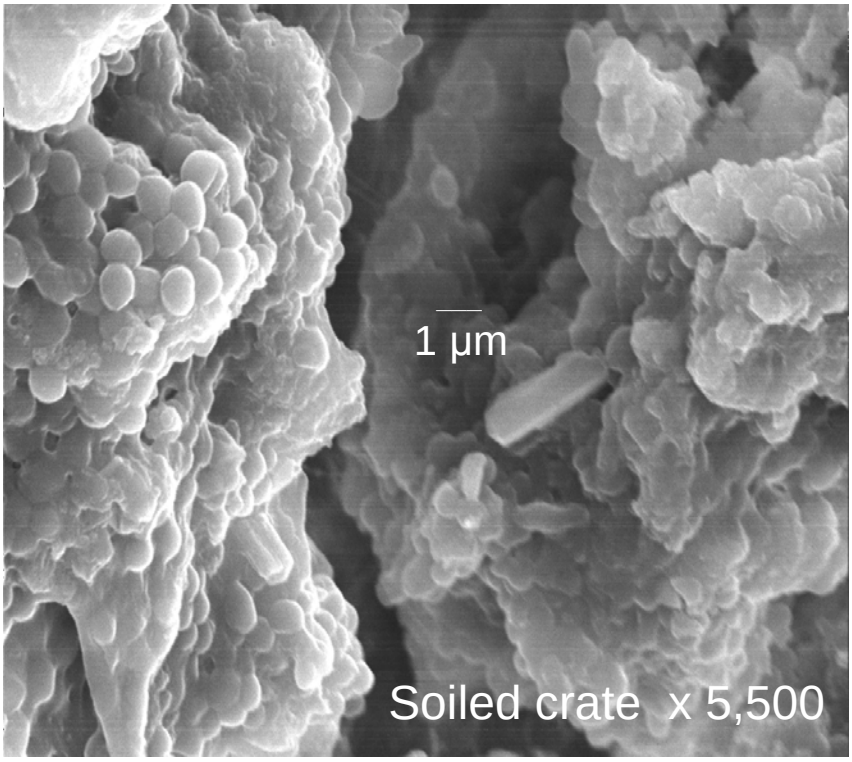


Figure 5. High magnification by SEM of a fissure in the bio-film matrix showing spherical and rod shaped bacteria.

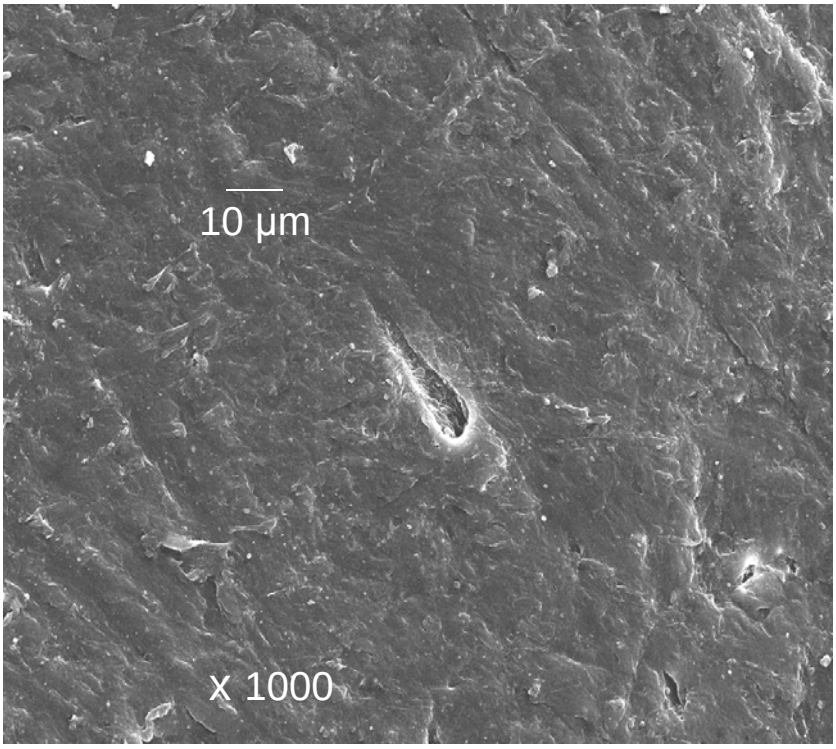


Figure 6. Surface appearance by SEM of soiled crate after pressure sprayed with water at 9 bar. Note the depression where an object appears to have been torn out by the spray.

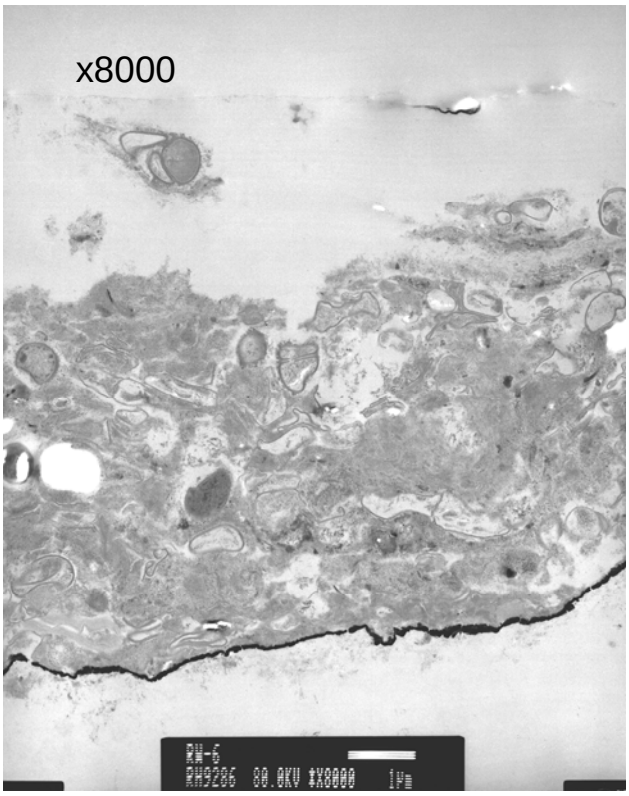


Figure 7. A thin TEM section of bio-film matrix removed from crate section surface showing indefinable structures within it, probably made up of organic material including polysaccharides and mineral salts. The black line at the bottom of the section is the gold layer that was deposited on the bio-film matrix for the SEM and is thus the surface of the bio-film matrix as it was on the crate section.

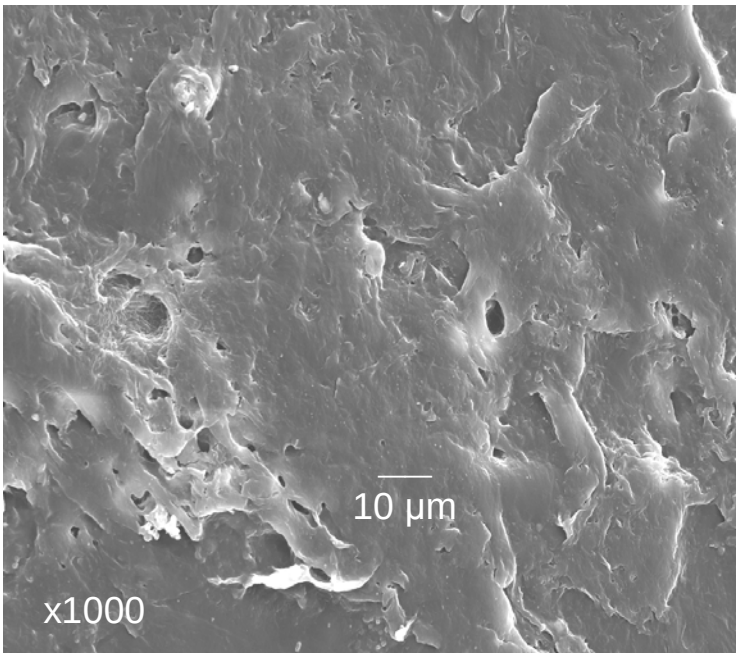


Figure 8. Surface appearance by SEM of the bio-film matrix that was treated with 60 seconds of ultrasonics at 50 C,. The surface appears to have been smoothed and perforated slightly perhaps as a result of the removal of looser material on the surface

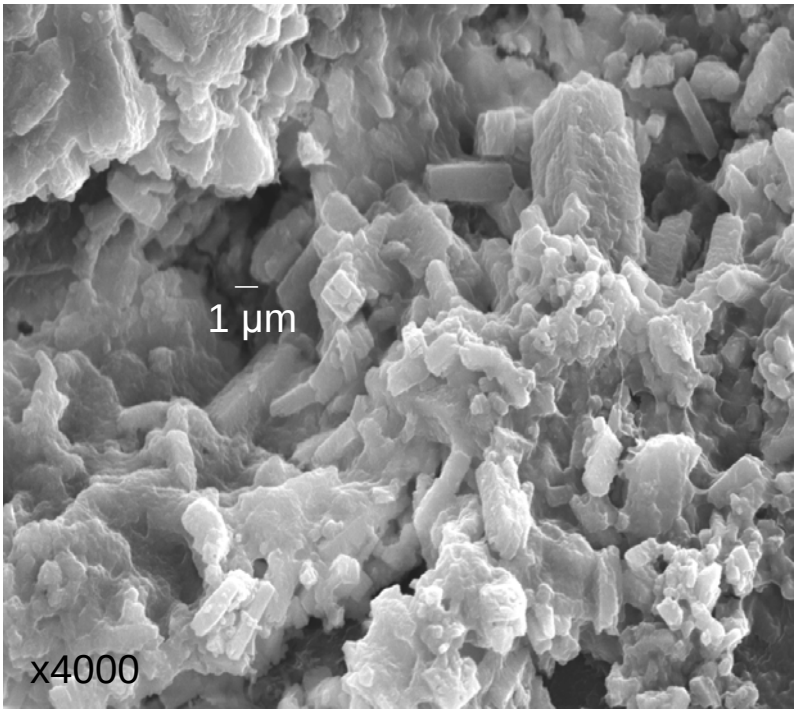


Figure 9. Appearance of bio-film matrix at higher magnification by SEM on an older crate after ultrasonic treatment for 60 seconds. A mixture of organic material and salt crystals are evident.

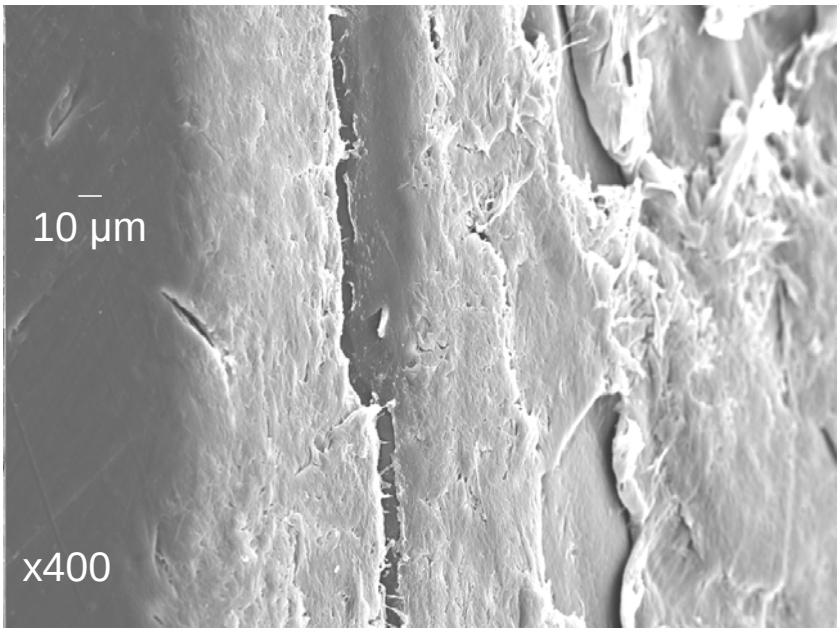


Figure 10. Low power magnification by SEM of the bio-film matrix following vigorous abrasion with a cotton swab. There are suggestions in the right of the SEM picture that the bio-film matrix has been peeled away from the crate section surface.

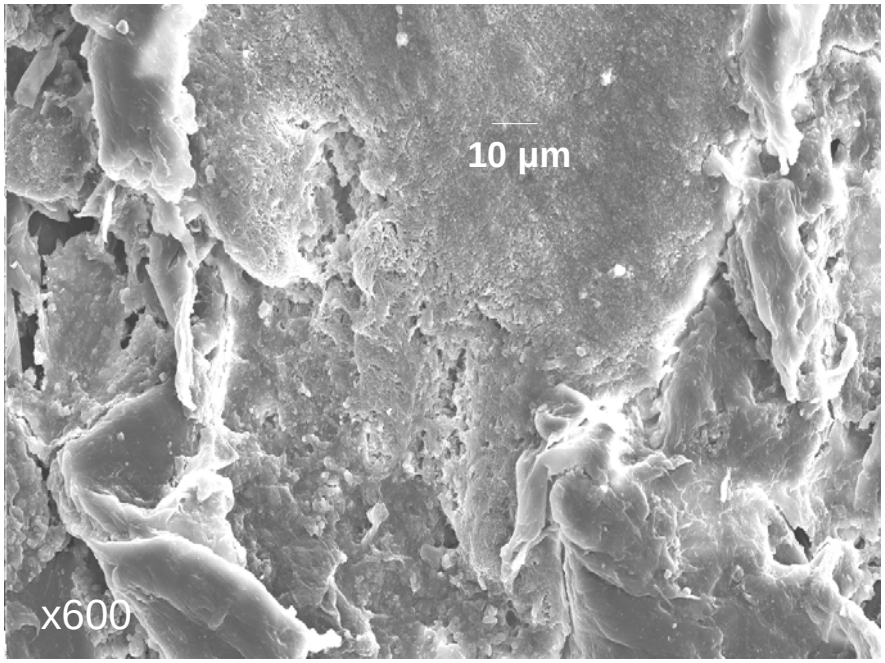


Figure 11. Appearance of bio-film matrix surface by SEM after treatment by 1% TWS at 60 C for 15 seconds. Some erosion of the surface is evident in the centre of the picture.

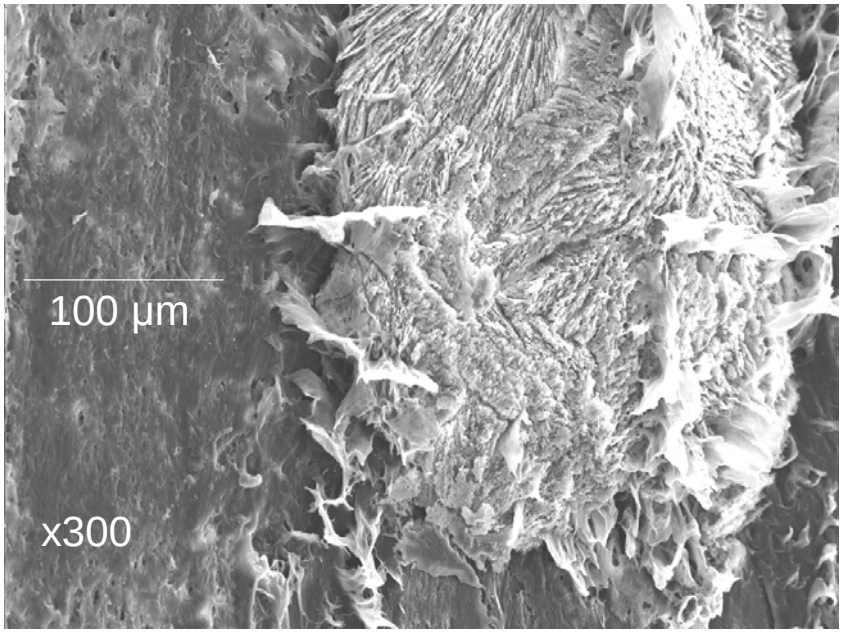


Figure 12. Typical appearance of salt crystals on the surface of an older closed floor crate section. These were not removed by all the chemical treatments applied.

APPENDIX 5

Microbiological Sampling Methods for Factory Trials

---

1 Visual assessment of crate cleanliness

Crates were visually scored for the total amount of organic debris in grams on three sides of each crate to be sampled:- the inside base, sides and the underside both prior to and following any treatments that occurred. The organic matter could not be removed from the crate for quantification purposes so estimations were made as to the amount present as 1 heaped teaspoon was found to weigh approximately 1 gram.

2 Sampling Methods

2.1 Crate sampling using swabbing technique

Four sterile jumbo cotton wool swabs (Medical Wire, MW104J) were moistened with Maximum Recovery Diluent (MRD, Oxoid CM733) and each was used to sample a quarter of the inside base of a crate. The swabs were wiped horizontally, vertically and diagonally across the surface before all 4 swabs were pooled into 10mls of MRD. The samples were transported back to the laboratory at 1oC where decimal dilutions were made.

2.2 Crate sampling using sponge technique

A small amount from 100mls (MRD) was used to wet a sterile sponge (103 x 185 x 5.8mm, Spongyl, Cat 95000087) contained within a sterile plastic bag. This was swabbed over the base of the crates horizontally, vertically and diagonally from bottom left to top right. The sponge was then returned to the plastic bag and the remainder of the 100mls of MRD was added. The bag was squeezed 60 times using both hands to transfer the majority any microbial cells present into the liquid phase. The sponge was wrung out thoroughly using clean gloves each time and the solution was transferred to a 100ml container to give a 1 x 10-2 dilution.

3 Culture Methods

3.1 APC and Enterobacteriaceae

Serial dilutions were completed and 100 l each of all 3 were inoculated onto different Plate Count Agar (PCA) (5g/l tryptone; 2.5g/l yeast extract; 1g/l glucose and 9g/l agar at pH7.0 + 0.2, Oxoid, CMO325) and Violet Red Bile Glucose (VRBG) agar (3g/l yeast extract; 7g/l peptone; 5g/lsodium chloride; 1.5g/l bile salts No. 3; 10g/l glucose; 0.03g/l neutral red, 0.002g/l crystal violet and 12g/l agar base at pH 7.4 + 0.2, Oxoid, CM0485). The PCA plates were incubated at 30oC for 48h and the VRBG plates were incubated at 37oC for 24h, after which the colony counts were determined. Characteristically Enterobacteriaceae appear as round purple colonies 1-2mm diameter surrounded by purple haloes but as recommended by the media manufacturer all red colonies were counted.

3.2 Camplyobacter

During selected trials, 100?l of each dilution was directly inoculated onto modified charcoal cefoperazone desoxycholate agar (mCCDA), which comprised campylobacter blood-free selective agar base (Oxoid, CM739) and campylobacter selective supplement (Oxoid, SR155). Plates were incubated at 42oC for 48h under microaerophillic conditions (88% N_2, 5% O_2, 5% CO_2 and 2% H_2 by volume) achieved using microaerophillic gas packs (Oxoid, CN0035A) after which campylobacter colonies were determined. A selection of colonies were confirmed by positive oxidase reaction, microscopic appearance following Gram staining and lack of growth in air at 25 °C. Some colonies were confirmed using a latex agglutination kit (Campylobacter Test Kit; Oxidase DR 0150M).