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Report on:

Testing of food businesses' proposed alternative controls, which can be equally as (or more) effective in controlling the risk from E*. coli* O157 as the FSA guidance on the separate use of complex equipment.

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Executive summary

Research was carried out to determine the inactivation kinetic parameters that can be achieved using heat (both dry and wet) and chemicals (quaternary ammonium compounds (QAC), chlorine based and wipes) on surfaces inoculated with *E. coli* O157:H7. Best practice and verification methods were also investigated in relation to the alternative methods suggested in a survey of food business operators (FBOs), carried out by the Policy Studies Institute (PSI). In addition, investigations were carried out to assess the cross contamination risks that occur when vacuum packing products, both externally from pack to pack and internally from contaminated areas of the vacuum packer that are hard to reach/clean.

Thermal results

- When heated for sufficient time *E.coli* O157:H7 can be removed from the stainless steel surfaces.
- The D- values were much higher during dry heat treatments as opposed to wet heat treatments, e.g The D-value of *E. coli* O157:H7 at 65°C on stainless steel using dry heat was 136.8 minutes as opposed to 2.1 seconds using moist heating. This means that to receive a 4 log reduction in *E. coli* O157:H7 on stainless steel with dry heat at 65°C the item would need to be heated for approximately 547 minutes as opposed to 8.4 seconds with moist heating at the same temperature (Section 4.2.1).

Chemical results

Liquid application:

- The information obtained demonstrates that there is no practical difference between non-toxigenic and toxigenic *Escherichia coli* O157:H7 in susceptibility under the correct use conditions for disinfectants. (Appendix II).
- When used correctly disinfectants can consistently achieve a >4 log reductions of *E. coli* O157:H7 dried upon surfaces using the European standard test BS EN13697:2001 and that deviations from optimal conditions will result in a decrease in efficacy of the disinfectant and thus if used inappropriately disinfectants may not achieve the desired effect (Section 4.3.1).

Sanitiser wipes:

- The use of wipes containing disinfectants has been shown to produce an approximately 3 log reduction in contamination on temperature probes inoculated and that allowing contamination to dry on the probes before using a wipe results in a significant reduction in efficacy (Section 4.3.4).
- For optimal efficacy the probe should be wiped immediately after use, first to remove any excess contamination and then re-wiped with a new disinfectant wipe (Section 4.3.4).
- Whilst the wiping action appears to be primarily a mechanical removal of the contamination allowing a contact time for the disinfectant prior to re-use appears to improve efficacy (Section 4.3.4).

- Wipes, used on the surfaces of temperature probes, can be used as a risk reduction method but not a control method in the removal of *E. coli* O157:H7. It is recommended that for temperature probes used on both raw and RTE products a more effective means of decontamination should be used. Wiping the probe to remove excess contamination, followed by immersion in hot water (>80ºC) or a disinfectant, for an appropriate contact time has been shown to produce a >4 log reduction (Section 4.3.4).

Using the results of this study and risk assessments disinfection protocols can be set up and providing they are followed correctly the removal/destruction of *E. coli* O157:H7 is possible.

Vacuum packers

External cross-contamination:

- It was observed that the operator can spread contamination from product to gloves and then to contact surfaces such as lid, handle, control panel and outer and inner bags.
- Even when gloves are replaced with a clean pair, the contamination can still be spread to subsequently packed products (Section 4.4.1).

Internal cross-contamination:

- The machine was inoculated in different areas to assess the spread to internal products if the machine was contaminated in hard to reach/clean areas.
- Contamination was observed in 4 out of the first 50 bags packed (Section 4.4.2). This indicates that contamination can be spread from the internal areas of a vacuum packing machine into the contents of a bag.

Overall:

- The internal cross-contamination study was carried out aseptically, wherein the operator did not come into contact with the packaged agar discs. However, if the results of the internal cross-contamination studies were taken into account and the operator was also handling the product when the chamber is also contaminated (Section 4.4.2) then this again increases the risk of cross-contamination to the internal surfaces and contents.
- When the chamber shows contamination this is likely to be transferred onto the outer surface of the packaging and thus the operator's hands/gloves, which can then transfer onto the item to be packaged (Section 4.4.1).
- This work showed that internal contamination of a VP machine does lead to contamination of both the vacuum chamber and the internal contents of packed bags produced in that chamber.

Food businesses must practise good food hygiene at all times to make sure the food they produce is safe. The information obtained in this study was useful in relation to determining the inactivation kinetic parameters that can be achieved using heat (both dry and wet) and chemicals (quaternary ammonium compounds (QAC), chlorine based disinfectants) on surfaces inoculated with *E. coli* O157. Using the

results of this study, disinfection protocols can be set up and providing they are followed correctly the removal of *E. coli* O157:H7 is possible. The information observed on the efficacy of disinfection wipes on temperature probe surfaces, shows that they can be used as a risk reduction method but not a control method in the removal of *E. coli* O157:H7. Best practice is provided to outline the best method to use in the disinfection process. To ensure the optimal effectiveness of a disinfection treatment it is important that the item is free from all food debris. This may be achieved using mechanical and/or chemical treatment (e.g detergent/surfactant). Observations of temperature and chemical concentration verification methods readily available to small businesses, found that these can be a suitable method to verify the heat treatment achieved on surfaces during both wet and dry heat treatment and the chemical concentration used in a disinfectant solution.

For the purpose of this report:

Cleaning chemicals are defined as chemicals that are used to remove dirt from surfaces

Chemicals used in this study were a Quaternary ammonium based compound (sometimes referred to as **QAC** – or **BKC** (Benzalkonium chloride) and a Chlorine based compound (sometimes referred to as **Sodium Hypochlorite** (or **ClO^-**).

Disinfectants are defined as chemicals that when used at manufacturer recommended use concentrations are able to meet the requirements of BS EN 1276 and/or BS EN 13697

EN 13697:2001: Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants

Sanitizers are defined as chemicals that have properties of cleaning chemicals and are expected to kill bacteria but may or may not meet the BS EN 1276 and/or BS EN 13697 requirements

D-value, or decimal reduction time, in microbiology refers to the time required, at a given temperature, to achieve a 90% reduction in the population of a given microorganism.

Z-value is the change in temperature which is required in order to bring about a 10-fold change in the D-value.

Re-commissioning is a prescribed method to be undertaken which, if followed, should render the machine safe for use with RTE products.

Appendices and deliverables:

Appendix I relates to the work carried out in Deliverable 2: Thermal studies (wet and dry heat).

Appendix II relates to the work carried out in Deliverable 3 a: Chemicals applied as liquid.

Appendix III relates to the work carried out in Deliverable 3 b: Chemicals applied as wipes.

Appendix IV relates to the work carried out in Deliverable 4: Best practice.

Appendix V relates to the work carried out in Deliverable 5: Verification.

Appendix VI relates to the work carried out in Deliverable 6: Vacuum packer cross contamination.

Appendix VII relates to the 2nd experiment carried out in Deliverable 6 b: Vacuum packer internal cross contamination x 2.

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APPENDICES

1 STATEMENTS

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2 BACKGROUND AND AIMS

In response to the FSA call to carry out testing of food businesses' proposed alternative controls, which could be equally as (or more) effective in controlling the risk from *Escherichia coli O*157:H7 as the FSA guidance on the separate use of complex equipment, Campden BRI submitted a proposal which included research into the alternative methods of decontamination of such complex equipment.

During a survey carried out on several food businesses by an external research company on behalf of the FSA, the following cleaning/disinfection approaches were mentioned as 'alternative' controls to the use of dual equipment[1]:

In relation to mixers, vacuum packers, over-wrappers, scales and slicers which can be dismantled, potentially safe alternatives used the following:

- Removal of food debris using single use sanitised cloths or vigorous rub under hot water.
- Heat disinfection – Submergence in hot water or placing in a dishwasher at a temperature of 60 °C or higher.
- Chemical disinfection – either submerging parts in a recommended dilution of liquid disinfectant for a recommended period of time or applying a sanitiser/disinfectant to the surface e.g spray.
- Some businesses used the 'double clean' method – using a sanitiser to clean, followed by a wipe with a single use cloth and then sanitised again.

In relation to the cleaning/disinfection of temperature probes the survey suggested the potentially safe alternatives used the following:

- After use, wipe with sanitiser/antibacterial wipes
- Placement in boiling water and clean with detergent
- Apply sanitiser again either e.g. wipe or spray or allow to sit in liquid sanitiser for recommended period of time
- Sanitise again, immediately before next use

In relation to drying stage – businesses used a variety of approaches including air drying, paper towels, and single use cloths (Air drying is the official recommended method if the equipment is hot (e.g. after hot dishwasher) and the area is clean and free form aerial contamination. There is a risk that wet equipment might be put away if people are in a hurry).

The study, carried out by the Policy Studies Institute (PSI), stated that "While some businesses reportedly followed each of these multiple steps, others only followed some steps and could not

[1] PSI (2013) Small food businesses' proposed alternative controls to the separate use of complex equipment, to control the risk from *E. coli* O157, p 1-76

therefore be described as adhering strictly to potentially 'safe' practices. It should also be noted practices that are reported, and those that are actually followed may diverge, particularly in the context of busy environments. If each of these stages is followed carefully they may represent a safe 'alternative' approach to complete separation and therefore warrant testing in a laboratory setting[2]."

Further to the alternative cleaning/disinfection method controls suggested by the participants, survey respondents also expressed a concern as to whether vacuum packers present a cross-contamination risk when used for the dual packing of raw and ready-to-eat products.

Research was carried out to determine the inactivation kinetic parameters that can be achieved using heat (both dry and wet) and chemicals (quaternary ammonium compounds (QAC), chlorine based and wipes) on surfaces inoculated with *E.coli* O157:H7. Best practice and verification methods were also investigated in relation to the alternative methods suggested. Investigations were also carried out to assess the cross contamination risks that occurs both externally from pack to pack and internally from contaminated areas of the vacuum packer that are hard to reach/clean.

3 METHODS

The alternative methods, proposed by food business operators, outlined the use of heat disinfection and/or chemical disinfection to decontaminate complex pieces of equipment. Efficacy, best practice and verification methods were investigated in relation to the alternative methods suggested. Research was also carried out to assess the cross contamination risks between vacuum packers and food items.

3.1 Comparison of toxigenic and non-toxigenic *Escherichia coli* O157:H7

Studies were carried out to ensure that non-toxigenic *Escherichia coli* O157:H7 was suitable for use in the practical studies.

D-values of representative examples of *Escherichia coli* O157:H7 toxigenic and non-toxigenic strains attached to stainless steel discs were determined using both moist and dry heating at a single process temperature. These experiments provided comparative data on the thermal inactivation of these strains under each heating condition, allowing subsequent use of non-toxigenic *E.coli* O157:H7 for the remaining heating studies (further details in Appendix I).

Research was also carried out to determine if *E. coli* O157:H7 toxigenic and non toxigenic strains had similar resistance under treatment with chemical disinfectants (further details in Appendix II).

[2] PSI (2013) Small food businesses' proposed alternative controls to the separate use of complex equipment, to control the risk from *E. coli* O157, p 1-76

3.2 Thermal disinfection

3.2.1 Thermal disinfection efficacy

D- and z-values for *E. coli* O157:H7 attached to stainless steel surfaces exposed to either dry or moist heating were determined. These data allow calculation of time and temperature combinations required to provide a specific reduction in this organism when attached to stainless steel. Sterile stainless steel discs (2 cm) with grade 2 finish on both sides were used to simulate stainless steel surfaces in this study. The discs were inoculated with known amounts of non-toxigenic *E. coli* O157:H7 and inoculum was allowed to dry at controlled humidity for 3 days. Inoculated discs were then subjected to dry and moist heat treatments as shown in Table 1. The interference of various substances such as protein and fats, both common in a food business operator's (FBO's) premises were not assessed. Levels of protein and fat are known to increase microbial heat resistance when heated in foods[3]. Different soilants will effect heat resistance to varying degrees and therefore the scope of this research was restricted to baseline heat resistance data on these organisms attached to stainless steel surfaces in the absence of soilants. However, it is standard industry practice to remove physical debris before commencing any disinfection process.

Table 1: Treatment temperatures used for both wet and dry heat treatment on stainless steel.

Dry heat (°C)	Wet heat (°C)
65	45
75	50
85	55
95	60
105	65

A D-value of *E.coli* O157:H7 was carried out using moist heating at 50°C on a plastic surface of a type commonly used in the food industry. Comparison of this data with data obtained for this organism treated under identical conditions on stainless steel was used to determine whether surface type has an influence on the thermal inactivation *E. coli* O157:H7 (Detailed description of work shown in Appendix I).

3.2.2 Thermal disinfection best practice

Using the results obtained from laboratory research into the thermal treatment of surfaces it was possible to draw up suitable best practice for the decontamination of applicable surfaces. The research was split into stages: The first stage investigated the practicalities of the methods suggested and the second stage developed best practice information as a result of this, combined with the laboratory results.

[3] Gaze, J. E. (2006) Pasteurisation: A food industry practical guide. Campden BRI Guideline 51 (Second edition)

The practicalities of the methods suggested were observed. For example, suitable food contact surfaces were subjected to heat treatments of both wet (submergence in a heated water bath) and dry (oven heating) conditions to observe the advantages and limitations of the method. The main complex item used was a Lan Elec; 812 slicer. This item was disassembled and the parts were placed in a hot water bath set at 65.2°C and an oven at 93°C. Temperatures were chosen based on both the results of the kinetic D- and z- values obtained in the *E.coli* thermal treatment research and the readings that could be observed on available temperature indicator labels (Omega, non-Reversible TL-4 Range Series (77-93°C/170-200°F)). Data loggers were used to measure the surface temperature of the items and temperature indicator strips were also used to verify the surface temperatures reached. The machine consisted of both metal and plastic parts. Using the information observed and details from the research carried out into the inactivation kinetic parameters that can be achieved using heat (both dry and wet) and chemicals on surfaces inoculated with *E. coli* O157:H7, best practice information could be provided (more details in Appendix IV).

3.2.3 Thermal disinfection verification

To ensure surfaces reach a desired temperature, temperature indicator strips can provide a quick and cost effective verification tool. The accuracy of temperature indicator labels were investigated using a BSI Standard (BS 7908:1999: Packaging - Temperature and time-temperature indicators - Performance specification and reference testing). The labels were assumed to be equivalent of BS708 Class 2 and the standard was carried out accordingly (more details in Appendix V)..

3.3 Chemical disinfection

3.3.1 Chemical disinfection efficacy

As chemical disinfectants have been used for many years in food manufacturing and food service, their performance with respect to concentration, contact time, temperature and interfering substances on some organisms is well established. The novelty in this work is in the use of *E. coli* O157:H7, which is not included with the range of organisms used in standard disinfectant tests, and the assessment of how it responds to disinfectants. Investigations were carried out into chemical type, concentration, contact time and method of application required to provide a 4 log reduction (specified by EN13697 criteria) of *E. coli* O157:H7 on suggested surfaces. This research investigated the efficacy of chemicals and the method of application (e.g. liquid and wipes) using the principles of the European disinfectant surface test EN 13697:2001.

The chemicals used in disinfection can be broadly described as either non oxidisers such as Quaternary Ammonium Compounds (QAC) or oxidisers such as chlorine based chemistries. Their efficacy is governed by their concentration, contact time, temperature and presence of interfering

substances such as organic material. The chemicals investigated were commercially available to the food service sector and included a Quaternary ammonium based compound (QAC – Benzalkonium chloride (BKC) and a Chlorine based compound (Sodium Hypochlorite (ClO^-).

The research aimed to ensure that when used optimally a 4 log reduction in *E. coli* O157:H7 on different surfaces can be achieved and also provide information as to how deviation from the optimum will influence disinfection efficacy. It is important to remember that the numerous variables affect the efficacy of the chemical treatment. High and low ranges of variables (detailed in Table 2) were used to show the suitability of using the correct treatment to gain the desired result. Surfaces were inoculated with non-toxigenic *E. coli* O157:H7, in various levels of soil and removed using some of the variables shown in Table 2. Interfering substance levels of 3 g/L and 30g/L were used to demonstrate the decrease in effectiveness of disinfectants in the presence of increasing amounts of organic material 0.3, 3 and 30g/L; thus the importance of cleaning surfaces prior to their use (Detailed description of work shown in Appendix II).

Table 2: Variables used during the chemical disinfection research. Also shown are recommended variables.

Chlorine	QAC
Concentration: 0.024–24% (~27 - 9200 ppm) **Recommended: 2.4 %**	Concentration: 0.0125–12.5% or (~3.75 - 3750 ppm) **Recommended: 1.25 %**
Contact time: 0.5 - 5 minutes **Recommended: 5 minutes**	Contact time: 0.5 - 5 minutes **Recommended: 5 minutes**
Temperature: 4 - 20°C) **Recommended: Ambient** (18 - 25°C)	Temperature: 4 - 20°C **Recommended: Ambient** (18-25°C)
Organic matter: 3 -150g/L BA **Recommended: Clean (<3 g/L)**	Organic matter: 3 -150g/L BA **Recommended: Clean (<3 g/L)**
Surfaces (stainless steel and plastic)	Surfaces (stainless steel and plastic)

There are currently no standard disinfection efficacy tests for disinfectant wipes and their use. Wipes were tested using the principles of EN 13697, but incorporating a mechanical motion to represent the wiping action used with these methods.

This research investigated the efficacy of disinfection wipes on temperature probe surfaces. The aim was to determine the effects of disinfection when wiping occurred (immediately after contamination or after contamination was dry), number of wipes, contact time after wiping, condition of wipes (wet/dry) and after immersion in a known alternative method of disinfection (recommended conditions of Chlorine, QAC or hot water).

It is important to remember that the numerous variables affect the efficacy of the wiping treatment. High and low ranges of variables (detailed in Table 3) were used to show the suitability of using the correct treatment to gain the desired result. Surfaces were inoculated with non-toxigenic *E. coli* O157:H7, in various levels of soil and removed using some of the variables shown in Table 3. (Detailed description of work shown in Appendix III).

Table 3: Variables used during the chemical wipe disinfection research. Also shown are recommended variables.

Surface	Alcohol Wipe	QAC Wipe
Temperature probes	Number of wipes applied (1 - 2) **Recommended: At least 2**	Number of wipes applied (1 - 2) **Recommended: At least 2**
	Wet or dry wipes **Recommended: Wet**	Wet or dry wipes **Recommended: Wet**
	Organic matter (3 - 150g/L BA) **Recommended: Clean (<3 g/L)**	Organic matter (3 - 150g/L BA) **Recommended: Clean (<3 g/L)**

3.3.2 Chemical disinfection Best practice

Using the results obtained from laboratory research into the chemical treatment of surfaces it was possible to draw up suitable best practice for the decontamination of applicable surfaces.

3.3.3 Chemical disinfection concentration verification

During chemical disinfection, the concentration of the solution can be easily verified using chemical test strips. Several rapid testing strip methods were used to test the concentration of both chlorine and Quaternary ammonium compound (QAC) solutions that may be used for disinfection purposes.

Solutions of various concentrations of chemicals (Chlorine and QAC) were made up and tested using verification strips from various manufactures. The concentrations of chlorine (Sodium Hypochlorite (ClO^-)) were also verified using analytical methods (ASTM D2022-89 (2008)). The concentrations of QAC (Benzalkonium chloride (BKC)) were determined using information from the manufacturer

(concentrations shown in Table 4 – (ClO^- was measured analytically and indicates a slight deviation from percentage measured by hand) (more details in Appendix V).

Table 4: Concentration of chemicals used (% and ppm)

	Chlorine Concentration		QAC Concentration	
	(%)	~(ppm)	(%)	~(ppm)
Undiluted	100	43400	100	30000
10 times recommended solution	24	9200	12.5	3750
Recommended solution	**2.4**	**1130**	**1.25**	**375**
1/10 of recommended solution	0.24	113	0.125	37.5
1/100 of recommended solution	0.024	27	0.0125	3.75
Water	0	0	0	0

3.4 Vacuum packer cross contamination studies

Investigations of cross–contamination to the outer and inner surfaces of vacuum packed products was demonstrated via two methods: (More details in Appendix VI)

3.4.1 External cross contamination

External cross contamination was investigated using UV detectable microbial simulants (GlitterBug Potion, 240ml; Brevis Corporation) to represent contamination on a piece of meat. One piece of meat, heavily contaminated with the UV simulant, was placed in a vacuum bag and vacuum packed. The operator continued to pack 4 more pieces of meat after handling the 1st contaminated piece of meat. The operator changed gloves and then continued to pack 5 more bags of meat using the same method as before. The machine was not wiped down in between uses. An ultra-violet (UV) light (Revealite™; Cleanitise) was used to show the contamination transferred from the 1st contaminated meat onto the proceeding packaged products and operators gloves. This method was carried out using two different vacuum packing machines, of different sizes and ages relevant to food service, shown in Figure 1.

Figure 1: Vacuum packer machines used in the study; (a) Multivac Gastrovac and (b) Multivac C200

3.4.2 Internal cross-contamination

The research into the **internal cross-contamination** of the items packed in a vacuum packer involved inoculating the vacuum packing machine (Figure 1 a) with *Serratia marcescens* subsp. *marcescens. marcescens* is a species of rod-shaped gram-negative bacteria, in the family Enterobacteriaceae. *S. marcescens* produces the red pigment prodigiosin, which facilitates easy detection by the presence of a red colouration. Due to the sensitive nature of the research, the investigation was carried out in 2 separate trials. In the 2nd trial 2 new machines were inoculated in the same manner and details are shown in Appendix VII.

Preliminary trials were completed to test suitability of culture and method (Appendix VI).

Vacuum packer trial

Two TSA plates were opened, placed in the chamber of the vac-packer (Multivac Gastrovac) and exposed to the vacuum packing process (see Figure 2). The vacuum packer internal parts were then inoculated with an overnight culture of *Serratia marcescens* (Figure 3 a and b). Areas of inoculation were decided following previous FSA research (Guidance on re-commissioning vacuum packers and weighing scales). The vacuum packer was then reconstructed and the chamber area cleaned with alcohol wipes (Azowipe, disinfectant wipes; Synergy Health plc) before starting. One hundred TSA discs (plates removed aseptically) were vacuum packed in 200 mm x 300 mm vacuum pouches (The Food Machinery Company Ltd).

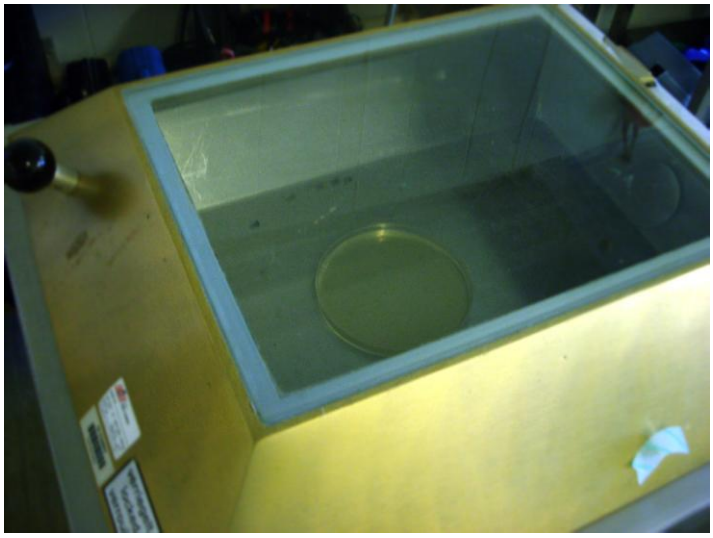

Figure 2: Open control plate test carried out in duplicate before and after packing 300 agar discs contained in vacuum pouches.

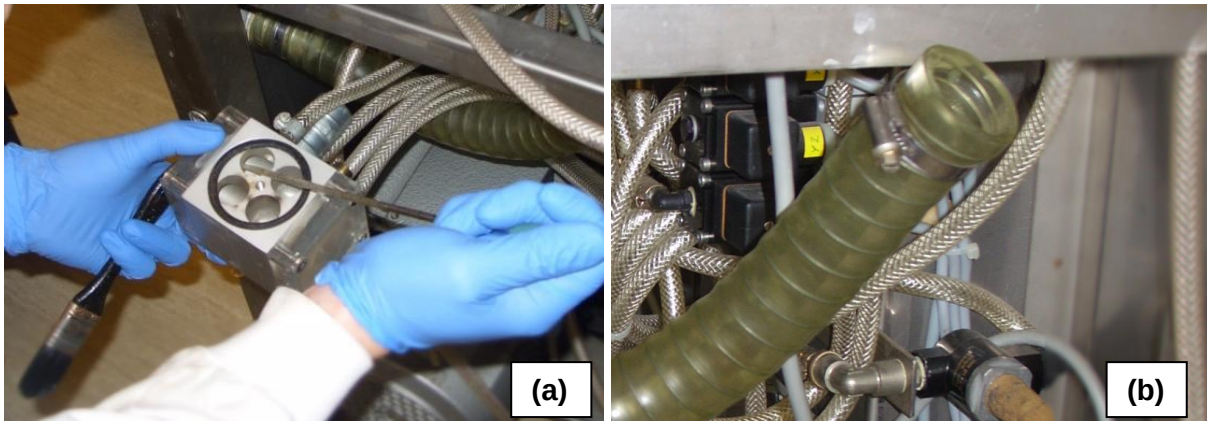

Figure 3: Inoculation of; (a) the suction pipe manifold and (b) the suction pipe

The machine was again taken apart and two further areas were inoculated; the rear side of the air outlet cover plate (Figure 4 a) and the inner surfaces of each gas flushing nozzle (Figure 4 b).

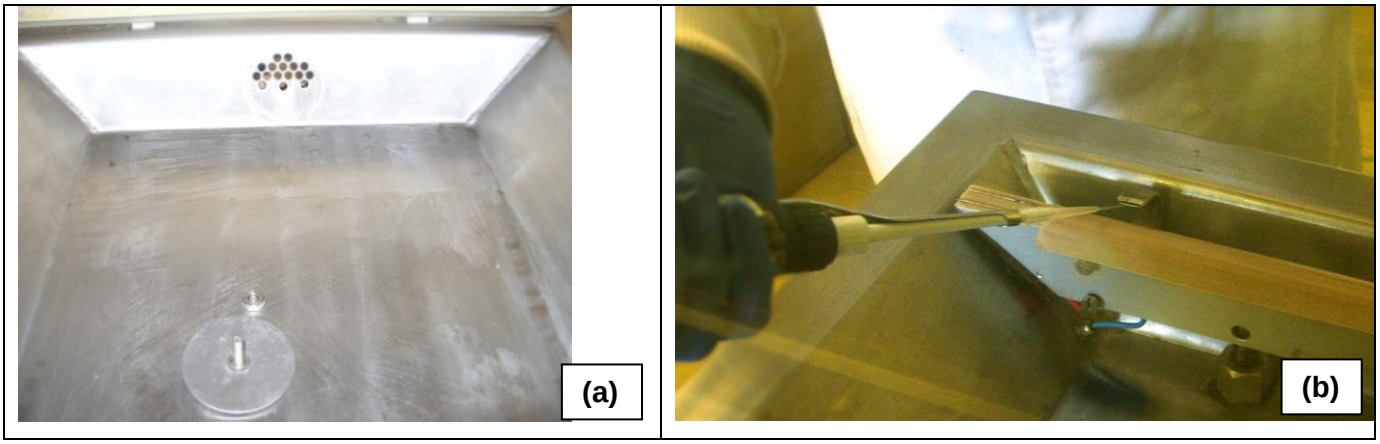

Figure 4: Inoculation of: (a) the rear side of the air outlet cover plate and (b) the inner surfaces of each gas flushing nozzle

The inside of the machine was cleaned with alcohol wipes before vacuum packing a further 200 TSA discs. As a final test, 2 further TSA plates were opened and placed in the vacuum packer and exposed to the vacuum packing process (with no bag – Figure 2).

4 RESULTS AND DISCUSSION

4.1 Comparison of toxigenic and non-toxigenic *Escherichia coli* O157:H7

The D-values determined for both toxigenic and non-toxigenic *Escherichia coli* were found to be comparable. Whilst the heat resistance of the toxigenic strain is slightly higher than the non-toxigenic strain, this difference could easily be accounted for by the application of a margin of safety in the calculation of hold time required to produce a suitable log reduction. The addition of such a margin of safety would be considered good practice in any case, as it would cover the effect of strain to strain variation in heat resistance of *Escherichia coli* O157:H7 (More details in Appendix I)

There was found to be no practical difference between the susceptibility of non toxigenic *E. coli* O157:H7 and the toxigenic *E. coli* O157:H7 to each of the two disinfectants at the concentrations tested and under the obligatory test conditions. (More details in Appendix II). This verified the use of non-toxigenic strains as suitable for this study.

4.2 Thermal disinfection

4.2.1 Thermal disinfection efficacy

4.2.1.1 Dry heat treatment

The D- and z-values calculated for *Escherichia coli* O157:H7 using dry heat are displayed in Table 5 and illustrated in Figure 5. D-values determined on stainless steel surfaces treated with dry heat were found to be considerably higher than might be expected for *E. coli* O157:H7 in a typical moist process. For example, the D-value of *E. coli* O157:H7 at 65°C in distilled water was found to be 0.05 minutes in a study by Spinks *et al.* (2006)[4], compared with 136.8 minutes on stainless steel treated with dry heat. Furthermore, a moist process of 70°C for 2 minutes, applied to short shelf-life chilled foods, would be expected to achieve a >6 log reduction in *E. coli*, whereas these results show that on stainless steel surfaces treated with dry heat at 70°C, a hold time of 322 minutes would be required to produce the same log reduction in this organism.

[4] Spinks, T. A., Dunstan, R.H., Harrison, T., Coombes, P. and Kuczera, G. (2006) Thermal inactivation of water-borne pathogenic and indicator bacteria at sub-boiling temperatures, Water Research, **40**, 1326 – 1332

Table 5: Dry heat D-values determined for *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel.

Process temperature (°C)	D-value (minutes)
65	136.8
75	35.1
85	22.6
95	8.7
105	3.9
z-value (C°)	**27.1**

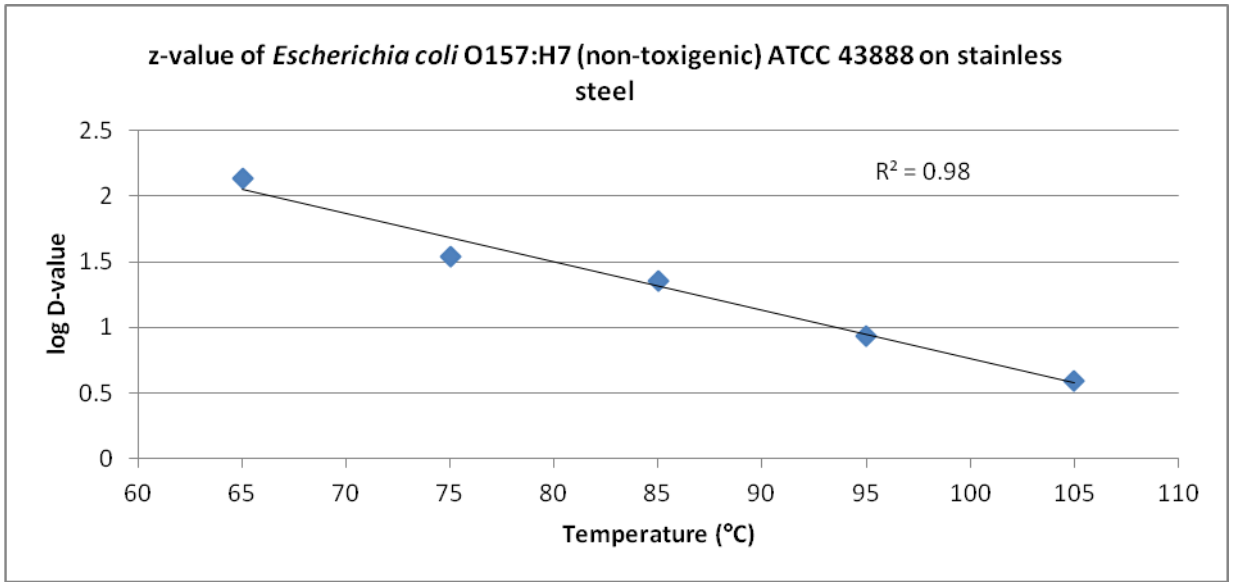

Figure 5: Dry Heat: z-value of *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel surfaces

The D- and z-values calculated for *E. coli* O157:H7 using cells dried on to stainless steel surfaces and then heated in water (moist heat) is displayed in Table 6 and illustrated in Figure 6. The D-values were similar to those described by Spinks *et al.* (2006)[5], when heated at 65ºC. However, the z–value of 21.8C° determined for this organism on stainless steel was only slightly lower than the z-value determined using dry heat (27.1C°). This z-value is considerably higher than would be expected for this organism in a normal moist situation, where the z-value might be 5-7C°[6]. An explanation for this z value may be attributed to the fact that the organisms were dried on to stainless steel discs and would then experience a degree of sensitivity according to stresses related to the cellular wall rehydration during heating. Consequently the environmental conditions may be closer to dry heating as is reflected in the z value. We would err on the side of caution when extrapolating to the lower temperatures during

[5] Spinks, T. A., Dunstan, R.H., Harrison, T., Coombes, P. and Kuczera, G. (2006) Thermal inactivation of water-borne pathogenic and indicator bacteria at sub-boiling temperatures, Water Research, **40**, 1326 – 1332

[6] Gaze, J. E. (2006) Pasteurisation: A food industry practical guide. Campden BRI Guideline 51, Second edition

industrial use and would therefore only refer to the temperature/time combination of 65ºC for 8.4 seconds.

Table 6: Wet heat D-values determined for *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel.

Process temperature (°C)	D-value (seconds)
45	21.7
50	10.5
55	5.4
60	3.4
65	2.1
z-value (C°)	**21.8**

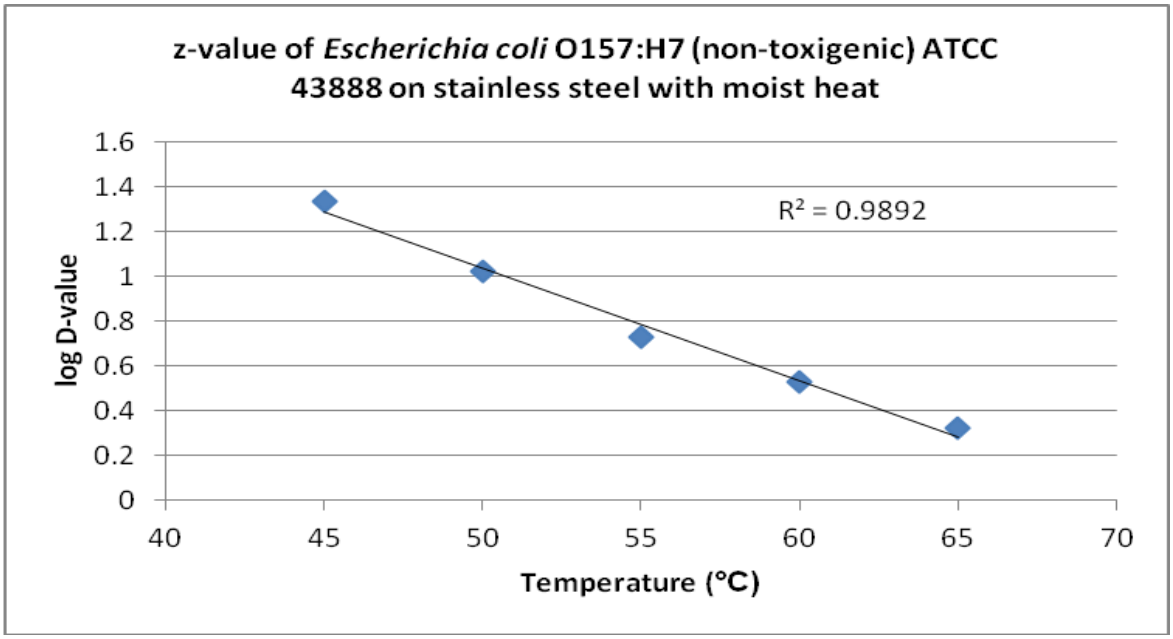

Figure 6: Moist Heat: z-value of *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel surfaces

4.2.1.2 Wet heat treatment on plastic

The effect of moist heating on *Escherichia coli* O157:H7, attached to either stainless steel or plastic surfaces was investigated. D-values at 50°C using moist heat on plastic and stainless steel discs were compared to assess the effect of surface-type on heat resistance of *E.coli* O157:H7 ATCC 43888. D-values were calculated to be 10.0 and 10.5 seconds on plastic and stainless steel surfaces respectively, showing that the heat resistance of the organism does not appear to differ greatly when attached to either of these surface types.

4.2.2 Thermal disinfection best practice

4.2.2.1 Oven studies: Dry heat best practice

The results of laboratory dry heat studies carried out (details in Section 4.2.1.1) show that the resistance of *E. coli* O157:H7 attached to stainless steel towards dry heat is very high compared to that in moist heat in foods or in wet conditions(details in Section 4.2.1.2).

Dry heat best practice:

Evaluate the suitability of the food contact item to the intended dry heat treatment. If the heat is likely to cause damage to the item this method is not suitable.

Before use of a heat treatment the item to be treated must be clean and free from all food debris. This may be achieved using mechanical and/or chemical treatment (e.g detergent/surfactant).

Parts of the equipment which come into direct contact with food shall be placed into an oven at temperatures and times shown in Table 7 or equivalent temperatures and time.

It must also be noted that depending on the type of surface the time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

The treatment can be verified using temperature indicator strips to ensure the surface reaches the specific temperature and then timed until the duration of holding time is met.

Table 7: Holding time suggestions to reduce *E. coli* O157:H7 contamination on stainless steel using dry heat.

Process temperature (°C)	D- value (Minutes)	Holding time to achieve a 4 log reduction (Minutes)* (in line with BS EN 13697:2001[7])*
65	136.8	547.2
75	35.1	140.4
85	22.6	90.4
95	8.7	34.8
105	3.9	15.6

**D-value, or decimal reduction time, in microbiology refers to the time required, at a given temperature, to achieve a 90% (or 1 log) reduction in the population of a given microorganism. To obtain a 4 log reduction the D-value was multiplied by 4.*

4.2.2.2 Hot water submergence: Wet heat best practice

The results of laboratory wet heat studies carried out (details in Section 4.2.2) show that the heat resistance of *E. coli* O157:H7 attached to stainless steel towards wet heat is much lower compared to dry heat treatments (details in Section 4.2.1).

[7] BS EN 13697:2001. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants

Wet heat best practice:

Evaluate the suitability of the food contact item to the wet conditions. If water is likely to ingress into hard to reach areas or damage is likely, this method is not suitable.

Before heat treatment the item to be treated must be clean and free from all food debris. This may be achieved using mechanical and/or chemical treatment (e.g detergent/surfactant).

If wet heat treatments are used to decontaminate stainless steel, these should follow the minimum heat treatment at holding times shown in Table 8 or higher. It must also be noted that depending on the type of surface the time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

The treatment can be verified using temperature indicator strips to ensure the surface reaches the specific temperature and then timed until the duration of holding time is met.

Table 8: Holding time suggestions to reduce *E. coli* O157:H7 contamination on stainless steel using moist heat.

Process temperature (°C)	D-value (seconds)	Holding time to achieve a 4 log reduction (seconds)* (in line with BS EN 13697:2001)[8]
65	2.1	8.4

**D-value, or decimal reduction time, in microbiology refers to the time required, at a given temperature, to achieve a 90% (or 1 log) reduction in the population of a given microorganism. To obtain a 4 log reduction the D-value was multiplied by 4.*

4.2.2.3 Overall thermal treatment best practice:

Thermal treatment best practice:

Hands should be washed prior to cleaning/disinfecting the equipment.

Disassemble the equipment.

Remove all food debris from the food contact item using single use sanitised cloths. Mechanical action and/or chemical treatment (e.g detergent/surfactant) can be applied to help remove any extra food debris.

Parts of the equipment which come into direct contact with food shall be placed into an oven or heated water at temperatures and times shown in Tables 7 and 8 respectively, or higher/equivalent temperatures and times as calculated from wet and dry heat z-values.

The time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

If suitable, instead of using dry or wet heat, a dishwasher at a temperatures of 60°C or higher can be used to clean the food contact items. The time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

[8] BS EN 13697:2001. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants

The temperature of the surface of the equipment can be verified using temperature indicator strips. When the surface temperature has been reached, maintain the item at the correspondent time for that temperature.
When the treatment time has been completed, remove the items from the oven or hot water safely and allow to cool on a clean, heat proof surface. Wet items should be air dried or dried with disposable towels.
Once dried or cooled the items should be reassembled with clean hands and moved to a clean area.

4.2.3 Thermal disinfection verification

All temperature indicator strips used in this study were commercial products and thus expected to perform as stated in their use instructions. The aim of this research was to investigate the reliability of verification methods, readily available to small businesses, for the purposes of monitoring the efficacy of *E. coli* O157:H7 decontamination techniques for food service equipment (Details of the verification report: Appendix V).

The verification methods may be a good technique:

- To monitor and record the disinfection practices used.
- To assist during disinfection training.
- To verify that the method is correct on changing operator or the disinfection method.
- To validate chemical dosing when a disinfectant method is used for the first time.

During the assessments of the temperature labels it was observed that the labels were a suitable method to verify the heat treatment achieved on surfaces during both wet and dry heat treatment. It was found that the labels tend to change colour when close to the threshold temperature (this change is usually quite slow). At or above the threshold temperature they tend to change faster. Some of the labels were reversible after the heat treatment indicating that they would not be suitable as proof of validation of a disinfectant method. It may be advisable to use photographic evidence if used for verification of results.

The advantage of the thermal label method is that the operator does not require training as the method is quite straight forward.

4.3 Chemical disinfection

4.3.1 Chemical efficacy applied as a liquid

As shown in Appendix II there no practical difference between the susceptibility of the non toxigenic *E. coli* O157:H7 and the toxigenic *E. coli* O157:H7 to each of the two disinfectants at the manufacturer's recommended concentration and two lower concentrations under the obligatory test conditions. Various

factors affect the efficacy of disinfectants such as concentration of chemical, contact time, interfering substances on the surface, temperature and surface type[9][10]. Research was carried out to assess whether *E. coli* O157:H7 (non toxigenic) responds to disinfectants as expected.

The efficacy of the disinfectants were investigated using the principles of the European bactericidal surface test EN 13697:2001. Whilst the manufacturer's recommended concentrations along with the tests obligatory parameters were tested, these parameters (disinfectant concentration, contact time, interfering substance concentration, temperature and the test surface) were altered to demonstrate the effects of deviating from the optimal use conditions. (Information on the variables used are shown in Table 2; Section 3.3.1).

Table 9 (a)-(f): Results comparing QAC and chlorine using various parameters and variables as described below (Results shown are individual replicate resultsfor each test).

KEY:	PASS (meets the 4 log reduction EN13697 criteria)	FAIL (fails 4log reduction EN13697 criteria)

a) Parameters used: Surface: Stainless steel, Temperature: 20°C, Interfering substances: 0.3g/L BA (clean), Time: 5 minutes
Variable: EN standard test strain and a Non-toxigenic strain of *E. coli* O157:H7

		QAC	Chlorine
		log reduction	log reduction
Organism	***E. coli* (EN Standard)** Recommended	>4.3	>5.1
Disinfectant Concentration		>4.3	>5.1
		>4.3	>5.1
Organism	*E. coli* O157:H7 non Toxigenic Recommended	>4.2	>4.3
Disinfectant Concentration		>4.2	>4.3
		>4.2	>4.3

Legend Results are of 3 replicates per test

[9] Maillard, J-Y. 2012. Factors affecting the activities of Microbicides. In:. Eds Fraise, A. P. Maillard, J-Y. Sattar, S. A. eds *Russell, Hugo & Ayliffes Principles and Practice of Disinfection, and Preservation and Sterilization.* Wiley-Blackwell pp 71
[10] Staniforth, L. 2012. Evaluation of antimicrobial efficacy. In:. Eds Fraise, A. P. Maillard, J-Y. Sattar, S. A. eds *Russell, Hugo & Ayliffes Principles and Practice of Disinfection, and Preservation and Sterilization.* Wiley-Blackwell pp 236

b) Parameters used: Strain: *E. coli* O157:H7 non toxigenic, Surface: Stainless steel, Temperature: 20°C, Interfering substances: 0.3g/L BA (clean), Time: 5 minutes
Variable: Disinfectant concentration

		QAC	Chlorine
		log reduction	log reduction
Disinfectant Conc	**10 times recommended**	>4.2	>4.0
		>4.2	>4.0
		>4.2	>4.0
	1/10th recommended QAC	>4.2	0.2
		2.3	0.3
	1/100th recommended ClO^{-}	>4.2	0.7

Legend Results are of 3 replicates per test

c) Parameters used: Strain: *E. coli* O157:H7 non toxigenic, Surface: Stainless steel, Temperature: 20°C, Interfering substances: 0.3g/L BA (clean), Disinfectant Concentration: Recommended
Variable: Time

		QAC	Chlorine
		log reduction	log reduction
Time	**1 minute**	3.1	1.7
		>4.2	1.0
		>4.2	0.7
	30 seconds	1.5	1.4
		1.8	1.8
		1.9	1.6

Legend Results are of 3 replicates per test

d) Parameters used: Strain: *E. coli* O157:H7 non toxigenic, Surface: Stainless steel, Temperature: 20°C, Time: 5 minutes, Disinfectant Concentration: Recommended
Variable: Interfering substances

		QAC	Chlorine
		log reduction	log reduction
Interfering substance	**3g/L**	2.7	>5.3
		2.2	>5.3
		3.3	>5.3
	30g/L	0.9	0.7
		0.5	0.7
		0.8	1.3

Legend Results of 3 replicates per test

e) Parameters used: Strain: *E. coli* O157:H7 non toxigenic, Surface: Stainless steel, Interfering substances: 0.3g/L BA (clean), Time: 5 minutes, Disinfectant Conc: Recommended.
Variable: Temperature

		QAC	Chlorine
		log reduction	log reduction
Temperature	**4°C**	2.3	>5.5
		>4.0	>5.5
		>4.0	>5.5

Legend Results of 3 replicates per test

f) Parameters used: Strain: *E. coli* O157:H7 non toxigenic, Temperature: 20°C, Interfering substances: 0.3g/L BA (clean), Time: 5 minutes, Disinfectant Concentration: Recommended.
Variable: Surface

		QAC	Chlorine
		log reduction	log reduction
Surface	**Plastic (smooth)**	>4.0	>5.3
		>4.0	1.9
		>4.0	2.7
	Plastic (rough)	>4.0	4.0
		>4.0	3.4
		>4.0	2.6

Legend Results of 3 replicates per test
Rough surface representative of textured surfaces and/or smooth surfaces that have been damage

Table 9 (a) demonstrates that both strains of *E. coli* (EN standard test strain of *E. coli* and a non-toxigenic strain of *E. coli* O157:H7) are susceptible to the disinfectants (>4 log reduction) when tested at their recommended concentrations under obligatory EN13697:2001 clean conditions.

The use of higher than recommended disinfectant concentrations (Table 9 (b)) has no practical negative effect upon efficacy, however, the increased chemical concentrations have implications upon health and safety and may be incompatible with some materials such as mild steel. The use of a more dilute disinfectant demonstrates that this does reduce the efficacy of the disinfectants with the chlorine based disinfectant (1/100th manufacturers recommended) not achieving the 4 log reduction required in 3 out of 3 replicates and the QAC based disinfectant (1/10th manufacturers recommended) not achieving the 4 log reduction in 1 out of 3 replicates. Thus concentrations less than the recommended have an increased risk of failing to achieve a 4 log reduction. Care needs to be taken to ensure that the correct disinfectant concentration is achieved.

Table 9 (c) shows that contact time has a significant effect upon disinfectant efficacy with a 5 minute contact time demonstrating a consistent >4log reduction for both disinfectants and a 30 second contact time at the concentrations tested demonstrating a consistent <2 log reduction. Disinfectants should always be left on a surface for their recommended contact time before being rinsed/wiped off.

Increasing levels of organic matter/interfering substance, as shown in Table 9 (d), demonstrated a negative affect upon disinfectant efficacy. Surfaces should always be cleaned of food soiling prior to disinfection.

Reduced temperature (4^0C $^{\pm}2$ ^{0}C) also demonstrated a negative effect upon efficacy, with 1 of the replicates for the QAC disinfectant failing (Table 9 (e)). In high care areas of the food industry (low temperature approximately 10ºC) disinfectants are often applied for increased contact times e.g. 20 minutes.

The plastic smooth surface used was typical of clear plastic used in machine shielding. In the experiments a degree of hydro-phobicity was noted and this may explain the two Chlorine based failures on this surface (Table 9 (f)). It was noted that when applied to a rough plastic surface the wetting action of the QAC disinfectant gave a more complete coverage and this is reflected in the results with QAC meeting the 4 log reduction criteria on all surface tested and the Chlorine based disinfectant having a range of log reductions from 2.6 to 3.95.

4.3.2 Chemical disinfection applied as a liquid best practice

Results shown in Table 9 (a) – (f) indicate that the disinfectants tested controlled *E. coli* on stainless steel (defined as passing the disinfection test) at its manufacturer's recommended concentration and under the obligatory conditions of the BS EN 13697:2001 test: (contact time 5 minutes; temperature 18 - 25°C and clean conditions 0.3 g/L BA. Both worked well at a 10 times strength of the recommended concentration and showed loss of effectiveness at lower than recommended dilutions. Shortening contact times (<5 minutes), lowering temperatures (4°C) and increasing interfering substance concentrations were shown to have a negative effect upon the disinfectants efficacy

The results in Table 9 show that *Escherichia coli* O157:H7 attached to stainless steel and plastic can be reduced by more than 4 logs when treated under the correct conditions

4.3.2.1 Chemical disinfection best practice:

Chemical disinfection best practice:

Hands should be washed prior to cleaning and disinfecting the equipment.

Disassemble the equipment as much as possible

Before use of any disinfection treatment, the item to be treated must be clean and free from all food debris. This may be achieved using mechanical and/or chemical treatment (e.g detergent/surfactant).

Evaluate the suitability of the food contact item to the treatment conditions; If the method is likely to damage the article an alternative method should be sought.

Ensure the disinfectant selected is suitable for the surfaces to be disinfected.

Once the contact time has passed the disinfectant can be rinsed off using clean (potable) water.

The item can be dried using air drying methods, or disposable towels.

Once dried the items should be reassembled with clean hands and moved to a clean area.

Disinfection of food contact areas can be undertaken with chemicals, providing they are suitable for such areas and meet the criteria of BS EN 1276 and/or BS EN 13697.

Manufacturer's instructions should be followed when using any chemical.

Suitable training is important to ensure disinfection is carried out in the correct manner, for example using the correct concentrations, temperature and contact times.

The chemical concentrations can be verified using indicator strips to ensure the concentration applied to the surface is as desired.

4.3.3 Chemical applied as a liquid concentration verification

All chemical indicator strips used in this study were commercial products and thus expected to perform as stated in their use instructions. The aim of this research was to investigate the reliability of verification methods, readily available to small businesses, for the purposes of monitoring the efficacy of *E. coli* O157:H7 decontamination techniques for food service equipment (Details of the verification report: Appendix V).

The verification methods may be a good technique:

- To monitor and record the disinfection practices used.
- To assist during disinfection training.
- To verify that the method is correct on changing operator or the disinfection method.
- To validate chemical dosing when a disinfectant method is used for the first time.

Rapid chemical testing strips were assessed for their suitability to test the concentration of a disinfectant solution. They were found to be quite accurate as long as the correct detection level strip

was used. These results may vary depending on the type of chemical method used and recommended concentration suggested. Photographic evidence could be used if required for verification of results.

The disadvantage of this method is that it requires the operative to be able to assess and understand the chemical concentrations required and choose the correct detection level of test strip. For example, being able to calculate the level of the active ingredient (HOCl or QAC) in the manufacturer's recommended in-use concentration in parts per million (ppm) from the manufacturer's stated in-use concentration as a percentage dilution.

4.3.4 Chemical disinfectant wipe efficacy

The research in this study investigated the efficacy of sanitising wipes on temperature probe surfaces. The aim was to determine the level of disinfection when wiping occurred (immediately after contamination or after contamination was dry), number of wipes, contact time after wiping, condition of wipes (wet/dry) and after immersion in a known alternative method of disinfection (Chlorine, QAC or hot water) (more details in Appendix III for).

Table 10: Results of efficacy of wipes against a visibly dry challenge of *E. coli* Non toxigenic O157:H7 ATCC 43888

Interfering substance level	Active	Treatment	Mean Log of bacteria left on probe	Reduction
30g/L Bovine albumin solution	Control	0 wipe	3.31	NA
	Alcohol	1 wipe	3.34	-0.03
		2 wipes	3.11	0.2
	QAC	1 wipe	3.29	0.02
		2 wipes	2.75	0.56
150g/L Bovine albumin solution	Control	0 wipe	5.34	
		1 wipe	4.23	1.11
	alcohol	2 wipes	4.33	1.01
	QAC	1 wipe	3.74	1.6
		2 wipes	3.13	2.21

Legend:
Results are means of 3 replicates.
Increase in log recovery compared to the control is within experimental variation (<0.5 log)
30g/L BA is 10 times the dirty surface conditions in EN13697:2001, 150g/L BA is the maximum BA concentration in solution and represents the worst-case interfering substance scenario achievable in this method

Table 11: Summary of results from efficacy of wet and dry disinfectant wipes.

Interfering substance level	Condition of wipe	Active	Test	Mean Log of bacteria left on probe	Reduction
	NA	Control	0 wipe	6.45	NA
150g/L Bovine albumin solution	Wet	Alcohol	1 wipe	4.35	2.11
			2 wipes	3.74	2.71
		QAC	1 wipe	3.98	2.47
			2 wipes	<2.79	>3.66
	Dry	Alcohol	1 wipe	4.26	2.19
			2 wipes	4.01	2.45
		QAC	1 wipe	3.96	2.49
			2 wipes	3.23	3.23
		Tissue	1 wipe	3.68	2.77
			2 wipes	4.19	2.27

Legend:
The wet results are from the means of duplicate tests containing 3 replicates
Results are means of 3 replicates
Increase in log recovery compared to the control is with experimental variation (<0.5 log)
150g/L BA is the maximum BA concentration in solution and represents the worst-case interfering substance scenario achievable in this method

Table 12: Summary of effect of contact time upon efficacy

Interfering substance level	Additional contact time (after wipes)	Active	Test	Mean Log of bacteria left on probe	Reduction
150g/L Bovine albumin solution	5min	control	0 wipe	5.73	NA
		alcohol	1 wipe	3.08	2.65
			2 wipes	<2.54	>3.19
		QAC	1 wipe	3.00	2.73
			2 wipes	1.52	4.21

Legend
Results are means of 3 replicates
150g/L BA is the maximum BA concentration in solution and represents the worst-case interfering substance scenario achievable in this method

Table 13: Summary of efficacy of immersion of contaminated probes into treatments

Interfering substance level	Treatment after dry wipe	Mean Log of bacteria left on probe	Reduction
150g/L Bovine albumin solution	Control	5.5	NA
	5min ClO-	<1.2	>4.3
	5min QAC	<1.4	>4.1
	2min 80^0C ±2 ^{0}C Water	<1.2	>4.3

Legend
Results are means of 3 replicates
150g/L BA is the maximum BA concentration in solution and represents the worst-case interfering substance scenario achievable in this method

The results, shown in Table 10, indicate that when the challenge (inoculum) was dried onto the probe surface the removal was minimal (<1 log reduction). During the comparison of wet (new from container) and dry (dried at $37^0C \pm 1^0C$) wipes, there also appeared to be little difference between the log reductions achieved (Table 11). For both wet and dry wipes the alcohol did appear to be consistently less effective than the QAC wipes this may be due to the lack of a cleaning surfactant in the alcohol wipes for the wet wipes and the evaporation of the active (alcohol is more volatile than QAC) for the dry wipes. The inclusion of tissue paper (dry) demonstrated that a significant amount of removal may be due to mechanical removal from the probes.

Results shown in Table 12 indicate that whilst there appears to be a small improvement in log reduction achieved when wet disinfectant wipes are used upon a wet challenge and left for a contact time of 5 minutes compared with previous results (Table 11) it is unclear the significance of this difference and further testing would be required to confirm.

Table 13 indicates that based upon the results of immersion in a disinfectant at a concentration that would meet the EN13697:2001 criteria or hot water, demonstrated a mean log reduction of greater than 4 log reductions.

4.3.5 Chemical disinfectant wipe best practice

4.3.5.1 Sanitiser wipes

There is currently no standard test that simulates the physical removal of dirt or microbes by wipes. Most tests are carried out on the disinfectant within the wipe via suspension tests, although some wipes may be tested via a wiping action on inoculated surfaces.

Best practice recommends that surfaces should be physically clean prior to disinfection in order for any disinfectant to be effective. To provide more effective disinfectant of probes, they should first have food debris removed. This may be achieved using mechanical and/or chemical treatment (e.g detergents/surfactant), before either wiping with a disinfectant wipe (if a reduction of 3 log (99.9%) is acceptable) or dipping in a recommended concentration of a chemical disinfectant or hot water for a suitable contact time (if a reduction of >4 log (99.99%) is required). The probe can then be wiped with a disinfectant wipe before allowing to air dry. Suitable training is important to ensure disinfection is carried out in the correct manner, for example; correct storage of the wipes so that they do not dry out and not re-using the same wipe.

The research provides no evidence that disinfectant wipes alone result in a 4 log reduction of *E. coli.* Therefore, wipes could be used as a risk reduction method for the removal of food residue with some microbial load reduction. However, wipes should not be used as a control for *E. coli* O157:H7. Best practice advice would be to disinfect probes using wipes straight after a contamination incident. It

would be suggested that wipes are only appropriate for use between products of the same nature (for example only between raw foods). A further step should be used to disinfect when probing RTE products after probing raw products. This may involve hot water or chemical submergence and probes should always be wiped again if they have not been used for some time prior to probing foods.

4.3.5.2 Sanitiser wipes: Best practice

Wipes best practice:

When disinfecting probes, wipes should be used straight after the use of the probe (optimum time of use).

The probe should be given one wipe to remove debris and then wiped with a second clean wipe to sanitise. The probe should be stored in a clean area between uses and sanitised again before further use.

If a probe is to be used for probing RTE products following the probing of raw products, it must first be cleaned of food debris (using mechanical and/or chemical treatment such as detergents or surfactants) before being disinfected via heat (immersion in hot water (>80ºC) or dish-washed at (60°C) or immersing in chemical disinfectants at the manufacturers recommended concentration and contact time.

To maintain their effectiveness the following key points should be considered;

Wipe manufacturer's instructions for storage guidance (where to store and length of storage life)

Stock rotation and age of wipes to ensure they have not dried out or expired.

The risk of cross contamination from the exterior of containers/packets in which the wipes are stored.

Ensure all staff who will use wipes have received training on how and when to use them to help guarantee consistency and efficacy of use in practice.

4.4 Vacuum packer cross contamination studies

4.4.1 External cross-contamination:

The spread of the UV simulant was visible under UV light conditions and in the images shows up as illuminated areas. Figures 7 and 8 respectively, show the contamination that was visible on the control panel and chamber area of both machines, after packing the initially contaminated product. Figure 9 shows the contamination on the gloves after vacuum packing 1 contaminated item followed by 4 consecutive items and then; after changing gloves and continuing to pack 5 further items. These results show that contamination that is passed onto a surface, can spread on to clean hands/gloves from a previous contamination event.

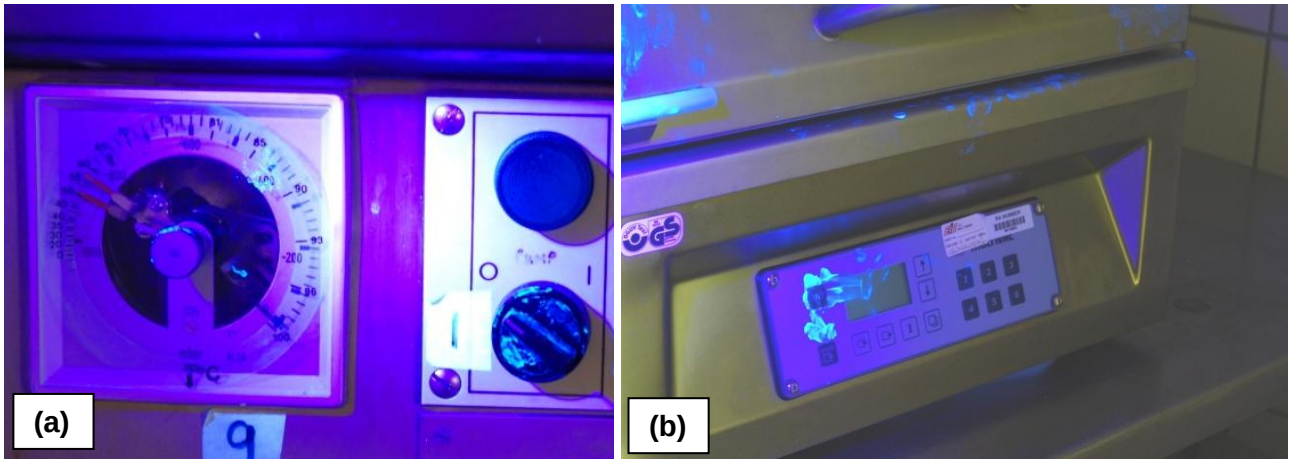

Figure 7: Control panel contamination after vacuum packing 1 contaminated item followed by 9 consecutive items; (a) Multivac Gastrovac; (b) Multivac C200.

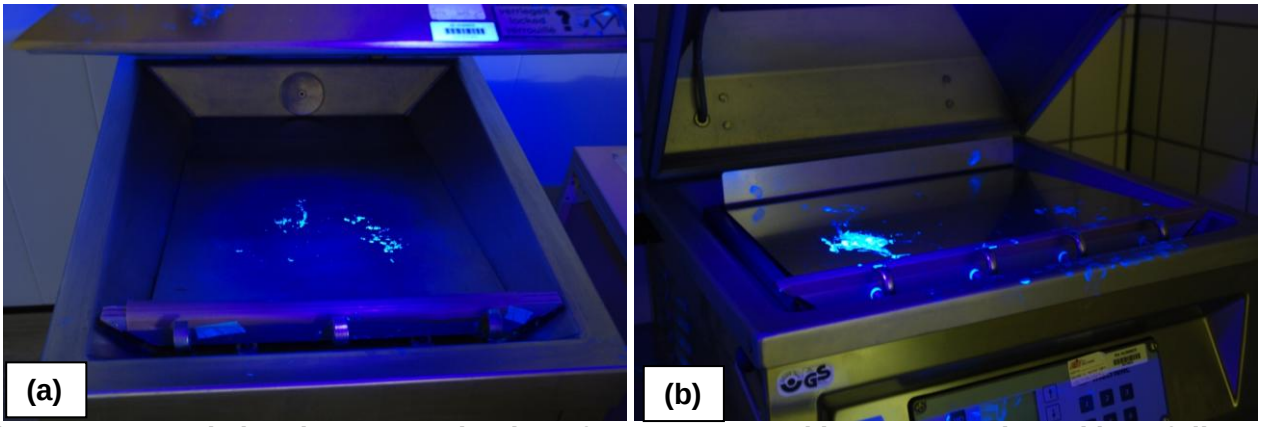

Figure 8: Internal chamber contamination after vacuum packing 1 contaminated item followed by 9 consecutive items; (a) Multivac Gastrovac; (b) Multivac C200.

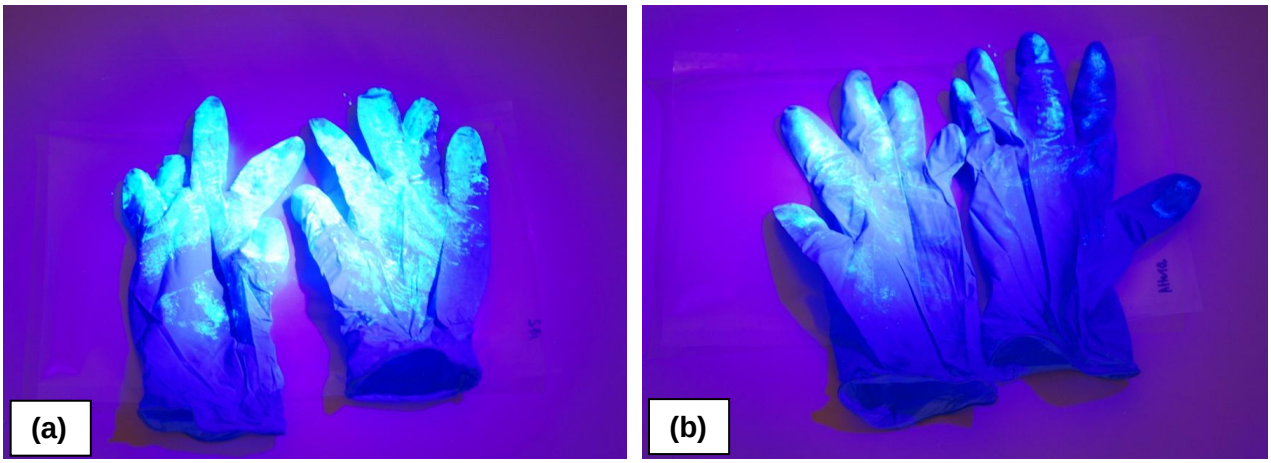

Figure 9: (a) Glove contamination after vacuum packing 1 contaminated item followed by 4 consecutive items and then; (b) after changing gloves and continuing to pack 5 further items.

Table 14 details the results and comments on the contamination spread from 1 initial heavily contaminated piece of meat (sample number 1), followed by proceeding packets of clean product (sample numbers 2-5). The operator changed gloves and continued to pack items (sample numbers 6-10). Sample numbers 1-5 appeared heavily contaminated with the UV simulant. When the operator changed their gloves the contamination was visibly lower, though still present. The operator's replaced gloves (Figure 9 b) had most likely been re-contaminated via contact with the exterior surfaces and the control panel of the vacuum packers (Figures 7). This contamination was then evident on the subsequent packed product and bags (Table 14: sample 6-10). The spread of the UV simulants represents the contamination that may occur after vacuum packing a contaminated piece of raw meat. The further samples packed (Table 14: samples 2-10) represent the cross contamination that can occur when the machine has not been thoroughly cleaned between use. Figure 9 (b) represents the contamination that can spread onto operators gloves/hands when using a contaminated machine. The results indicate that if an item, heavily soiled with microorganisms, was packed and a cleaning step was not suitably carried out afterwards, there is the potential for the spread of contamination from the machine onto subsequent items packed in the machine.

The contamination, if somewhat small, on the subsequently packed products following the initial soiling event, represents the spread of *E. coli* O157. It is well known that even low levels of *E. coli* O157 contamination could result in serious public health risks if there were to be contamination of ready-to-eat food.

Table 14: Results of external cross contamination study; UV contamination spread from initial heavily contaminated piece of meat (sample number 1), followed by proceeding packets of product (2-5). The operator changed gloves and continued to pack items (6-10).

Sample numbers	Result	Comments	Image
1	**Positive**	Highly contaminated	
2	**Positive**	Highly contaminated	
3	**Positive**	Highly contaminated	
4	**Positive**	Highly contaminated	
5	**Positive**	Highly contaminated	

Sample numbers	Result	Comments	Image
6	**Positive**	Lesser contamination; most noticeable on the bag surface	
7	**Positive**	Contamination visible on food product	
8	**Positive**	Contamination visible on food product	
9	**Positive**	Contamination not obvious on food product but visible on bag surface	
10	**Positive**	Contamination visible on food product	

4.4.2 Internal cross contamination

Serratia marcescens was firstly inoculated onto the suction pipe manifold and suction pipe (Figure 3 (a) and (b) respectively). This area is where air exits and enters the chamber during a vacuum packing event. The results in Table 15 indicate that 4 packed agar discs, out of the first 50 bags packed, were contaminated by the initial inoculation. These results indicate that if an item highly contaminated with *E. coli* O157 was packed in the machine, and this contamination reached the difficult to clean parts such as the exhaust manifold or exhaust tube, this has the potential to spread to subsequent products packed in the same machine. There appeared to be no further cross contamination after sample number 49. This may be due to the nature of the area in which the *Serratia marcescens* was inoculated, as the air entering and exiting the chamber may have desiccated the inoculation, either resulting in a loss of inoculum viability or preventing it from spreading further.

Inoculation of the air outlet cover plate and the inner surfaces of each gas flushing nozzle (Figure 4 (a) and (b) respectively) did not seem to cause further cross contamination. This suggests that the gas flush nozzles, even when heavily contaminated, may not cross-contaminate product being packed when gas pack flushing is not in use.

Table 15: Results of vacuum packer cross contamination study.

Sample numbers	Result	API Confirmation top match
Start control plate 1	Negative	-
Start control plate 2	Negative	-
1-3	Negative	-
4	**Positive**	*Serratia marcescens*
5-14	Negative	-
15	**Positive**	*Serratia marcescens*
16	Negative	-
17	**Positive**	*Serratia marcescens*
18-48	Negative	-
49	**Positive**	*Serratia marcescens*
50-300	Negative	-
End control plate 1	**Positive**	*Serratia marcescens*
End control plate 2	Negative	-

The final control agar plate (no bag) placed in the chamber did however show a positive result (Table 15; End control plate 1). This suggests that the contamination was still viable in the chamber although not reaching the inside of the packages.

The internal cross-contamination studies were carried out aseptically, wherein the operator did not come into contact with the packaged agar discs. However, if the results of the external cross-contamination studies were taken into account and the operator was also handling the product when the chamber is also contaminated (as shown in Table 15; End control plate 1) then this again increases the risk of cross-contamination to the internal surfaces and contents. Also, when the chamber shows contamination this is likely to be transferred onto the outer surface of the packaging and thus the operator's hands/gloves, which can then transfer onto the packaged item as shown in the external cross-contamination results (Table 15).

A separate study carried out on 2 new machines found no contamination that there was no contamination to the sealed products. There was, however, evidence to show that the chamber itself was contaminated. This may increase the risk of cross-contamination of the product (Appendix VII).

The results of this research illustrate how bacteria, such as *E. coli* O157, that are invisible to the naked eye, can be easily spread to food without the operator's realisation. These bacteria could cause serious public health issues. If food businesses handle raw food, which could be contaminated with *E. coli* O157, in the same area as ready-to-eat food, there will be a greater risk.

5 CONCLUSION

The alternative methods, proposed by food business operators, outlined the use of heat disinfection and/or chemical disinfection to decontaminate complex pieces of equipment. Research was carried out to determine the inactivation kinetic parameters that can be achieved using heat (both dry and wet) and disinfectant chemicals (quaternary ammonium compounds (QAC), chlorine based and wipes) on surfaces inoculated with *E.coli* O157:H7. Best practice and verification methods were also investigated in relation to the alternative methods suggested. Research was also carried out to assess the cross contamination risks between vacuum packers and food items.

5.1 Thermal disinfection

The research found that the thermal inactivation of *E. coli* O157:H7 attached to stainless steel surfaces is linear and thus the D- and z-values calculated using both dry and moist heating can be used to predict reduction in this organism on stainless steel surfaces expected at given times and temperatures. As observed for *Salmonella* spp. in current research (current Campden BRI project number 128882) the D-values observed for surface-attached *E.coli* O157:H7 on exposure to dry heat treatments were much higher than D-values observed for moist heating. The D-value of *E. coli* O157:H7 at 65°C on stainless steel using dry heat was 136.8 minutes as opposed to 2.1 seconds using moist heating. This means that to receive a 4 log reduction in *E. coli* O157:H7 on stainless steel with

dry heat at 65°C the item would need to be heated for approximately 547 minutes as opposed to 8.4 seconds with moist heating at the same temperature.

The results obtained allowed the development of best practice procedures for thermal disinfection of equipment. Correct training on disinfection protocol is essential. A protocol may differ between parts of the equipment. Verification of thermal disinfection methods can be easily carried out using temperature indicator labels widely available on the market. It was observed that these labels were a suitable method to verify the heat treatment achieved on surfaces during both wet and dry heat treatment. Some of the labels were reversible after the heat treatment indicating that they would not be suitable as proof of validation of a disinfection method. It may be advisable to use photographic evidence if used for verification of results. The advantage of the thermal label method is that the operator does not require training as the method is quite straight forward.

5.2 Chemical disinfection

5.2.1 Chemical applied as a liquid

The research found that QAC controlled (passed the disinfectant test) *E. coli* on stainless steel at its in-use manufacturer's recommended concentration and under the recommended conditions of the BS EN 13697 test: contact time (5 minutes); temperature (20°C) and visibly clean surfaces (0.3 g/L BA). Chlorine also controlled *E. coli* on stainless steel at its in-use manufacturer's recommended concentration and under the recommended conditions of the BS EN 13697 test: Contact time (5 minutes); Temperature (20°C) and visibly clean surfaces (0.3 g/L BA). Outside these recommended conditions both chemicals showed certain weaknesses on the stainless steel surfaces. This research highlights the importance of using the manufacturer's recommended conditions to achieve the best results in regard to removal of *E.coli.* When using chemical methods the smoothness of the surface and hydrophobicity must be taken into consideration to assure the chemical treatment can achieve a suitable contact with the surface.

The results obtained allowed the development of best practice guidance. It is important to follow the manufacturer's advice for disinfection. (This relates to both chemical and surface suitability). Correct training on disinfection protocol is essential. A protocol may differ between parts of the equipment.

Verification of concentration of the cleaning chemicals can be carried out using rapid chemical testing strips widely available on the market. It was observed that these labels were a suitable method to verify the concentration of a cleaning chemical; however it may require further training to ensure the user understands the testing method and uses the correct detection level. The efficacy may also depend on the type of chemical method used and recommended concentration suggested.

5.2.2 Chemical disinfectant wipes

There is currently no standard test that simulates the physical removal of dirt or microbes by wipes. Most tests are carried out on the disinfectant within the wipe via suspension tests, although some wipes may be tested via a wiping action on inoculated surfaces. Best practice recommends that surfaces should be physically clean prior to disinfection in order for any disinfectant to be effective. Suitable training is important to ensure cleaning is carried out in the correct manner, for example; correct storage of the wipes so that they do not dry out and not re-using the same wipe.

The use of wipes appears to have a physical removal action with a minor disinfectant action. The volume of disinfectant applied to each surface using a wipe appears to be very low and, if in frequent use, the disinfectant may not have sufficient contact time with the surface. When the probe was given a contact time of 5 minutes after the application of the disinfectant wipe, there was an increase in achieved mean log reduction however; further work needs to be performed to demonstrate if this is significant (Detailed information in Appendix III).

The research provided demonstrates that the disinfectant wipes alone do not result in a >3 log reduction of *E. coli* on probe surfaces. Therefore, wipes could be used as a risk reduction method for the removal of food residue with some microbial load reduction; however, they should not be used as a control for *E. coli* O157:H7 when used in raw and RTE products.
Best practice advice would be to disinfect probes using wipes straight after a contamination incident. It would be suggested that wipes are only appropriate for use between products of the same nature (for example only between raw foods). A further step should be used to disinfect when probing RTE products after probing raw products. This may involve hot water or chemical submergence and probes should always be wiped again if they have not been used for some time prior to probing foods.

5.3 Vacuum packer cross contamination studies

During the external cross contamination studies, UV simulants were used to represent a contamination event and the spread that can occur following packing of one heavily contaminated product. It was observed that the operator can spread contamination from product to gloves and then to contact surfaces such as lid, handle, control panel and outer and inner bags. Even when gloves are replaced with a clean pair, the contamination was still spread to subsequently packed products. During internal cross-contamination studies, the machine was inoculated in different areas to assess the spread to internal products if the machine was contaminated in hard to reach/clean areas. Contamination was observed in 4 out of the first 50 bags packed (Table 15). This signifies the risk that is posed when machines are used for dual purposes such as raw and ready to eat (RTE) products.

The internal cross-contamination study was carried out aseptically, wherein the operator did not come into contact with the packaged agar discs. However, if the results of the internal cross-contamination studies were taken into account and the operator was also handling the product when the chamber is also contaminated (as shown in Table 15; End control plate 1) then this again increases the risk of cross-contamination to the internal surfaces and contents. Also, when the chamber shows contamination this is likely to be transferred onto the outer surface of the packaging and thus the operator's hands/gloves, which can then transfer onto the packaged item as shown in Table 14.

The results of this research illustrate how bacteria, such as *E. coli* O157, that are invisible to the naked eye, can be easily spread to food without the operator's realisation. These bacteria could cause serious public health issues. If food businesses handle raw food, which could be contaminated with *E. coli* O157, in the same area as ready-to-eat food, there will be a greater risk.

Theoretically it would be possible to reuse a vacuum packer for packing RTE products after packing raw products, but all internal and external vacuum packer surfaces would need to receive thorough cleaning and disinfection process. This would require extensive dismantling of the machine, thorough cleaning and disinfection of all parts and rebuilding and testing the operation of the machine. This is most unlikely, however, to be undertaken in practice.

Appendix I
Thermal Studies

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Confidential report for:

Food Standards Agency

FAO: Marc Wormald

Report on:

Deliverable 2: Heat resistance to dry and moist heating of *Escherichia coli* O157:H7 attached to stainless steel surfaces

Work performed by Campden BRI (Chipping Campden) Limited
Report number: MICRO/REP/131759/objective 2 ◆ Issue date: 02 April 2014

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Our ref: Appendix I
Page count: 7

MATERIALS & METHODS

Microorganisms

Two verocytoxin-negative reference strains of *Escherichia coli* O157:H7 were used in this study:

Escherichia coli O157:H7 strain ATCC 43888, originally isolated from human faeces.
Escherichia coli O157:H7 strain NCTC 12900

One verocytotoxin (VT1 and VT2)-positive reference strain of *Escherichia coli* O157:H7 was used in the study:

Escherichia coli O157:H7 strain NCTC 12079, isolated from human faeces.

Maintenance & growth of microorganisms

Microorganisms were stored at <-80°C on preservative beads until required. On each occasion that the organisms were required, a bead was removed from frozen stock and streaked onto a Tryptone Soya Agar (TSA, Oxoid, CM0131) plate. Plates were incubated at 37±1°C for 24 hours and checked for purity and colony morphology. Individual colonies were then used to inoculate Tryptone Soya Broth (TSB, Oxoid, CM0129).

TSB broths were incubated for 24 hours at 37±1°C. 1mL aliquots of TSB culture were then dispensed onto the surface of 140mm TSA plates and spread using a sterile spreader. TSA plates were incubated at 37±1°C for 24 hours and bacterial growth was harvested by dispensing 10mL of 0.1% peptone onto the surface of each plate, loosening bacterial growth using a sterile spreader and collecting the subsequent bacterial suspension with a sterile 10mL pipette. Bacterial suspensions from all such plates were pooled together and mixed thoroughly to create an inoculum.

Preparation of stainless steel discs

2cm diameter stainless steel discs 304 with grade 2 finish on both sides (Resurgem Engineering Ltd) were used to simulate stainless steel surfaces in the study.

Prior to inoculation, discs were submerged in 5% Decon 90 solution (Decon Laboratories) for 60 minutes. They were then transferred into another container of sterile distilled water (SDW), rinsed for 10 seconds by agitation and then transferred into fresh SDW for a further 10 seconds. Discs were immersed in iso-propanol for 15 minutes and transferred to a laminar flow cabinet to dry. Finally, discs were sealed in paper autoclave bags and autoclaved at 121.1°C for 15 minutes to sterilise.

Preparation of plastic discs

2cm diameter discs were cut from a sheet of plastic (conveyor belt material) and used to simulate plastic surfaces in the study. Prior to inoculation, discs were autoclaved at 121.1°C for 15 minutes to sterilise.

Inoculation of stainless steel or plastic discs

Sterilised discs were laid out in 140mm petri dishes and 50µL of inoculum was applied to one surface of each disc using an automatic pipette. Inoculum was dried onto discs at 30°C for 80 minutes and all discs were transferred to sealed boxes maintained at 33% relative humidity using $MgCl_2$. Discs were left in these boxes for 3 days at ambient temperature before heating.

Determination of dry heat resistance of *Escherichia coli* O157:H7 on stainless steel discs

An aluminium block measuring 200mm x 150mm (thickness 35mm) was submerged in a heated glycerol bath. The surface of the block was coated with a thin layer of silicone heat transfer compound (HTS, Electrolube) to facilitate the transfer of heat between the block and the stainless steel discs.

Surface temperature was measured using a 2cm stainless steel disc with a 1mm groove filed into the surface. A platinum wire temperature probe was affixed to this groove and the groove was back-filled with HTS allowing measurement of surface temperature. This temperature probe assembly was placed onto the surface of the block in the centre. The temperature of the block was confirmed using a handheld thermal imaging camera (FLIR Systems Inc., FLIR-E49001). 3 x 2cm stainless steel discs were spray-painted black with heat resistant paint to facilitate accurate temperature measurement. Discs were placed on the surface at the block at the centre and at the outermost corners. The apparatus used to carry out D-value determinations is depicted in figure 1. Point surface temperature readings were taken from each disc using the thermal imaging camera once the block temperature had stabilised and used to confirm the data logger measurements. A typical thermal imager temperature measurement is shown in figure 2.

For each triplicate D-value, once the process temperature had been achieved and stabilised across the block, 30 inoculated steel discs were added to the surface of the block, inoculated side upwards. At time intervals, triplicate samples were removed from the block using sterile forceps, residual HTS was removed from the back of each disc using an Azowipe™ (Synergy Health (UK) Ltd.) and each disc was placed inoculated side down into a 60mL sterilin pot containing 10mL Maximum Recovery Diluent (Lab M, LAB103) and a single layer of sterile glass beads (VWR, 3321249). Samples were then transferred to an orbital shaker platform for ≥10 minutes to dislodge any surviving cells from the surface of the discs.

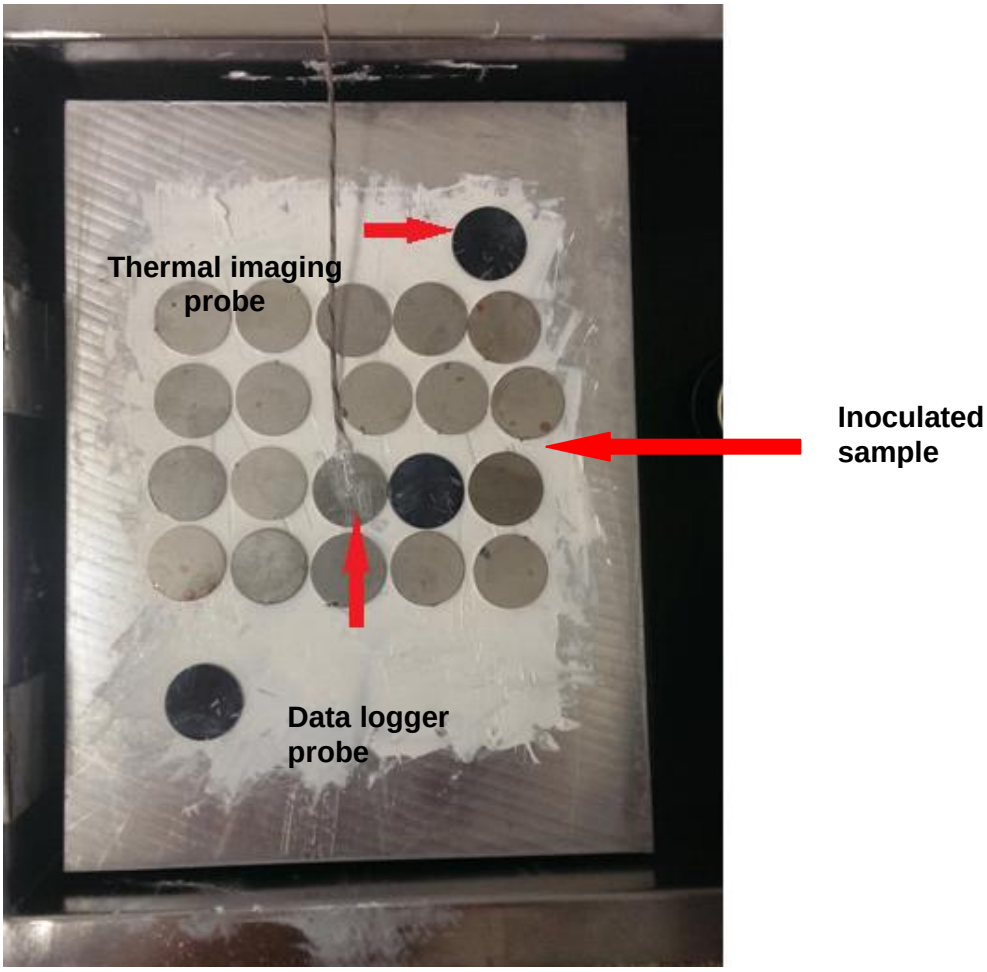

Figure 1. Apparatus used to determine D-value on surfaces using dry heat

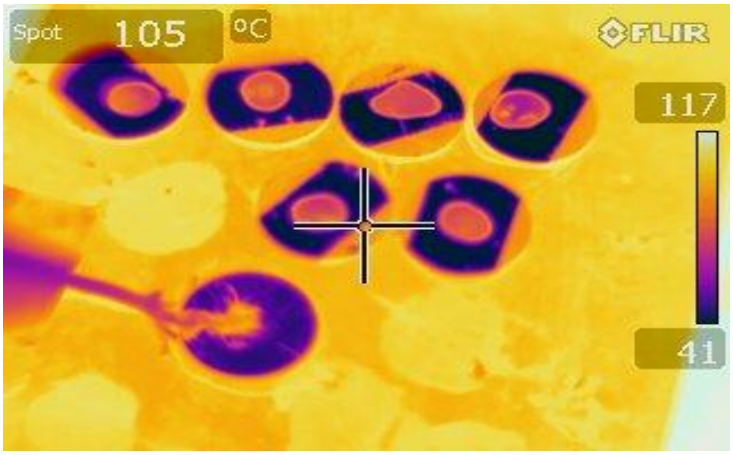

Figure 2. Temperature measurement using thermal imaging

Determination of moist heat resistance of *Escherichia coli* O157:H7 on stainless steel discs

For each D-value determination, 30 x 30mL plastic universals containing 5mL of sterile distilled water (SDW) were placed into a rack and pre-heated to the desired process temperature in a laboratory water bath. One tube was added which had a temperature probe inserted into the SDW to monitor the sample temperature. At each time point, triplicate inoculated stainless steel discs were processed. Due to the short time intervals involved, samples were processed one at a time.

For each sample, a single inoculated stainless steel disc was transferred into a pre-heated tube containing 5mL SDW using forceps so that the entire surface of the disc was immersed. Once the hold time had elapsed, the entire contents of the sample tube were immediately emptied into a refrigerated 60mL sterile pot containing 5mL double-strength Maximum Recovery Diluent (dsMRD) and a single layer of sterile glass beads. Discs were oriented such that the inoculated side was in contact with the glass beads and samples were then agitated on an orbital shaker platform for ≥10 minutes to dislodge any surviving cells from the surface of the discs.

Analysis of samples.

For each sample, a serial dilution was created by transferring 1mL aliquots of sample and into further 9mL aliquots of MRD (Oxoid, EB0348D) and repeating this process for each successive dilution. 1mL aliquots of each dilution to be plated were transferred into duplicate petri dishes and ~20mL molten TSA was added to each plate. Plates were swirled to mix inoculum with growth medium, allowed to set and then incubated at 37±1°C for 24 hours.

In order to ensure the efficiency of recovery from each disc, the surface of each disc rinsed with 10mL MRD to remove residual bacterial suspension and then each disc was placed onto the surface of a TSA plate. 100µL of MRD was then added to the surface of each disc and a pipette tip was used to scratch the discs to physically dislodge any remaining cells. Plates were then over layered with further TSA, mixed, allowed to set, inverted and incubated at 37±1°C for 24 hours.

After incubation, bacterial colonies on each plate were counted and used to determine the number of surviving microorganisms on each disc. For each process temperature assessed, the log number of survivors per disc (log CFU/disc) was then plotted against time and D-value was calculated from the line of best fit through the data using linear regression analysis.

Dry heat D-values were calculated at 65, 75, 85, 95 and 105°C.

Moist heat D-values were calculated at 45, 50, 55, 60 and 65°C

Log D-value was plotted against process temperature and the z-value was determined from the line of best fit through the data using linear regression analysis.

RESULTS & DISCUSSION

Dry heat resistance of *Escherichia coli* O157:H7 attached to stainless steel surfaces

Comparison of heat resistance of 2 non-toxigenic strains of *Escherichia coli* O157:H7

Comparison of D-values on stainless steel surfaces using dry heat for *E.coli* O157:H7 strains ATCC43888 and NCTC 12900 at 85°C gave values of 22.6 minutes and 21.1 minutes respectively. Strain ATCC 43888 was therefore selected for use in all further D-value determinations due to it's slightly higher heat resistance.

Determination of D- and z-values for *E.coli* O157:H7 ATCC43888 on stainless steel using dry heat

The D- and z-values calculated for *Escherichia coli* O157:H7 using dry heat are displayed in Table 1. and illustrated in figure 3. D-values determined on stainless steel surfaces treated with dry heat were found to be considerably higher than might be expected for *E.coli* O157:H7 in a typical moist process. For example, the D-value of *E.coli* O157:H7 at 65°C in distilled water was found to be 0.05 minutes in a study by Spinks *et al.* (2006), compared with 136.8 minutes on stainless steel treated with dry heat. Furthermore, a moist process of 70°C for 2 minutes, applied to short shelf-life chilled foods, would be expected to achieve a >6 log reduction in *E.coli*, whereas these results show that on stainless steel surfaces treated with dry heat at 70°C, a hold time of 322 minutes would be required to produce the same reduction in this organism.

Table 1. Dry heat D-values determined for *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel.

Process temperature (°C)	D-value (minutes)
65	136.8
75	35.1
85	22.6
95	8.7
105	3.9
z-value (C°)	**27.1**

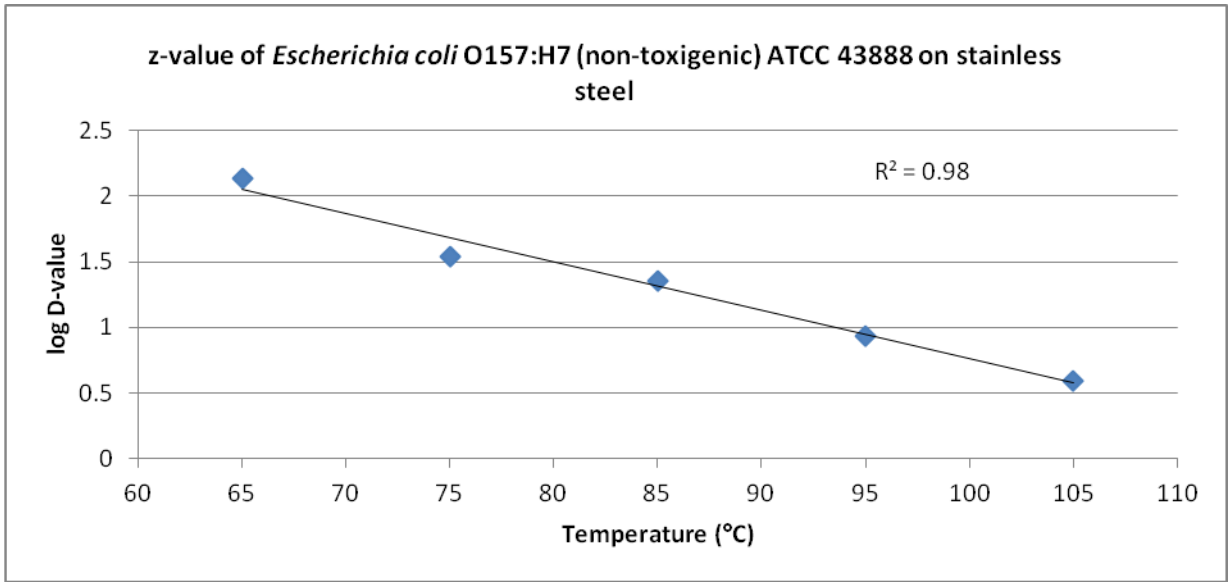

Figure 3. z-value of *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel surfaces (dry heat)

Moist heat resistance of *Escherichia coli* O157:H7 attached to stainless steel surfaces

Comparison of heat resistance of 2 non-toxigenic strains of *Escherichia coli* O157:H7

Comparison of D-values on stainless steel surfaces using moist heat for *E.coli* O157:H7 strains ATCC43888 and NCTC 12900 at 60°C gave values of 3.4 seconds and 5.0 seconds respectively, showing that the heat resistance of these two strains is comparable. Given their similar heat resistance properties, strain ATCC 43888 was therefore selected for use in all further D-value determinations due to its use in the dry heating and disinfectant trials.

Determination of D- and z-values for *E.coli* O157:H7 ATCC43888 on stainless steel using moist heat

The D- and z-values calculated for *Escherichia coli* O157:H7 using cells dried on to stainless steel surfaces and then heated in water (moist heat) are displayed in Table 2. and illustrated in figure 4. The D-values were similar to those described by Spinks *et al.* (2006)[1], when heated at 65ºC. However, the z–value of 21.8C° determined for this organism on stainless steel was only slightly lower than the z-value determined using dry heat (27.1C°). This z-value is considerably higher than would be expected for this organism in a normal moist situation, where the z-value might be 5-7C°.

Table 2. Moist heat D-values determined for *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel.

Process temperature (°C)	D-value (seconds)
45	21.7
50	10.5
55	5.4
60	3.4
65	2.1
z-value (C°)	**21.8**

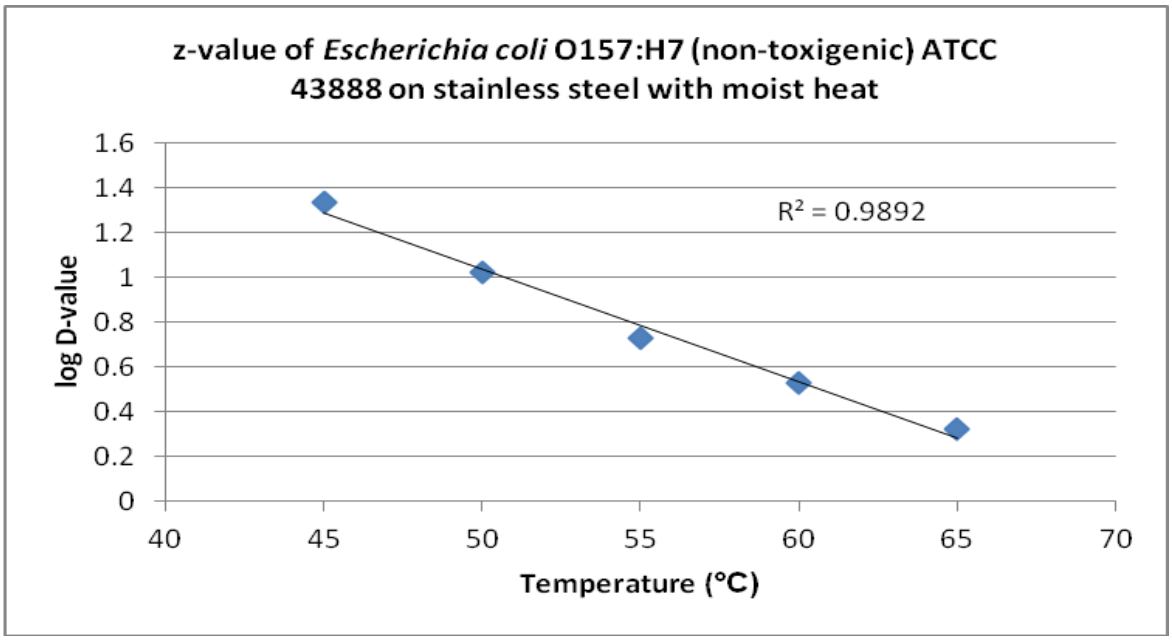

Figure 4. z-value of *Escherichia coli* O157:H7 ATCC 43888 attached to stainless steel surfaces (moist heat)

[1] Spinks, T. A., Dunstan, R.H., Harrison, T., Coombes, P. and Kuczera, G. (2006) Thermal inactivation of water-borne pathogenic and indicator bacteria at sub-boiling temperatures, Water Research, **40**, 1326 – 1332

Comparison of the effect of moist heating on *Escherichia coli* O157:H7 attached to either stainless steel or plastic surfaces

D-values at 50°C using moist heat on plastic and stainless steel discs were compared to assess the effect of surface-type on heat resistance of *E.coli* O157:H7 ATCC 43888. D-values were calculated to be 10.0 and 10.5 seconds on plastic and stainless steel surfaces respectively, showing that the heat resistance of the organism does not appear to differ greatly when attached to either of these surface types.

Comparison of the effect of moist and dry heating on non-toxigenic and toxigenic *Escherichia coli* O157:H7 attached to stainless steel

D-values of verocytotoxigenic-*Escherichia coli* NCTC 12079 (VT1 and VT2 positive) dried onto stainless steel discs were carried out using dry and moist heating. Dry heating at 85°C gave a D-value for *E.coli* NCTC 12079 of 28 minutes. The D-value previously determined for *E.coli* ATCC 43888 (non-toxigenic) was 22.6 minutes.

Moist heating at 50°C gave a D-value for *E.coli* NCTC 12079 of 35.1 seconds. The D_{50}-value determined for *E.coli* ATCC 43888 for moist heat is 10.5 seconds.

The D-values determined for both toxigenic and non-toxigenic *Escherichia coli* are comparable. Whilst the heat resistance of the toxigenic strain is slightly higher than the non-toxigenic strain, this difference could easily be accounted for by the application of a margin of safety in the calculation of hold time required to produce a suitable log reduction. The addition of such a margin of safety would be considered good practice in any case, as it would cover the effect of strain to strain variation in heat resistance of *Escherichia coli* O157:H7

CONCLUSIONS

The results of this study show that the heat resistance of *Escherichia coli* O157:H7 attached to stainless steel towards dry heat is very high compared to the moist heat resistance in foods. For example, the D-value of *E.coli* O157:H7 at 65°C in distilled water was found to be 0.05 minutes in a study by Spinks *et al.* (2006), compared with 136.8 minutes on stainless steel treated with dry heat. Furthermore, a moist process of 70°C for 2 minutes, applied to short shelf-life chilled foods, would be expected to achieve a >6 log reduction in *E.coli*, whereas these results show that on stainless steel surfaces treated with dry heat at 70°C, a hold time of 322 minutes would be required to produce the same reduction in this organism.

When heated under moist conditions, however, the D-values determined for this organism attached to stainless steel discs are considerably lower, in the range 21.7-2.1 seconds for process temperatures of 45-65°C respectively. The z-value calculated for *E.coli* O157:H7 on stainless steel surfaces was 21.8C°, which is very much higher than would be expected for this organism in a normal moist process (e.g. in phosphate buffer or water), where a z-value of 5-7C° would be considered typical. Comparison of the D_{50}-values of *E.coli* O157:H7 attached to stainless steel and plastic surfaces showed that no difference in the resistance was evident between organisms dried onto each of these surface types.

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Appendix II
Chemical – Liquid

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Confidential report for:

FSA project

FAO: Marc Wormald

Report on:

Deliverable 3 (a): Study for the Food Standards Agency upon the use of two commonly available disinfectants against *E. coli* O157 using Campden BRI Method Reference TES-MB-211 based on BS EN 13697:2001[1] with described variations

Work performed by Campden BRI (Chipping Campden) Limited
Report number: MB/REP/131759/disinfectant tests ◆ Issue date: 01/04/2014

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Report issued and authorised by:

Campden BRI (Chipping Campden) Limited
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SUMMARY

Based upon the results shown in Tables 2 & 3 both disinfectants were shown to be effective when tested at the manufacturers recommended concentrations under the clean obligatory test conditions of 0.3g/L Bovine Albumin, ambient temperature (22^0C±3 ^{0}C), with a five minute contact time on stainless steel (EN13697:2001[1]) against toxigenic and non toxigenic strains of *Escherichia coli* O157:H7.

There was no practical difference noted in susceptibility between *E. coli* toxigenic O157:H7 NCTC 12079 and *E. coli* non toxigenic O157 ATCC 43888 to 3 concentrations of each disinfectant type.

Variations from the obligatory conditions were shown to have an effect upon efficacy.

The variations studied in this work were disinfectant concentration, surface type, temperature, interfering substance concentration and contact time, which are listed and described in the results (Table 2).

INTRODUCTION

Campden BRI were approached by the Food Standards Agency (FSA) and requested to provide supporting data for the FSA document control of cross contamination of *Escherichia coli* O157:H7. The work was designed to support the FSA stance that disinfectants be used at concentrations and under use conditions that would meet the requirements of EN 13697:2001[1]. Further more, it was stipulated that the disinfectants were to be commercially and readily available and commonly used in the food industry. It was agreed that a Quaternary Ammonium Compound (QAC) specifically a Benzalkonium chloride (BKC) and a Chlorine specifically Sodium Hypochlorite based disinfectants would be used in testing as examples of those available to and commonly used by the food industry.

Method

PRODUCT DETAILS

Campden BRI Sample Code	Benzalkonium chloride based disinfectant (BKC) DT53 Sodium Hypochlorite based disinfectant (ClO^-) DT55		
Name of the Product	Undisclosed	Product Batch Code	Undisclosed
Product Manufacturer	Undisclosed		
Date of product arrival at Campden BRI	DT53 Benzalkonium chloride (BKC) 4/2/14 DT55 Sodium Hypochlorite (ClO^-) 19/2/14		
Condition & appearance of product on receipt	Satisfactory		
Storage conditions of product	Dark ambient		
Active substances	DT53 Benzalkonium chloride (BKC) DT55 Sodium Hypochlorite (ClO^-)		
Product diluent recommended by the manufacturer for use	Water		

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Providing services under an ISO 9001 registered quality management system

EXPERIMENTAL DETAILS & CONDITIONS

Date of tests	February 2014 until March 2014
Test organisms	*Escherichia coli* toxigenic O157:H7 NCTC 12079 *E. coli* Non toxigenic O157:H7 ATCC 43888 *E. coli* EN standard challenge organism ATCC 10536
Test temperature	Ambient (18-25°C) unless specified differently
Contact time	5 minutes unless specified differently
Interfering substance	Bovine Albumin was used to simulate soiling
Incubation temperature	37^0C±1 ^{0}C
Product diluent used during the test	Water of Standard Hardness
Product concentrations	Chlorine based disinfectant manufacturers recommended use concentration was 2.4% BKC based disinfectant manufacturers recommended use concentration was 1.25%
Appearance of diluted product	Both were transparent slightly coloured solutions
Neutraliser	MB-MR-8-241
Variations/ Variables	Organism, Disinfectant concentration, Surface type, Temperature, Interfering substance concentration, Contact Time. These are listed and described in the results Tables 1 & 2.

Legend:
NCTC National Collection of Type Cultures
ATCC American Type Culture Collection

The method TES-MB-211 based upon BS EN 13697:2001[1] was followed with described variations to demonstrate:
1. If there was any practical difference between the toxigenic *E. coli* O157:H7 and the non toxigenic *E. coli* O157 (results shown in Table 1).

This was carried out within a Biohazard Containment Level 3 laboratory; the only variation to the standard method used was that for safety reasons the inoculated stainless steel discs (both toxigenic and non toxigenic) were dried in a sealed containers containing silica gel crystals within a 37^0C ±1 ^{0}C incubator.

2. The effects upon disinfectant efficacy on a non toxigenic *E. coli* O157:H7 by altering the test variables such as test surface, test temperature, interfering substance concentrations and contact time. Each test was performed in triplicate and each replicate was reported individually (Results shown in Table 2).

RESULTS & DISCUSSION

Table 1: Comparison of toxigenic and non toxigenic test strains susceptibility under EN13697:2001

Test variables		Log reduction achieved			
		BKC		ClO^-	
Organism	*Escherichia coli* Non toxigenic O157:H7	Concentration	Log reduction achieved	Concentration	Log reduction achieved
Surface Temperature Interfering substance Contact Time	Stainless steel $22^0C \pm 3\ ^0C$ 0.3g/L BA (Clean) 5 minutes	Recommended	>4.6	Recommended	>4.6
		$1/10^{th}$ recommended	>4.6	$1/20^{th}$ recommended	3.6
		$1/100^{th}$ recommended	<1	$1/100^{th}$ recommended	<1
Organism	*E. coli* Toxigenic O157:H7	Concentration	Log reduction achieved	Concentration	Log reduction achieved
Surface Temperature Interfering substance Contact Time	Stainless steel $22^0C \pm 3\ ^0C$ 0.3g/L BA (Clean) 5 minutes	Recommended	>4.3	Recommended	>4.3
		$1/10^{th}$ recommended	>4.3	$1/20^{th}$ recommended	2.2
		$1/100^{th}$ recommended	<1	$1/100^{th}$ recommended	<1

Legend:
BA Bovine Albumin
Validation of tests for EN13697:2001 were deemed valid
Log reductions calculated from control surfaces and quoted as 1 d.p.
> Indicates surviving organisms were below the limit of detection (No surviving organisms recovered)

Based upon the results above there is no practical difference between the susceptibility of non toxigenic *E. coli* O157:H7 and the toxigenic *E. coli* O157:H7 to each of the two disinfectants at the concentrations tested and under the obligatory test conditions.

Table 2: Effects upon disinfectant efficacy by altering test variables as described

Test variables		Log reduction achieved (Green indicates a 4 log reduction or greater (0 d.p.)) (Red indicates a <4 log reduction (0 d.p.))	
		BKC	ClO^-
Organism	***E. coli* (EN 13697 Standard)**	>4.3	>5.1
Disinfectant Concentration	Recommended	>4.3	>5.1
Surface	Stainless steel	>4.3	>5.1
Temperature	$22^0C \pm 3\ ^0C$		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	5 minutes		
Organism	*E. coli* O157:H7 Non toxigenic	>4.2	>4.3
Disinfectant Concentration	Recommended	>4.2	>4.3
Surface	Stainless steel	>4.2	>4.3
Temperature	$22^0C \pm 3\ ^0C$		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	5 minutes		
Results demonstrate that both disinfectants are effective (>4 log reduction) when tested at recommended concentrations under obligatory EN13697:2001 clean conditions against both the EN standard test strain of *E. coli* and a non-toxigenic strain of *E. coli* O157.			
Organism	*E. coli* O157:H7 Non toxigenic	>4.2	>4.0
Disinfectant Concentration	**10 times recommended**	>4.2	>4.0
Surface	Stainless steel	>4.2	>4.0
Temperature	$22^0C \pm 3\ ^0C$		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	5 minutes		
The use of higher than recommended concentrations has no practical negative effect upon efficacy, however, the increased chemical concentrations do have implications upon Health & Safety and may be incompatible with some materials such as mild steel.			
Organism	*E. coli* O157:H7 Non toxigenic	>4.2	0.2
Disinfectant Concentration	**$1/10^{th}$ recommended BKC $1/100^{th}$ recommended ClO^-**	2.3	0.3
Surface	Stainless steel	>4.2	0.7
Temperature	$22^0C \pm 3\ ^0C$		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	5 minutes		
The use of a more dilute disinfectant demonstrates that this does reduce the efficacy of the disinfectants with the chlorine based disinfectant ($1/100^{th}$ manufacturers recommended) not achieving the 4 log reduction required in 3 out of 3 replicates and the BKC based disinfectant ($1/10^{th}$ manufacturers recommended) not achieving the 4 log reduction in 1 out of 3 replicates. Thus concentrations less than the recommended have an increased risk of failing and this is considered to be dependent upon the disinfectant type and dilution factor.			

Table 2: Effects upon disinfectant efficacy by altering test variables as described (continued)

Test variables		Log reduction achieved (Green indicates a 4 log reduction or greater (0 d.p.)) (Red indicates a <4log reduction (0 d.p.))	
		BKC	ClO^-
Organism	*E. coli* O157:H7 Non toxigenic	>3.5	>5.3
Disinfectant Concentration	Recommended	>3.5	1.9
Surface	**Plastic (smooth) ($2cm^2$)**	>3.5	2.7
Temperature	$22^0C±3^0C$		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	5 minutes		
The plastic smooth surface (Appendix 1: plates 1 & 2) was typical of clear plastic used in machine shielding. In the experiments a degree of hydro-phobicity was noted and this may explain the two Chlorine based failures on this surface.			
Organism	*E. coli* O157:H7 Non toxigenic	>3.8	4.0
Disinfectant Concentration	Recommended	>3.8	3.4
Surface	**Plastic (rough) ($2cm^2$)**	>3.8	2.6
Temperature	$22^0C±3\ ^0C$		
Interfering substance	0.3g/L BA (Clean)		
Time	5 minutes		
It was noted that when applied to a rough plastic surface (Appendix 1:plates 1 & 3) the wetting action of the BKC disinfectant gave a more complete coverage and this is reflected in the results with BKC meeting the 4 log reduction (0 d.p.) criteria in 3 out of 3 replicates and the Chlorine based disinfectant having a range of log reductions from 2.6 to 4.0			
Organism	*E. coli* O157:H7 Non toxigenic	2.3	>5.5
Disinfectant Concentration	Recommended	>4.0	>5.5
Surface	Stainless steel	>4.0	>5.5
Temperature	**$4^0C±2\ ^0C$**		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	5 minutes		
Reduced temperature ($4^0C±2^0C$) was shown to have a negative effect upon efficacy with 1 of the replicates for the BKC disinfectant failing.			
Organism	*E. coli* O157:H7 Non toxigenic	2.7	>5.3
Disinfectant Concentration	Recommended	2.2	>5.3
Surface	Stainless steel	3.3	>5.3
Temperature	$22^0C±3\ ^0C$		
Interfering substance	**3g/L**		
Contact Time	5 minutes		
Organism	*E. coli* O157:H7 Non toxigenic	0.9	0.7
Disinfectant Concentration	Recommended	0.5	0.7
Surface	Stainless steel	0.8	1.3
Temperature	$22^0C±3\ ^0C$		
Interfering substance	**30g/L**		
Time	5 minutes		
Increasing levels of organic matter/ interfering substance as shown above has a negative effect upon disinfectant efficacy.			

Table 2: Effects upon disinfectant efficacy by altering test variables as described (continued)

Test variables		Log reduction achieved (Green indicates a 4 log reduction or greater (0 d.p.)) (Red indicates a <4log reduction (0 d.p.))	
		BKC	ClO^-
Organism	*E. coli* O157:H7 Non toxigenic	3.1	1.7
Disinfectant Concentration	Recommended	>4.2	1.0
Surface	Stainless steel	>4.2	0.7
Temperature	$22^0C \pm 3\ ^0C$		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	**1 minute**		
Organism	*E. coli* O157:H7 Non toxigenic	1.5	1.4
Disinfectant Concentration	Recommended	1.8	1.8
Surface	Stainless steel	1.9	1.6
Temperature	$22^0C \pm 3\ ^0C$		
Interfering substance	0.3g/L BA (Clean)		
Contact Time	**30 seconds**		
As shown above contact time has a significant effect upon disinfectant efficacy with a 5 minute contact time demonstrating a consistent >4 log reduction for both disinfectants and a 30 second contact time at the concentrations tested demonstrating a consistent <2 log reduction.			

Legend
Validation of tests for EN13697:2001 were deemed valid
Log reductions quoted to 1 d.p.
> Indicates surviving organisms were below the limit of detection (No surviving organisms recovered)
The variation in each test from the manufacturers recommended and/or test obligatory conditions is in ***bold***

It is well known that the efficacy of disinfectants is dependent upon a number of factors; such as concentration of disinfectant, level of interfering substance or soil, the temperature of disinfection and the contact time, these have been investigated as detailed in Table 2.

The results in Tables 1 & 2 demonstrated that for both disinfectants when used as recommended by the manufacturer, achieve a 4 log reduction on stainless steel within 5 minutes. However, problems were highlighted when used upon different materials, at below recommended concentrations, contact times and temperature or in the presence of increased interfering substances.

CONCLUSIONS

Both disinfectants were shown to be effective when tested at the manufacturers recommended concentrations under the clean obligatory test conditions of 0.3g/L BA, ambient temperature ($22^0C \pm 3\ ^0C$), with a five minute contact time on stainless steel (EN13697:2001[1]) against toxigenic and non toxigenic strains of *E. coli* O157:H7 (Table 1). .

Variations from the obligatory conditions were shown to have an effect upon the efficacy of both disinfectants tested.

Appendices

Appendix 1: Plates
Plate 1: Plastic surfaces used in project

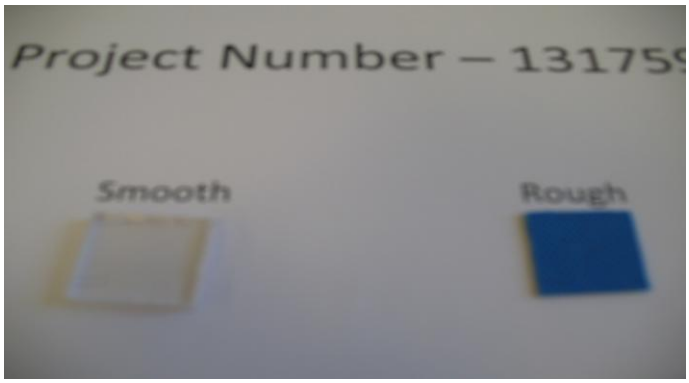
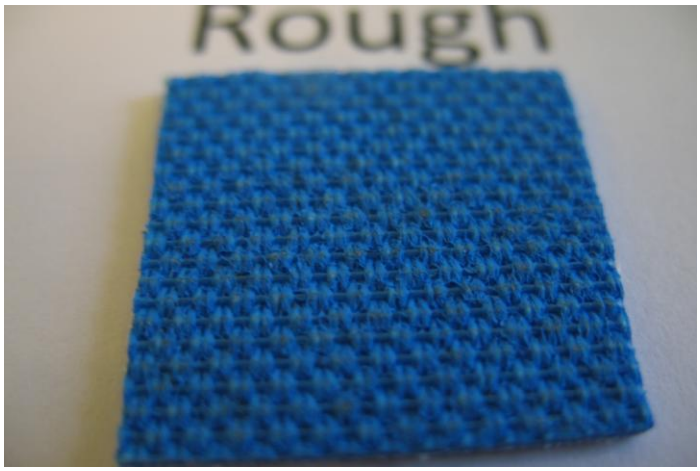


Plate 2: Smooth plastic surface

Plate 3: Rough plastic surface flexible material used in grips, mats and conveyor belts



Appendix 2: Reference

1. **BS EN 13697:2001** Chemical disinfectants and antiseptics. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2/step 2)

Appendix III

Chemical – Wipes

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Report on:

Deliverable 3 (b): Study for the Food Standards Agency upon the disinfection of Temperature probes using examples of available methods.

Work performed by Campden BRI (Chipping Campden) Limited
Report number: MB/REP/131759/Probes ◆ Issue date: 01/04/2014

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Our ref: Appendix III
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SUMMARY

The use of “disinfectant wipes” to decontaminate artificially contaminated temperature probes was assessed.

The efficacy of two disinfectant wipes was shown to be most dependent upon how the challenge was presented (dry/wet) and there was little practical difference in log reductions achieved between wet and dry wipes..

The results imply that the reduction of contamination from the probes was mechanical in action with the use of 2 wipes being consistently more effective in terms of mean log reduction achieved, than the use of 1 wipe (using a fresh disinfectant wipe for each wipe), with a contact time of 5 minutes there appears to be a slight further increase in log reduction (approx. 0.5 log). The volume of disinfectant applied to surface could not be measured as it was contained in each moist wipe, but it was estimated to be less than the 0.1mL used in the EN13697:2001[1] disinfection carrier test. The interfering substance levels were 50 times higher than that used in EN13697:2001 to simulate product contamination.

The use of a dry wipe to remove excess contamination and then immersion into a disinfectant or hot water (80^0C) was shown to produce a mean log reduction of >4.

1. BACKGROUND

Campden BRI were approached by the FSA and requested to provide supporting data for the FSA document control of cross contamination of *E. coli O157:H7,* the work was designed to investigate the disinfection of temperature probes. It was agreed to investigate the use of "probe wipes" or "disinfectant wipes". Two types of wipe were chosen an Alcohol based wipe and a Quaternary Ammonium Compound (QAC) specifically a Benzalkonium chloride (BKC) based wipe. The efficacy of the disinfectants (Chlorine and BKC) and hot water (80^0C ±2 ^{0}C) by immersing the contaminated probes was also investigated.

2. SAMPLES/MATERIALS

PRODUCT DETAILS

Campden BRI Sample Code	Benzalkonium chloride based disinfectant wipes (BKC) DT62 Alcohol based disinfectant wipes DT63 Benzalkonium chloride based disinfectant (BKC) DT53 Sodium Hypochlorite based disinfectant (ClO^-) DT55		
Name of the Product	Undisclosed	Product Batch Code	Undisclosed
Product Manufacturer	Undisclosed		
Date of product arrival at Campden BRI	DT53 Benzalkonium chloride disinfectant (BKC) 4/2/14 DT55 Sodium Hypochlorite disinfectant (ClO^-) 19/2/14 DT62 BKC wipes 3/3/14 DT63 Alcohol wipes 3/3/14		
Condition & appearance of product on receipt	Satisfactory		
Storage conditions of product	Dark ambient		
Active substances	DT53/DT62 Benzalkonium chloride (BKC) DT55 Sodium Hypochlorite (ClO^-) DT63 Alcohol		
Product diluent recommended by the manufacturer for use	Wipes used as supplied Disinfectants supplied as concentrates diluted in water to manufacturers recommendations		

Abbreviations: <=less than, >=greater than, ND=not detected, NM=not measured, NT=not tested, LOD=limit of detection, LLOQ=lower limit of quantification, ULOQ=upper limit of quantification

EXPERIMENTAL DETAILS & CONDITIONS

Date of tests	March 2014 – April 2014
Test organism	*E. coli* Non toxigenic O157:H7 ATCC 43888 Culture & Recovery media TSA Incubation 48h @ 37°C±2ºC A 24hr culture on Nutrient Agar incubated at 37°C± 2°C was used to prepare the inoculum. A minimum of 2 subcultures and a maximum of 3 subcultures from any storage state (-75°C on Cryobeads) was used. The inoculum in MRD was adjusted using spectrophotometer @ λ 420nm to achieve the required concentration
Test temperature	Ambient (18-25°C)
Contact time	<2 minutes or 5 minutes
Interfering substance	Solutions of 60 g/L and 300 g/L of bovine albumin fraction V (suitable for microbiological purposes) dissolved in sterile distilled water(SDW) were aseptically prepared in 40 ml volumes
Incubation temperature	37^0C±1 ^{0}C
Product concentrations	Wipes used as supplied Chlorine based disinfectant at manufacturers recommended use concentration of 2.4% BKC based disinfectant at manufacturers recommended use concentration of 1.25%
Appearance of diluted product	Both were transparent slightly coloured solutions
Neutraliser	MB-MR-8-241
Variations/ Variables	No wipes, dry or wet challenge, use of dry or wet wipes, immersion in disinfectant or “hot water”

Legend:
NCTC National Collection of Type Cultures
TSA Tryptone Soya Agar - Oxoid CM 0131
MRD Maximum Recovery Diluent 9mL -Oxoid EB0348D

3. METHODS

Preparation of test surfaces

Probes were cleaned and disinfected in accordance with BS EN 13697:200[1] i.e. using Decon to degrease and 70% alcohol to disinfect, after each stage (degrease and disinfect) the surfaces were washed in Sterile Distilled Water. They were then packaged and autoclaved to sterilise.

Challenge Inoculum preparation

The challenge inoculum was a 1:1 mixture of interfering substance (Bovine Albumin 60 g/L or 300g/L) and inoculum (organism suspension in MRD).

Inoculation and treatment of test surfaces

Clean sterile probes were immersed up to ½ way in the challenge inoculum and then removed (to simulate temperature probe in a sauce).

In **Test 1** after inoculation the probes were put to one side and allowed to visibly dry (<30 seconds). They were then wiped (see Plates 1 & 2) with a wet disinfectant wipe (either once or twice using fresh wipes each time). Un-wiped probes were used as a control The surviving organisms were recovered from the whole of the probe using a pre-moistened (neutralizer) swab and the swab placed in 10mL of MRD + 1mL neutralizer.

In **Test 2** the probes were immediately wiped (see Plates 1 & 2) after inoculation with a wet disinfectant wipe (either once or twice using fresh wipes each time). Un-wiped probes were used as a control As an additional control a dry tissue was included in the test regime.
The surviving organisms were recovered from the whole of the probe using a pre-moistened (neutralizer) swab and the swab placed in 10mL of MRD + 1mL neutralizer.

In **Test 3** the probes were immediately wiped (see Plates 1 & 2) after inoculation with **either** a wet disinfectant wipe **or** a dry (incubated at 37^0C±1 ^{0}C over night, dry to the touch) disinfectant wipe (either once or twice using fresh wipes each time). The surviving organisms were recovered from the whole of the probe using a pre-moistened (neutralizer) swab and the swab placed in 10mL of MRD + 1mL neutralizer. Un-wiped probes were used as a control.

In **Test 4** the probes were immediately wiped (see Plates 1 & 2) after inoculation with a wet disinfectant wipe (either once or twice using fresh wipes each time). Un-wiped probes were used as a control The probes were left for 5 minutes and then the surviving organisms were recovered from the whole of the probe using a pre-moistened (neutralizer) and the swab placed in 10mL of MRD + 1mL neutralizer.

In **Test 5** the probes were immediately wiped (see Plates 1 & 2) after inoculation with a dry tissue once and then immersed (full length-Plate 4) in one of the following treatments.

a. No treatment control (after wiping)
b. Na Hypochlorite based disinfectant at manufacturers recommended concentration for 5 minutes
c. BKC based disinfectant at manufacturers recommended concentration for 5 minutes
d. Hot water at 80^0C±2 ^{0}C for 2 minutes (temperature was not maintained)

The surviving organisms were recovered from the whole of the probe using a pre-moistened (neutralizer) and the swab placed in 10mL of MRD + 1mL neutralizer.

Samples were enumerated by preparing serial dilutions in MRD to 10^{-6} for controls (0 wipes or no immersion) and to 10^{-4} for test surfaces in neutraliser. From each serially diluted sample, 1 ml aliquots of appropriate dilutions were plated in duplicate into sterile Petri dishes. Approximately 15 ml of TSA, cooled to 45 ± 1°C, was added to each Petri dish. The sample inoculum was mixed with the TSA and allowed to solidify. After solidification, plates were incubated under suitable conditions for the target organism.

After recovery of the surviving organisms from the probes, the probes were stabbed into bottles containing solidified TSA and incubated at suitable conditions (Plate 3). This was a control to validate the recovery process in case of low recovery levels from the swabs (Appendices 2 Tables 6.2.1 – 6.2.4). In the event that no organisms were recovered from the probes by the swab technique this would be confirmed by no growth on the stabbed probes. If there was no recovery from the probe using swab technique but growth on the stab probes >100CFU then this would invalidate the swab recovery method of detection. The stabbed probes confirmed the results from the swab recovery throughout the testing.

Abbreviations: <=less than, >=greater than, ND=not detected, NM=not measured, NT=not tested, LOD=limit of detection, LLOQ=lower limit of quantification, ULOQ=upper limit of quantification

4. RESULTS

Based upon the results shown above Table 1, there is an overall correlation in log reduction and number of wipes for both types of disinfectant wipe. There is a correlation between interfering substance concentration and log reduction achieved, with the higher interfering substance levels giving the greatest log reductions, this may be due to the survivability/level of organisms on the initial surfaces (control) or that the organisms being bound to the interfering substance which is easier to remove (more work is required to understand this correlation). There was also a greater log reduction achieved under both interfering substance concentrations from the use of BKC based wipes this may be due to the surfactant/cleaning ability of BKC containing wipes. The Alcohol wipes did not contain surfactants that may help with removal of the contamination (interfering substance and organisms) from the surface.

Table 1:Results of efficacy of wipes against a visibly dry challenge (*Summary of results from Appendices 2 Table 6.2.1*)

Interfering substance level	Active	Treatment	Mean Log	Mean Log reduction	STD (log)
30g/L Bovine albumin solution	Control	0 wipes	3.31	NA	0.63
	Alcohol	1 wipe	3.34	-0.03	1.25
		2 wipes	3.11	0.2	0.18
	BKC	1wipe	3.29	0.02	0.19
		2 wipes	2.75	0.56	0.14
150g/L Bovine albumin solution	Control	0 wipes	5.34	NA	0.33
	Alcohol	1wipe	4.23	1.11	1.1
		2 wipes	4.33	1.01	0.2
	BKC	1wipe	3.74	1.6	0.14
		2 wipes	3.13	2.21	1.76

Legend
STD log Standard Deviation of log recovery
BKC Benzalkonium chloride

Abbreviations: <=less than, >=greater than, ND=not detected, NM=not measured, NT=not tested, LOD=limit of detection, LLOQ=lower limit of quantification, ULOQ=upper limit of quantification

Log reduction = mean log control – mean log recovered for each treatment

There appears to be little practical difference between wet wipes (new from container) and dry wipes (dried at $37^0C \pm 1^0C$) and the use of dry tissue paper in log reduction achieved. The alcohol wipes appear to be less effective than the BKC wipes this may be due to the lack of a cleaning surfactant or the evaporation of the active (Table 2).

Table 2: Summary of results from Tests 2 & 3 efficacy of wet and dry disinfectant wipes (*Summary of Appendices 2 Tables 6.2.2 & 6.2.3*)

Interfering substance level	Condition of wipe	Active	Treatment	Mean Log	Mean log reduction	STDEV log
150g/L Bovine albumin solution	NA	Control	0 wipes	6.45	NA	0.12
	Wet	Alcohol	1 wipe	4.35	2.11	0.47
			2 wipes	3.74	2.71	1.44
		BKC	1 wipe	3.98	2.47	1.63
			2 wipes	<2.79	>3.66	NA
	Dry	Alcohol	1 wipe	4.26	2.19	0.30
			2 wipes	4.01	2.45	0.15
		BKC	1 wipe	3.96	2.49	0.20
			2 wipes	3.23	3.23	0.90
		Tissue	1 wipe	3.68	2.77	0.17
			2 wipes	4.19	2.27	0.19

Legend
BKC Benzalkonium chloride
NA Not applicable due to less than results in raw data
Log reduction = mean log control – mean log recovered for each treatment

Whilst there appears to be a small improvement in log reduction achieved when wet disinfectant wipes are used upon a wet challenge and left for a contact time of 5 minutes compared with previous results (Table 3) The significance of this difference is unclear and further testing would be required to confirm.

Table 3 Summary of Test 4 effect of contact time upon efficacy (*Summary of Appendices 2 Table 6.2.4*)

Interfering substance level	additional contact time (after wipes)	Active	Treatment	Mean log recovered	reduction	SD log
150g/L Bovine albumin solution	5min	Control	0 wipes	5.73	NA	0.12
		Alcohol	1 wipe	3.08	2.65	0.83
			2 wipes	<2.54	>3.19	NA
		BKC	1 wipe	3.00	2.73	0.62
			2 wipes	1.52	4.21	0.24

Legend
BKC Benzalkonium chloride
NA Not applicable due to less than results in raw data
Log reduction = mean log recovered (control) – mean log recovered (for each treatment)

Based upon the results (Table 4), immersion in a disinfectant at a concentration that would meet the EN13697:2001[1] criteria or hot water demonstrated mean log reductions of greater than 4.

Table 4 Summary of Test 5 efficacy of immersion contaminated probes into disinfectant or "hot water" (*Summary of Appendices 2 Table 6.2.5*)

Interfering substance level	Treatment after dry wipe	Mean Log	Mean log reduction	SD log
150g/L Bovine albumin solution	Control	5.5	NA	0.2
	5min ClO-	<1.2	>4.3	NA
	5min BKC	<1.4	>4.1	NA
	2min 80^0C ±2 ^{0}C Water	<1.2	>4.3	NA

Legend
BKC Benzalkonium chloride at manufactures recommended use concentration
ClO^- Sodium Hypochlorite at manufactures recommended use concentration
NA Not applicable due to less than results in raw data
Log reduction = mean log control – mean log recovered for each treatment

5. CONCLUSION

Based upon the results shown in Result Tables 1-3 the greatest influence upon wipe efficacy appears to be the challenge (interfering substance and organism) dryness (Table 1). When the challenge is dried on to the probe it becomes more difficult to remove with disinfectant wipes.

As expected the use of 2 wipes was consistently more effective in terms of mean log reduction achieved, than the use of 1 wipe (using a fresh disinfectant wipe for each wipe).

There was little practical difference (< 1log) in efficacy between wet and dry Alcohol wipes and tissue paper (Table 2). There was an increase in achieved mean log reduction if the disinfectant applied to the wipe was allowed a contact time of 5 minutes (Table 3), however, further work needs to be performed to demonstrate if this is significant.

There was little practical difference (<1 log) in efficacy between wet and dry BKC wipes and tissue paper (Table 2). There was an increase in achieved mean log reduction if the disinfectant applied to the wipe was allowed a contact time of 5 minutes (Table 3), however, further work needs to be performed to demonstrate if this is significant.

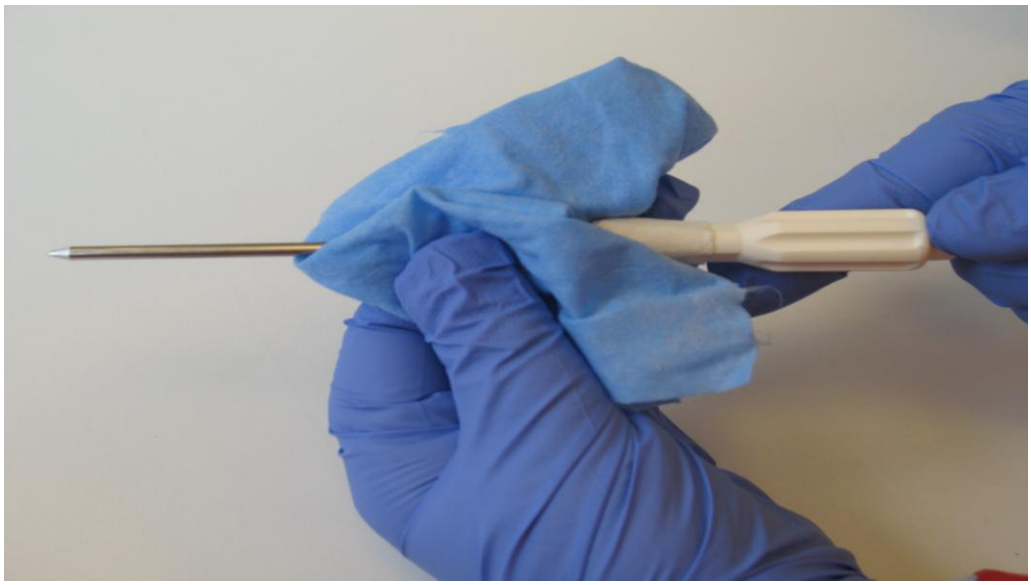
The immersion of "dry wiped" probes into a treatment solution (disinfectant solution at manufactures recommended concentration or hot water) for a specified contact time demonstrated a mean log reduction of >4.

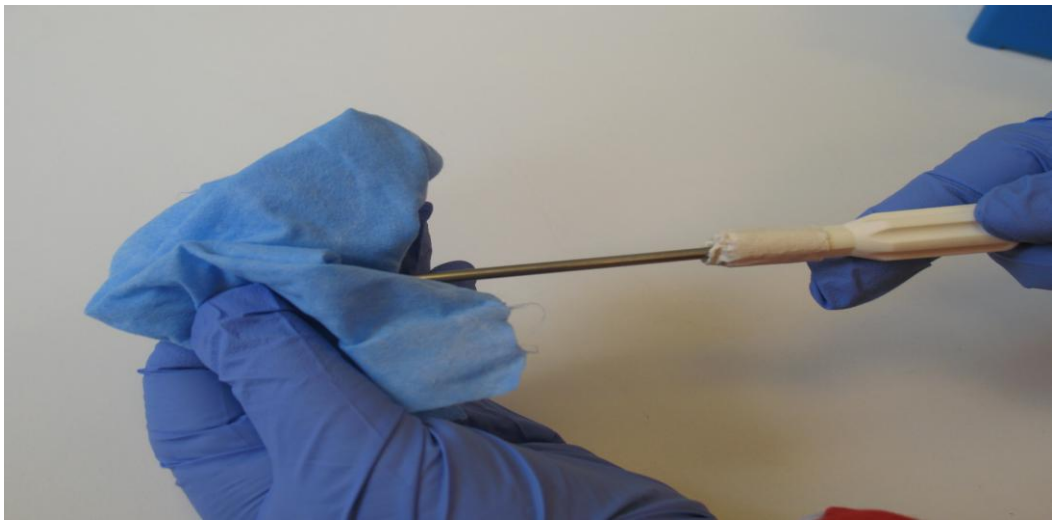
Based upon the results above disinfectant wipes demonstrate varying degrees of efficacy depending upon state and level of the challenge, the state of the wipes (dry/ wet) and the contact time given to the applied disinfectant. However, a mean log reduction of >4 was only achieved by immersion of the probe in a treatment (disinfectant or water ≥80^0C) for an appropriate time.

6. Appendices

Appendices 1 (plates)

Plates 1 and 2 wiping probe

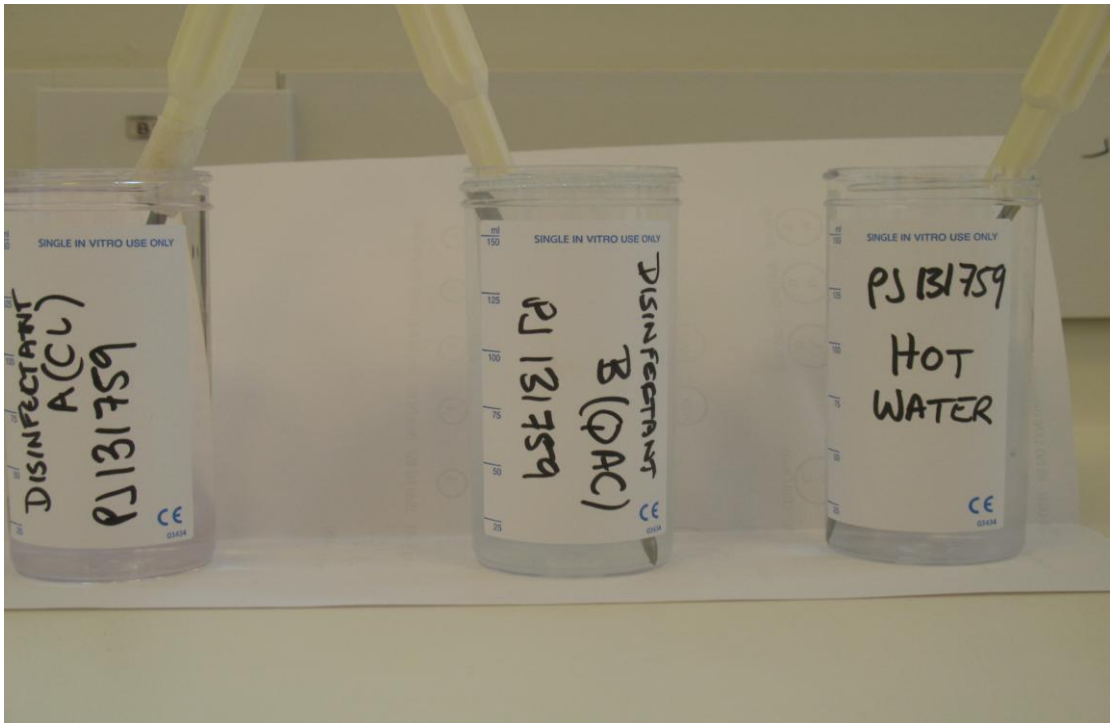




Probes were wiped from top (Handle) to bottom

Plate 3: Probes in agar after sampling

Plate 4 Probes in Disinfectant Hot Water



Probes fully immersed in disinfectant solution (BKC or ClO^-) or “hot water” (80±2 ^{0}C)

Abbreviations: <=less than, >=greater than, ND=not detected, NM=not measured, NT=not tested, LOD=limit of detection, LLOQ=lower limit of quantification, ULOQ=upper limit of quantification

Appendices 2: Results

Table 6.2.1 Test1 Results

Interfering substance level	Active	Treatment	Growth on Probe	CFU/swab	Log CFU/swab	Mean Log	Mean Log reduction	STD log
30g/L Bovine albumin solution	Control	0 wipes	+	2.27E+03	3.36	3.31	NA	0.63
			+	8.18E+03	3.91			
			+	4.55E+02	2.66			
	alcohol	1 wipe	+	1.77E+02	2.25	3.34	-0.03	1.25
			+	1.17E+03	3.07			
			+	5.14E+04	4.71			
		2 wipes	+	8.91E+02	2.95	3.11	0.20	0.18
			+	2.03E+03	3.31			
			+	1.21E+03	3.08			
	BKC	1 wipe	+	2.30E+03	3.36	3.29	0.02	0.19
			+	1.21E+03	3.08			
			+	2.72E+03	3.43			
		2 wipes	+	4.27E+02	2.63	2.75	0.56	0.14
			+	8.05E+02	2.91			
			+	5.14E+02	2.71			
150g/L Bovine albumin solution	Control	0 wipes	+	1.50E+05	5.18	5.34	NA	0.33
			+	5.14E+05	5.71			
			+	1.31E+05	5.12			
	alcohol	1 wipe	+	1.09E+05	5.04	4.23	1.11	1.10
			+	4.86E+04	4.69			
			+	9.59E+02	2.98			
		2 wipes	+	3.41E+04	4.53	4.33	1.01	0.20
			+	1.37E+04	4.14			
			+	2.14E+04	4.33			
	BKC	1 wipe	+	4.41E+03	3.64	3.74	1.60	0.14
			+	7.00E+03	3.85			
			NT	NT	NT			
		2 wipes	+	2.39E+04	4.38	3.13	2.21	1.76
			+	7.73E+01	1.89			
			NT	NT	NT			

Legend:
BKC Benzalkonium chloride
NA Not applicable
NT Not tested
+ visible growth in the probes stabbed into TSA (Appendix 1: plate 3)
Log reduction = mean log control – mean log recovered for each treatment
STD log Standard deviation of log recovery

Table 6.2.2 Test 2 Results

Interfering substance level	Active	test	Growth on Probe	CFU/swab	Log CFU/swab	Mean Log	reduction	STD log
150g/L Bovine albumin solution	Control	control	+	2.38E+06	6.38	6.49	NA	0.16
			+	2.68E+06	6.43			
			+	4.73E+06	6.67			
	alcohol	1 wipe	+	2.15E+04	4.33	4.58	1.91	0.23
			+	4.00E+04	4.60			
			+	6.28E+04	4.80			
		2 wipes	+	5.05E+03	3.70	3.93	2.56	0.32
			+	6.05E+03	3.78			
			+	2.00E+04	4.30			
	BKC	1 wipe	+	4.96E+04	4.70	4.19	2.30	0.51
			+	4.75E+03	3.68			
			+	1.59E+04	4.20			
		2 wipes	+	<10	<1.00	>3.06	>3.43	>1.19
			+	1.09E+03	3.04			
			+	1.22E+03	3.09			
	Tissue	1 wipe	+	6.35E+03	3.80	3.68	2.81	0.17
			+	3.60E+03	3.56			
		2 wipes	+	1.12E+04	4.05	4.18	2.31	0.19
			+	2.08E+04	4.32			

Legend:
BKC Benzalkonium chloride
NA Not applicable
NT Not tested
+ visible growth in the probes stabbed into TSA (Appendix 1: plate 3)
Log reduction = mean log control – mean log recovered for each treatment
STD log Standard deviation of log recovery

Table 6.2.3 Test 3 Results

Interfering substance level	Condition of wipe	Active	Test	Growth on Probe	CFU/swab	Log CFU/swab	Mean Log	Mean log reduction	STD log
150g/L Bovine albumin solution	NA	Control	0 wipe	+	3.18E+06	6.50	6.41	NA	0.09
				+	2.55E+06	6.41			
				+	2.14E+06	6.33			
	Wet	Alcohol	1 wipe	+	2.21E+04	4.34	4.11	2.30	0.58
				+	3.45E+04	4.54			
				+	2.86E+03	3.46			
			2 wipes	+	5.41E+03	3.73	3.55	2.86	0.22
				+	4.09E+03	3.61			
				+	2.00E+03	3.30			
		BKC	1 wipe	+	9.82E+03	3.99	3.77	2.64	0.90
				+	3.50E+04	4.54			
				+	6.14E+02	2.79			
			2 wipes	+	6.82E+02	2.83	3.21	3.20	0.42
				+	1.37E+03	3.14			
				+	4.55E+03	3.66			
	Dry	Alcohol	1 wipe	+	1.12E+04	4.05	4.26	2.15	0.30
				+	2.95E+04	4.47			
			2 wipes	+	7.91E+03	3.90	4.00	2.41	0.15
				+	1.29E+04	4.11			
		BKC	1 wipe	+	6.59E+03	3.82	3.96	2.45	0.20
				+	1.27E+04	4.10			
			2 wipes	+	7.23E+03	3.86	3.22	3.19	0.90
				+	3.86E+02	2.59			

Legend:
BKC Benzalkonium chloride
NA Not applicable
+ visible growth in the probes stabbed into TSA (Appendix 1: plate 3)
Log reduction = mean log control – mean log recovered for each treatment
STD log Standard deviation of log recovery

Table 6.2.4 Test 4 Results

Interfering substance level	additional contact time (after wipes)	Active	test	Growth on Probe	CFU/swab	Log CFU/swab	Mean Log	Mean log reduction	STD log
150g/L Bovine albumin solution	5min	control	0 wipes	+	7.00E+05	5.85	5.73	NA	0.12
				+	4.00E+05	5.60			
				+	5.36E+05	5.73			
		alcohol	1wipe	+	2.14E+02	2.33	3.08	2.65	0.83
				+	9.25E+03	3.97			
				+	8.91E+02	2.95			
			2wipes	+	7.15E+03	3.85	<2.54	>3.19	>1.64
				-	<5	<0.7			
				+	1.17E+03	3.07			
		BKC	1wipe	+	5.64E+02	2.75	3.00	2.73	0.62
				+	3.45E+02	2.54			
				+	5.05E+03	3.70			
			2wipes	+	1.82E+01	1.26	1.52	4.21	0.24
				+	3.64E+01	1.56			
				+	5.45E+01	1.74			

Legend:
BKC Benzalkonium chloride
NA Not applicable
+ visible growth in the probes stabbed into TSA (Appendix 1: plate 3)
- no *visible growth in the probes stabbed into TSA (Appendix 1: plate 3)*
Log reduction = mean log control – mean log recovered for each treatment
STD log Standard deviation of log recovery

Table 6.2.5 Test 5 Results

Interfering substance level	Treatment after dry wipe	CFU/swab	Log CFU/swab	Mean Log	Mean log reduction	STD log
150g/L Bovine albumin solution	Control	2.10E+05	5.3	5.5	NA	0.2
		3.95E+05	5.6			
		3.45E+05	5.5			
	5min ClO-	1.00E+01	1.0	<1.2	>4.3	0.6
		<5	<0.7			
		8.00E+01	1.9			
	5min BKC	<5	<0.7	<1.4	>4.1	0.8
		1.55E+02	2.2			
		2.50E+01	1.4			
	2min 80^{0}C ±2 ^{0}C	5.00E+00	<0.7	<1.2	>4.3	0.5
		6.00E+01	1.9			
		1.50E+01	1.2			

Legend:
BKC Benzalkonium chloride
ClO$^-$ Sodium Hypochlorite
NA Not applicable
Log reduction = mean log control – mean log recovered for each treatment
STD log Standard deviation of log recovery

Appendices 3: Reference

1. **BS EN 13697:2001** Chemical disinfectants and antiseptics. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2/step 2)

Abbreviations: <=less than, >=greater than, ND=not detected, NM=not measured, NT=not tested, LOD=limit of detection, LLOQ=lower limit of quantification, ULOQ=upper limit of quantification

Appendix IV
Best practice

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Report on:

Deliverable 4: Decontamination Best Practice

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We'd value your feedback: FMT Client Feedback

Report credentials:

Report number: FMT/REP131759/4
Issue date: 3rd April 2014
Report authorised on behalf of Campden BRI by:
Dr Roy Betts, Head of Department - Microbiology

Our ref: Appendix IV
Page count: 14

1 BACKGROUND AND AIM

Research was carried out to determine the inactivation kinetic parameters that can be achieved using heat (both dry and wet) and chemicals (quaternary ammonium compounds (QAC), chlorine based and wipes) on surfaces inoculated with *E.coli* O157 (detailed in Deliverables 2 and 3 respectively). The aim of this document (Deliverable 4) was to discuss the decontamination best practice in relation to the methods that were proposed and the results obtained in laboratory research.

2 METHODS

The alternative methods, proposed by food business owners, outlined the use of heat disinfection and/or chemical disinfection to decontaminate complex pieces of equipment. This deliverable focused on the methods that can be applied to various surfaces of the equipment. The research was split into stages: The first stage investigated the practicalities of the methods suggested and the second stage developed best practice information as a result of stage 1 and previous deliverables.

2.1 Stage 1: Practicalities of the methods

The practicalities of the methods suggested were observed. For example, for the heat treatment methods, common items and parts of equipment were subjected to heat treatments of both wet (submergence in a heated water bath) and dry (oven heating) conditions to observe the advantages and limitations of the method.

2.2 Stage 2: Best practice

Using the information gathered in Stage 1 and details from the research carried out into the inactivation kinetic parameters that can be achieved using heat (both dry and wet) and chemicals on surfaces inoculated with *E.coli* O157 (detailed in Deliverable 2 and 3 respectively) best practice information could be provided.

3 RESULTS AND DISCUSSION

3.1 Heat treatment:

Studies were carried out on wet and dry decontamination methods. The practicalities of the methods were observed and best practice was developed. The Heat treatments investigated were hot water submergence and oven based studies.

3.1.1 Hot water submergence: Stage 1 – Practicalities

A Lan Elec; 812 slicer (Figure 1) was disassembled and the parts were placed in a hot water bath set at 65.2°C. This temperature was selected based on both the results of the kinetic D and z values obtained in Deliverable 2 and the readings that could be easily observed on temperature indicator labels. The machine consisted of both metal and plastic parts as shown in Figure 2 (a). Data loggers were used to measure the surface temperature of the items and temperature indicator strips were also used to verify the surface temperatures reached (TI labels; Thermax 8 Level, irreversible indicator labels; Range A (37-65^{o}C/99-149^{o}F) and T.D.I.; Dishwashing temperature strips (65, 71, 82^{o}C / 150,160,180^{o}F) from TMC Hallcrest, Flintshire, UK).

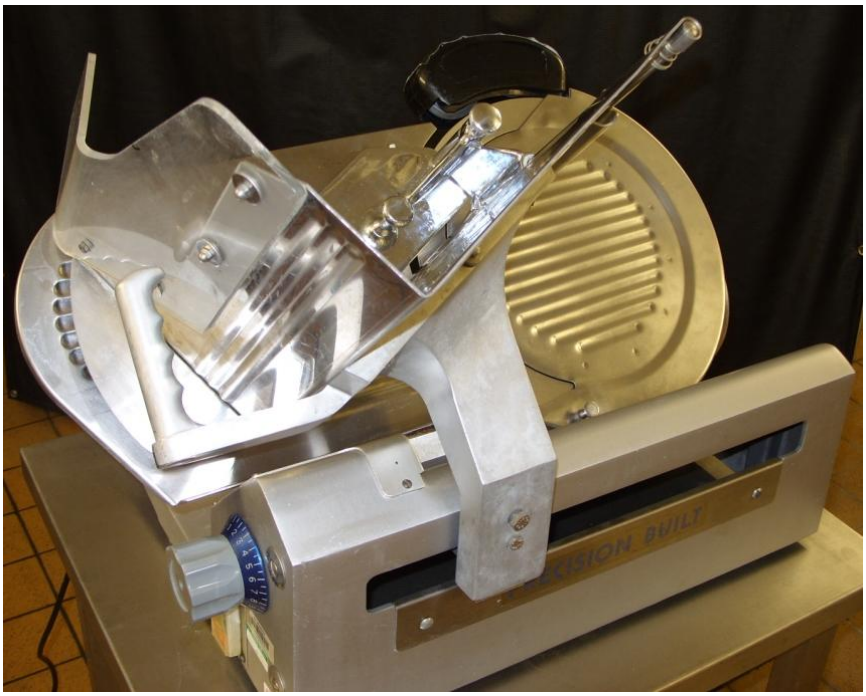


Figure 1: Lan Elec 812 Slicer observed in the studies

The graph shown in Figure 3 indicates the temperature rise observed on the surface of the plastic and metal part of the chosen slicer item (See Figure 2 a). Table 1 compares the surface temperatures observed using a data logger and temperature indicator strips (54-65°C).

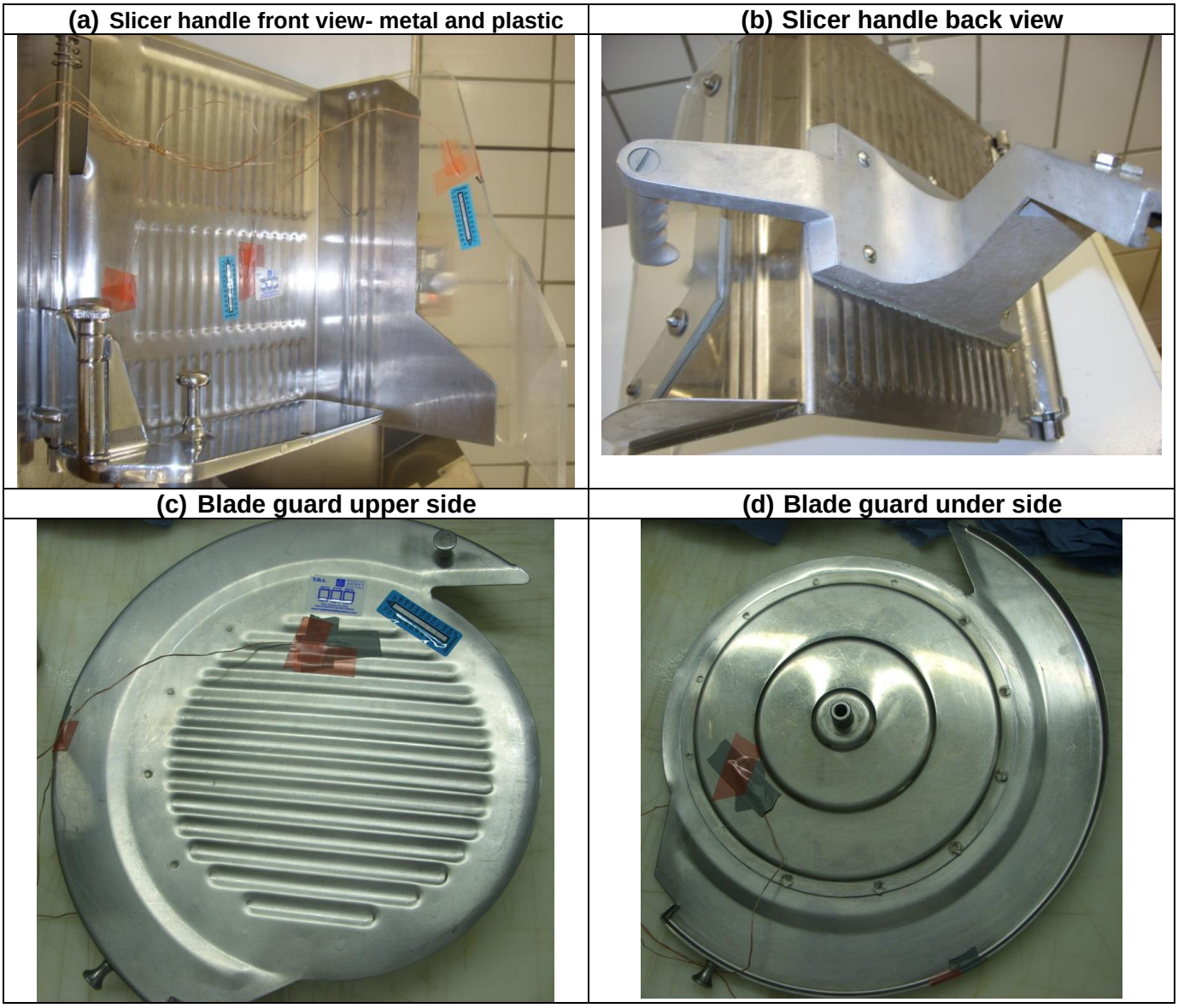


Figure 2: Lan Elec 812 Slicer parts observed in the studies

Table 1: Approximate timings of when temperatures were achieved on the surfaces (Figure 2a) during placement in a water bath set at 65.2°C. Results are those obtained using data loggers and thermal indicator labels. Temperatures observed 54 - 71 °C on TI labels and 65-82° on a T.D.I label. Times shown in minutes and seconds; x indicates unknown timing.

		Data logger (10 second intervals) Temperature °C				TI labels (visual assessment) Temperature °C				TDI (visual assessment)
No.		**54**	**60**	**62**	**65**	**54**	**60**	**62**	**65**	**65**
1	Water	<00:10	<00:10	<00:10	<00:10	No labels				
2	Plastic guard on slicer handle	00:20	01:00	01:20	02:40	00:19	x	01:00	02:09	No label
3	Thick metal centre of slicer handle	00:20	00:30	00:40	01:50	x	00:19	00:50	01:39	01:11

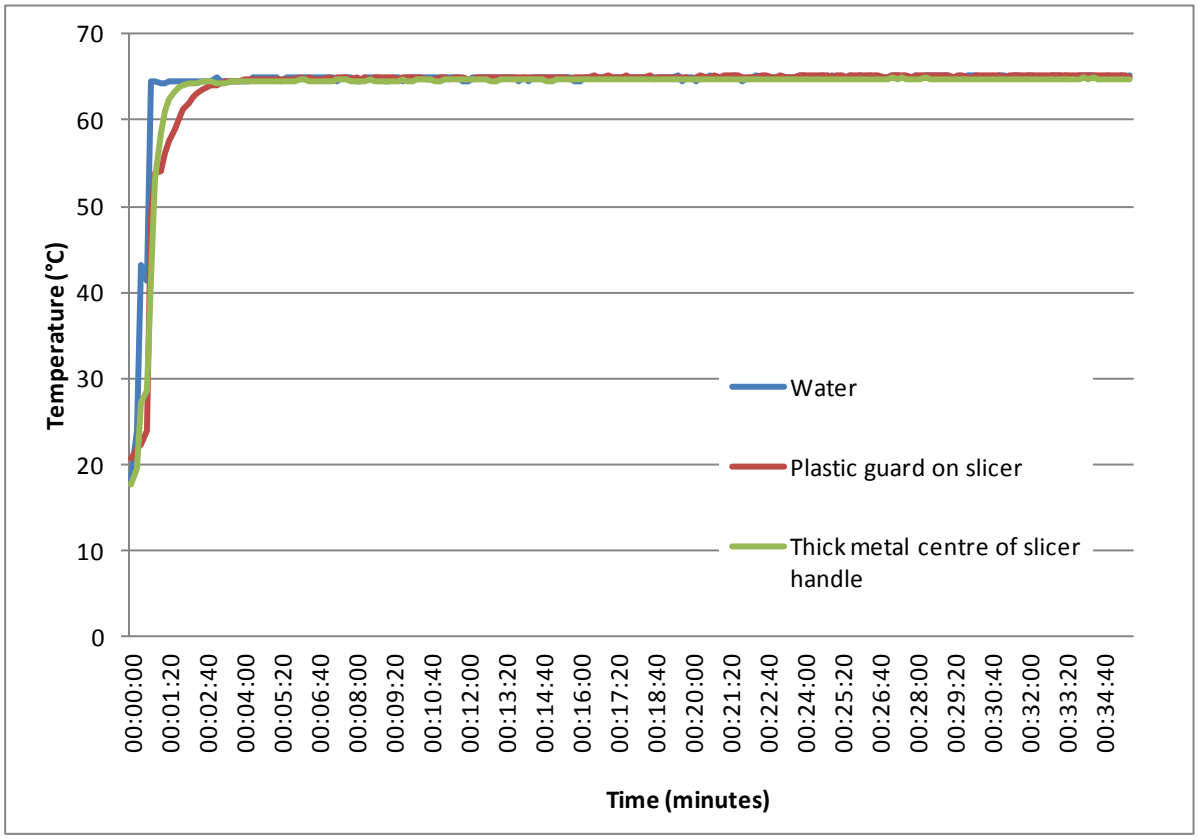


Figure 3: Data logger readings on different surfaces during placement in a water bath set at 65.2°C over 35 minutes

The data logger readings were taken at 10 second intervals, so these results may be underestimates of when actual temperatures were reached. According to the results, shown in Figure 3 and Table 1, it took less then 3 minutes for both items to reach 65 °C. According to Table 1, it appears that the surface of the metal part of the slicer handle heated up quicker then the plastic surface. It reached 65 °C between 1 minute 39 and 1 minute 50 after being placed in the hot water bath. The plastic guard on the slicer took between 2 minutes 9 seconds and 2 minutes 40 seconds to reach 65 °C. These results suggest that considerable care should be taken to identify the length of time a surface takes to get to temperature. This may depend on the thickness and nature of the item to be heat treated.

According to the thermal label and data logger results, in Table 1, the plastic guard on the slicer handle took between 02:09 (TI labels) and 02:40 (data logger) to reach 65°C. This was a difference of 31 seconds. The thick metal centre of the slicer handle took between 01:11 (TDI labels), 01:39 (TI labels) and 1:50 (data logger) to reach 65°C. This was a difference of 39 and 11 seconds when comparing the TI and TDI labels respectively with the data logger results. The results suggest that the thermal labels give a good indicator of the time at which the surface reaches the desired temperature. In some cases the labels underestimated the temperature when compared to the data loggers; however, they can be used as a simple visual method to confirm surface temperature has been reached.

During this study it was also observed that the configuration of the item treated by this method may affect the suitability of the treatment for decontamination purposes. The close up image of the back of the slicer handle (Figure 4 b) and the under side of the blade guard (Figure 4 d) show that during submergence, water became trapped in the unreachable areas of the equipment. Metal to metal connections have the potential to allow ingress of water as shown in these Figure 4 (b) and (d). This example poses a risk to the subsequent product sliced with this machine, as stagnant water may be harboured in difficult areas and released during consequent use of the machine. The water released has the potential to allow the growth of harmful microorganism. Upon release, this water may cause cross contamination to the sliced product.

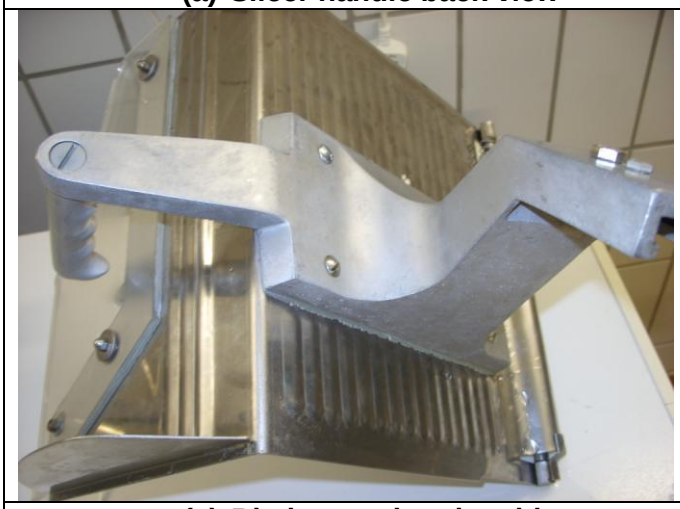
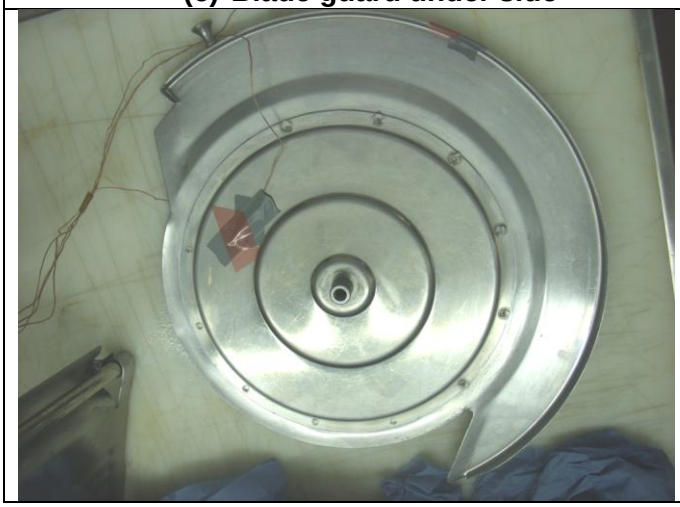
(a) Slicer handle back view	(b) Slicer handle back – close up
	
(c) Blade guard under side	**(d) Blade guard under side – close up**
	

Figure 4: Water ingress observed on slicer parts after submergence in water.

3.1.2 Hot water submergence: Stage 2 – Best practice

The results of laboratory studies carried out at Campden BRI (details in Deliverable 2) show that the D- and z-values calculated for *Escherichia coli* O157:H7 using moist heat were found to be much lower than those determined for dry heat. However, the z–value of 21.8°C determined for this organism on

stainless steel was only slightly lower than the z-value determined using dry heat (27.1°C). This z-value is considerably higher than would be expected for this organism in a normal moist situation, where the z-value might be 5-7°C.

Hot water submergence best practice:

Evaluate the suitability of the food contact item to the wet conditions. If water is likely to ingress into hard to reach areas or damage is likely, this method is not suitable.

Before heat treatment the item to be treated must be clean and free from all food debris. This may be achieved using mechanical and/or chemical treatment (e.g detergent/surfactant).

If wet heat treatments are used to decontaminate stainless steel, these should follow the minimum heat treatment at holding times shown in Table 2 or higher. It must also be noted that depending on the type of surface the time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

The treatment can be verified using temperature indicator strips to ensure the surface reaches the specific temperature and then timed until the duration of holding time is met.

Table 2: Holding time suggestions to reduce *E. coli* 0157:H7 contamination on stainless steel using moist heat.

Process temperature (°C)	D-value (seconds)	Holding time to achieve a 4 log reduction (seconds)* (in line with BS EN 13697:2001)[1]
65	2.1	8.4

**D-value, or decimal reduction time, in microbiology refers to the time required, at a given temperature, to achieve a 90% (or 1 log) reduction in the population of a given microorganism. To obtain a 4 log reduction the D-value was multiplied by 4.*

3.1.3 Oven studies: Stage 1 – Practicalities

A Lan Elec; 812 slicer (Figure 1) was disassembled and the parts were placed in an oven set at 93°C. This temperature was selected based on both the results of the kinetic D and Z values obtained in Deliverable 2 and the readings that could be easily observed on temperature indicator labels. The machine consisted of both metal and plastic parts as shown in Figure 2 (a). Data loggers were used to measure the surface temperature of the items and temperature indicator strips were also used to verify the surface temperatures reached (TI labels; Thermax 8 Level, irreversible indicator labels; Range A (37-65°C/99-149°F) and Omega; non-Reversible TL-4 Range Series (77-93°C/170-200°F).

[1] BS EN 13697:2001. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants

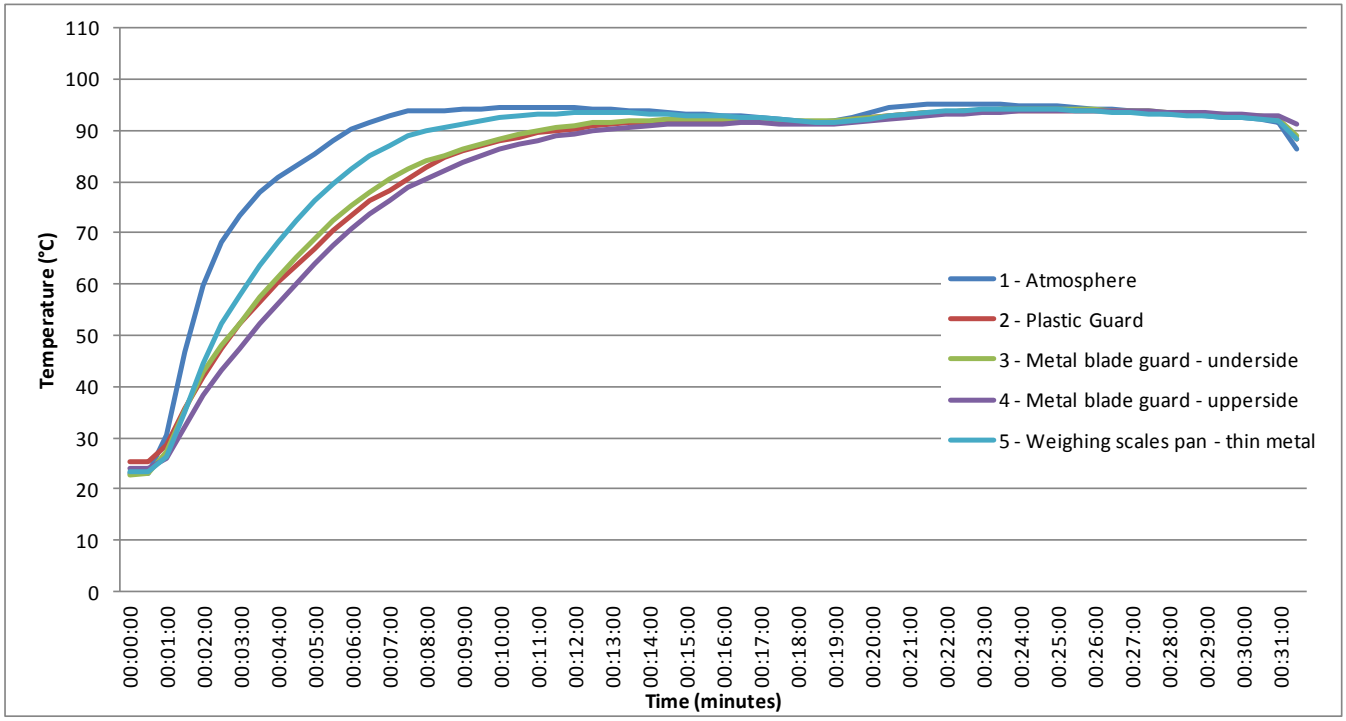


Figure 5: Data logger readings on various surfaces during placement in an oven set at 93°C over 30 minutes.

Table 3: Approximate timings of when temperatures were achieved on the various surfaces during placement in an oven set at 93 °C. Results are those obtained using data loggers and thermal indicator label readings 62 - 71 °C. Times shown in minutes and seconds; x indicates unknown timing.

	Approximate timings of when temperatures reached	Data logger (30 second intervals)			TI labels (visual assessment)		
No.		**62**	**65**	**71**	**62**	**65**	**71**
1	**Atmosphere**	01:30	01:30	02:00	No labels		
3	**Blade guard under side (Figure 2d)**	03:30	03:30	04:30	03:33	04:00	04:41
5	**Weighing scales pan (not shown)**	02:30	03:00	03:30	02:00	x	02:55

Table 4: Approximate timings of when temperatures were achieved on the various surfaces during placement in an oven set at 93°C. Results are those obtained using data loggers and thermal label readings 77 - 93 °C. Times shown in minutes and seconds; x indicates unknown timing

	Approximate timings of when temperatures Reached	Data logger (30 second intervals)				Omega labels (visual assessment)			
No.		**77°C**	**82°C**	**88°C**	**93°C**	**77°C**	**82°C**	**88°C**	**93°C**
1	**Atmosphere**	02:30	03:30	04:30	06:00	No labels			
2	**Plastic guard on slicer handle (Figure 2a)**	06:00	07:00	09:00	20:00	05:00	06:20	08:00	19:40
3	**Blade guard under side (Figure 2d)**	05:30	06:30	09:00	20:00	05:55	x	x	19:38
4	**Blade guard upper side (Figure 2c)**	06:30	07:30	10:00	21:00	No labels			
5	**Weighing scales pan (not shown)**	04:30	05.00	06:30	10:00	04:00	04:44	05:40	09:00

The graph shown in Figure 5 indicates the temperature rise observed on the surface of the plastic and metal items. Tables 3 and 4 compare the surface temperatures observed using a data logger and temperature indicator strips (62-93°C).

The data logger readings were taken at 30 second intervals, so these results may be underestimates of when actual temperatures were reached. According to the results, shown in Figure 5 and Table 4, it took ~21 minutes for all items to reach 93 °C. The thin metal pan of the weighing scales was the fastest to heat up. It reached 93°C between 9 and 10 minutes after being placed in the oven. The plastic guard of the slicer and upper and lower parts of the slicer blade guard took approximately double this time. These results suggest that considerable care should be taken to identify the length of time a surface takes to get to temperature. This may depend on the thickness and nature of the item to be heat treated.

According to the thermal label and data logger results, in Table 4, the plastic guard on the slicer handle took between 19 minutes 40 seconds (Omega labels) and 20 minutes (data logger) to reach 93°C. This was a difference of 20 seconds. Similar results were obtained for the blade guard under side (a difference of 12 seconds). The weighing pan was made of thin metal and this showed a faster rate of heating (10 minutes using the data logger) which was reflected also using the thermal temperature labels (9 minutes). The results suggest that the thermal labels give a good indicator of the time at which the surface reaches the desired temperature. In some cases the labels underestimated the temperature when compared to the data loggers; however, they can be used as a simple visual method to confirm surface temperature has been reached.

3.1.4 Oven studies: Stage 2 – Best practice

The results of laboratory studies carried out at Campden BRI (Details in deliverable 2) show that the heat resistance of *E. coli* O157:H7 attached to stainless steel towards dry heat is very high compared against its moist heat resistance in foods. For example, the D-value of *E. coli* O157:H7 at 65°C in distilled water was found to be 0.05 minutes in a study by Spinks *et al.* (2006), compared with 136.8 minutes on stainless steel treated with dry heat at the same temperature. Furthermore, a moist process of 70°C for 2 minutes, applied to short shelf-life chilled foods, would be expected to achieve a >6 log reduction in *E. coli*, whereas the results obtained show that, on stainless steel surfaces treated with dry heat at 70°C, a hold time of 322 minutes would be required to produce the same reduction in this organism.

Dry heat best practice:

Evaluate the suitability of the food contact item to the intended dry heat treatment. If the heat is likely to cause damage to the item this method is not suitable.

Before use of a heat treatment the item to be treated must be clean and free from all food debris. This may be achieved using mechanical and/or chemical treatment (e.g detergent/surfactant).

Parts of the equipment which come into direct contact with food shall be placed into an oven at temperatures and times shown in Table 5 or equivalent temperatures and time.

It must also be noted that depending on the type of surface the time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

The treatment can be verified using temperature indicator strips to ensure the surface reaches the specific temperature and then timed until the duration of holding time is met.

Table 5: Holding time suggestions to reduce *E. coli* 0157:H7 contamination on stainless steel using dry heat.

Process temperature (°C)	D value (Minutes)	Holding time to achieve a 4 log reduction (Minutes)* (in line with BS EN 13697:2001[2])
65	136.8	547.2
75	35.1	140.4
85	22.6	90.4
95	8.7	34.8
105	3.9	15.6

**D-value, or decimal reduction time, in microbiology refers to the time required, at a given temperature, to achieve a 90% (or 1 log) reduction in the population of a given microorganism. To obtain a 4 log reduction the D-value was multiplied by 4.*

[2] BS EN 13697:2001. Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants

3.1.5 Overall Thermal treatment best practice:

Thermal treatment best practice:

Hands should be washed prior to cleaning/disinfecting the equipment.

Disassemble the equipment.

Remove all food debris from the food contact item using single use sanitised cloths. Mechanical action and/or chemical treatment (e.g detergent/surfactant) can be applied to help remove any extra food debris.

Parts of the equipment which come into direct contact with food shall be placed into an oven or heated water at temperatures and times shown in Tables 5 and 4 respectively, or equivalent temperatures and times as calculated from wet and dry heat z-values.

The time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

If suitable, instead of using dry or wet heat, a dishwasher at a temperatures of 60°C or higher can be used to clean the food contact items. The time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time.

The temperature of the surface of the equipment can be verified using temperature indicator strips. When the surface temperature has been reached, maintain the item at the correspondent time for that temperature.

When the treatment time has been completed, remove the items from the oven or hot water safely and allow to cool on a clean, heat proof surface. Wet items should be air dried or dried with disposable towels.

Once dried or cooled the items should be reassembled with clean hands and moved to a clean area.

3.2 Chemical spray treatment:

Studies were carried out on chemical decontamination methods. The practicalities of the methods were observed and best practice was developed. The chemicals investigated were a Quaternary ammonium based compound (QAC – Benzalkonium chloride (BKC) and a Chlorine based compound (Sodium Hypochlorite (ClO^-). The comparison results obtained are shown in Appendix II

3.2.1 QAC: Stage 1 – Practicalities

The results in Appendix II show that *Escherichia coli* O157:H7 attached to stainless steel and plastic can be reduced by more than 4 logs when treated under the correct conditions

Chemical liquid application best practice:

Hands should be washed prior to cleaning and disinfecting the equipment.
Disassemble the equipment as much as possible
Before use of any disinfection treatment, the item to be treated must be clean and free from all food debris. This may be achieved using mechanical and/or chemical treatment (e.g detergent/surfactant).
Evaluate the suitability of the food contact item to the treatment conditions; If the method is likely to damage the article an alternative method should be sought.
Ensure the disinfectant selected is suitable for the surfaces to be disinfected.
Once the contact time has passed the disinfectant can be rinsed off using clean (potable) water.
The item can be dried using air drying methods, or disposable towels.
Once dried the items should be reassembled with clean hands and moved to a clean area.
Disinfection of food contact areas can be undertaken with chemicals, providing they are suitable for such areas and meet the criteria of BS EN 1276 and/or BS EN 13697.
Manufacturer's instructions should be followed when using any chemical.
Suitable training is important to ensure disinfection is carried out in the correct manner, for example using the correct concentrations, temperature and contact times.
The chemical concentrations can be verified using indicator strips to ensure the concentration applied to the surface is as desired.

3.3 Wipes

3.3.1 Sanitiser wipes: Stage 1 – Practicalities

The research (Details in Appendix III) provides no evidence that disinfectant wipes alone result in a 4 log reduction of *E. coli.* Therefore, wipes could be used as a risk reduction method for the removal of food residue with some microbial load reduction. However, wipes should not be used as a control for *E. coli* O157:H7. Best practice advice would be to disinfect probes using wipes straight after a contamination incident. It would be suggested that wipes are only appropriate for use between products of the same nature (for example only between raw foods). A further step should be used to disinfect when probing RTE products after probing raw products. This may involve hot water (>80°C) or chemical submergence and probes should always be wiped again if they have not been used for some time prior to probing foods.

3.3.2 Sanitiser wipes: Stage 2 – Best practice

Wipes best practice:
When disinfecting probes, wipes should be used straight after the use of the probe (optimum time of use).
The probe should be given one wipe to remove debris and then wiped with a second clean wipe to sanitise. The probe should be stored in a clean area between uses and sanitised again before further use.

If a probe is to be used for probing RTE products following the probing of raw products, it must first be cleaned of food debris (using mechanical and/or chemical treatment such as detergents or surfactants) before being disinfected via heat (immersion in hot water (>80ºC) or dish-washed at (60°C) or immersing in chemical disinfectants at the manufacturers recommended concentration and contact time.

To maintain their effectiveness the following key points should be considered;
Wipe manufacturer's instructions for storage guidance (where to store and length of storage life)
Stock rotation and age of wipes to ensure they have not dried out or expired.
The risk of cross contamination from the exterior of containers/packets in which the wipes are stored.

Ensure all staff who will use wipes have received training on how and when to use them to help guarantee consistency and efficacy of use in practice.

4 CONCLUSION

From the results and discussion it was observed that the alternative methods suggested are suitable for certain items of equipment as long as the methods of application are used as recommended. The efficacy of the method also depends on the equipment to be disinfected. To ensure the optimal effectiveness of a disinfection treatment it is important that the item is free from all food debris before application. This may be achieved using mechanical and/or chemical treatment (e.g. detergent/surfactant).

For wet heat treatments (e.g. hot water submergence methods) the equipment must be suitable for water immersion and without connections that may cause water ingress that may later release onto the food product. During dry heat treatments (e.g. oven heat) the materials of construction of the equipment shall be considered so that the heat does not damage the item. During the heat treatment methods it must also be noted that depending on the type of surface the time taken for the surface to come up to temperature must be taken into consideration and added to the total treatment time (which, in practical terms, may be considerable (hours)).

When using chemical methods the smoothness of the surface and hydrophobicity must be taken into consideration to assure the chemical treatment can achieve a suitable contact with the surface. Wipes can be used to assist in the disinfection of temperature probes if used directly after a contamination incident or, better still, after submergence in disinfectant at a concentration that would meet the EN13697:2001[1] criteria or hot water.

It is important to follow the manufactures advice for disinfection. (This relates to both chemical and surface suitability). Correct training on disinfection protocol is essential. A protocol may differ between parts of the equipment.

There is currently no standard test that simulates the physical removal of dirt or microbes by wipes. Best practice recommends that surfaces should be physically clean prior to disinfection in order for any disinfectant to be effective. Suitable training is important to ensure cleaning is carried out in the correct manner, for example; correct storage of the wipes so that they do not dry out and not re-using the same wipe. Wipes are appropriate to use when disinfecting probes between product of the same nature (for example RTE foods), however a further step should be used to disinfect between raw and RTE. This step may involve prior chemical or hot water submergence before the use of disinfection wipes.

Appendix V
Verification

Campden BRI group:
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Report on:

Deliverable 5: Recommend decontamination verification procedures for the 'alternative' methods developed.

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We'd value your feedback: FMT Client Feedback

Report credentials:

Report number: FMT/REP131759/5
Issue date: 27th March 2014
Report authorised on behalf of Campden BRI by: Dr John Holah
Business Development Project Manager - Hygiene

Our ref: Appendix V
Page count: 15

1 BACKGROUND AND AIM

The aim of this deliverable was to provide guidance on how businesses can validate the performance of their decontamination method, against the proposed recommendations, with a focus on methods that could be readily achievable by small businesses.

2 METHODS

This research investigated the reliability of temperature and chemical concentration verification methods readily available to small businesses. To ensure surfaces reach a desired temperature, temperature indicator strips can provide a quick and cost effective verification tool. Standard tests were carried out to assess the accuracy of these verification methods to ensure they are suitable to verify the temperatures required when using the alternative methods suggested.

During chemical treatment, the concentration of a cleaning solution can be easily verified using chemical test strips. Several rapid testing strip methods were used to test the concentration of both chlorine and Quaternary ammonium compound (QAC) solutions that may be used for disinfection purposes.

2.1 Thermal indicator standard test

The aim of this assessment was to determine the accuracy of temperature indicator labels using the BSI Standard (BS 7908:1999: Packaging - Temperature and time-temperature indicators - Performance specification and reference testing).

Four types of temperature indicator labels were tested; (1) Thermax 8 Level, irreversible indicator labels (TI labels), Range A (37-65^{o}C/99-149^{o}F), (2) Thermax 8 Level, irreversible indicator labels (TI labels), Range B (71-110^{o}C/160-230^{o}F), from Temperature Indicators Ltd, Kent, UK; (3) TDI Dishwashing temperature strips (65, 71, 82^{o}C / 150,160,180^{o}F) from TMC Hallcrest, Flintshire, UK and (4) Omega, non-Reversible TL-4 Range Series (77-93°C/170-200°F).

The temperatures tested on the TI label: Range A were 60°C (end point 1 – EP1) and 65°C (end point 2 – EP2). The temperatures tested on the TI label: Range B were 88°C (end point 1 – EP1) and 93°C (end point 2 – EP2). The manufactures stated an accuracy of ±2% of the temperature in °F. This gave ranges of 58.4-61.6°C for EP1; Range A, 63.3-66.7°C for EP2; Range A and 85.7-89.9°C for EP1; Range B and 90.6-94.9°C for EP2; Range B. Due to the level of accuracy, the labels were assumed to be equivalent to BS708 Class 2 and the standard was carried out accordingly.

The temperatures tested on the TDI labels were 65°C (EP1) and 71°C (EP2). The accuracy was not given with the instructions however the same labels claimed they are accurate within ±1°C when researched online. This gave ranges of 64-66°C for EP1 and 70-72°C for EP2. Due to the level of

accuracy, the labels were assumed to be equivalent to BS708 Class 2 and the standard was carried out accordingly.

The temperatures tested on the Omega labels were 88°C (EP1) and 93°C (EP2). The accuracy was not given with the instructions. The labels were assumed to be equivalent of BS708 Class 2 and the standard was carried out accordingly.

The labels (two at a time) were placed onto the aluminium surface of a temperature controlled isotherm block. As they were treated as Class 2 labels they were tested at 1.5°C either side of the end point temperature in increments of 0.5°C. See Figure 1 for details and Table 1 for temperatures tested on each label and the increments used.

The labels were left at each temperature for at least 20 minutes so the block could stabilise. When the label reached its end point, the set and surface temperatures were recorded. Observations were also made throughout the trials. A thermal imaging camera was used to measure the surface temperatures of the block and for calibration. After the assessment the labels were left to cool to room temperature on the block and were observed to determine whether or not they were irreversible. This was observed as loss of the colour that had developed upon reaching the indicated temperature.

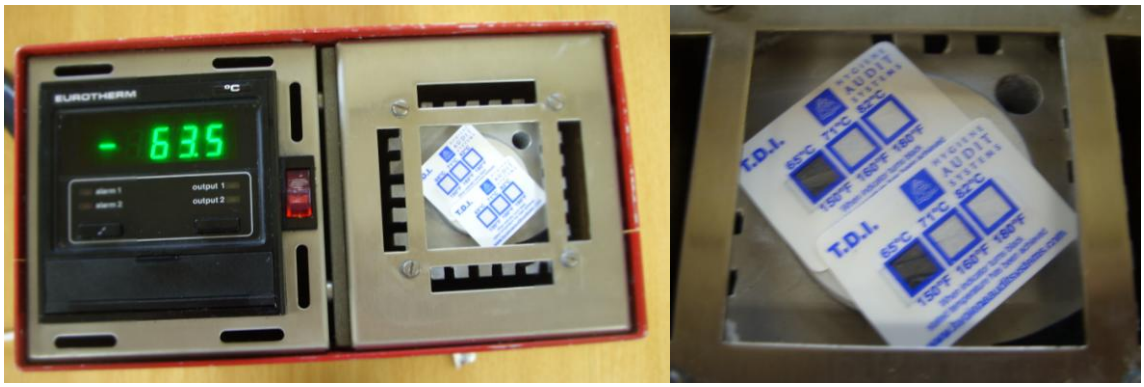


Figure 1: layout of the experiment; example showing TDI label testing

Table 1: Temperatures tested on each label and the increments used

End Point Temperature (°C)	Increments used (°C)	Labels tested
60	58.5	TI labels Range A
	59.0	
	59.5	
	60.0	
	60.5	
	61.0	
	61.5	
65	63.5	TI labels Range A and T.D.I labels
	64.0	
	64.5	
	65.0	
	65.5	
	66.0	
	66.5	
71	69.5	T.D.I labels
	70.0	
	70.5	
	71.0	
	71.5	
	72.0	
	72.5	
88	86.5	TI labels Range B and Omega labels
	87.0	
	87.5	
	88.0	
	88.5	
	89.0	
	89.5	
93	91.5	TI labels Range B and Omega labels
	92.0	
	92.5	
	93.0	
	93.5	
	94.0	
	94.5	

2.2 Chemical test strips: Test Kits for Chemical Compounds

The aim of this assessment was to determine the accuracy of chemical testing strips. Solutions of various concentrations of chemicals (Chlorine and QAC) were made up and tested using verification strips from various manufactures. The concentrations of chlorine (Domestos- extended germ kill; original bleach with CTAC; Unilever) were also verified using analytical methods (ASTM D2022-89 (2008) NON-UKAS). The concentrations of QAC (Suma BAC – D10; Johnson Diversey) were determined using information from the manufacturer.

Chlorine solution: The ingredients label stated that the Domestos bleach was made up of 4.5% sodium hypochlorite (present as hypochlorous acid, HOCL, and typically referred to as ‘free chlorine’). Chemical analysis of the neat bleach solution stated that the available chlorine was 4.34%. This indicates that a 100% solution of bleach was 43400 ppm (parts per million). Dilutions of the chlorine

solution were 24% (measured as 9200 ppm), 2.4% (measured as 1130 ppm), 0.24% (measured as 130 ppm) and 0.024% (measured as 27 ppm) (See Table 2).

QAC solutions: According to the manufacturer of the D-10, a 1 % dilution would have 300ppm QAC. The concentrations were made up as neat 100% (30000 ppm), 12.5 % (~3750 ppm), 1.25 % (~375 ppm), 0.125 % (~37.5 ppm) and 0.0125 % (~3.75 ppm) (See Table 2).

Table 2: Concentration of chemicals used (% and ppm)

	Chlorine Concentration		**QAC Concentration**	
	(%)	~(ppm)	(%)	~(ppm)
Neat	100	43400	100	30000
10 x Recommended solution	24	9200	12.5	3750
Recommended solution	**2.4**	**1130**	**1.25**	**375**
1/10 of recommended solution	0.24	113	0.125	37.5
1/100 of recommended solution	0.024	27	0.0125	3.75
Water	0	0	0	0

Rapid testing methods were used to verify the concentration of the chemical solutions. The chlorine analysis tests (Table 3) consisted of 4 types of detection limits; low (0-10 ppm), medium (0-200 ppm) and high (0-1000 ppm) and very high (0-10000 ppm). The QAC analysis tests (Table 4) consisted of 1 detection limit; medium (0-400 ppm).

Table 3: Details of Chlorine (HOCl) rapid test methods:

Type	**Name**	**Company**	**Detection levels (ppm)**
Low (a)	Sansafe – Free chlorine water check	Industrial Test Systems Inc.	0 - 6
Low (b)	Pool and spa test strips – Free chlorine, Bromine, Alkalinity and pH	Insta-test – LaMotte	0-10
Medium (a)	Hydrion - Chlorine test paper	Cole Parmer – MicroEssential laboratory	10-200
Medium (b)	Chlorine test papers	Cole Parmer – La Motte	10 -200
Medium (c)	Chlor-Assure (25-200ppm)	Labtek services	25-200
High (a)	Water works – Free chlorine check –	Industrial Test Systems Inc.	0-750

	Ultra-high		
High (b)	High Range – Free chlorine	Insta-test – LaMotte	0-800
High (c)	High level chlorine – (0-1000pm)	Labtek services	0-1000
Very High (a)	Activate – high level chlorine test strips	Cole Parmer – Deardoff Fitzsimmons corporation	0-10000

Table 4: Details of QAC rapid test methods:

Assigned code:	Name	Company	Detection levels (ppm)
QAC (a)	Hydrion - Quaternary Ammonium compound test paper	Cole Parmer – MicroEssential laboratory	0-400
QAC (b)	QAC QR test strips	Cole Parmer –La Motte	0 -400
QAC (c)	QAC (0-400ppm)	Labtek Services	0-400

3 RESULTS AND DISCUSSION

3.1 Thermal indicator standard test

Table 5: Results obtained during testing of the TDI labels. End point setting 1 (EP1) was 65°C and End point setting 2 (EP2) was 71°C. The table includes information on reversibility of the labels, the surface and set temperatures at each end point and the observations made during the test.

Label	Irreversible?		End Point 1	End Point 2	Observations
	EP1	EP2	Surface Temp	Surface Temp	
1	Y	Y	65.1	70.0	Time to change at; EP1: 1min 21sec; EP2 1min 23sec
2	Y	Y	65.0	70.1	Time to change at; EP1 1min 15sec; EP2 1min 34sec
3	Y	Y	64.7	70.0	
4	Y	Y	64.6	70.0	This label took a longer time to reach both end points in comparison to label 3.
5	Y	Y	64.8	70.0	
6	Y	Y	64.8	70.0	
7	Y	Y	64.7	69.9	The indicator for EP2 started getting dark at a set temperature of 70°C.
8	Y	Y	64.8	69.9	
9	Y	Y	64.8	69.9	
10	Y	Y	64.8	69.9	
Mean			**64.81**	**69.97**	
Standard deviation			**0.145**	**0.067**	

During the study of the **TDI label** results (Table 5) it was observed that the labels were quite consistent with each other. When looking at the measured surface temperature results for EP1, it can be observed

that they were within the ±1°C stated by the manufacturer. The results for the EP2 showed that the label 1, 3, 4, 5 and 6 were -1°C below the set temperature (just within the ±1°C threshold), with a few results falling at 1.1°C below the set point (labels 7-10 - 0.1°C outside the threshold). This could, however, be due to fluctuations in the block temperature as calibrations showed it could flicker by ~0.1°C which could potentially mean these labels changed colour within the stated range. If the temperature is slightly above the set point the colour change is likely to change rapidly. If the machine is running just below the set point it may take longer to change colour (in some cases up to 10 minutes: results not shown).

Table 6: Results obtained during testing of the TI labels: Range A. End point setting 1 (EP1) was 60°C, End point setting 2 (EP2) was 65°C. The table includes information on reversibility of the labels, the surface and set temperatures at each end point and the observations made during the test.

Label	Irreversible?		End Point 1	End Point 2	Observations
	EP1	EP2	Surface Temp	Surface Temp	
1	Y	N	59.2	63.0	EP1: The 60°C box started filling in at a set temperature of 58.5°C. EP2: The indicator for 65°C almost reached its endpoint at a set temperature of 63.5°C
2	Y	N	58.7	63.9	EP1: The 60°C box started filling in at a set temperature of 58.5°C. Prolonged time at 60°C caused it to reach its endpoint. The rate at which this happened was slower then label 1
3	Y	N	58.7	64.5	Unlike labels 1 & 2 the indicator for EP2 did not start darkening until a set temp of 65°C was reached. At EP2 this label took a lot longer to turn compared to label 4.
4	Y	N	58.7	64.4	Unlike labels 1 & 2 the indicator for EP2 did not start darkening until a set temp of 65°C was reached.
5	Y	N	58.7	63.7	
6	Y	N	59.3	64.7	
7	Y	N	59.3	63.9	
8	Y	N	59.3	63.9	At EP2 this label took about 6 minutes longer to reach the endpoint than label 7.
9	Y	N	59.2	63.9	
10	Y	N	59.1	63.9	
Mean			**59.02**	**63.98**	
Standard deviation			**0.282**	**0.476**	

During the study of the **TI labels; Range A** (Table 6), it was observed that they were less consistent when compared with the TDI labels, but nearly all of the labels fell within the ranges stated in the

method (manufactures stated an accuracy of ±2% of the temperature in °F: 58.4-61.6°C for EP1 and 63.3-66.7°C for EP2). The exception was EP2 on label 1 which turned at 63.0°C (0.3°C below the stated range). This may be due to temperature fluctuations in the block; however it is out by further than the normal fluctuation range.

There were issues with irreversibility of the TI labels at the higher temperatures tested. It was observed, however, that whilst they started to reverse slowly during cooling down it was still clear they had reached their end point. It was only once the labels had been peeled from the aluminium block that it became difficult to determine whether they had reached the set temperature or not. This suggests that they can be used for the purpose of validating surface temperatures during hot water treatment performance, however, they would not be suitable to keep as records due to their colour loss; it may be best to photograph the labels before removal and save the image as a record.

Table 7: Results obtained during testing of the TI labels: Range B. End point setting 1 (EP1) was 88°C and End point setting 2 (EP2) was 93°C. The table includes information on reversibility of the labels, the surface and set temperatures at each end point and the observations made during the test.

	Irreversible?		End Point 1	End Point 2	Observations
Label	EP1	EP2	Surface Temp	Surface Temp	
1	Y	N	89.0	93.1	EP1 and EP2: One degree before the set point the colour change began to happen. The colour did not fully change until stated. At EP2: The labels went back to a grey/white colour once removed from the heating block
2	Y	N	89.5	94.8	
3	Y	N	89.4	93.8	
4	Y	N	89.4	94.3	
5	Y	N	87.0	93.6	
6	Y	N	87.4	93.6	
7	Y	N	88.9	94.1	
8	Y	N	88.5	94.6	
9	Y	N	89.2	94.3	
10	Y	N	89.2	94.3	
Mean			88.75	94.05	
Standard deviation			0.87	0.52	

During the study of the **TI labels; Range B** (Table 7), it was observed that both labels fell within the ranges stated in the method (manufactures stated an accuracy of ±2% of the temperature in °F: 85.7-89.9°C for EP1 and 90.6-94.9°C for EP2). Again, there was issues with irreversibility of the TI labels at the higher temperatures tested. Once the labels had been peeled from the aluminium block the colour changed returned to the original pale colour. This suggests that they can be used for the purpose of validating surface temperatures during hot water treatment performance, however, they would not be suitable to keep as records; it may be best to photograph the labels before removal and save the image as a record.

Table 8: Results obtained during testing of the Omega labels. End point setting 1 (EP1) was 88°C and End point setting 2 (EP2) was 93°C. The table includes information on reversibility of the labels, the surface and set temperatures at each end point and the observations made during the test.

Label	Irreversible?		End Point 1	End Point 2	Observations
	EP1	EP2	Surface Temp	Surface Temp	
1	Y	Y	88.2	94.8	EP1: Started to observe colour change at ~86.5- 87°C EP2: The colour change was a slightly lighter grey/black to the other temperature points.
2	Y	Y	88.2	94.8	
3	Y	Y	88.8	94.8	
4	Y	Y	89.3	94.8	
5	Y	Y	88.2	94.9	
6	Y	Y	88.2	94.9	
7	Y	Y	88.4	94.8	
8	Y	Y	88.4	94.8	
9	Y	Y	89.0	94.8	
10	Y	Y	88.5	94.8	
Mean			88.52	94.82	
Standard deviation			0.388	0.042	

During the study of the **Omega label** results (Table 8) it was observed that, the labels were quite consistent with each other. When looking at the measured surface temperature results, for EP1 it can be observed that they indicated the surface temperature within 1°C of the temperature set point.

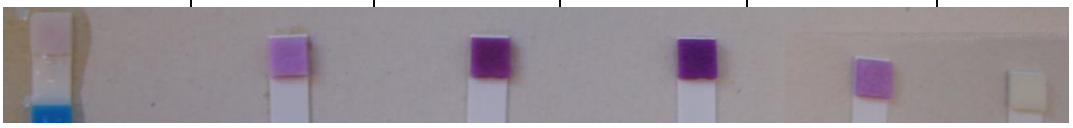
At EP2 the labels indicated the surface temperature within 1.8°C of the temperature set point. This was just within the stated accuracy of ±2% of the temperature in °F.

3.2 Chemical concentration tests

Studies were carried out to assess the methods used to verify concentrations of cleaning solutions. The studies were carried out on various concentrations of Chlorine and QAC. The chlorine analysis tests consisted of 3 types of detection limits; Low (0-10 ppm), medium (0-200 ppm) and high (0-10000 ppm). The QAC analysis tests consisted of 1 detection limit; medium (0-400 ppm).

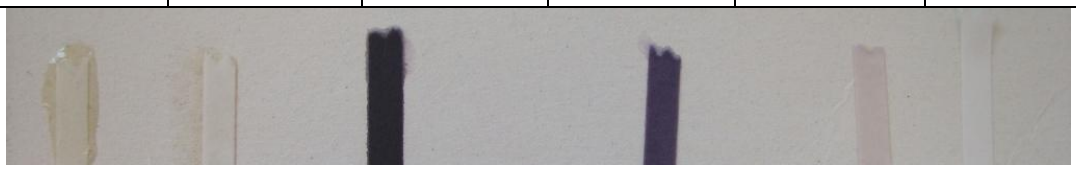
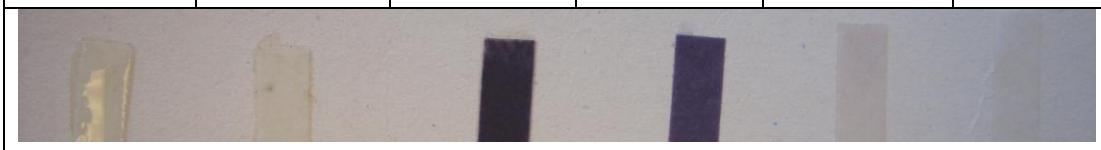
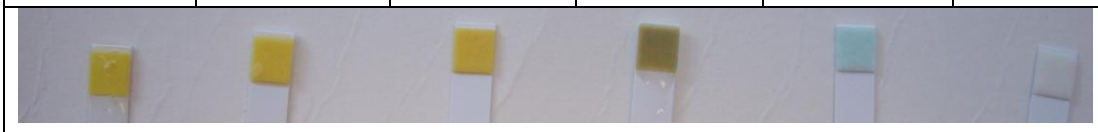
3.2.1 Chlorine test strips

Table 9 (a): Readings (ppm) obtained on the low level detection chlorine test strips (0-10ppm) once dipped in chlorine at various concentrations. (More information on label types shown in Table 3).

Label type	Concentration of Chlorine (HOCl) (% and ppm)					
	100	**24**	**2.4**	**0.24**	**0.024**	**0 (Water)**
	43400	**9200**	**1130**	**113**	**27**	**0 (Water)**
Low (a)	Bleached light purple - 1	Bleached purple - 3	**10**	10	3-5	0
						
Low (b)	Brown orange - Unknown	Brown orange - Unknown	**Black – unknown**	>6	0.8-1.2	0
						

The results shown in Table 9 (a) indicate that using low detection methods the strips gave false negatives (Low a) at very high concentrations of chlorine (24 and 100%) and Low (b) gave unknown results after dipping in concentrations above 2.4%. The results indicate that this is not a suitable method to use to test recommended concentrations of chlorine which are normally around 1000 ppm.

Table 9 (b): Readings (ppm) obtained on the medium level detection chlorine test strips (0-200 ppm) once dipped in chlorine at various concentrations. (More information on label types shown in Table 3).

Label type	Concentration of Chlorine (HOCl) (% and ppm)					
	100	**24**	**2.4**	**0.24**	**0.024**	**0 (Water)**
	43400	**9200**	**1130**	**113**	**27**	**0 (Water)**
Medium (a)	0 Bleached	0 Bleached	**200**	100	10	0
						
Medium (b)	0 Bleached	0 Bleached	**200**	100	10	0
						
Medium (c)	>100	>100	**>100**	50-100	0-25	0
						

The results shown in Table 9 (b) indicate that, using medium detection methods, the strips gave false negatives (medium a and b) at very high concentrations of Chlorine (24 and 100%). It was also difficult to decipher the difference between concentrations above 2.4% following testing with Medium (c) product. The results were most accurate for concentrations of 0 - 2.4% using method s (a) and (b). When a dark purple colour is obtained using methods (a) and (b) it can be a suitable way to ascertain if the concentration is correct. If the strips stay white or turn white under solutions of chlorine used for disinfection than this may indicate that either too little or too much chlorine respectively has been added and the formulation should be re-assessed.

The results shown in Table 9 (c) indicate that, when using high detection (0-1000 pmm) methods, the strips gave the most suitable results at concentrations between 0.24 and 2.4%, above which the concentration was out of range for the test strips. The results shown in Table 9 (d) indicate that, when using very high detection (0-10000 pmm) methods, the strips gave the most suitable results at concentrations between 2.4 and 100%, below which the concentration was out of range of the test strips. These results indicate that the most suitable method to test that the recommended concentration is very high chlorine detection strips (Table 9 d).

Table 9 (c): Readings (ppm) obtained on the high level detection chlorine test strips (0-1000 ppm) once dipped in chlorine at various concentrations. (More information on label types shown in Table 3).

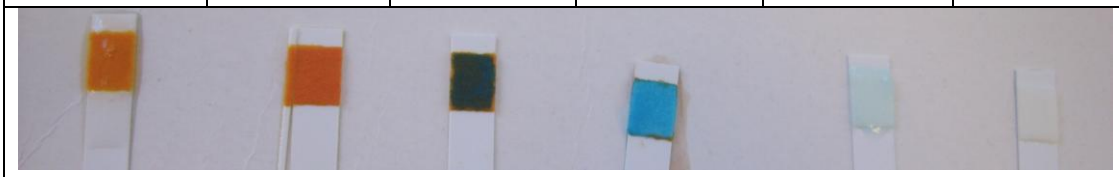
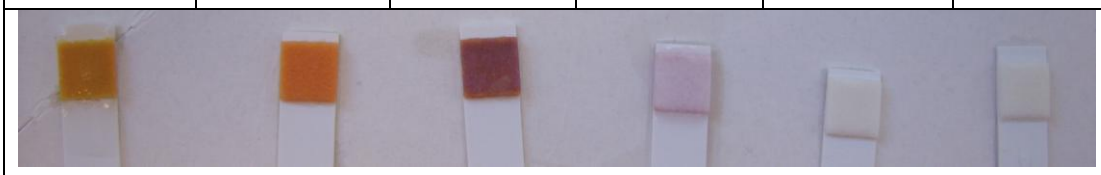
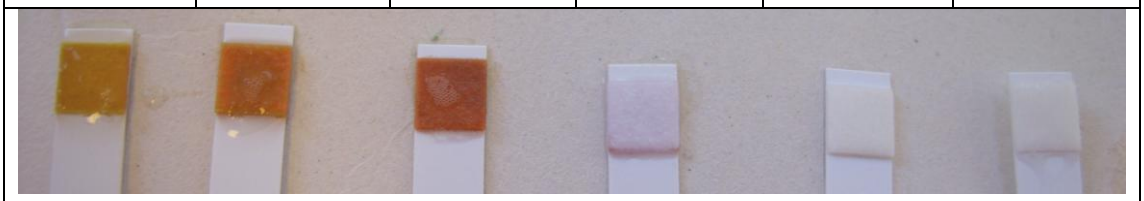
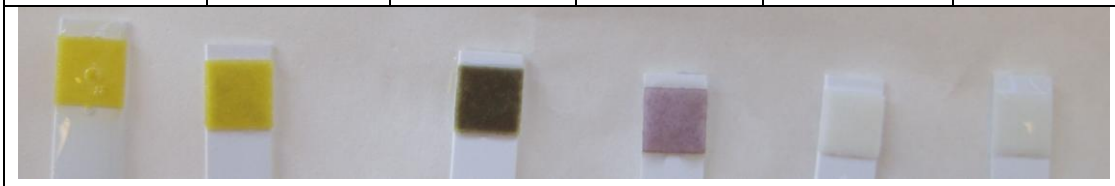
Label type	Concentration of Chlorine (HOCl) (% and ppm)					
	100	**24**	**2.4**	**0.24**	**0.024**	**0 (Water)**
	43400	**9200**	**1130**	**113**	**27**	**0 (Water)**
High (a)	Orange - Unknown	Orange - Unknown	**>750**	50-100	0-25	0
						
High (b)	Orange - Unknown	Orange-red - Unknown	**500-800**	100-250	0	0
						
High (c)	Orange – Unknown	Orange-red – unknown	**500-1000**	100-250	0	0
						

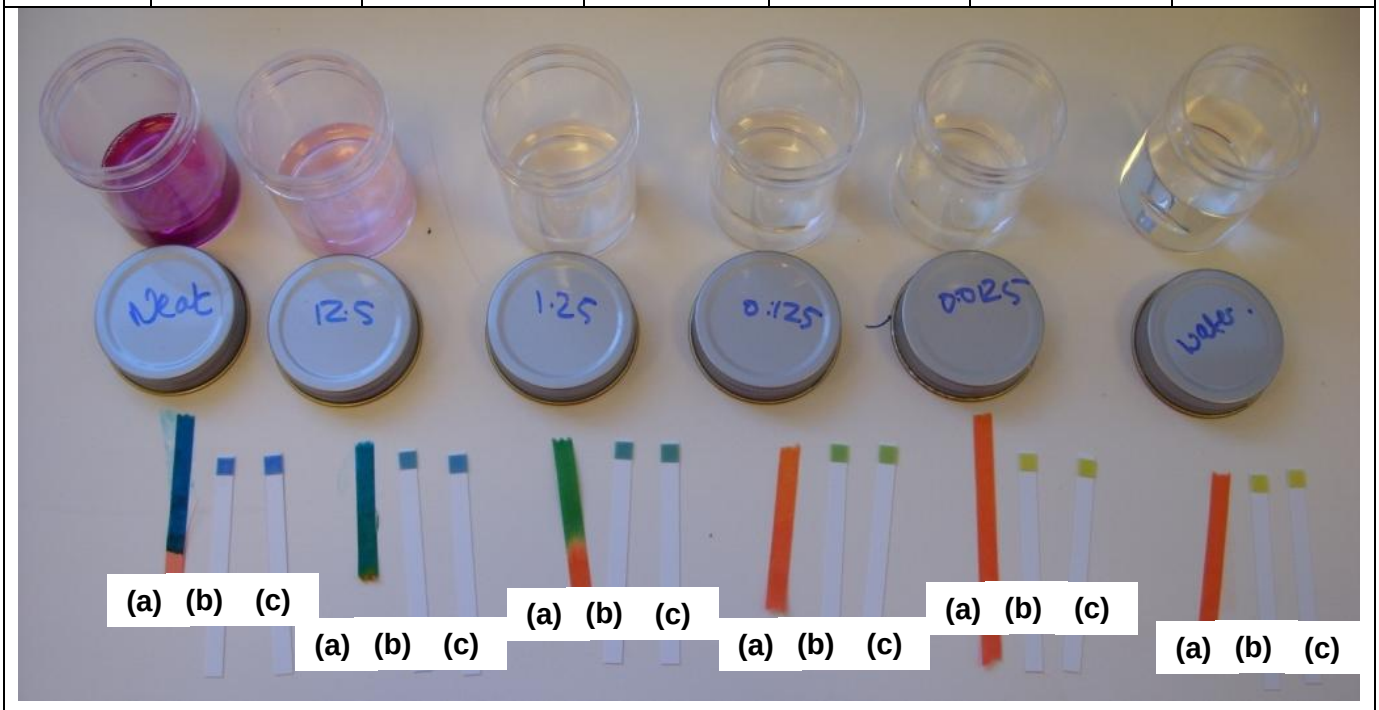
Table 9 (d): Readings (ppm) obtained on the very high level detection chlorine test strips (0-10000 ppm) once dipped in chlorine at various concentrations. (More information on label types shown in Table 3).

Label type	Concentration of Chlorine (HOCl) (% and ppm)					
	100	**24**	**2.4**	**0.24**	**0.024**	**0 (Water)**
	43400	**9200**	**1130**	**113**	**27**	**0 (Water)**
Very High High (a)	10000	7500-10000	**1000**	Purple – unknown	0	0
						

3.2.2 QAC test strips

Table 10: Readings (ppm) obtained on the medium detection QAC test strips (0-400 ppm) once dipped in QAC solutions at various concentrations. (More information on label types shown in Table 4).

Label type	Concentration of QAC solution (% and ppm)					
	100	**12.5**	**1.25**	**0.125**	**0.0125**	**0 (Water)**
	30000	**3750**	**375**	**37.5**	**3.75**	**0 (Water)**
QAC (a)	Blue - unknown	Blue - unknown	**300-400**	0-100	0	0
QAC (b)	Blue - unknown	Blue - unknown	200-400	0-100	0	0
QAC (c)	Blue - unknown	Blue - unknown	200-400	50-100	0	0



The results shown in Table 10 indicate that using medium detection methods the strips gave unknown results above concentrations of QAC (1.25 %). The results were quite similar over all concentrations. The colour scale provided with the testing strips went from yellow green (orange in the case of QAC (a)), at low concentrations of QAC, to dark green at the highest concentrations. When a blue colour was obtained this indicated concentrations were above 400 ppm. This can be a suitable way to ascertain if the concentration of a disinfectant solution is correct. If the strips stay yellow or orange when tested in disinfectant solution of QAC than this indicates that the concentration is too low. If the

strips turn dark blue this may indicate that there is too much QAC and the formulation should be re-assessed.

Whilst studies were not undertaken to assess reversibility of the colour change for the test strips, some of the manufacturers' instructions recommended that the test strips are read immediately after dipping and disposed of after a short period (e.g. 40 seconds; Very high a). As noted above for temperature indicators, it may be advisable to use photographic evidence of the chemical strip colour change for record purposes.

4 DISCUSSION/CONCLUSION

All temperature and chemical indicator strips used in this study were commercial products and thus expected to perform as stated in their use instructions. The aim of this research was to investigate the reliability of verification methods, readily available to small businesses, for the purposes of monitoring the efficacy of *E. coli* O157 decontamination techniques for food service equipment.

The verification methods may be a good technique:

- To monitor and record the disinfection practices used.
- To assist during disinfection training.
- To verify that the method is correct on changing operator or the disinfection method.
- To validate chemical dosing when a disinfectant method is used for the first time.

During the assessments of the temperature labels it was observed that the labels were a suitable method to verify the heat treatment achieved on surfaces during both wet and dry heat treatment. It was found that the labels tend to change colour when close to the threshold temperature (this change is usually quite slow). At or above the threshold temperature they tend to change faster. Some of the labels were reversible after the heat treatment indicating that they would not be suitable as proof of validation of a disinfectant method. It may be advisable to use photographic evidence if used for verification of results.

Rapid chemical testing strips were also assessed for their suitability to test the concentration of a disinfectant solution. They were found to be quite accurate as long as the correct detection level strip was used. These results may vary depending on the type of chemical method used and recommended concentration suggested. Again photographic evidence could be used if required for verification of results.

The advantage of the thermal label method is that the operator does not require training as the method is quite straight forward. Using the chemical treatment test strip method requires the operative to be

able to assess and understand the chemical concentrations and choose the correct detection level of test strip. For example, being able to calculate the level of the active ingredient (HOCl or QAC) in the manufacturer's recommended in-use concentration in ppm from the manufacturer's stated in-use concentration as a percentage dilution.

Appendix VI

Vacuum packer cross contamination

Campden BRI group:
Campden BRI (registered no. 510618)
Campden BRI (Chipping Campden) Limited (registered no. 3836922)
Campden BRI (Nutfield) (registered no. 2690377)

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food and drink innovation

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Report on:

Deliverable 6: Vacuum packer cross-contamination

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We'd value your feedback: FMT Client Feedback

Report credentials:

Report number: FMT/REP131759/1
Issue date: 28th February 2014
Report authorised on behalf of Campden BRI by: Craig Leadley
Innovative Processing Technologies Section Manager

Our ref: Appendix VI
Page count: 13

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1 BACKGROUND AND AIM

In response to the FSA call to carry out testing of food businesses' proposed alternative controls, which could be equally as (or more) effective in controlling the risk from E*. coli O*157 as the FSA guidance on the separate use of complex equipment, Campden BRI submitted a proposal which included research into the cross-contamination risks associated with vacuum packers. Concern has been expressed as to whether vacuum packers present a cross-contamination risk when used for the dual packing of raw and ready-to-eat products. The aim of this research was to assess the cross contamination risks that occurs both externally from pack to pack and internally from contaminated areas of the vacuum packer that are hard to reach/clean.

2 METHODS

Cross–contamination to the outer and inner surfaces of vacuum packed products was demonstrated via two methods detailed below: Test 1: External cross contamination and Test 2: Internal cross-contamination.

Test 1: External cross-contamination:

UV detectable microbial simulants (GlitterBug Potion, 240ml; Brevis Corporation) were used to represent contamination on a piece of meat. One piece of meat, heavily contaminated with the UV simulant was placed in a vacuum bag and vacuum packed. The operator continued to pack 4 more pieces of meat after handling the 1st contaminated piece of meat. The operator changed gloves and then continued to pack 5 more bags of meat using the same method as before. The machine was not wiped down in between uses. An ultra-violet (UV) light (Revealite™; Cleanitise) was used to show the contamination transferred from the 1st contaminated meat onto the proceeding packaged products.

This method was carried out using two different vacuum packing machines, shown in Figure 1.

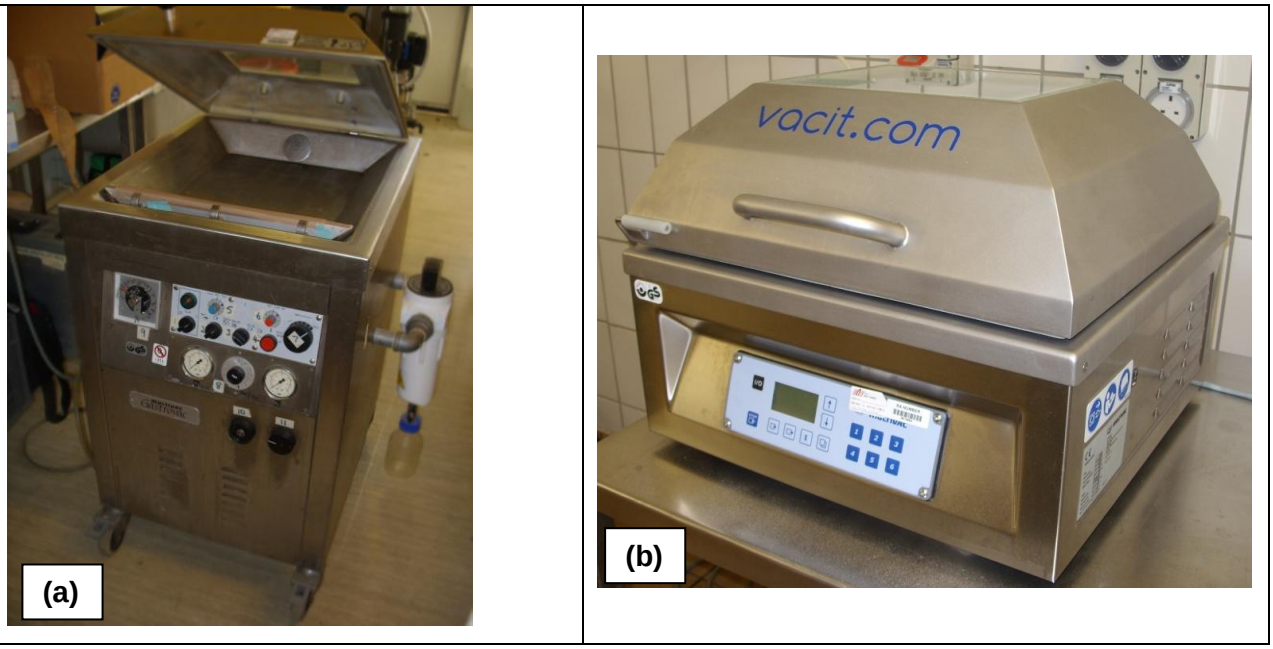


Figure 1: Vacuum packer machines used in the study; **(a)** Multivac Gastrovac and **(b)** Multivac C200

Test 2: Internal cross contamination

The research into the internal cross-contamination of the items packed in a vacuum packer involved inoculating the vacuum packing machine with *Serratia marcescens. Serratia marcescens* is a species of rod-shaped gram-negative bacteria, in the family Enterobacteriaceae. It has similar properties to *E. coli* so was suitable for the required test, but in addition, is readily visible via its red colony colouration on growth media.

Preliminary trials to test suitability of culture and method:

A culture of *Serratia marcescens* NCTC 13382 was obtained from the National Collection of Type Cultures. The organism was cultured overnight at 25°C in 10mL Tryptone Soya Broth (TSB) and this culture was streaked onto 2 x Tryptone Soya Agar (TSA) plates. TSA plates were incubated both aerobically and anaerobically at 25°C for 24 hours. Aerobic colonies showed strong red pigmentation, whereas anaerobically grown colonies where non-pigmented, suggesting that the pigment prodigiosin is not produced by this organism in the absence of oxygen. Anaerobic plates were re-incubated aerobically at 25°C for 24 hours and colonies showed red pigmentation.

A further overnight culture of *S. marcescens* was prepared and a serial dilution was made in Maximum Recovery Diluent (MRD). 0.1mL aliquots of relevant dilutions were spread across the surface of duplicate 90mm TSA plates and duplicate 140mm TSA plates. Ten 90mm plates were incubated aerobically and ten 140mm plates were allowed to dry before being dislodged into a vac packer bag using a quadloop and sealed in a vacuum-packer (Multivac Gastrovac). Plates were incubated at 25°C for 48 hours.

After 48 hours incubation, aerobic plates were counted to establish inoculum level. Vac-packed bags showed non-pigmented growth due to the anaerobic conditions. The vacuum in the bags packed with 140mm inoculated plates was released by aseptically opening each pack using a scalpel and samples were re-incubated at 25°C and checked at intervals. After 24 hours, red pigmentation was observed in all inoculated vac-packer bags, corresponding to a minimum inoculation level of 3-4 CFU/plate, suggesting that the method was sensitive enough to detect low numbers of contaminating organisms.

Vacuum packer trial

Three hundred and four 140mm TSA plates were pre-poured. Two TSA plates were opened, placed in the chamber of the vac-packer (Multivac Gastrovac) and exposed to the vacuum packing process (see Figure 6. The vacuum packer internal parts were then inoculated with an overnight culture of *Serratia marcescens*. Areas of inoculation were decided following previous FSA research (Guidance on re-commissioning vacuum packers and weighing scales). 1mL of culture was applied to the suction pipe manifold and spread over the surfaces with a sterile paint brush (Figure 2). A further 1mL of culture

was spread on the inner surface of the suction pipe (Figure 3). The vacuum packer was then reconstructed and the chamber area cleaned with alcohol wipes (Azowipe, disinfectant wipes; Synergy Health plc) before starting. One hundred TSA discs were vacuum packed in 200 mm x 300 mm vacuum pouches (The Food Machinery Company Ltd).

Figure 2: Inoculation of the suction pipe manifold

Figure 3: Suction pipe

After 100 TSA discs were vacuum packed, the machine was again taken apart and two further areas were inoculated; the rear side of the air outlet cover plate (Figure 4) and the inner surfaces of each gas flushing nozzle (Figure 5).

The inside of the machine was cleaned with alcohol wipes before vacuum packing a further 200 TSA discs. As a final test, 2 further TSA plates were opened and placed in the vacuum packer and exposed to the vacuum packing process (with no bag – Figure 6). All plates and vacuum packed agar discs were incubated at 25°C for 48 hours. After 48 hours, each bag was opened aseptically using a scalpel

and re-incubated at 25°C. Samples were checked after 24 hours and 96 hours for the presence of red pigmentation. Growth from positive packs was streaked onto TSA for confirmation by API (Analytical Profile Index) biochemical testing.

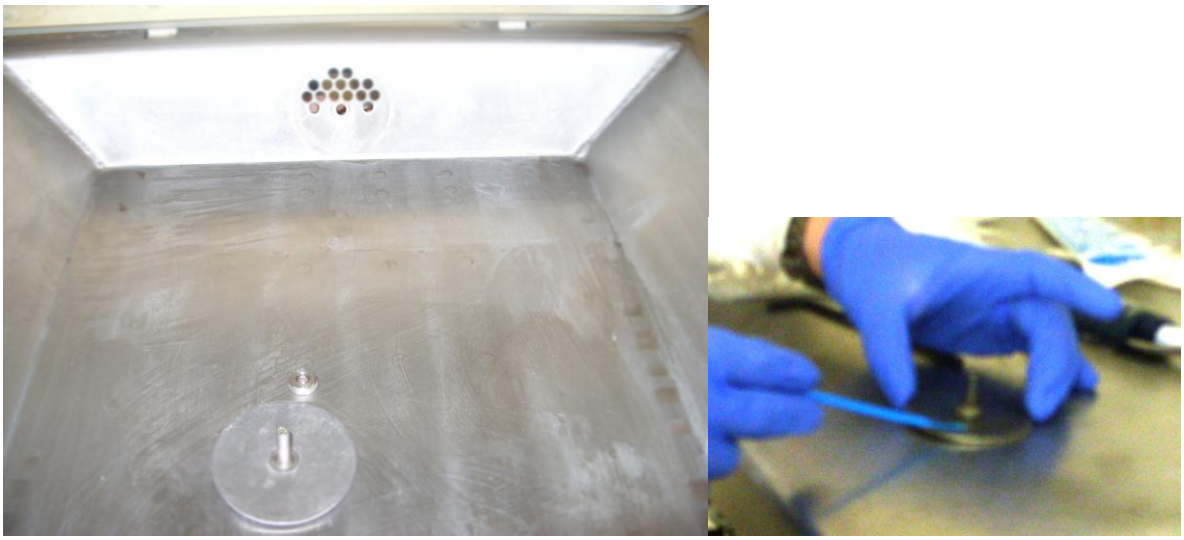


Figure 4: The rear side of the air outlet cover plate and inoculation method

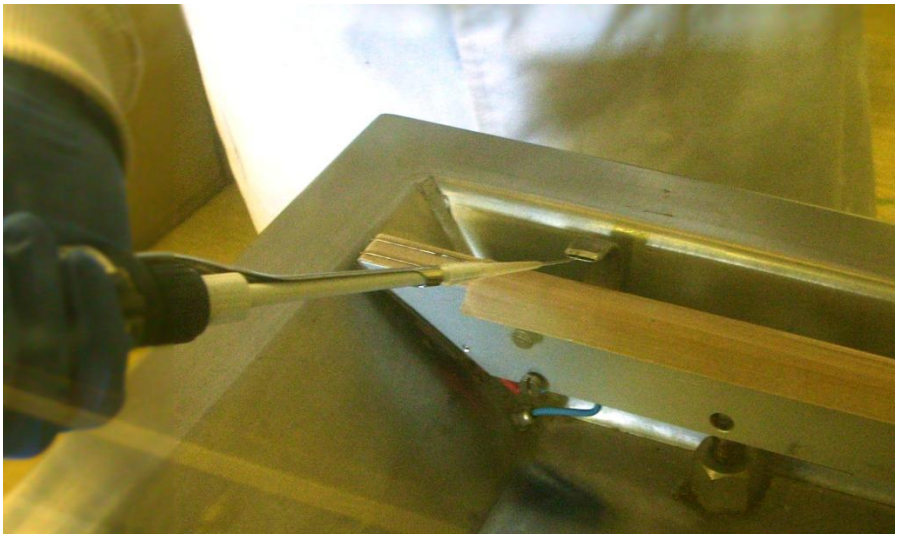


Figure 5: Inoculation of the inner surfaces of each gas flushing nozzle

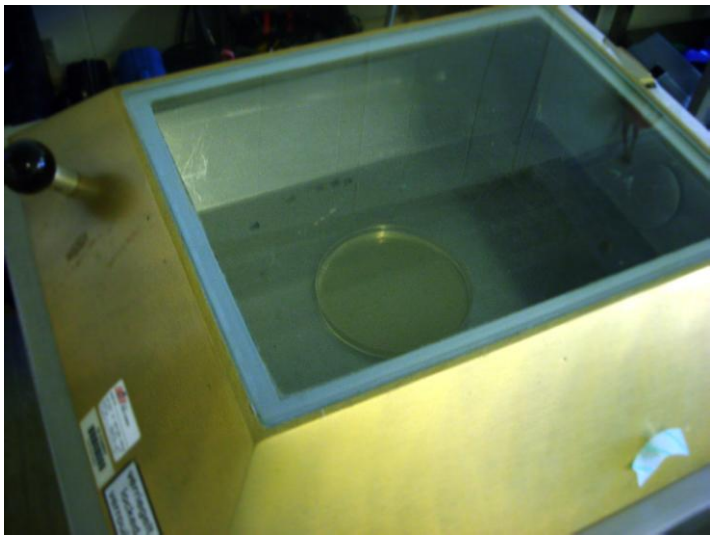


Figure 6: Control plate test carried out in duplicate before and after packing 300 agar filled bags.

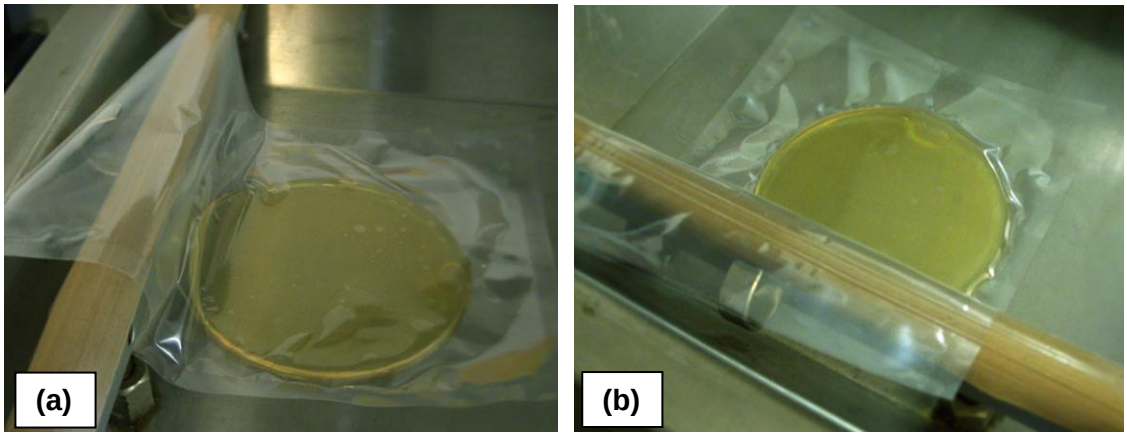


Figure 7: Agar filled bags; **(a)** before and **(b)** after vacuum packing

3 RESULTS

Test 1: External cross-contamination:

The spread of the UV simulant was visible under UV light conditions and in the images shows up as illuminated areas. Figures 8 and 9 respectively, show the contamination that was visible on the control panel and chamber area of both machines, after packing the above described products. Figure 11 shows the contamination on the gloves after vacuum packing 1 contaminated item followed by 4 consecutive items and then; after changing gloves and continuing to pack 5 further items. These results show that contamination that is passed onto a surface can spread on to clean hands/gloves from a previous contamination event.

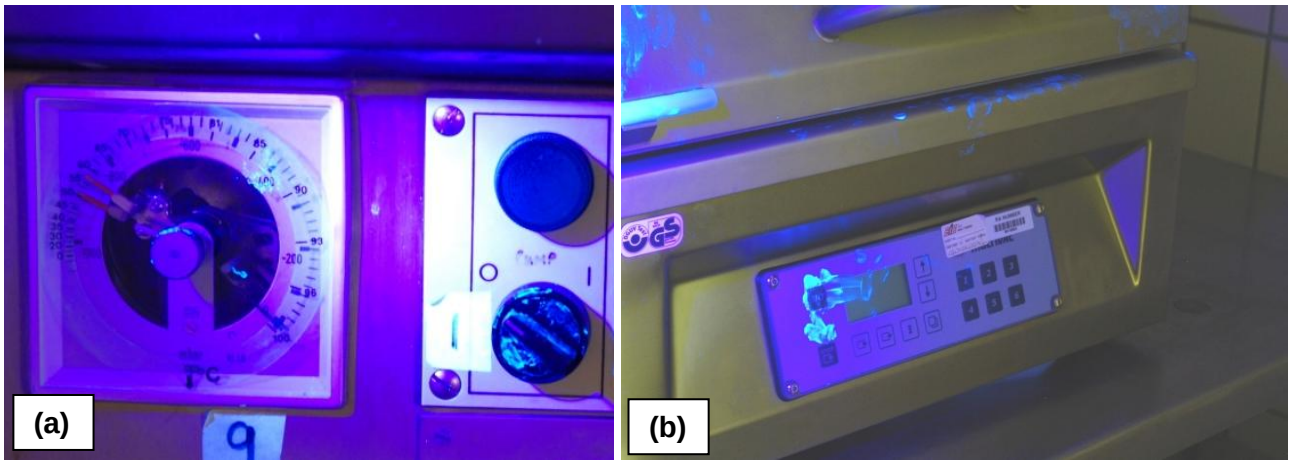


Figure 8: Control panel contamination after vacuum packing 1 contaminated item followed by 9 consecutive items; **(a)** Multivac Gastrovac; **(b)** Multivac C200.. .

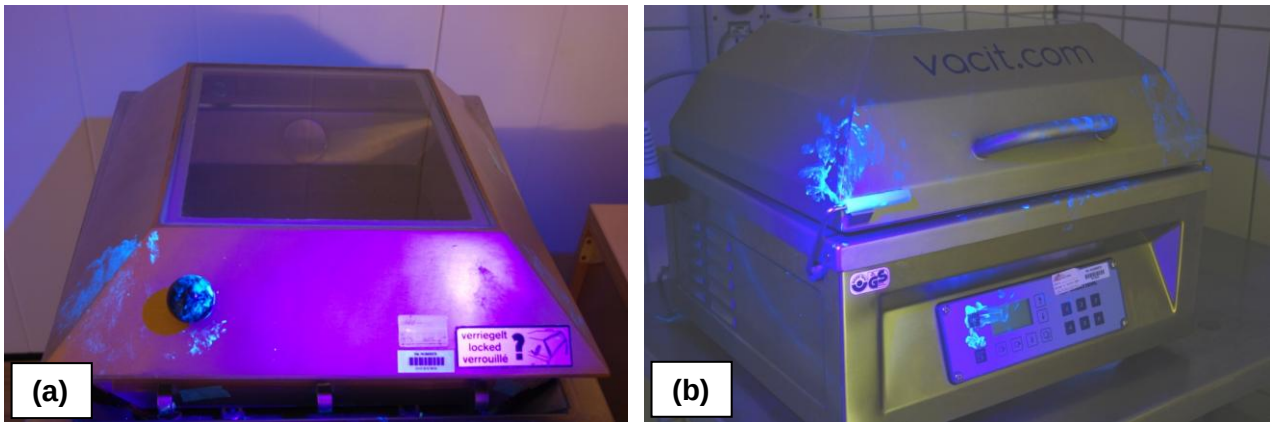


Figure 9: External contamination after vacuum packing 1 contaminated item followed by 9 consecutive items; **(a)** Multivac Gastrovac; **(b)** Multivac C200.

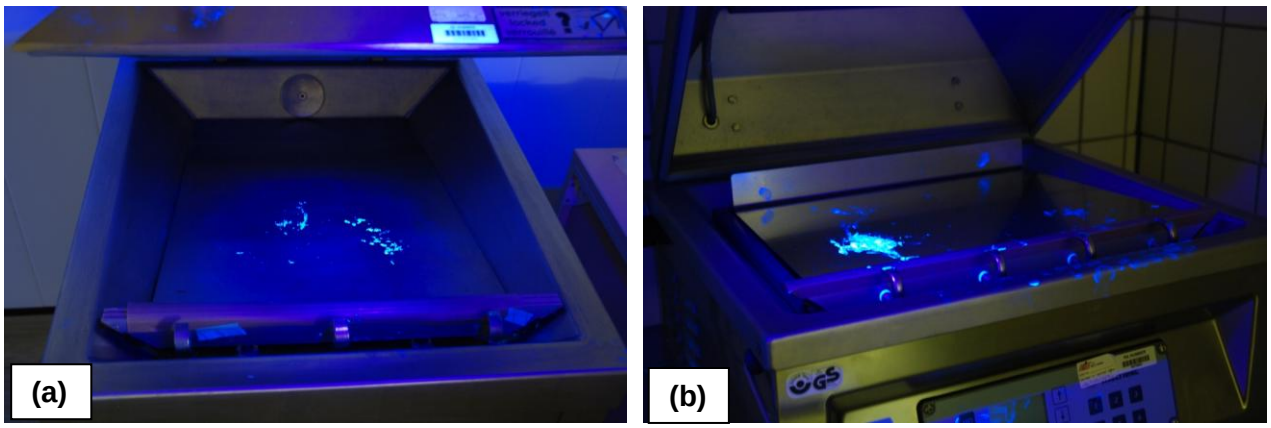


Figure 10: Internal chamber contamination after vacuum packing 1 contaminated item followed by 9 consecutive items; **(a)** Multivac Gastrovac; **(b)** Multivac C200.

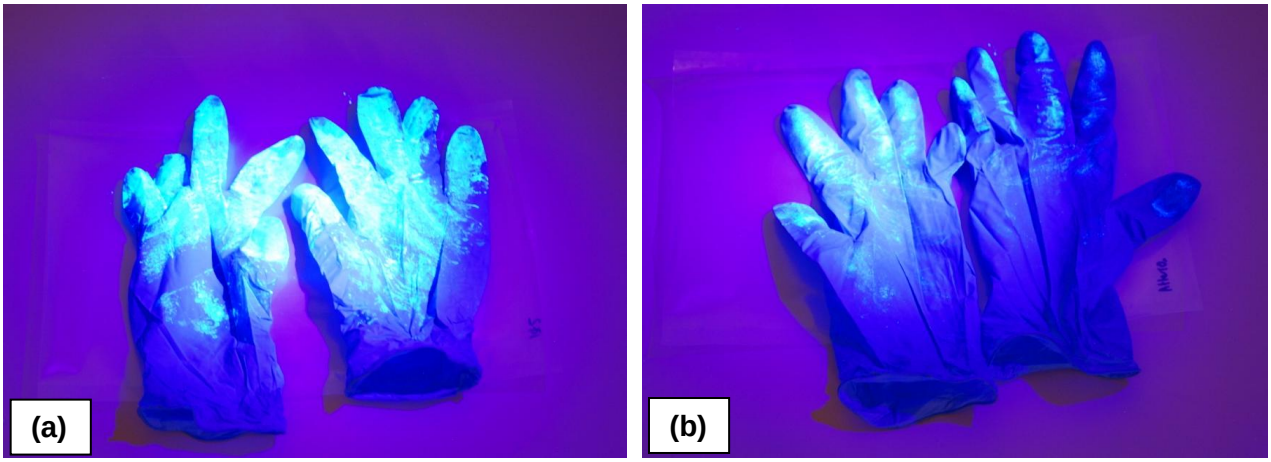
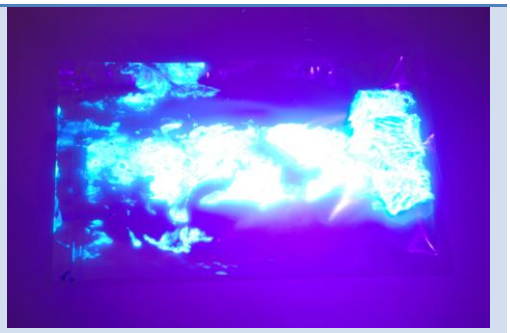


Figure 11: (a) Glove contamination after vacuum packing 1 contaminated item followed by 4 consecutive items and then; **(b)** after changing gloves and continuing to pack 5 further items.

Table 1 details the results and comments on the contamination spread from 1 initial heavily contaminated piece of meat (sample number 1), followed by proceeding packets of clean product (sample numbers 2-5). The operator changed gloves and continued to pack items (sample numbers 6-10). Sample numbers 1-5 appeared heavily contaminated with the UV simulant. When the operator changed their gloves the contamination was visibly lower, however still present. The operator's replaced gloves (Figure 10 b) had most likely been re-contaminated via contact with the exterior surfaces and the control panel of the vacuum packers (Figures 7 and 8). This contamination was then evident on the subsequent packed product and bags (Table 1; sample 6-10). The spread of the UV simulants represents the contamination that may occur after vacuum packing a contaminated piece of raw meat. The further samples packed (Table 1; samples 2-10) represent the cross contamination that can occur when the machine has not been thoroughly cleaned between use. Figure 11 (b) represents the contamination that can spread onto operators gloves/hands when using a contaminated machine. The results indicate that if an item, heavily soiled with microorganisms, was packed and a cleaning step was not suitably carried out afterwards, this has the potential to spread the contamination onto subsequent items packed in the machine.

Table 1: Results of external cross contamination study; UV contamination spread from initial heavily contaminated piece of meat (sample number 1), followed by proceeding packets of product (2-5). The operator changed gloves and continued to pack items (6-10).

Sample numbers	Result	Comments	Image
1	**Positive**	Highly contaminated	
2	**Positive**	Highly contaminated	
3	**Positive**	Highly contaminated	
4	**Positive**	Highly contaminated	
5	**Positive**	Highly contaminated	

6	**Positive**	Lesser contamination; most noticeable on the bag surface	
7	**Positive**	Contamination visible on food product	
8	**Positive**	Contamination visible on food product	
9	**Positive**	Contamination not obvious on food product but visible on bag surface	
10	**Positive**	Contamination visible on food product	

The contamination, if somewhat small, on the subsequently packed products following the initial soiling event, represents the spread of *E. coli* O157. It is well known that even low levels of *E. coli* O157 contamination could result in serious public health risks if there were to be contamination of ready-to-eat food.

Test 2: Internal cross contamination

The *Serratia marcescens* was firstly inoculated onto the suction pipe manifold and suction pipe (Figure 2 and 3 respectively). This area is where air exits and enters the chamber during a vacuum packing event. The results in Table 2 indicate that 4 packed agar discs, out of the first 50 bags packed, were contaminated by the initial inoculation. These results indicate that if an item highly contaminated with *E.coli* O157 was packed in the machine, and this contamination reached the difficult to clean parts such as the exhaust manifold or exhaust tube, this has the potential to spread to subsequent products packed in the same machine. There appeared to be no further cross contamination after sample number 49. This may be due to the nature of the area in which the *Serratia marcescens* was inoculated, as the air entering and exiting the chamber may have desiccated the inoculation, either resulting in a loss of inoculum viability or permitting it from spreading further.

After 100 TSA discs were vacuum packed, the machine was again taken apart and two further areas were inoculated; the rear side of the air outlet cover plate and the inner surfaces of each gas flushing nozzle (Figure 3 and 4 respectively). Inoculation of these parts did not seem to cause further cross contamination to the agar discs in the succeeding 200 packs.

Table 2: Results of vacuum packer cross contamination study.

Sample numbers	Result	API Confirmation top match
Start control plate 1	Negative	-
Start control plate 2	Negative	-
1-3	Negative	-
4	**Positive**	*Serratia marcescens*
5-14	Negative	-
15	**Positive**	*Serratia marcescens*
16	Negative	-
17	**Positive**	*Serratia marcescens*
18-48	Negative	-
49	**Positive**	*Serratia marcescens*
50-300	Negative	-
End control plate 1	**Positive**	*Serratia marcescens*
End control plate 2	Negative	-

The final control agar plate placed in the chamber did however show a positive result (Table 2; End control plate 1). This suggests that the contamination was still viable in the chamber although not reaching the inside of the packages.

4 DISCUSSION/CONCLUSION

The aim of this research was to assess the cross contamination that occurs both externally from pack to pack and internally from contaminated areas that are hard to reach/clean. Cross contamination to the outer and inner surfaces of vacuum packed products was demonstrated via two methods discussed earlier; Test 1: External cross contamination and Test 2: Internal cross-contamination.

In Test 1, UV simulants were used to represent a contamination event and the spread that can occur following packing of one heavily contaminated product. It was observed that the operator can spread contamination from product to gloves and then to contact surfaces such as lid, handle, control panel and outer and inner bags (Figure 8-10). Even when gloves are replaced with a clean pair, the contamination was still spread to further products packed (See Table 1). In Test 2, the machine was inoculated in different areas to assess the spread to internal products if the machine was contaminated in hard to reach/clean areas. Contamination was observed in 4 out of the first 50 bags packed. This signifies the risk that is posed when machines are used for dual purposes such as raw and ready to eat (RTE) products.

Test 2 was carried out aseptically, wherein the operator did not come into contact with the packaged agar discs. However, if the results of Test 1 are taken into account and the operator was also handling the product when the chamber is also contaminated (as shown in Table 2; End control plate 1) then this again increases the risk of cross-contamination to the internal surfaces and contents. Also, when the chamber shows contamination this is likely to be transferred onto the outer surface of the packaging and thus the operator's hands/gloves, which can then transfer onto the packaged item as shown in Test 1; Table 1.

The results of this research illustrate how bacteria, such as *E. coli* O157, that are invisible to the naked eye, can be easily spread to food without the operator's realisation. These bacteria could cause serious public health issues. If food businesses handle raw food, which could be contaminated with *E. coli* O157, in the same establishment as ready-to-eat food, there will be a greater risk.

Theoretically it would be possible to reuse a vacuum packer for packing RTE products after packing raw products, but all internal and external vacuum packer surfaces would need to receive thorough cleaning and disinfection process. This would require extensive dismantling of the machine, thorough cleaning and disinfection of all parts and rebuilding and testing the operation of the machine. This is most unlikely, however, to be undertaken in practice.

Food businesses must practise good food hygiene at all times to make sure the food they produce is safe. Following the results of this research and the impracticability of total machine dismantling and

decontamination, it would be advisable to avoid using a vacuum packer for both raw and RTE food items. In addition, if a vacuum packer has been contaminated, it must undergo a stringent re-commissioning cleaning to ensure it is then safe for use with RTE products.

Appendix VII

Vacuum packer internal cross contamination x 2

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Report on:

Repeat of Deliverable 6 with 2 new Vacuum packer machines

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1 BACKGROUND AND AIM

The aim of this research was to assess the cross contamination risks that occur internally from perceived difficult to clean, contaminated areas of two additional vacuum packers.

2 METHODS

The research into the internal cross-contamination of the items packed in a vacuum packer involved inoculating the vacuum packing machine with *Serratia marcescens.* This has similar properties to *E. coli* so was suitable for the required test, but in addition, is readily visible via its red colony colouration on growth media. Two new machines were used for the test (shown in Figure 1).

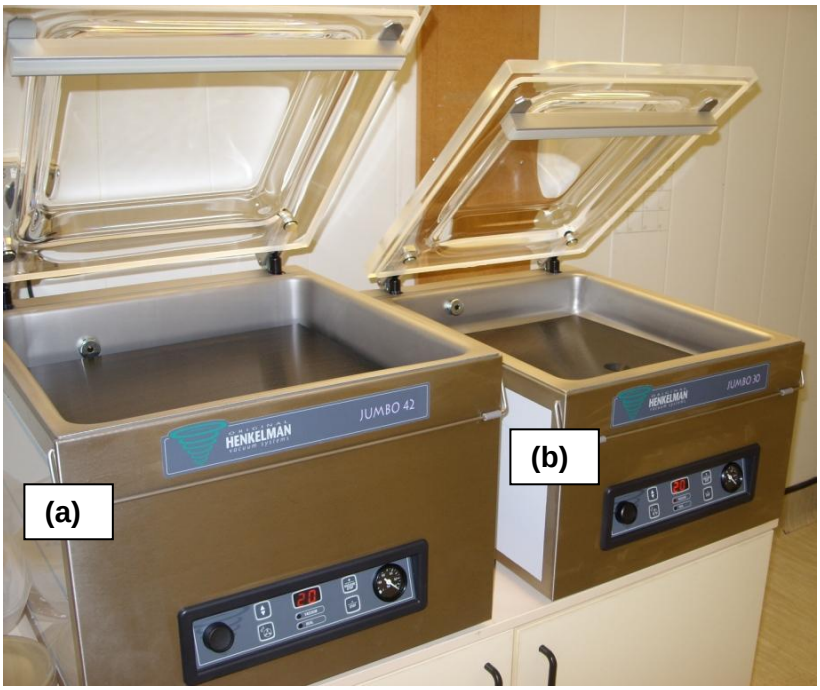

Figure 1: Machines used in the experiment; **(a)** Henkelmann Jumbo 42 **(b)** Henkelmann Jumbo 30

Six hundred and twenty three 140mm TSA plates were pre-poured in a HEPA-filtered laminar flow cabinet.

Controls

For each vacuum packer, before inoculation, three TSA plates were opened, placed in the chamber and exposed to the vacuum packing process (Referred to as pre-inoculation controls 1-3 plates). Three further TSA discs were placed in vacuum packer bags and packed in each vacuum packer (Referred to as pre-inoculation controls 1-3 discs).

Inoculation

Each vacuum packer was inoculated with an overnight 25°C culture of *Serratia marcescens* (7.98 log CFU/g). For each machine, the following sites were inoculated:

- Exhaust manifold and suction pipe (0.5mL spread with sterile inoculation loop) (Figure 2(a)).

- Dead space below sealing bar (2 x 50µL using automatic pipette) (Figure 2(b)).
- Air outlet cover plate (0.25mL via pipette) (Figure 2 (d))

Both vacuum packers were then reconstructed and the chamber area cleaned with alcohol wipes (Azowipe, disinfectant wipes; Synergy Health plc) before starting. For each inoculated vac-packer, 300 TSA discs were aseptically dislodged from petri dishes, transferred to vacuum packer bags and vacuum packed[1]. Three further TSA plates were then opened and placed in the vacuum packer chamber whilst a vacuum was applied after packaging of all TSA discs was complete. This was to determine whether any contamination remained in the air in the vacuum chamber (Referred to as post-packing controls 1-3 plates). All plates and vacuum packed agar discs were incubated at 25°C for 48 hours. Each bag was then opened aseptically using a scalpel and re-incubated at 25°C. Samples were checked after 24 hours and 96 hours for the presence of red pigmentation. Growth from positive packs was streaked onto TSA for confirmation by API biochemical testing.

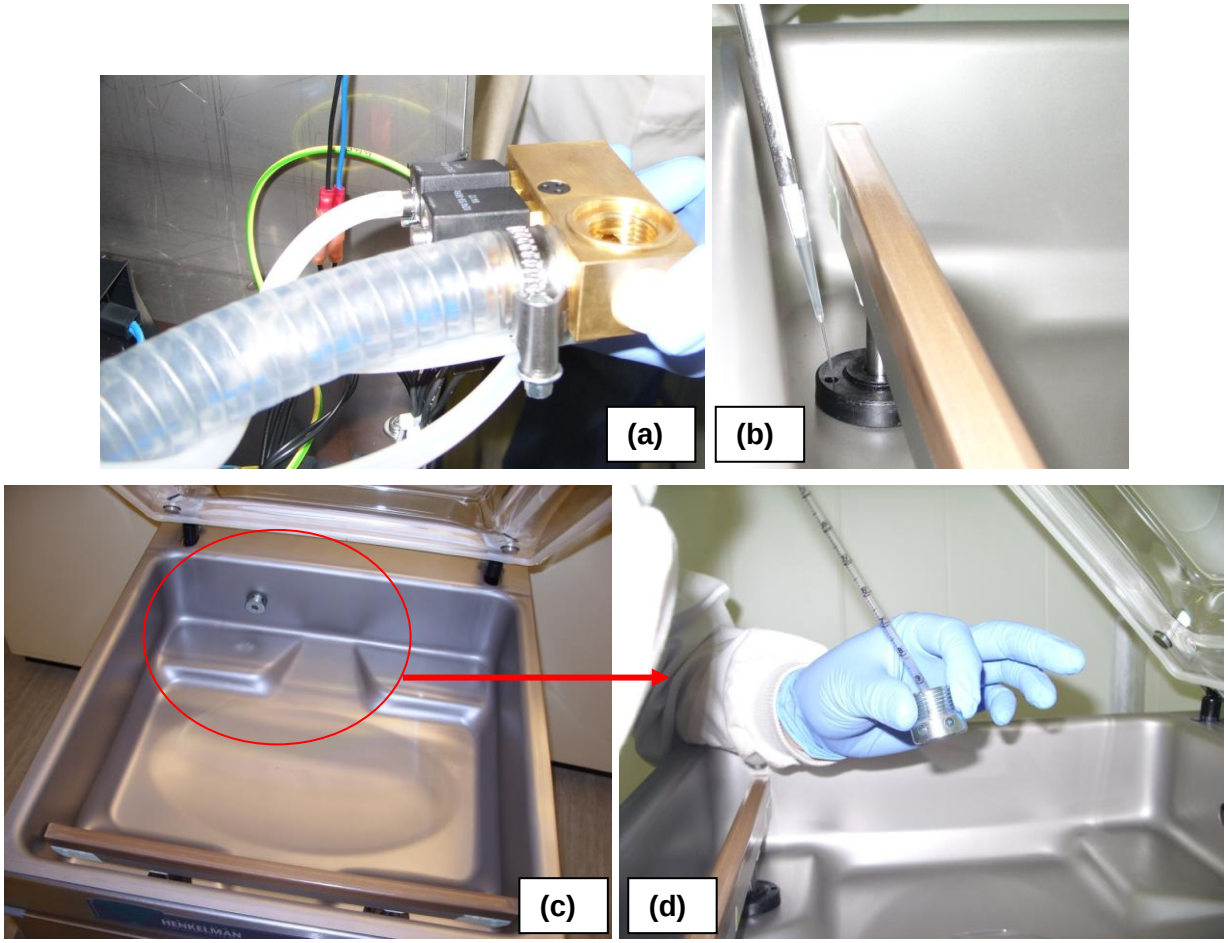

Figure 2: Areas of inoculation;**(a)** suction pipe and suction pipe manifold **(b)** dead space under sealing bar **(c)** Vacuum chamber image indicating the location of the air outlet cover plate **(d)** inoculation of the air outlet cover plate.

[1] **NB.** One TSA disc was inadvertently placed into the vacuum chamber backwards such that the bag was sealed at the wrong end. This sample was incubated with the trial samples to determine the potential effect on cross contamination of such an operator mistake when vacuum-packaging meats.

3 RESULTS

The results in Table 1 indicate that no cross-contamination occurred in agar discs packed in either machines (Table 1: samples 1-300). The only cross-contamination observed was on an agar disc within a bag that was not sealed correctly (Table 1: Sample placed backwards in vac-packer). This indicates that heavy contamination of the machine may be sufficient to contaminate the inside of a bag that has been mishandled. It also indicates that, under such conditions of heavy soiling, the air in the chamber may become contaminated allowing potential cross contamination of products.

Table 1: Results of vacuum packer cross contamination study.

Vacuum packer	Sample numbers	Result	API Confirmation top match
-	TSA sterility 1-3	-	-
J30	Pre-inoculation controls 1-3 (plates)	-	-
	Pre-inoculation controls 1-3 (discs)	-	-
	Samples 1-300	-	-
	Post-packaging controls 1-3	-	-
	Sample placed backwards in vac-packer	+	*Serratia marcescens* Very good ID (99.8%)
J42	Pre-inoculation controls 1-3 (plates)	-	-
	Pre-inoculation controls 1-3 (discs)	-	-
	Samples 1-300	-	-
	Post-packaging controls 1-3	-	-

4 DISCUSSION/CONCLUSION

The test revealed that there was no contamination to the sealed products. There was, however, evidence to show that the chamber itself was contaminated. This may increase the risk of cross-contamination of the product.

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