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SUMMARY OVERVIEW

Clinical invasive infection by *Listeria monocytogenes* is called listeriosis and it is rare in healthy humans. However, there are subsections of the population that are vulnerable to invasive infection, including the immuno-compromised, the elderly and pregnant women. Overall, there was 12.7% mortality across 26 EU Member States (MS) and Norway for *L. monocytogenes* infections for the period January 2010 to January 2012. The Food Standards Agency (FSA) regards targeting *Listeria* food safety advice at those consumers most at risk as important in tackling this problem. However, finding ways to reduce exposure of vulnerable consumers to *Listeria* in ready-to-eat foods remains important. In terms of the number of microbiological incidents reported to the Agency each year, those involving *Listeria* are the second most frequent after incidents involving *Salmonella.* Approximately 30% of incidents involving *Listeria* reported to the FSA between 2005 and 2011 involved ready-to-eat sliced meats. Whilst few of these incidents were associated with known cases of listeriosis, this food type was linked to two UK listeriosis outbreaks in 2009 and 2010. The frequency of incidents indicated that more work was required to understand the sampling and testing regimes used by manufacturers, particularly Small to Medium-sized Enterprises (SMEs) of ready-to-eat foods and their approaches to controlling *Listeria* in the food supply chain.

Accordingly, a critical review of literature relating to *L. monocytogenes* contamination of cooked sliced meats (CSM) was undertaken from a variety of academic and other bibliographical sources. The review literature was identified using a systematic approach, with an assessment of publication quality adapted from the Oxford System. The review purpose was to assess the likelihood of CSM contamination by *L. monocytogenes*. Additionally, key production and processing practices that could influence the *L. monocytogenes* prevalence and numbers associated with ready-to-eat CSM through the processing and retailing chain were identified. There was some evidence of a higher prevalence of *L. monocytogenes* in meat processing environments during the summer months as a consequence of increased production.

Based on outbreak data and a review of the literature, including the conclusions of surveillance studies, it is apparent that there are two main failings required for an outbreak of listeriosis. The first is contamination of the final product with *L. monocytogenes*. When such contamination occurs, only very low numbers of *L. monocytogenes* are typically transferred to the product. The second failing is

inadequate refrigeration, between production and consumption, which allows the multiplication of *L. monocytogenes* from initially low numbers, to levels sufficient to cause infection. Thus, the contamination of CSM with populations of *L. monocytogenes* high enough to lead to human illness in vulnerable groups is an issue for which responsibility is shared. The stakeholders are food business operators (FBOs) who can produce contaminated product, and the retailers, wholesalers, distributers, caterers and consumers who can inadequately refrigerate contaminated products.

L. monocytogenes can be introduced into CSM processing environments by several routes, including being present on contaminated ingredients such as raw meat and packing materials. *L. monocytogenes* is a ubiquitous environmental bacterium and CSM processing environments are therefore under continuous risk of colonisation by *L. monocytogenes*. The *L. monocytogenes* strains found on final products tend to be different to those isolated from raw material. The main, but not sole, contamination mechanism is by transfer of *L. monocytogenes* strains from raw materials into niches in the plant environment and subsequent transfer from these niches into final products. Effective cleaning (the removal of soil) followed by sanitising (the killing of microorganisms), or the application of heat can remove *L. monocytogenes* from CSM processing environments.

One of the main conclusions from the literature review was that effective thermal processing, i.e. cooking at or above 72^{o}C, was the main critical control point (CCP) for the elimination of *L. monocytogenes* in CSM. If the thermal process phase was successfully completed, earlier process stages prior to cooking were largely unimportant in terms of final product risk for *Listeria*.

Since thermal processing is normally the only CCP for *L. monocytogenes*, post-cooking contamination of effectively-cooked CSM was identified as a major hazard. Post-cook contamination can occur if slicing and packing machines are imperfectly cleaned, or become re-contaminated with *L. monocytogenes* after effective cleaning. After slicing contaminated meat, slicer blades can transfer *L. monocytogenes* sporadically to as many as 150 slices of previously uncontaminated meat, with low numbers of cells being transferred to each slice. However, meat and fat residues on these and other food contact surfaces can interfere with the antimicrobial actions of a number of common sanitising chemicals. Therefore, the efficient cleaning of slicing and packing equipment prior to sanitizing is a key requirement for the complete removal of *L. monocytogenes* from food contact surfaces.

There was evidence that contaminated workers hands could spread *L. monocytogenes* around processing environments. Machinery controls and door handles were of particular concern.

Although *L. monocytogenes* is psychrotrophic (i.e. can survive and grow at refrigeration temperatures), effective chilling impedes the multiplication of *L. monocytogenes* on packed finished product. Recent surveillance by the FSA (FS241042) identified that there was an issue of inadequate refrigeration in some open-faced refrigerated display cabinets in some SME retailers. This may have been associated with the replacement of specialist fluorescent tubes (specifically designed to radiate very little heat into refrigerated display cabinets), with standard fluorescent tubes, which operated at higher temperatures (30^{o}C) and consequently radiated larger amounts of heat into refrigerated display units. Under such circumstances, the temperatures achieved inside some SME refrigerated display cabinets were sufficiently high to allow significantly faster multiplication of *L. monocytogenes*. In contrast, a previous UK survey of retail CSM (FSA project B18024) determined that the temperatures of product on display in major retail outlets were satisfactory.

There is a lack of recent information on the typical temperatures inside domestic refrigerators in the UK. Historic information showed a significant number of UK domestic fridges did not maintain food at a low enough temperatures to prevent significant *L. monocytogenes* multiplication. Some strains of *L. monocytogenes* can grow at temperatures as low as -1.5°C. Predictive modelling (e.g. ComBase) showed that *L. monocytogenes* can grow twice as fast at 8°C as at 5°C under ideal pH and water activity (a_w) conditions. Thus, inadequate domestic refrigerated storage may allow *L. monocytogenes* proliferation on contaminated CSM, from low levels to numbers capable of causing illness. Major CSM manufacturers, and major retailers, have already recognised the potential issues involved with consumer failures to ensure adequate refrigeration. Shelf life determination protocols devised by such companies use simulated domestic storage temperatures of 7-8^{o}C. In addition, some protocols take account that chilled foods may be subject to higher temperatures after purchase, for example, up to 22°C, during transportation to the home.

The possibility of additional CCPs in CSM production has also been explored. Post manufacture interventions such as high intensity pulsed light or high-pressure processing (HPP) treatments can reduce the *L. monocytogenes* populations on CSM. However, both of these treatments may also cause adverse organoleptic changes in CSM. High intensity pulsed light treatments, which are sufficient to significantly reduce *L. monocytogenes* numbers may cause lipid oxidation of the meat, resulting in off

odours indistinguishable from those associated with spoiled product. Less information is available on organoleptic damage resulting from HPP treatments, as most HPP studies have focused on microbiological aspects. Given the wide range of CSM products, any consequences of HPP would be required to be evaluated on a product-by-product basis.

Other studies of the survival and growth of *L. monocytogenes* on food surfaces concluded that product textures or surface types affect *L. monocytogenes* growth rates and/or the effectiveness of anti-listerial interventions. These product-specific differences may relate to surface texture, with rougher surfaces and larger surface areas, protecting *L. monocytogenes* from interventions such as organic acidic washes. Although some beneficial chemical interventions were identified, there were legal barriers to application on CSM sold within the European Union.

As part of this study, a series of visits were undertaken to commercial CSM manufacturers, large retailer store delicatessen counters and SME retailers that sliced cooked meats in store across the UK. These visits aimed to gather information on any practices that might influence the risks of *Listeria* contamination of CSM during manufacture and sale from the viewpoint of manufacturers and retailers. These visits also sought to identify any perceived barriers to the delivery of procedures required for the effective management of *L. monocytogenes* on CSM.

Many of the smaller manufacturers visited were not fully aware of the requirements of the Microbiological Criteria for Foodstuffs Regulation (EC) 2073/2005 regarding shelf life analysis or environmental testing in relation to *L. monocytogenes*. Once such requirements were explained, manufacturers became concerned about the costs of laboratory testing associated with meeting their obligations. Smaller CSM manufacturers based their product shelf lives on previous estimates of the time taken for their products to spoil, advice from environmental health officers (EHOs), and/or shelf life estimates for similar products offered for sale by other (larger) retailers.

Larger CSM manufacturers and retailers tended to employ specialist technical staff to manage microbiological risks (including risks from *L. monocytogenes*). Consequently, larger CSM manufacturers had defined protocols and routine testing regimes in place for continually monitoring the *L. monocytogenes* colonisation status of high-care processing areas and products. Larger CSM

manufacturers had developed their own shelf life protocols for *L. monocytogenes*, or applied shelf life protocols specified by major retailers. Most of the CSM manufacturers visited (large and small) supported the idea of an FSA and BRC standardised shelf life protocol with specific storage temperatures for *L. monocytogenes* and CSM to complement the existing general guidance (CFA, 2010). For larger manufacturers, such a standardised protocol would reduce the amount of testing required in meeting the current differing requirements of their retailer customers. A standardised, easy to follow, test protocol would also address the identified gap in technical knowledge among some SME CSM manufacturers. From a national retailer viewpoint, a standardised protocol was not appropriate. Several retailers stated their protocols were tailored to their unique distribution and retail chains. For example, some retailers have different refrigerated displays set to different temperatures for different products. Furthermore, retailer technologists have the authority to modify a retailer's standard protocol on a product-specific basis.

Wide ranges of processing practices were observed across the business sizes visited. Smaller producers of CSM processors often lacked sufficient space to separate physically the different processing stages, although the literature suggests that such compartmentalisation is beneficial. Recognising that effective cooking was a CCP, larger businesses had adopted a range of practices aimed at preventing contamination of cooked product. These included double bagging of final product packaging with removal, and appropriate disposal, of the outer bag on entry to the post-thermal processed (i.e. high-care) areas of the plant. The most effective measure to prevent post-cooking contamination with *L. monocytogenes* was restriction of the amounts of water used in cleaning high-risk areas during routine processing. The largest processors removed equipment from the high-risk areas for cleaning and sanitising. In addition, they cleaned and sanitised plant environments on a weekly basis. Across the range of processors, final product chillers were rarely empty; limiting the type of cleaning that could be undertaken in these areas.

Delicatessen counters in larger retailers had standard operating procedures for slicer cleaning and sanitation, including blade removal, and the use of appropriate personal protective equipment (PPE). A number of national retailers using multiple slicers in-store restricted the range of products which could be sliced on each slicer, as a general strategy to reduce cross-contamination. In relation to delicatessen CSM shelf life, national retailers either provided no advice on potential shelf life after slicing, or suggested very short shelf lives of, for example, two or three days (usually including the day of sale). Expiry dates for unsliced meats were as determined by the original meat manufacturer, or set by

retailers as a specific number of days (e.g. four days) after opening of individual meat packages. In larger retail stores, daily checks were undertaken to ensure the unsliced meats on retail display were still within their expiry dates. Several retailers displayed unsliced meat expiry dates on the retail-facing side of the cards used to inform customers of product names and prices.

Most of the SME in-store slicing retailers did not test their environment for *L. monocytogenes*, relying on tests undertaken by local authority (LA) environmental health officers (EHOs). Small retailers tended to adopt the product shelf lives provided by the original CSM manufacturer, although some small retailers were observed to suspend the original manufacturer's shelf life for any period that the meat was stored frozen. Some SMEs were using slicing equipment which appeared to be very old (several decades), which were not designed to be easily disassembled to achieve effective cleaning and sanitation. The cleaning instructions supplied with some of these older items, focused principally on visible cleanliness and advised the use of detergent only. In the absence of an effective sanitising step, viable bacteria could remain on washed equipment, especially if contained within biofilms. A range of cleaning and sanitation practices was observed in the treatment of in-store SME slicers. Probably the least effective of these, observed in a small number of SMEs, involved washing older slicers with hot soapy water (i.e. domestic dish soap), in accordance with the slicer manufacturer's instructions. Some of the SME retailers visited displayed a limited understanding of the difference between cleaning and sanitising chemicals and the importance of the adequate sanitiser contact times. In contrast, all of the SME stores in one region (SW England) reported that EHOs strongly enforced regimes for the cleaning and sanitising of slicing equipment, and provided SMEs with an approved list of cleaning and sanitising chemicals. The approved list however was drawn up to help with compliance to the FSA guidance on *E. coli* O157 cross contamination rather than *L. monocytogenes*. The approved list is supplied by EHOs to the businesses they enforce to ensure cleaner/disinfectant compliance with British Standards BS EN 1276 or BS EN 13 697. A number of SME employees reported that slicer blades were sharp and blade removal for cleaning could be hazardous, which may have been a disincentive for effective cleaning.

L. monocytogenes can evolve increased resistance to sanitising chemicals of the type used in CSM processing and retailing environments after repeated sub-lethal exposure. Increased resistance to sanitising chemicals can occur because of the establishment of biofilm in the absence of adequate cleaning and sanitation. Biofilms, once formed, can reduce the efficiency of sanitising chemicals. However, the increased resistance reported by the literature is not high enough to interfere with effective routine sanitation. Excessive dilution of sanitising chemicals is a potential cause of repeated

sub-lethal exposure to chemicals. This study found no evidence that sanitising chemicals were diluted to excess in any of the sectors visited.

The opinions of more than 90 EHOs, responsible for enforcement in CSM processing plants and SME stores that slice cooked meats, were obtained using a structured questionnaire. The questionnaire aimed to identify any issues of concern relating to *L. monocytogenes*, and identify what EHOs thought FBOs needed to help them reduce the risks from *L. monocytogenes* on CSM. Information was also collected from EHOs on the additional training and/or other resources they needed to support FBOs and enforce relevant consumer protection laws. There were significantly more responses to the EHO-focussed questions compared with the FBO-focussed questions. However, there was good agreement between the six most important priorities for both viewpoints. There was an overall perceived need for increased support and guidance. The top six priorities are listed as Table 1.

Table 1 Six highest priorities identified by EHOs, for themselves and for FBOs relating to *L. monocytogenes* and CSM. The mean appraisal score was awarded from a range from 0-5.

EHO Priority	EHOs' perceived needs for EHOs (mean appraisal score)	FBO Priority	EHOs' perceived needs for FBOs (mean appraisal score)
1	Shelf life determination (4.41)	1	Shelf life determination (4.65)
2	Plant cleaning and sanitisation (4.05)	2	Plant cleaning and sanitation (4.50)
3	Monitoring salt/other parameters (4.04)	3	Monitoring salt/other parameters (4.37)
4	Control of key hazard organisms (4.02)	4 equal	Control of key hazard organisms (4.31)
5	Post-process handling (4.01)	4 equal	Plant high risk (4.31)
6	Changing product formulation (3.82)	6	Post-process handling (4.24)

All of the top six topics for each group (EHOs and FBOs) are the subject of longstanding legal requirements, covered by regional regulations installed for compliance with EC 852/2004 and/or Microbiological Criteria for Foodstuffs Regulation 2073/2005. These findings suggest persistent shortcomings in the activities of some FBO.

Guidance on shelf life determination was identified as the clear priority for both groups (EHOs and FBOs). Guidance for FBOs was a higher priority (i.e. scored higher) than guidance for EHOs. Some EHO

comments indicated that either they were not aware of the existing BRC/CFA (CFA, 2010) shelf life guidance and/or that they felt that existing guidance should be revised to make some key points clearer. Other EHOs felt that current shelf life guidance was adequate.

Additional plant cleaning and sanitisation guidance was ranked as the second most important priority for both groups (EHOs, and FBOs). We note that the FSA has previously funded work to investigate gaps in these areas and has included advice on cleaning and sanitisation in its *E. coli* cross-contamination guidance. However, it would appear that further efforts are required to fill an important information and training knowledge gap amongst some EHOs and FBOs.

Guidance on monitoring salt and other product parameters was ranked as the third most important priority by both groups (EHOs, and FBOs) and again scoring more highly among FBOs. Of particular concern for some EHOs were physicochemical parameters such as pH and a_w and how these influenced *L. monocytogenes* populations.

Control of key hazardous organisms was ranked fourth for each group, with manufacturer-focused information on plant high-risk areas scoring equal fourth for FBOs. Hazard organism control includes consideration of the parameters required for the growth and survival of *L. monocytogenes*, and could potentially include the use of predictive modelling (e.g. www.combase.cc). An important finding of this study was that physicochemical properties (e.g. pH and water activity) and predictive modelling were not routinely used to inform shelf life analysis in any of the SME FBOs that were visited.

An open-ended question seeking concerns about environmental and food sampling from an EHO perspective gained the highest number of responses, covering a broad number of topics and indicating that sampling was viewed as a key concern.

Many EHOs referred unprompted to Regulation 2073/2005 as an area warranting support. In particular, it was noted that there were concerns with the section of the regulation concerned with how to determine likely numbers of *L. monocytogenes* at end of shelf life.

Basic training for food handlers was ranked seventh overall by EHOs, and was the priority personnel issue for FBO employees. Such basic training of FBO employees is a key element for the safe production of food and as such, the perception of some EHOs that there were issues in this area was

unexpected. In combination, these findings highlight key gaps in the practical implementation of 852/2004 almost ten years after its introduction.

The relatively high mean score given to raw materials acceptance criteria (3.46) indicates that EHOs feel the need for support in this area. Raw materials were determined to be a key route of contamination into processing plants and thus were an important strategic consideration in the prevention of plant environmental contamination and the establishment of persistent *L. monocytogenes*.

EHOs were asked if they had any preference for the format of additional information they felt they required. Training courses or workshops were the most favoured options, closely followed by written online guidance (Table 2), with no significant differences between the response scores for these two options (t-test; P=0.78).

Table 2 Ranked responses showing the preferred format for the identified required guidance

Format of guidance	Mean score	Number of responses	Ranking
Guidance training course or workshop	4.20	90	1
Guidance web written	4.15	89	2
Guidance web interactive	3.78	89	3
Guidance booklet pamphlet	3.42	85	4

The FSA will sponsor sixteen EHO and industry training workshops scheduled throughout the UK during June and July 2014. Although these are targeted at *L. monocytogenes* and the SME smoked fish sector, there was overlap between the current study in areas such as effective cleaning and shelf life determination of RTE foods. The FSA workshops will generalise in these areas so that they will go some way towards helping to address the identified EHO information requirements.

STUDY RECOMMENDATIONS

Based on the experiences gained by undertaking this study, the following recommendations were prioritised in order of importance to assist the producers and retailers of RTE meats, and the local authority representatives responsible for overseeing these businesses:

- Local authorities should more assertively ensure that processors implement appropriate sampling and testing regimes for *L. monocytogenes*, including environmental and shelf life testing, to make sure that food business operators (FBOs) are able to demonstrate compliance with Regulation 2073/2005 and that sampling regimes take a risk-based approach which reflects the size and nature of businesses.
- Some businesses were using slicing equipment that was never designed to be straightforwardly disassembled to achieve effective cleaning and sanitation. Such businesses should be advised that older or poorly-designed equipment could represent a significant risk to consumers. The cleaning instructions supplied originally with some older slicers were sometimes inadequate. Some manufacturers recommended only washing in dish soap, without the use of a sanitiser. Consequently, there would be merit for EHOs to also inform SME retailers that the original manufacturer's cleaning instructions were no longer considered adequate.
- There may also be merit in assembling information describing simple and effective decontamination procedures (e.g. heating in a domestic oven at a defined temperature for a defined time) for heat-stable objects such as slicer blades that have never been routinely sanitised and thus may harbour *L. monocytogenes* biofilms.
- An easy to follow standardised shelf life protocol for CSM with regard to *L. monocytogenes* could be prepared to complement the general guidance already prepared by CFA and BRC. Alternatively, two sets of European Commission guidance documents for shelf life determination for 1.) laboratories and 2.) FBOs should be promoted. A number of EHOs and smaller processors were not aware of existing guidance on shelf life determination with regard to *L. monocytogenes*. Dissemination of a standard protocol would address an identified knowledge gap in SMEs that lacked the technical expertise required to devise a shelf life protocol. Larger manufacturers generally supported a standard protocol as a way to reduce the amount of testing required to comply with several different retailer supply criteria. There were barriers to the widespread **exclusive** adoption of a standardised protocol. Large retailers have distribution chains that use slightly different temperatures and therefore may require customised supply protocols to reflect these different distribution chain temperatures.

- Raw materials constituted a major route of *L. monocytogenes* entry into CSM plants. Therefore, guidance describing good practices for the acceptance of raw materials (e.g. inspection of fat on red meat to determine whether yellowing, indicative of temperature abuse, had occurred) should be compiled from available sources or awareness of existing guidance should be raised. Such targeted advice could be supplied to EHOs (or other interested parties), for further distribution to smaller processors.

- The ISO testing protocol for *L. monocytogenes* stipulated by EC 2073/2005 constituted a significant concern in terms of cost to SME processors. Therefore, a cheaper methodology could be validated for equivalence to the ISO standard. The validation would allow a less expensive test to be used legally, which would help address the testing cost concerns identified from the SME processors. Furthermore, an alternative test would also allow larger manufacturers to undertake more comprehensive surveillance of processing environments for the current costs.

- Encouragement should be made of the use of predictive microbiological modelling as an approach for shelf life determination. EHOs should be routinely trained in the interpretation of model outputs to help them determine whether predictive modelling carried out by food business operators (or on the FBO's behalf by relevant experts) provides appropriate evidence of whether *L. monocytogenes* may exceed the 100 cfu/g limit during a product's shelf life.. A model-based approach is an exceptionally cost-effective way of predicting likely product safety, determining an appropriate shelf life. In addition, for products that support the growth of *L. monocytogenes*, this type of data would support more widespread compliance with EC 2073/2005. However, a model-based approach would require that EHOs and FBOs received training and guidance on measuring pH, a_w and storage temperature of the products of SMEs.

- EHO in-service training should be reviewed to ensure that it meets the demands of the above recommendations. Further, there was also a need to ensure that environmental health degree courses and CPD activities adequately covered the information shortfalls identified in this study. It was considered important that all EHOs were cognisant of *L. monocytogenes* control and the statutory requirements. Some of the EHOs interviewed indicated they may require additional training in order to provide them with the technical information they required to support and assist businesses struggling to comply with their statutory obligations. Feedback from EHOs indicated that workshops, and online information, were the preferred mechanisms for the supply of such training.

ABBREVIATIONS COMMONLY USED IN THE REPORT

ASC	Acidified sodium chlorite
AFSSA	Agence Française de Securité Sanitare des Aliments
BC	Benzalkonium chloride
BMPA	British Meat Processors Association
BRC	British Retail Consortium
CCP	Critical control point
CFA	Chilled Food Association
CFU	Colony forming units
CSM	Cooked, sliced meats
CPC	Cetylpyridinium chloride
DNA	Deoxyribonucleic acid
DRT	Decimal reduction time
EHO	Environmental health officers
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation (of the United Nations)
FBO	Food business operators
FSA	Food Standards Agency
GRAS	Generally regarded as safe
HC	High care
HR	High risk
HPP	High-pressure processing
LA	Local Authority
LA	Lactic acid
LAB	Lactic acid bacteria
LeA	Levulinic acid
LED	Light emitting diode
MAP	Modified atmosphere packaging
MS	Member states

NPLM Non-persistent *L. monocytogenes*

NSWFA New South Wales Food Authority

PABA p-aminobenzoic acid

PB Potassium benzoate

PFU Plaque forming units

PHE Public Health England

PL Potassium lactate

PLM Persistent *L. monocytogenes*

PFGE Pulsed field gel electrophoresis

PPE Personal protective equipment

PS Potassium sorbate

PVC Polyvinyl chloride

PVOH Polyvinyl alcohol

QAC Quaternary ammonium compounds

QMRA Quantitative microbiological risk assessment

RAPD Randomly amplified polymorphic DNA

RLU Relative luminance units

RTE Ready-to-eat

SD Sodium diacetate

SDS Sodium dodecyl sulphate

SL Sodium lactate

SME Small to medium-sized enterprises

SOP Standard operating procedure

UN United Nations

VP Vacuum packed

WHO World Health Organisation (of the United Nations)

w/w A percentage concentration expressed as weight divided by weight

w/v A percentage concentration expressed as weight divided by volume

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1 INTRODUCTION

Non-invasive infection with *L. monocytogenes* can occur in the general human population and the illness is termed febrile gastroenteritis. The symptoms of febrile gastroenteritis are comparatively mild and therefore are likely not to be reported to clinicians (Miettinen et al, 1999). Clinical invasive infection by *Listeria monocytogenes* is called listeriosis and it is rare in healthy humans. However, there are subsections of the population that are vulnerable to invasive infection, including the immuno-compromised, the elderly and pregnant women (Lyytikainen et al, 2006). In vulnerable populations, an invasive infection commonly spreads through the blood circulatory system or the central nervous system and consequently listeriosis usually presents as bacteraemia, septicaemia or meningitis. In pregnant women, the mother is rarely affected, but the condition can result in spontaneous abortion, stillbirth of the foetus or the delivery of a severely ill child due to the infection. In contrast to febrile gastroenteritis, invasive infection by *L. monocytogenes* is serious because the mortality level in the vulnerable populace can be as high 30% (Farber, 2000; Gillespie 2010). Overall, there was 12.7% mortality across 26 EU Member States (MS) and Norway for *L. monocytogenes* infections for the period January 2010 to January 2012 (EFSA, 2013).

L. monocytogenes is ubiquitous on vegetation and in soils and surface waters (Strawn et al 2013) and consequently is routinely isolated from foods such as fresh produce and cold smoked fish that are not cooked prior to consumption (FAO/WHO, 2004). The opinion of the FAO/WHO (2004) is that it is likely most consumers routinely ingest small numbers of *L. monocytogenes*.

EFSA (2013) reported that the prevalence of cooked sliced meat (CSM) products contaminated with >100 cfu/g *L. monocytogenes* at the end of shelf life in the EU was 2.07% (72 positive samples out of 3470). The proportion (and number) of meat products samples with a *L. monocytogenes* count exceeding 100 cfu/g at time of purchase was 0.43 % (15 samples). The latter 15 samples originated from nine MS, and the distribution of the animal species of the origin of the meat product for those samples was: eight pork; one beef; two broiler; two poultry; one turkey; and one mixed species. Twelve were reported as 'cold, cooked meat product', two as 'pate' and one as 'sausage'. All, except one, were sliced meat products. Seven samples were packaged in modified atmosphere, two in normal atmosphere, five in vacuum and one in 'other'.

The UK retail chilled cooked sliced meat market in the UK is worth over £2 billion per annum and has seen, over the past five years, a year-on-year increase of 25% (£2,143 million in 2012 compared with

£1,717 million in 2007). The market value excludes use of cooked sliced meat in sandwiches, rolls and baguettes, the total UK market for which in 2012 was £4,109 million, of which £681 million was pre-packed and sold through retail (Chilled Food Association, 2013).

The pre-packed chilled meats sector has existed for some 40 years in industrialised countries. The vast majority of chilled pre-packed meat products are sold in modified atmosphere packaging (MAP) or vacuum packing (VP). In the UK, approximately 70% of pre-packed sliced cooked meat is sold in MAP and 30% in VP (British Retail Consortium (BRC), personal communication; Kaarin Goodburn 20/02/2013).

In addition to legal food safety/hygiene (EC 178/2002 and EC 853/2004) and labelling (EC 2000/13 in force until 12/12/14 and 1169/2011 in force from 12/12/14) requirements, there are assurance standards provided by some trade associations (e.g. British Meat Processor Association (BMPA), Chilled Food Association (CFA, 2006)). These standards describe processing requirements likely to reduce CSM contamination and the multiplication of *Listeria*. Technical expertise is required to comply with assurance standards and there is concomitant auditing by independent third parties. Thus, assurance schemes tend to be adopted mainly by larger CSM manufacturers. Manufacturers that do not adopt an assurance scheme are subject to periodic LA checks to ensure legal compliance.

2 AN OVERVIEW OF A TYPICAL PROCESS FOR THE MANUFACTURE OF COOKED SLICED MEAT (CSM) IN THE UNITED KINGDOM

One of the first stages of this study was for the project team to visit a small number of CSM processing plants and a slaughterhouse, all operating under commercial processing conditions. The purpose of the visits was to prepare an overview of a typical CSM manufacturing process that was illustrated with photographs. The approach was intended to ensure that a reader who was not familiar with CSM manufacture, and who may not have visited a CSM plant previously, could understand the various sections of this report and how they related to CSM manufacture.

2.1 BACKGROUND: CARCASS DRESSING, CUTTING AND DEBONING AND RAW MATERIALS USED FOR CSM MANUFACTURE

In the UK, the output at the end of the slaughter process is a carcass, which for red meat species (cattle, sheep and pigs) is commonly whole or sawn down the backbone to create two halves of carcass. A decision to split a carcass is largely dependent on the carcass weight, with heavier masses being split. If split, pig carcass halves can be incompletely sawn such that they are still joined at the neck and head, or one half of the carcass can retain the un-sawn head. In the UK, heads can be partly severed from the carcase side to which they were attached (Figure 1).

Figure 1 Heat removal from split pig carcasses after slaughter and dressing (processing to remove organs and inedible parts of the carcass)

After at least overnight chilling, red meat carcasses/carcass halves are sawn or otherwise cut into smaller sections of carcass called primals, for easier handling. The cut sites used for sectioning carcasses into primals are dependent on both customer requirements and country of carcass origin. Cutting can be undertaken in a cutting hall attached to a slaughterhouse or by specialist meat processors who typically buy chilled carcasses or primals and debone the meat before selling it on.

Although numbers have declined in recent years, there are still red meat (cattle, sheep and pig) slaughterhouses in the UK, which have on-site cutting and deboning halls. One of the primal cuts for porcine carcasses is the ham, which comprises the area of the rear leg between the rectum and the patella (knee joint). The ham primal is most commonly used for pork CSM manufacture, although the loin (especially the tenderloins, which run down either side of the spine in the peritoneal cavity) and front shoulder (commonly referred to as the picnic shoulder) can also be used.

A number of sections of beef carcasses can be used for CSM manufacture including the brisket, sirloin, tenderloin and rump. For white meat (chicken and turkey), breast muscle is exclusively used for CSM manufacture. Meat of ovine origin is not used commonly for the manufacture of CSM in the UK. The small number of processors that manufacture CSM from sheep primals tend to use rolled cuts of flank and brisket (the underside areas of the carcass between the front and rear legs). CSM manufacturers in the UK tend to buy in deboned primals. At the economy end of manufacture, soluble myosin (muscle tissue) can be extruded into a casing of an appropriate shape and used as a raw ingredient for the manufacture of CSM products such as luncheon meat and chicken roll.

2.2 FLOW DIAGRAM OF A TYPICAL CSM PROCESS

Although there are manufacturer-specific differences between CSM processes, there was a set of key stages (Heinz and Hautzinger 2007) that were uniformly presented at almost all of the CSM manufacturers visited. These key stages are shown as Figure 2, which can be considered a generic description of a typical CSM process.

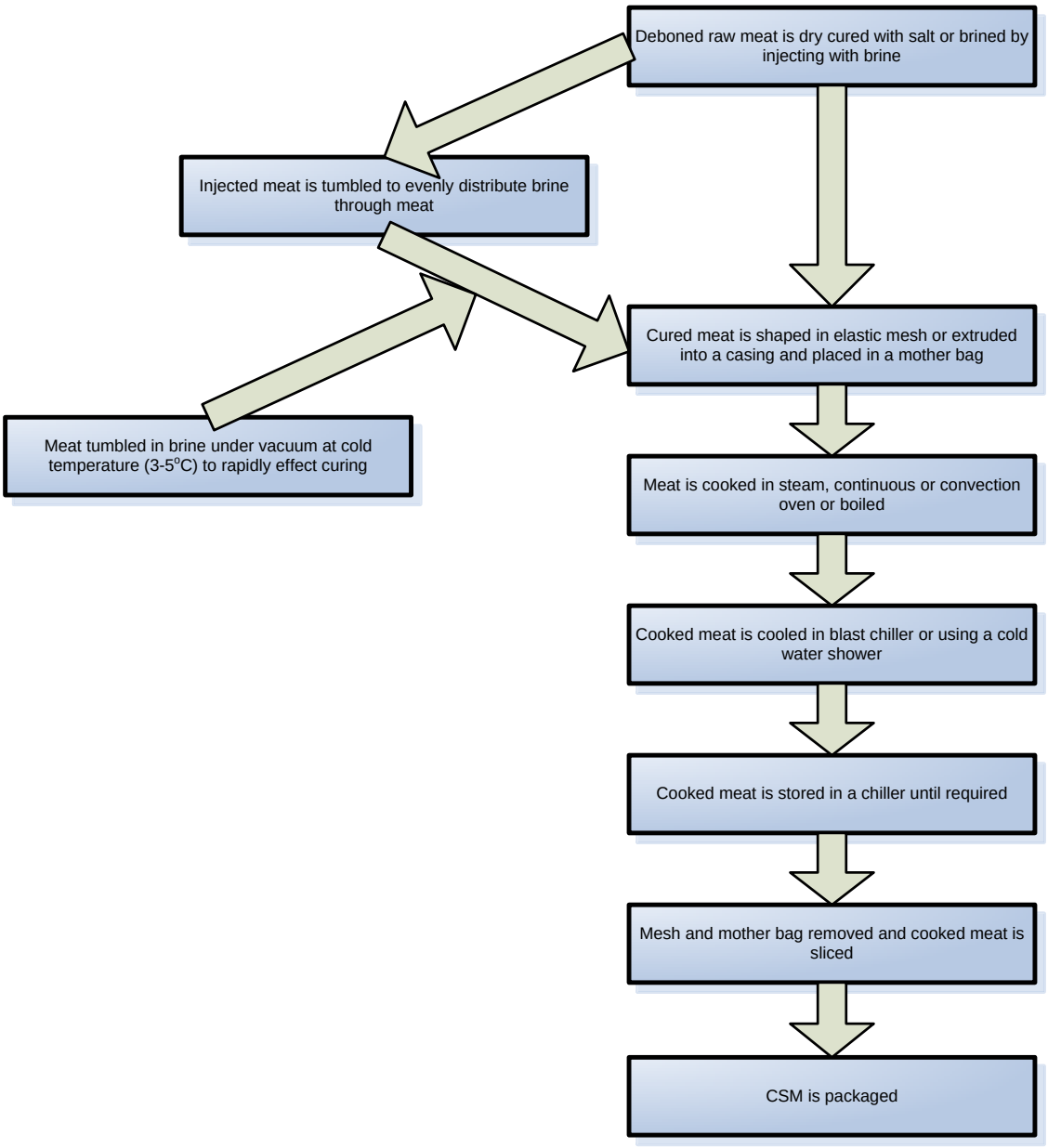


Figure 2 A flow diagram depicting a generic overview of typical CSM manufacturing processes

2.3 A CASE STUDY OF A TYPICAL MANUFACTURE PROCESS FOR COOKED SLICED HAM

The sliced ham processor visited processed 250 hams each week and employed 12 people, ten of whom handled food. The plant exclusively manufactured a variety of sliced ham products and bought in raw hams already pre-boned. The hams were typically sourced from Ireland, Spain and Belgium and no UK-sourced hams were used (because they tended to be more expensive). The hams were transported in a refrigerated lorry, which had a target transit temperature of 0-4^{o}C and sounded an alarm if the temperature became too warm. The temperature of each batch of hams was checked on arrival and a batch was rejected if it was warmer than 5^{o}C. After arrival and temperature/organoleptic checking, the hams were stored in an instrumented and alarmed chiller set to 0^{o}C until they were required. If the chiller temperature exceeded 5^{o}C, the technical manager, plant engineer and other key employees were sent text messages to their mobile phones by an automated alarm system.

2.3.1 TRIMMING AND INSPECTION

The first stage of processing was for the hams to be removed from the storage chiller and manually trimmed using a small knife to remove any excess fat. At the same time, the ham was visibly checked to ensure there were no undesirable detritus from previous processes (e.g. bone fragments or untrimmed tendons).

2.3.2 BRINING AND CURING

Immediately after trimming, the hams were cured using one of a variety of brines used by the plant. The plant used a mixture of salt (NaCl), sugars (brown and/or crystallised honey), nitrates and nitrites, ascorbic acid and sodium phosphate. The brine ingredients in the correct proportions for each product were prepared by another company to the CSM manufacturer's recipe and were supplied to the plant in 10 kg bags. The basic process of brine preparation was 10 kg of brine mix (which included salt) and 10 kg of salt per 100l of mains water. Adding the salt after the brine mix was important to ensure the brine dissolved fully. The final NaCl concentration of the brines typically ranged from 14-18% (w/v).

Brine was freshly prepared each day and sometimes multiple fresh batches were required. The brine was injected into the hams under pressure (around two bars) to effect curing (Figure 3). Injection was always into the muscle side of the ham (rather than the rind side). The technical manager at the plant did not know if there was any reason for the preference. Other technical managers shared the same injection side preference and commented that fat was more likely to block injection needles compared with muscle. It was noted that the injection process was not gentle and the ham was subjected to significant amounts of pressure as the brine was delivered. Typically, injection forced the ham into the injection conveyor and caused impressions to be formed in the rind (Figure 4). Injection pressures have a potential to cause physical damage to hams and so injection pressures were typically kept below 2.2 bars. The goal for successful brine injection was a weight gain to the meat of around 14% (w/w), which was fairly typical for most CSM processors.

Figure 3 A set of injection needles used to transport brine under pressure into the centre of a pre-boned ham

The brine injected hams were then tumbled overnight for 12-16 hours to distribute the brine evenly throughout the hams, extract soluble proteins, and remove any excess brine from the meat (Figure 5). One other consequence of tumbling after brine injection was that the muscle tissue relaxed its post-mortem rigour and the hams became easier to form into a shape suitable for slicing.

To ensure the hams were an appropriate shape for slicing, after brining and tumbling, they were wrapped in elastic netting that forced the meat into a roughly cylindrical shape (Figure 6). The hams were then packed into a cooking bag prior to baking. Alternative shaping methods observed at other plants included the extrusion of meat into casings using either a ram or high pressure.

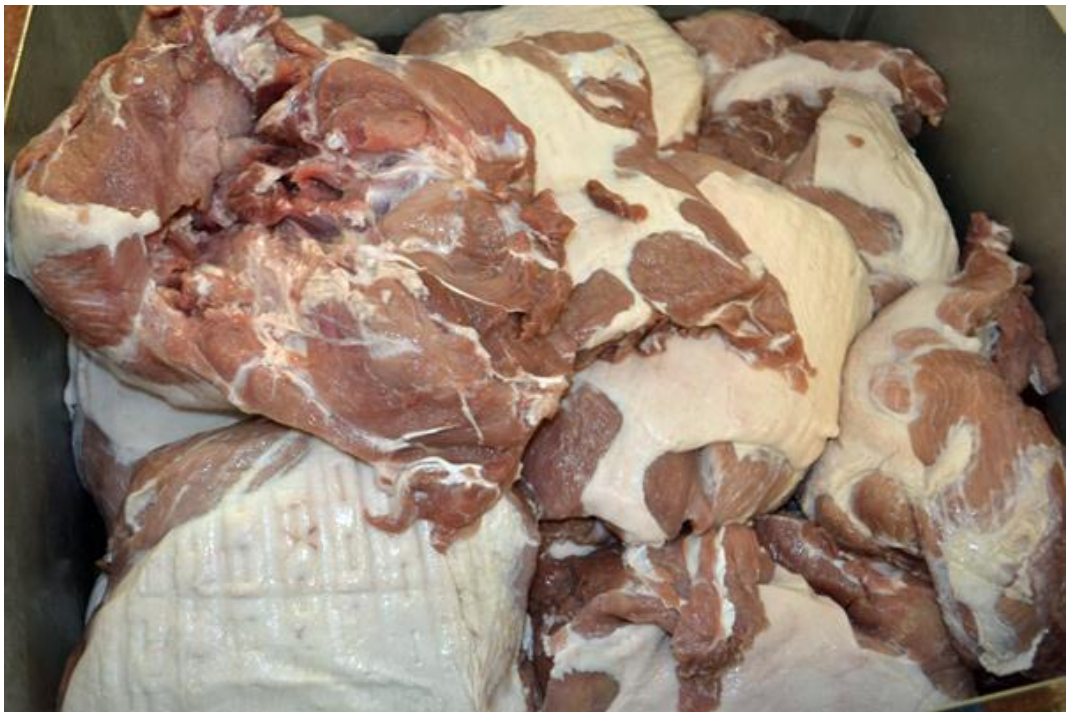


Figure 4 Hams showing belt indentations in the rind as a consequence of the pressures required to inject brine

Figure 5 Hams after tumbling inside a rotating baffled drum for 16 hours prior to unloading

Figure 6 Hams in cook bags shaped to rough cylinders by elastic string mesh to ensure a shape which could be effectively sliced

2.3.2.1 THE PURPOSE OF CURING AND BRINING

The application of dry salts or liquid brine (salt and/or sugar dissolved in water) to meat was undertaken for a number of reasons. These included historical justifications such as the fact that salt was an effective preservation method in the days before widespread domestic refrigeration. The basis of the preservation was that the high concentrations of salt in brine (up to 20% w/v) caused osmotic stress and dehydration of microorganisms, inhibiting their growth and extending shelf life by reducing microbial spoilage. Sugar and salt application to most meat improves flavour. The presence of nitrite in brine is principally to cure pork into ham and to allow the development of flavour and the characteristic red/pink ham colour due to nitrosomyoglobin formation. It is important to note that only ham is cured with dissolved mixtures of nitrite, salt and sugar. Other meats such as beef or chicken were brined using only salt and sugar (and no nitrite). Other additions to basic brine include sodium ascorbate, which acts as an antioxidant that helps prevent lipid oxidation and the development of rancid flavours. In curing brines that contain nitrite, ascorbate can also help inhibit the formation of carcinogenic nitrosamine compounds during cooking.

Phosphate can also be added to brine for a number of purposes. Phosphates help buffer and otherwise regulate the pH of the meat. In addition, muscle fibres expand and retain enhanced volumes of water on exposure to phosphate ions. Thus, the water content of some economy meats can be raised by the use of phosphates. Most commonly however, phosphates are added to meat to help reduce water loss

during cooking. Phosphates also help extract soluble protein from meat and can be used to increase protein yields in tumbled meat residues destined for extrusion as formulated meats such as luncheon meat. Sufficient protein in a formulated meat batter is a requirement for effective binding with other ingredients such as chopped ham pieces and rusk.

2.3.2.1.1 NITRITE

The Miscellaneous Food Additives and the Sweeteners in Food Regulations (2007) (and devolved equivalents) apply to enforce European Council and Parliament Directive 2006/52/EC. 2006/52/EC only permits the use of sodium nitrite (E250) as a preservation agent if sold in combination with sodium chloride (NaCl) or a salt substitute. Sodium nitrite is approved for use as a preservative for cured meat products. In the UK, the Miscellaneous Food Additives and the Sweeteners in Food Regulations permit the use of nitrate only at product-specific, variable concentrations of up to 250 mg sodium nitrite per kg of meat.

Sodium, and to a lesser extent potassium, nitrite has an antibacterial action, and is applied often to meat as a preserving agent in conjunction with sodium or potassium nitrate. Microbial metabolism will reduce nitrate to nitrite, and the inclusion of nitrate is a strategy for maintaining nitrite concentrations in meat over time (Adams and Moss, 2000). Nitrite plays a key role in controlling the growth of the pathogen *Clostridium botulinum*, by preventing the successful outgrowth of germinating spores. Nitrite contributes to the flavour of cured products and confers a reddish colour owing to it binding reversibly to myoglobin in cured meats to form nitrosomyoglobin. On cooking, nitrosomyoglobin is irreversibly converted to nitrosylhaemochrome, the compound that confers the pink colour characteristic of cooked hams (Adams and Moss, 2000).

During the cooking of cured products such as ham, nitrosamines can form, which are carcinogenic (McCutcheon, 1984). Where cured meats are to be cooked, for example in the production of cooked hams, anti-oxidant additives such as ascorbate (vitamin C) can be included to help inhibit nitrosamine production (Rywotycki, 2002).

2.3.2.1.2 BRINE PREPARATION

The first sliced ham plant visited used pre-packed brine mixtures dissolved before the addition of extra salt. However, it was apparent from other plants that prepared their own brine from component ingredients, that the addition of the chemicals was undertaken in a defined order. A particular concern was that the brine was clear after mixing (because cloudy brine meant undissolved chemicals and lower concentrations of inhibitors such as nitrite being injected into the meat). If all of the brine components were to be added to water at the same time, some would not dissolve.

Instructions in the standard operating procedures (SOP) of one CSM plant added all of the phosphate- and nitrite-containing ingredients initially to a vat of vigorously stirred mains water. After waiting for the phosphate and nitrate to dissolve, the sugars were added. After the sugars had dissolved, ascorbate was added and the brine was used only after the vitamin had completely dissolved and the solution was clear.

2.3.2.1.3 LIVE BRINING

Wiltshire curing is also known as live brining. It is a traditional curing process that dates back to a time when supplies of sodium or potassium nitrite were scarce, although saltpetre (potassium nitrate) was readily available. Traditionally, the bacteria in live brine converted the saltpetre component into nitrite as a preservation strategy for ham. Live brines contain a range of halotolerant (salt tolerant) bacterial species including *Acinetobacter*, *Alcaligenes*, *Arthrobacter, Corynebacterium, Lactobacillus, Micrococcus*, *Vibrio* and yeasts (Gardiner, 1971). The original source of the microorganisms present in a live brine was the indigenous microflora of previously-cured hams. After a brining was completed, the water, salt, sugar and nitrate (and in modern brines, nitrite) were replenished and the replenished brine was reused. One of larger throughput premises visited as part of this study had a live brine that had been in continuous use since the 1940s. Commonly, *Micrococcus roseus* (Hinrichsen et al 1984) confers a pink colour to a high percentage of live brines used in the UK. A traditional Wiltshire process involved soaking hams in live brine for three or four days at 4^{o}C to achieve curing.

2.3.2.1.4 SHAPING AND PACKING INTO COOK BAGS

In the factory visited initially, hams were shaped into rough cylinders using a tube of elastic string mesh to ensure the cylindrical shape was retained (Figure 6). The purpose of the shaping was to ensure hams that could be effectively sliced. In other processing plants, alternative strategies to achieve the same end were observed. A common approach was to extrude meat into a casing contained in a dye of the required shape using a steel ram. Variations on that theme also included pumping soluble protein and pieces of meat into casings of the required shape. Dependent on the type of casing and the oven used, the shaped meats were either cooked in the casing, or placed in a cook bag prior to cooking. An important consideration for the use of cook bags was water (and consequent weight) loss from the meat during the thermal process.

2.3.2.1.5 THERMAL PROCESSING

The water content of the brine injected into the meat helped keep the meat moist and minimised water loss from the meat during cooking.

At the site visited, baking of the hams was undertaken for 12-16 hours in a 'steam' oven. Water vapour was injected into the oven and the oven was heated to 74^{o}C. The cooking process was treated as a critical control point (CCP) by the plant and so the oven temperatures were monitored. In order for a batch of hams to have been cooked successfully, two hams in the centre of the oven were required to reach 74^{o}C at their cores for at least ten minutes. The cooking time was largely set as the time required to meet the CCP criterion. The basis of the CCP criterion was BRC guidance "with an additional safety margin". The BRC advises 70^{o}C for at least two minutes.

2.3.3 CHILLING

After cooking, the hams were showered with mains water inside the oven for up to four hours (Figure 7). The purpose of the shower was to lower the temperature of the hams before they were moved to a high risk chiller adjacent to the oven room for storage until slicing (Figure 8). It was not common for cooked meats to be cooled using mains water. Most CSM manufacturers in the UK use a blast chiller to quickly remove heat. Hams were held in the high care chiller for up to one week before slicing. Since most commercial meat chillers in the UK were designed to remove moisture from the air, the hams were consequently stored in their watertight cooking bags to prevent excessive water loss from the meat during the storage.

Figure 7 Hams were showered in mains water after overnight cooking as a way of lowering the temperature of the meat prior to chilled storage

Figure 8 Hams stored in a high risk chiller inside their cook bags to prevent excessive drying of the meat

2.3.4 SLICING AND PACKING

When a ham was required for slicing and packaging, it was removed from the high risk chiller using a second exit that led directly into the slicing and packaging area, which the plant termed the high risk area (section 12.3.3). The cook bag and elastic mesh were removed using a knife that was sanitised on a daily basis. Liquid and jelly that accumulated around some of the hams during cooking was allowed to drain through a hole in centre of the work table and into a waste container (Figure 9). For some of the premium products manufactured at the plant, any rind and excess fat (which was soft after cooking), was removed by scraping a knife along affected parts of the ham. For the majority of products, the fat was not removed. After cook bag and elastic mesh removal, the hams had the appearance as shown in Figure 10. The hams were then sliced using an automated slicing machine (Figure 11) before being weighed into standard vacuum packs (VP) and heat-sealed under vacuum (Figure 12).

2.3.5 COATED HAMS

For certain products (e.g. crumbed, peppered or glazed ham), the coating of pepper, breadcrumbs or glaze was applied immediately before the slicing stage of the process. All of the coatings were applied by rolling the ham in a shallow tray containing a thin layer (1-2cm) of the required coating. At other operations, the hams were observed to be coated in melted gelatin prior to coating, which improved adhesion. For glazed products (e.g. honey roast ham), the glaze was set by flash-roasting the coated ham in a hot air oven (250^{o}C) for five minutes.

Figure 9 A self-draining work table used for the removal and disposal of elastic mesh and cook bag from the hams

Figure 10 Hams after removal of the elastic mesh and cook bag

Figure 11 A log of ham being loaded into an automated slicer

Figure 12 Sliced ham prior to being heat sealed under vacuum

2.3.6 VARIATIONS TO STANDARD PROCESSES, THE RECYCLING OF COOKED MEAT AND THE ROLE OF CHILLED VACUUM TUMBLING

Although the initial visit to a CSM plant was beneficial prior to undertaking a review of the literature, subsequent visits to other plants revealed the first process observed was atypical in terms of the method of cooling, the plant layout and because it did not manufacture CSM from batter. In the UK, the vast majority of CSM manufacturers cool meat in blast chillers and do not shower with mains water. Furthermore, the original plant visited undertook operations in two physical buildings. One building was used for the raw meat operations. The prepared meats were loaded onto a forklift truck and conveyed to the second building for cooking and slicing. Although compartmentalised working areas are common in larger CSM plants, and desirable in terms of *L. monocytogenes* control, in other plants it was not typical to convey prepared raw materials by public road to be cooked.

Finally, a comment should be made regarding the recycling of cooked meats and its use in the manufacture of economy meats such as luncheon meat and chicken roll. Although this aspect of a typical process was missing from the initial process that was viewed, a significant number of UK CSM manufacturers use chilled vacuum tumbling and recycle product as part of their processes. The basic process involved mixing defined quantities of meat and brine (or cure) inside a large tumbler. Typically, tumblers were loaded by vacuuming meat and brine into a tumbling chamber. The temperature of the meat and brine was lowered to around 5-6^{o}C and the mixture was gently massaged and tumbled. The duration of tumbling was dependent on the meat species, the size of individual pieces of meat, and the total mass inside the tumbler. Typical tumbling times ranged from a few hours for small quantities of chicken breast to 24 hours for four tonnes of hams. Vacuum tumbling was observed for ham, turkey and chicken. In the UK, beef is not commonly vacuum tumbled. A significant advantage to chilled vacuum tumbling was that meat cured quickly compared with a standard tumble at atmospheric pressure. However, the vacuum tumbling process caused changes to the physical structure of the meat. Typically, some of the meat proteins were solubilised, and after tumbling the brine had become a semi-solid gelatinous mass. The material was called batter, and it was the batter that was the basis of economy meat products.

In a typical CSM process, logs of meat are unable to be fully sliced. The end pieces (called ends or heels) that cannot be sliced, are recycled by chopping and mixing with batter. The chopped meat and batter mixes were typically extruded into casings and cooked to generate products such as luncheon

meat. However, logs of luncheon meat were also unable to be fully sliced and so the ends of these logs were also recycled by chopping and mixing with batter. One CSM manufacturer has developed processing practices and custom equipment that allows the creation and handling of logs of CSM that are up to 4m long in an attempt to reduce the quantity of ends that are recycled. In terms of microbiological risk from *L. monocytogenes*, recycling did not pose much of a hazard because the meat was cooked fully during each round of recycling. It is beyond the scope of this report to comment on any possible implications of recycling for spore forming bacteria such as *Clostridium botulinum*, although we note that nitrite can inhibit spore germination normally triggered by heat shock.

3 A SYSTEMATIC SEARCH OF THE PEER-REVIEWED LITERATURE

The objective of the publications overview section of this report was to critically review and evaluate the literature relating to *L. monocytogenes* and CSM. Four main areas were targeted:

- The identification of human listeriosis outbreaks caused by consumption of contaminated CSM
- The transfer and persistence of *L. monocytogenes* in processing plant environments
- The transfer and persistence of *L. monocytogenes* in retail environments with an emphasis on in-store slicing
- Interventions which prevented or reduced the multiplication of *L. monocytogenes* on CSM

The approach adopted was based on the methodology of a systematic review (Jadad et al., 2000). Publications were independently scored by two reviewers and an average score of three or more out of five was required for inclusion. Assessment scores were the subjective opinions of each reviewer and took into account: the numbers of samples tested for surveillance, the similarity of climate to the UK, the appropriateness of the laboratory testing method(s), replication for laboratory-based interventions, appropriateness of cultured strain selection/use of naturally contaminated product; and the appropriateness of any model system to commercial processing conditions and practices. Only those sections of the scoring scheme that had relevance to a publication were completed. The Thompson ISI electronic database, PubMed and Medline Ovid were searched with a defined search string. The construction of the search string was iterative, with the search parameters refined and new keywords identified from the previous round of searching. In total, seven rounds of searching were undertaken. After the final round of searching, selection criteria were used to remove irrelevant references and to select based on title, keywords and abstracts, a secondary library of relevant literature.

The Boolean search string used was:

"*Listeria monocytogenes*" AND "Meat" AND ("sliced" OR "cold cut*" OR "cooked") AND ("beef" OR "chicken" OR "turkey" OR "luncheon" OR "mortadella" OR "bresaola" OR "salami" OR "pepperoni" OR "pastrami" OR "corned" OR "meatloaf" OR "outbreak" OR "disease" OR "intervention" OR "treatment" OR "decontamination" OR "persistence")

The search string provided 428 hits from the Thompson ISI electronic database, 130 hits from PubMed and 66 hits from Medline. The titles and abstracts of these 624 papers were screened for relevance using the following criteria for rejection:

(1) the publication did not relate to a CSM,
(2) the contaminating organism was not *L. monocytogenes* or a relevant *Listeria* species
(3) the publication language was not English, German, Portuguese, Polish or French,
(4) the reference was a duplicate of another reference and
(5) the amount of replication, laboratory testing protocols and statistical analyses were not robust and appropriate in the collective opinion of the research team.

Duplicate publications were removed by importing the reference lists into Reference Manager 12 (Thompson ISI) and using the 'identify duplicates' function of the programme. A total of 255 references passed the relevance screening (Table 3). The final library is included in Section 15 of this document.

Table 3 Results of literature search

Source database and relevance criteria applied	Number of references
Pubmed	130
Thompson ISI web of Knowledge	428
Medline Ovid	66
Total after removing articles not on the acceptable languages list	613
Total after removing duplicates	348
Total after relevance screening	255

4 *L. MONOCYTOGENES* CONTAMINATION IN CSM PROCESSING AND RETAILING ENVIRONMENTS

4.1 GENERAL CONTAMINATION OF PROCESSING ENVIRONMENTS

A key finding of the extended studies of Tompkin et al (1992) was that there was a seasonal increase in the *L. monocytogenes* isolations from meat processing plants during the summer months. Tompkin (2002) considered that the seasonal elevation was due to increased production during summer, which made it more difficult to maintain processing control in the plant environment. Similar increased *L. monocytogenes* isolations have been observed during November and December in smoked fish plants as throughputs are increased to accommodate Christmas demand (Tompkin, 2002). Higher than usual throughputs can therefore be an important factor for increased *L. monocytogenes* contamination of CSM.

Samelis and Metaxopoulos (1999) undertook an extensive overview of environmental (and final product) contamination by *Listeria* spp. and *L. monocytogenes* in a commercial Greek CSM plant. The researchers visited the small CSM plant (4000 tonnes annual output) six times over a six month period, collecting swab and final product samples for testing. Although more than a decade old, the specific findings, reported in sections below, provide a very informative overview of how *Listeria* and *L. monocytogenes* can be distributed in CSM plants to contaminate final product.

In Norway, a multi-year study of three CSM processing plants (Heir et al 2004) determined that from 127, 143 and 49 environmental samples collected at the three plants respectively in 2001-2002 there were 13 (10%), 22 (15%) and 12 (24%) detections of *L. monocytogenes*. Typing of the isolates by PFGE (pulsed field gel electrophoresis) showed that some isolates with the same pulsotype were isolated from raw stored meat, the plant environment, including slicer blades, and from final sliced meat products over periods of several years. The authors conclude that the only reasonable explanation for their findings was that there were persistent *Listeria* in the environments of some plants. Furthermore, although effective cooking was a critical control point, there were mechanisms operating in the plants investigated that had allowed the contamination of cooled, cooked product. Heir et al (2004) identified a previously unrecognised outbreak of listeriosis caused by the same strain that was isolated from final product and the environment of one CSM plant.

Berrang et al (2005) collected samples from the processing environment, raw materials and final products of a single CSM plant over a six week period. Each sample site was examined for the presence of *L. monocytogenes* before and after routine processing. There was molecular characterisation of the *L. monocytogenes* isolates allowing strain differentiation. The study reported a number of key findings relating to *L. monocytogenes* in drains. *L. monocytogenes* was detected in floor drains on the raw product side of the plant before processing and in drains on both raw and cooked sides following eight-hours of processing operations. The majority of *L. monocytogenes* isolations were from the drains, and the isolations were more frequent in the side of the plant that handled raw meat. The authors considered that observation to be a possible indication that compartmentalisation of the CSM process was advantageous. The drains on the cooked side of the plant were never positive prior to the commencement of processing, but were contaminated frequently at the end of the processing day. When a drain was positive before operation, it was always positive after operation; however, the same subtype was not always detected after a shift was completed. It was possible to isolate up to five *L. monocytogenes* strains from a single drain after processing. The authors considered that factory workers or contaminated product, may have contaminated the post-cook drains or the transfer of equipment from the raw side to the cooked side (not stated what equipment was transferred). In all but two cases, the strain detected in drains on the cooked side was also detected somewhere on the raw side on that same day.

When the typed strains were compared against isolates from a previous visit the previous year to the same plant (Berrang et al 2002), a number of strains were identical. Berrang et al (2005) considered the possibility that the plant had harboured resident strains for over a year, but also acknowledged that the plant may be subject to continual re-introduction from an unknown source. Although Berrang et al (2005) commonly isolated *L. monocytogenes* from raw materials entering the plant, the bacterium was isolated only once from finished product. There were no isolations from food contact surfaces in the post-cook compartment of the plant during any of the visits. The Berrang (2005) study made clear the importance of floor drains as sources of *L. monocytogenes* in CSM plants during operations.

Keeratipibul and Techaruwichit (2012) used a PCR-based typing method to track the movements of *Listeria* spp. through a processing plant that manufactured cooked chicken meat. Although the study was general, the *Listeria* isolates were all identified to species level before PCR fingerprinting. The approach was a response to a relatively small number of *L. monocytogenes* isolations (1% of the

~13,000 samples collected) and justified by previous studies (Barros et al 2007), which assert the presence of one *Listeria* species is potentially indicative of the presence of another. Keeratipibul and Techaruwichit (2012) classified the sites of sample collections into one of three zones. Zone one was food contact surfaces, zone two were non-food contact surfaces in close proximity to zone one surfaces, and zone three samples had a distant proximity to zone one surfaces. The key findings of the Keeratipibul and Techaruwichit work was that at the start of the day's production, *Listeria* prevalence on zone one surfaces was low (0.8% of all samples taken), but above zero. Furthermore, surfaces in all of the zones were contaminated with *Listeria* during the days' production. The authors noted that the contamination of zone three surfaces increased rapidly and consistently throughout the course of a working day. A total of 415 *Listeria* isolated were fingerprinted to determine any relationship between contaminants in the final product and those in the processing environment. Some isolates were only present in final product, with no corresponding isolation from the plant environment. However, there was one *L. innocua* strain that was persistently found on surfaces in the plant throughout the sampling period. The surfaces that were most contaminated by that strain were a liquid N_2 chiller exhaust pipe, a metal detector conveyor belt and inside a drain inside a product freezer. More generally, *Listeria* was commonly isolated from conveyor belts and the gloved hands of various workers in zone one; conveyor belts, equipment control panels and packaging tables in zone two; and floors, walls and the hands of a worker carrying soiled equipment in zone three.

More recently, Syne et al (2013) undertook a general investigation of contamination routes in an RTE meat plant in Trinidad. The plant was the same one that was investigated by Gibbons et al (2006) after three batches of products were recalled as a consequence of *L. monocytogenes*-contamination. The plant manufactured cooked sliced bacon, chicken bologna and sausages. The microbiology undertaken was mostly for bacterial indicators, but included *Listeria* spp. and *L. monocytogenes*. Samples of air, water, raw and finished products and the plant environment were collected on two occasions from various stages of processing.

Fifty percent (10 of 20) of raw bacon and bologna batters were contaminated with *Listeria* spp., and four samples (20%) contained *L. monocytogenes* (Syne et al 2013). There were no isolations of *Listeria* or *L. monocytogenes* from cooked products. There were however isolations from the plant environment with one swab from the case stuffing equipment testing positive for Listeria spp. In addition, one each from a meat tumbler, post-cook slicing equipment and pre-cook batter pumping equipment also contained *Listeria* spp. Although the majority of the Syne study was focussed on

microbiological indicators, the authors noted that cooking was an effective CCP and that any microbiological issues were related to post-cook re-contamination. In particular, *Staphylococcus aureus* (a common commensal of human skin) was a common contaminant of post-cook product and post-cook food contact surfaces. The study provided further evidence that workers can cross-contaminate final product.

4.1.1 RAW MATERIALS RECEIVED

Samelis and Metaxopoulos (1999) tested the raw meats received at a Greek CSM processing plant. Fresh and frozen meat from a variety of species were tested, with the majority of the meats purchased from slaughterhouses located in Northern Continental Europe. The results revealed that 51% of the raw meat samples (n=51) were contaminated with *L. monocytogenes*. Although relatively few samples were taken, Samelis and Metaxopoulos (1999) reported that turkey necks and breasts and mechanically deboned pork were the sample types most likely to harbour *L. monocytogenes*. Samelis and Metaxopoulos (1999) also implicated pork lard as a primary source of *L. monocytogenes* contamination. None of the samples of casing, or granular or powdered ingredients contained *L. monocytogenes*.

Lunden et al (2003a) also tested samples of raw meat received at four CSM plants for *L. monocytogenes*. The Lunden study undertook PFGE (pulsed field gel electrophoresis) typing of isolates in order to determine if *L. monocytogenes* were persistent (PLM defined as five or more isolations over an interval of three or more months) or non-persistent (NPLM defined as fewer than five isolations in less than three months). A summary of the findings are reported as Table 4. A general conclusion of the Lunden work was that *L. monocytogenes* could be isolated from raw meat received into the environments of the four CSM plants sampled. A second key finding of the Lunden study was that, although CSM plants commonly received *L. monocytogenes*-contaminated raw meats, the pulsotypes isolated from final product tended to be PLM rather than the NPLM isolates present on the raw meat (Table 4).

Table 4 Number of persistent *L. monocytogenes* (PLM) and non-persistent *L. monocytogenes* (NPLM) isolated from raw and cooked meats in four CSM processing plants*

Meat status	PLM or NPLM	Number of pulsotypes (%)
Cooked	PLM NPLM	12 (71) 5 (29)
Raw	PLM NPLM	3 (23) 10 (77)

*Reproduced from Lunden et al (2003a).

Syne et al (2013) tested 20 batches of uncooked meats for *Listeria* spp. and *L. monocytogenes*. Ten of the pre-cooked samples collected during the production of bologna tested positive for *Listeria* spp. The samples were one raw meat pre-flaking, one post-flaking and all eight samples of uncooked emulsion (raw meat and filler). Furthermore, four of the emulsion samples were positive for *L. monocytogenes*.

4.1.2 PRE-PROCESS EQUIPMENT

L. monocytogenes present on incoming meat was considered to be the original source of *L. monocytogenes* isolated from processing equipment in the CSM plant (Samelis and Metaxopoulos, 1999). In particular cutting equipment designed for use on frozen pork shoulders was found routinely to harbour *L. monocytogenes*. Furthermore, when the affected equipment was cleaned and sanitised, the *L. monocytogenes* persisted. Samelis and Metaxopoulos (1999) believed that the persistence was evidence for strong attachment and possible biofilm formation. The authors discussed additional factors, which supported the persistence of *L. monocytogenes*. These included that some equipment parts had not been made from stainless steel or were not easily removable for daily cleaning purposes. There was specific note that 'notches and hidden spots' on some equipment consistently harboured *L. monocytogenes*.

4.1.3 MEAT TUMBLING

Samelis et al 1999 investigated changes in the microbiological flora during the manufacture of CSM. In raw ham, Gram-negative bacteria were dominant during butchering and brining. However, during

tumbling, Gram-positive microorganisms became predominant. Furthermore, at the plant investigated, *L. monocytogenes* was not isolated from raw meat immediately after brining, but was after tumbling and prior to moulding. An intensive programme of sampling and testing, which included swabs from the inside surfaces of the tumbler, indicated that there were areas inside the tumbler that were 'hot-spots' for *L. monocytogenes* (Samelis et al 1999). The authors reported that the machine was required to work continuously during periods of high demand for finished product. Time constraints meant that the tumbler was not effectively cleaned and sanitised during such periods and consequently different batches of ham could be cross-contaminated (Samelis et al 1999) by transfer from the tumbler surfaces.

There are further considerations for vacuum tumbling. For high throughput CSM plants in the UK, it is a common practice to tumble meat at low (3-6^{o}C) temperatures under vacuum. *Listeria* spp. and *L. monocytogenes* grow well under vacuum at low temperatures. Using naturally contaminated pork under commercial processing conditions, Samelis and Metaxopoulos (1999) observed *Listeria* spp. counts could exceed 10^{6} cfu/g meat after tumbling for 12h.

4.1.4 THERMAL PROCESSING AS A CRITICAL CONTROL POINT

Poorly-controlled thermal processes have been implicated as causes for the contamination of cold sliced meats by *L. monocytogenes* (Willis and Greenwood, 2003). Cooking is considered to be a true critical control point for the control of *L. monocytogenes* by a number of authors (Carlier et al 1996, Samelis and Metaxopoulos 1999, Zhu et al 2005). In theory, if cooking proceeds as intended, any risk from *L. monocytogenes* is eliminated - irrespective of how the meat had been processed prior to cooking. In general, CSM sold by larger UK retailers will have received a heat process of at least 70^{o}C for 2 min, or the calculated equivalent, as part of the terms of supply (BRC, personal communication). The heat treatment duration and time was designed to ensure at least a six-log reduction to *L. monocytogenes* with a comfortable margin of error of one decimal reduction. The BRC-recommended thermal process was derived scientifically (Gaze et al 1989, Mackey et al 1990). Although the BRC-issued advice was generally applicable, the cooking stage of CSM is quite complex and there are key papers, reviewed below, that describe atypical conditions, or possibly atypical strains, where additional care should be taken.

In France in the 1990s there were several high profile outbreaks of listeriosis caused by the same strain of *L. monocytogenes* (Section 6). Using the outbreak strain, Carlier et al. (1996) determined that, during the cooking stage of processing, moderately contaminated hams should be heated to a minimum core temperature of 65^{o}C. However, Carlier et al. (1996) also reported that when hams were inoculated with roughly 10^4 cfu *L. monocytogenes*/g meat, no *L. monocytogenes* were detectable by direct plating after cooking. However, after enrichment of the cooked sample, *L. monocytogenes* were recovered, indicating numbers of *L. monocytogenes* were reduced only to below the detection method of the quantitative testing method and not entirely eliminated.

Later studies that investigated the spread of *L. monocytogenes* under commercial processing conditions (Samelis and Metaxopoulos, 1999) noted there were differences in the core temperature required for safety in specific products. The reason for the differences was that the method used to cook CSM had an impact on *L. monocytogenes* survival. One cooking method for 'boiled' ham involved the use of submerging ham packed in a cook bag into a tank containing hot water. Samelis and Metaxopoulos (1999) observed that *L. monocytogenes* was able to survive, sporadically, the hot water process at temperatures up to 68.4^{o}C but was killed by baking in an oven at a similar temperature. In keeping with the findings of Carlier et al (1996) regarding increased risk for extensive contamination, *L. monocytogenes* multiplication to high concentrations of 10^6 cfu/g meat during tumbling for 12h under vacuum, was required for *L. monocytogenes* to survive the hot water immersion process. As a consequence of their observations, the authors increased their recommended safe cooking temperature to at least 72.6^{o}C for immersion cooking, to ensure the complete destruction of *L. monocytogenes* (Samelis and Metaxopoulos, 1999).

An important consideration for cooking is the rate of temperature increase. A number of studies have demonstrated that *L. monocytogenes* is more heat resistant compared to most non-spore forming food borne pathogens (Farber and Brown 1990, Lou and Yousef 1996, Doyle et al 2001). If the rate of temperature rise is low, *L. monocytogenes* has time to upregulate stress-response genes, express heat-shock proteins, and alter the lipid profile of the plasma membrane (Lou and Yousef 1996). In combination, these biochemical changes provide thermal protection to *L. monocytogenes* and increase the likelihood of survival at elevated temperatures.

As part of an investigation to determine the source of *L. monocytogenes* for a contaminated batch of CSM that was recalled in Trinidad, Gibbons et al (2006) reported a variety of factors had contributed to the issue. These included inadequate cooking of the CSM, which Gibbons et al (2006) believed was one probable cause of the contamination, along with post-processing contaminated from inadequately cleaned work surfaces.

4.1.5 POST-COOKING AND PRODUCT CONTAMINATION IN HIGH RISK AREAS

In Greece, Samelis and Metaxopoulos (1999) sampled processing equipment in a single plant on two different occasions. *Listeria* spp. and *L. monocytogenes* were isolated from food contact surfaces on slicing equipment on both sampling occasions.

A review of *L. monocytogenes* in general food processing environments (Tompkin, 2002) summarised *L. monocytogenes* contamination in a number of RTE food processes, including some of relevance to CSM. A summary of the reported hot spots relating to CSM are provided as Table 5. The Tompkins review (2002) also provided details of how the contamination was removed from commercial processing plants. That information can be used as the basis of a series of practical strategies to deal with the removal of *L. monocytogenes* from contaminated equipment.

These successful strategies can be summarised as:

1. Equipment was disassembled, cleaned, sanitised and rebuilt
2. Equipment was modified so that it could more easily be disassembled, cleaned, sanitised and rebuilt
3. Equipment was modified to remove hard to clean places that could harbour *L. monocytogenes* (e.g. spot welds between surfaces were fully welded to remove hollow areas between joined materials)
4. Sanitation was accomplished by placing the contaminated item in an oven, which supplied moist heat
5. Contaminated equipment was replaced

6. An antimicrobial (e.g. grease containing anti-listerial sodium benzoate) was applied to problem areas of the equipment

Lunden et al (2003a) also investigated *L. monocytogenes* contamination in red and white meat CSM manufacturing plants. The focus of the Lunden work was persistent (PLM) and non-persistent *L. monocytogenes* (NPLM) strains. Swab samples (the exact number was not stated) were taken over a period of several years in three-red, and one-white, Finnish meat processing plants. A total of 596 *L. monocytogenes* isolates were isolated from these swabs and typed using PFGE. Overall, there were 47 different pulsotypes, which were classified as PLM or NPLM depending on the number of isolations.

PLM and NPLM were found in all of the plants visited (Lunden et al, 2003a). All plants were contaminated with several NPLM and at least one PLM. There were 35 pulsotypes that were plant-specific, seven pulsotypes that were present in two plants and five pulsotypes that were isolated from three plants. Some pulsotypes were isolated from both red and white meat plants. Nine PLM pulsotypes were NPLM in another plant.

Lunden et al (2003a) also reported there were differences in the sampling locations harbouring PLM and NPLM. Unfortunately, there was a lack of detail as to the precise sampling locations inside the plant environments, although a general summary is provided as Table 6.

A general conclusion of the Lunden et al (2003a) study was that NPLM were most commonly isolated from the processing environment and PLM were isolated from processing equipment. However, almost half of the PLM pulsotypes were isolated from the plant environment, processing equipment and final products (Table 6). Final product contamination was most likely a consequence of PLM-contaminated food contact surfaces on processing equipment or indirect transfer from worker hands that were contaminated from equipment control panels. A summary of *L. monocytogenes* contaminated equipment sites and the paper author's assessment of the likelihood of cross contamination to CSM is shown as Table 7.

Table 5 Examples of sources of contamination by *Listeria* in RTE-food-processing operations and corrective actions that were taken (1989–2000)*

Product	Contaminated equipment	Contamination source	Corrective actions taken
Sliced luncheon meat	Slicer	Worn hydraulic seals at base of slicer, oil with water and product residue	Slicer stripped, cleaned sanitised; parts placed in oven and moist heat applied. Oil with sodium benzoate (anti-listerial) used for lubrication
Sliced ham from cans	Slicing/packaging line	Can opener and heavy wire safety cover	Cover modified so it could be removed (was not removable for employee safety concerns)
Sliced pepperoni	Slicer	Product detritus build-up inside safety cover on gear and drive belt; material further contaminated conveyor belt below	Cover changed so it could be removed for cleaning each night
Diced cooked meat/poultry	Dicer (multiple events)	Undetermined	Dicer moved into oven and moist heat applied or dicer covered in tarp and steam applied
Cooked sausage	Packaging machine	Crack in stainless steel covering on top edge of machine near product loading area	Area cleaned, sanitised and welded
Cooked products	Conveyors (multiple events)	Hollow rollers	Rollers replaced on detection; where possible conveyors replaced with sloping steel slides to prevent roller contamination
Hams	Brine chill tunnel	Damaged rubber seals on stainless steel door at end of tunnel	Damaged seals replaced, cleaning procedures tightened
Cooked turkey products	Conveyor between shrink tunnel and boxing	Worn conveyor made of rubber-coated fabric	New belt was fitted
Cooked turkey breast	1. Conveyor leading to packaging machine 2. Cooked product knock out table	1. Fabric conveyor belt material 2. Hand held knives for opening product	1. Conveyor replaced with stainless steel chute 2. Knifes cleaned and sanitised daily in automatic washer and not stored in employee lockers
Large cooked products	Bagging table	Air duct used for blowing bags open	Table modified to make duct accessible for nightly cleaning
Breaded products	1. Exit from spiral freezer 2. Spiral freezer	1. Wheel bearings for conveyor belt 2. Undetermined	1. Bearings removed and replaced 2. Cleaning frequency increased and equipment allowed to defrost before cleaning
Cooked burgers and links	1. Between freezer and packing machine 2. Wire mesh conveyor between oven and freezer	1. Overhead conveyor 2. Hollow support rods for conveyor	Hollow rods replaced with solid rods
Cooked linked sausage	Packaging machine	Steel rods for pushing product into carton	Push rods removed and cleaned on a daily basis

*reproduced from Tompkins 2002

Table 6 Sampling locations for persistent *L. monocytogenes* (PLM) and non-persistent *L. monocytogenes* (NPLM) in CSM plants*.

Sample location	PLM or NPLM	Number of pulsotypes
Processing environment only	PLM NPLM	0 9
Equipment only	PLM NPLM	0 16
Product only	PLM NPLM	1 9
Processing environment and product	PLM NPLM	0 1
Equipment and product	PLM NPLM	6 4
Processing environment and equipment	PLM NPLM	5 4
Processing environment, equipment and product	PLM NPLM	8 1

*Reproduced from Lunden et al (2003a).

Table 7 *L. monocytogenes* contamination sites of CSM processing equipment*

Processing machine	Contamination site	Direct / indirect food contact surface
Freezer	Spiral conveyor Supporting structures Surfaces[a]	Yes/- No/Likely Unknown
Slicing equipment	Blades Blade cover Control panel Motor Lubricant Ball-race screw Surfaces[a]	Yes/- Yes/- No/Likely No/Unlikely No/Unlikely No/Unlikely Unknown
Dicing machine	Blade Blade cover Surface under blade Product remains collector	Yes/- Yes/- No/Likely No/Unlikely
Peeling machine	Control panel Surface under the peeler Surfaces[a]	No/Likely No/Unlikely Unknown
Product scales (including MAP)	Funnel Surfaces[a]	Yes/- Unknown
Packing machine	Chamber Surfaces	Yes/- Unknown
Conveyor	Belt Supporting structures	Yes/- No/Likely

*Reproduced from Lunden et al (2003a). MAP is modified atmosphere packaging. [a]Denotes that the specific sampling sites were not known.

The observations of Lunden et al (2003) that processing equipment in Finland was persistently contaminated with the same *L. monocytogenes* pulsotypes as were isolated from final products, suggested poor equipment sanitation. Lunden et al (2003) suggested that part of the reason for the apparent sanitation issues were complex equipment designs, which made disassembly for cleaning difficult. Furthermore, some plant *L. monocytogenes* pulsotypes were categorised as persistent in one processing plant, and non-persistent in another. It is possible the PFGE typing did not recognise genetic adaptations in some strains, and that persistence as a consequence of factors such as increased resistance to sanitiser were the reason for the different *L. monocytogenes* fates. Heir et al (2004) were unable to distinguish quaternary ammonium-resistant and -sensitive strains by PFGE (Section 4.1.7.1).

Aarnisalo et al 2006 investigated post-cook contamination using a different strategy. The study made an assumption that plant technical managers see all the laboratory test results from their plants and have an intimate knowledge of their processes. Aarnisalo's strategy was to make use of this technical

knowledge and solicited the opinions of fourteen CSM plant technical managers using a postal questionnaire relating to *L. monocytogenes* contamination in Finnish plants. The responses indicated that for the meat and poultry CSM sector, the most hygienically problematic equipment was considered by plant operators to be packaging machines, conveyor belts and slicing machines. Further investigations revealed that the root cause was perceived to be poor equipment design with the worst cases being where parts of equipment could not be effectively cleaned and sanitised due to a lack of access.

In Switzerland, a food processing plant manufacturing sandwiches that used meat sliced in the plant was investigated. The investigation was required because there were frequent *L. monocytogenes* isolations from the finished products (Blatter et al 2010). A large number of swab samples were taken over the course of several visits to the plant. The problem areas identified included slicing equipment (bread slicers), conveyor belts and product handling tables. The establishment of verified, effective cleaning and sanitation procedures solved the problem of contamination with *L. monocytogenes*.

The studies summarised in this section make it clear that equipment in high risk areas can become contaminated with *L. monocytogenes*. In combination with the information in sections 4.1.1 to 4.1.4, there is a body of evidence that supports raw meat as a source of *L. monocytogenes* in CSM plants. *L. monocytogenes* on raw materials can cross contaminate into processing plant environments where some strains may establish persistent, plant-resident populations. Since effective cooking is a CCP (for *L. monocytogenes*), it is less common for the *L. monocytogenes* present on raw materials to persist until final product. Consequently, plant resident strains are the ones most commonly isolated from final product CSM (Lunden et al, 2003a). Plant resident strains can be prevented from becoming established and be removed once established by effective cleaning and sanitation. A number of publications highlight the need to disassemble equipment for effective sanitation (Tompkin 2002, Lunden et al 2013a). Where chemical decontamination strategies are ineffective the application of moist or dry heat can be used to achieve decontamination.

4.1.6 REFRIGERATION

In Greece, a survey of *Listeria* prevalence in the chillers of meat processing plants reported *L. monocytogenes* and *L. innocua* were isolated from the walls of 4.5% and 36.4%, respectively, of the

22 refrigerators sampled (Sergelidis et al 1997). One of the 22 refrigerator door handles tested was contaminated with *L. monocytogenes*.

Evans et al (2004) studied the refrigeration equipment in 15 food manufacturing plants in the UK including two that undertook CSM manufacture and one that was involved in raw and cooked poultry processing. The plants were visited twice between June and December 1994. Samples were taken from various areas including the chiller evaporator coils, drip trays and chiller walls. The study failed to detect any *Listeria* in any of the refrigerators sampled, indicating it is possible to operate refrigerators that do not harbour *L. monocytogenes*. However, the methodology to detect *Listeria* spp. has since been formalised in EC 2073/2005 around EN/ISO 11290-1, and the change in testing methodology may retrospectively affect the reported findings.

Some strains of *L. monocytogenes* can grow at temperatures as low as -1.5°C. Predictive modelling (e.g. ComBase) showed that *L. monocytogenes* can grow twice as fast at 8°C as at 5°C under ideal pH and water activity (aw) conditions. Thus, inadequate refrigeration at any point between production and consumption allows the multiplication of *L. monocytogenes* on contaminated CSM, from low levels to numbers capable of causing illness. Major CSM manufacturers, and major retailers, have already recognised the potential issues involved with consumer failures to ensure adequate refrigeration. A summary of domestic refrigeration temperatures is provided in sections 10.3 and 10.4.

The contamination of CSM with populations of *L. monocytogenes* high enough to lead to human illness in vulnerable groups is an issue for which responsibility must be shared by the FBOs who produce contaminated product, and the retailers, caterers and consumers who inadequately refrigerate these products.

4.1.7 CLEANING AND SANITATION

There was an epidemic outbreak of food borne listeriosis in 1992 in France (Goulet et al., 1993; section 6). As part of the epidemiological investigations into the outbreak, Salvat et al (1995) visited initially six

French plants under suspicion of causing the outbreak and a single control plant that was not under suspicion. Two hundred and seventy samples were collected from plant environments and final products. Sixty eight percent of the tests were positive for *L. monocytogenes* in the raw product preparation areas, with 33% positives in the finished product areas. The outbreak *L. monocytogenes* strain was identified in a single processing plant. Salvat et al (1995) considered that the major causes of product contamination were contact of cooked products with soiled surfaces, although there was also evidence of contamination from raw to final product. The authors concluded there was an inadequacy of cleaning and disinfection procedures at the plant that caused the outbreak.

Further investigations into CSM plant cleaning and sanitation was undertaken by Salvat et al (1995) as part of the study that identified the outbreak plant. A second round of visits were made to five plants to assess their cleaning and disinfection procedures. Seven percent of the samples collected from finished product contact surfaces were found to harbour *L. monocytogenes*. The authors considered that the cleaning and disinfection procedures were unable to reliably eliminate *L. monocytogenes* from the surfaces in the plants assessed (Salvat et al 1995).

Worsford and Griffiths (2001) undertook a series of assessments of cleaning and sanitation effectiveness in butchers' shops in Kent, Avon and Somerset. Cleaning effectiveness was assessed by visible cleanliness, the frequency of cleaning, and assays of selected foods and hands using adenosine triphosphate (ATP) bioluminescence. (ATP testing is a rapid method for verify general cleaning efficacy prior to the commencement of a day's manufacture. ATP is present in viable cells of all living organisms and its presence is indicative of ineffective cleaning and sanitation procedures). Worsford and Griffiths (2001) reported considerable variation in surface ATP results, both within and between butchers' shops. Despite the widely ranging results, the study reported that food and hand contact surfaces could be heavily contaminated during food production and service. *Ad hoc* interim cleaning during the day was beneficial and resulted in a significant improvement in cleanliness assessed both visually and by ATP assay. Although *ad hoc* cleaning was beneficial, these results were less satisfactory than those obtained by the use of best practice protocols. A lack of written cleaning schedules and post-clean records (including effectiveness of cleaning records), training in the correct use of cleaning products and awareness of the importance of cleaning hand contact sites were common issues (Worsford and Griffiths, 2001).

In general, effective equipment cleaning and sanitation according to validated schedules is essential because once *L. monocytogenes* becomes attached to a surface, their sessile growth can result in the generation of biofilms (Aarnisalo et al 2006). Although *Listeria* at the base of biofilms are attached to surfaces, bacteria in the upper sections of a biofilm can be motile and their movement is required to keep the biofilms oxygenated and adequately supplied with nutrients (Aarnisalo et al 2006). Motility is essential for the formation of *L. monocytogenes* biofilms (Lemon et al 2007) and motile bacteria within a biofilm can detach and contaminate food (Aarnisalo et al 2006).

As noted above (section 4.1.4), in 2003, RTE spiced ham and turkey ham manufactured in a single plant in Trinidad was recalled because the product was contaminated with *L. monocytogenes* (Gibbons et al 2006). An investigation concluded that lapses in cleaning and sanitation of food contact surfaces and unhygienic handling of products had caused the contamination. Similarly, in Switzerland, a food processing plant with *L. monocytogenes* product issues was studied to determine the key points at which *L. monocytogenes* could contaminate product (Blatter et al 2010). Identified problem areas included slicing equipment, conveyor belts and product handling tables. There was evidence that *L. monocytogenes* genotype 1/2a had persisted in the processing environment for more than nine months. After revision of the cleaning and disinfection procedures, *L. monocytogenes* was only infrequently isolated from slicers, conveyor belts or product (Blatter et al 2010). The finer details of how effective cleaning and sanitation were achieved were not reported by the study, but this work again provided evidence that inadequate cleaning regimes can allow *L. monocytogenes* to become resident in a processing plant.

Hoelzer et al (2012a) reviewed the literature relating to *L. monocytogenes* transfer onto product and its removal from equipment surfaces in meat plants as preparation for a risk assessment model. The review included information pooled from 37 studies, representing 2308 test samples, and concluded overall that transfer of *Listeria* spp. occurred routinely between equipment, workers and product. Any transfer usually involved a small number of cells, although a single point e.g. a contaminated cutting blade, would typically transfer low level contamination to many cut surfaces of product. A common theme from a number of reports relating to CSM contamination by *L. monocytogenes* is that product with a small amount of contamination that multiplied during storage under conditions of inadequate refrigeration (reviewed by Tompkin et al).

The same researchers (Hoelzer et al 2012b) also reviewed the literature relating to the effective sanitization of equipment and concluded that in the absence of protein residues, a five-log reduction in numbers of *L. monocytogenes* was achievable using hypochlorite and quaternary ammonium compounds. However, the presence of protein residues dramatically reduced the efficacy for both types of sanitizer and the other sanitisers reviewed (Hoelzer et al 2012b). Thus, irrespective of the active agent used in a particular sanitiser, effective cleaning is a pre-requisite for effective sanitation (Table 8).

Table 8 Efficacy of sanitizers in removing *L. monocytogenes* contamination from surfaces or suspensions (reproduced from Hoelzer et al 2012b)

Sanitiser type	In the absence of protein residues				In the presence of protein residues			
	No. of studies reviewed	No. of observations	Total No. of replicates	Mean reduction (log cfu)	No. of studies reviewed	No. of observations	Total No. of replicates	Mean reduction (log cfu)
Acid-anionic	3	39	78	7.1	1	4	32	5.3
Halogen	3	27	124	3.8	2	9	60	2.4
Hypochlorite	11	321	891	5.5	4	38	117	2.8
Peracetic acid	6	177	484	4.6	2	24	52	3.8
Quaternary ammonium	5	59	262	6.1	2	8	56	5.3

4.1.7.1 RESISTANCE TO SANITISING CHEMICALS

The emergence of food-related bacteria that are resistant to quaternary ammonium compounds QAC has been observed for at least fifteen years (Sundheim et al 1998), and resistance is not confined to QAC (Chapman 2003). Early studies by Aase et al (2000) revealed that one resistance mechanism employed by QAC-resistant bacteria was an ABC-type efflux pump, which could remove QAC and other toxic chemicals such as ethidium bromide from *L. monocytogenes* cells.

A review by Chapman (2003) summarised evidence that biofilms make a contribution to disinfectant resistance because they provide a mechanism for sub lethal exposure to sanitising compounds. There are at least two reports that have shown resistance to QAC-based disinfectants are more prevalent among food-borne *L. monocytogenes* isolates than isolates from pools of human, animal, faecal and environmental (e.g. soil) sources. (Aase et al 2000; Romanova et al 2002).

A more recent study by Dutta 2013 has revealed the basis of the transfer of resistance in some *L. monocytogenes* strains. Analysis of a 116-strain collection isolated from 30 clinical, food processing environments and foods revealed that all but one of the 71 benzalkonium chloride (BC)-resistant (BCR) isolates harboured a gene cluster called *bcrABC*. *bcrABC* was not detected amongst the BC-susceptible (BCS) *L. monocytogenes* isolates. The *bcrABC* sequences were highly conserved across the majority of the resistant strains. *brcABC* was most commonly carried on a large plasmid, pLM80, although in some strains *brcABC* has translocated into the *L. monocytogenes* chromosome. pLM80 was originally described in an *L. monocytogenes* outbreak strain isolated from contaminated hot dogs.

It is now well established that *L. monocytogenes* can become adapted to a range of sanitising chemicals in food processing areas. However, sanitiser resistance did not correlate with the ability of *L. monocytogenes* strains to persist in a plant. In addition, it is important to make clear that although increased resistance has been observed for compounds such as QAC, resistant *L. monocytogenes* can survive exposures at concentrations around 5-10 μg/ml. The low end of a typical commercial QAC application would be of the order of 200μg/ml. QAC-resistant *L. monocytogenes* however may be important if *L. monocytogenes* are growing in biofilms, which can reduce the effective sanitiser concentration experienced by some individual cells. Based on the information reviewed in this section, it may be prudent to advise CSM operators that it is a good practice to periodically change the active

agent in their sanitising chemical, in order to help prevent the emergence of resistant *L. monocytogenes*. Many processors in hard water areas inadvertently achieve a periodic sanitiser change by using an acid based sanitiser every few weeks, primarily to remove lime scale from equipment.

4.1.7.2 DECONTAMINATION USING HEAT

Tompkin (2002) has provided robust advice for the decontamination of processing equipment that is known, or suspected, to be contaminated with *L. monocytogenes*. Tompkin (2002) considered that most of the time, an adequate corrective action for the source of a positive *L. monocytogenes* isolation would be to disassemble the equipment and clean and sanitise the parts using specialist chemicals. On those occasions that such treatment was ineffective, Tompkin (2002) advises an equipment-specific application of heat.

For small pieces of equipment, immersion in a hot (>80^{o}C) water bath with detergent has been reported as effective (Tompkin 2002). For equipment that is larger, contains water-sensitive electronics or greased parts, heat can be applied by moving the equipment into a product-baking oven. A related, novel approach is proposed by Lindsay et al (2013) who favourably evaluated the use of a moist heat bread dough proofing machine as a way to apply heat and remove *L. monocytogenes* from CSM slicing equipment. The intention of the Lindsay study was to evaluate equipment that may already exist in stores that buy in pre-cooked meat for in-store slicing and sell sandwiches made from store-baked bread.

For equipment that is too large for an oven, Tompkins (2002) advises the electronics can be removed, the equipment covered in a heat resistant tarpaulin and steam applied from the bottom. The previous experiences of the project team are that inexpensive (£20-30) steam generators of the type used to remove domestic wallpaper perform exceptionally well when used to decontaminate industrial equipment. Tompkin considers that a target temperature of 71^{o}C should be achieved for at least 20 minutes to achieve effective equipment decontamination.

4.1.7.3 HOSES AND FACTORY DRAINS

Berrang and Franks (2012) inoculated *L. innocua* into floor drains to final concentrations of 10^4 cfu/cm^2 bound to the wall of the drain and 10^8 cfu/ml of liquid in the drain. The drains were then sprayed with a burst of low pressure (~70kPa) tap water for two seconds and any airborne listeria generated by the water impact were captured using an impaction sampler and also settle plates placed in the area around the drains. The work had robust replication and the authors observed that *Listeria* spp. was recovered from settle plates on the floor at distances of up to 4 m from the sprayed drain and from walls at heights as high as 2.4 m above the floor.

Although some authors have stated there is not much evidence for the airborne transfer of *L. monocytogenes* as an important issue for product contamination (Autio et al 1999), the Berrang group (Berrang et al 2013) undertook follow on work relating to drains. As before, model floor drains were inoculated with *L. innocua* as a model for *L. monocytogenes*. The inoculated drains were sprayed with a low pressure water hose. Broiler breast fillets (uncooked) were left uncovered on a table 2.4 m away from the drain. After 10 minutes, a different set of fillets were indirectly exposed by placement on the same table. The results showed 18 *L. innocua* cells were transferred on to each of the directly-exposed fillets. The indirectly exposed fillets were also contaminated with *L. innocua* at a level of around nine cells per fillet.

Although the experiments in the later publication used raw chicken, they demonstrate a basic transfer mechanism and it is not difficult to extrapolate that there is hazard when using even low pressure water for cleaning, if there is cooked meat in the vicinity. In some plants, it is a routine practice to remove detritus by herding it into drains using hoses during worker breaks. If such clean-downs were unavoidable, the publications justify advice that all cooked food should be removed from the hall prior to cleaning and not returned until at least 15 minutes after the end of any hose use. It is likely that the use of a dry squeegee to remove detritus is a better option during processing.

4.1.7.4 NOVEL EQUIPMENT DECONTAMINATION TREATMENTS

Leipold et al (2010) investigated the use of an electrical discharge to generate cool plasma at atmospheric pressure and room temperature. The resulting plasma was assessed as a method for decontaminating a rotating cutting blade of the type used in commercial slicers. The blade was inoculated with *L. innocua* (5×10^7 cfu/ml) and a five-log reduction was obtained after 340 seconds of plasma treatment. There were a number of potential criticisms of the study, which was undertaken from an engineering rather than a microbiological standpoint. For example, it is unclear from the methodology section of the paper how long after inoculation of the blade the treatment was applied. If the interval was only a few minutes, it is likely that the decline of planktonic *Listeria* was measured rather than sessile cells. The important issue for *L. monocytogenes* tends to be sessile biofilms (Aarnisalo et al 2006). Thus, the model used by the study may not be effective for the inactivation of *Listeria* spp. in biofilms as the charged plasma particles may cause the rupture of cells at the surface of the biofilms but not lower down. In addition, the provenance of the strain of *L. innocua* used was not reported (other than it was provided by the Danish Meat Research Institute as strain 0011). The temperature of the blade after treatment was below 30°C, and the authors considered that plasma was an effective decontamination treatment for slicer blades. Further work, ideally including multiple strains of *L. monocytogenes* growing as biofilms on the blade, would be required before the efficacy of cold plasma as a method for the decontamination of blades could be determined.

Inactivation of *L. monocytogenes* on slicers by levulinic acid (LeA) plus sodium dodecyl sulphate (SDS) was assessed by Chen et al (2014). The sanitizers were applied either as a liquid or as foam at three concentrations at a temperature of 21°C. Contaminated slicer surfaces sprayed with 1% LeA plus 0.1% SDS as a foam reduced *L. monocytogenes* populations by 6-8 log cfu/blade within one minute. Chen et al (2014) concluded that LeA-based sanitizers applied as foam can be a useful interim treatment to remove microbiological contamination from slicers.

4.1.8 WORK TABLES

During a general UK shopping basket survey, *Listeria monocytogenes* was isolated from a number of CSM samples (Willis and Greenwood, 2003). All of the isolates were of the same serotype and so the source was investigated. Investigations implicated a single Belgian CSM manufacturing plant, and

within the plant the primary source was found to be a contaminated cutting table, which was consequently replaced, ending the sporadic contamination of CSM.

One conclusion of an investigation into the source of *L. monocytogenes* in a recalled batch of CSM (Gibbons et al 2006) was that the presence of *L. monocytogenes* in biofilms on different work and food contact surfaces allowed continuous or occasional contamination of the finished CSM products (Gibbons et al 2006). More recently, Blatter et al (2010) reported that product handling tables were amongst the areas with significant potential for the contamination of product with *L. monocytogenes*.

4.1.9 THE BENEFITS OF COMPARTMENTALISATION IN CSM PLANTS

Lunden et al (2003) noted an effect for compartmentalisation on *L. monocytogenes* contamination. Two processing lines in a single plant had differing degrees of compartmentalisation and different degrees of contamination. Both processing lines produced a cooked pork and beef product independently of each other. The line that was less compartmentalised was found to be more extensively contaminated with *L. monocytogenes* for longer periods (Lunden et al 2003). A similar observation was made in two other plants, although to a lesser degree. Overall, the findings of Lunden et al (2003) demonstrate there is an advantage in terms of *L. monocytogenes* contamination if plants undertake different aspects of processing in physically separate areas.

4.1.10 PLANT EMPLOYEES

Heir et al (2004) sampled employee throats (n=70) and faeces (n=45) for *L. monocytogenes* but none of the workers harboured *L. monocytogenes*. It was concluded that *L. monocytogenes* carriage from *infected* employees was a minor risk factor for *L. monocytogenes* in final CSM products at the premises visited.

Contaminated employees and their food handling practices however are recognised as important for *L. monocytogenes* transfer. Lianou and Sofos (2007) have reviewed the key studies describing general

L. monocytogenes transmission. None of the papers reviewed related to CSM, but most describe transfer to and from other RTE food such as fresh produce. In summary, there is a realistic possibility that *L. monocytogenes* will be present on the hands of food workers and that the contamination can be transferred to food. There is a higher prevalence of food worker hand contamination compared with office workers, with *L. monocytogenes* being specifically isolated from the hands of delicatessen workers (Kerr et al 1993). In one delicatessen, genetically-identical *L. monocytogenes* strains were isolated from the hands of different workers. However, it was not clear if the result meant some strains of *L. monocytogenes* were suited to survival on hands or if certain strains dominated in some environments.

Worker contamination can occur by a number of routes (Lianou and Sofos 2007). The handling practices that have been reported as resulting in cross contamination to food are inadequate hand washing, touching unwrapped raw food without gloves, using common weighing scales for multiple foods, using the same work surfaces to handle raw and cooked foods, and handling RTE meat after handling raw meat without washing hands in between (Lianou and Sofos 2007). These two studies, in combination, make clear food worker handling practices play an important role in the transmission of *L. monocytogenes* both onto food, and around processing and retail environments.

4.1.11 THE COLONISATION OF A NEW CSM PLANT

A considered and important piece of work was reported by Berrang et al (2010). The study commenced from a viewpoint that a newly constructed commercial processing plant that manufactured diced cooked chicken would begin operations free of *L. monocytogenes,* but acquire plant resident strains at some point. In brief, the Berrang et al (2010) study monitored the colonisation process and the establishment of plant resident strains on a monthly basis over a period of 2 years.

A variety of samples were collected each month by the researchers. These included raw products, plant personnel, high traffic sections of floors, incoming fresh air and the external environment surrounding the plant. All *L. monocytogenes* isolates were typed using a sequence-based methodology.

At the beginning of the study, before any meat processing had commenced, the factory drains were completely clear of *L. monocytogenes.* One month later (i.e. at the second sampling occasion), the researchers were able to isolate *L. monocytogenes* from drains during and immediately after processing

and before clean up. When the plant had been operating for five months (the fourth sampling occasion), *L. monocytogenes* was isolated from the drains after cleaning and sanitation had been completed but before the commencement of the next day's processing. Over the study duration, *L. monocytogenes* was detected in drains on six occasions after cleaning and sanitation. On the raw meat side of the plant, the most frequent drain isolate was strain C_3. On the cooked meat side of the factory, the most frequent drain isolate was B_3.

High traffic floor surfaces in the factory were sampled to detect if *L. monocytogenes* was carried into the plant on the soles of worker's shoes. No *L. monocytogenes* was recovered from any floor swabs during the study duration. Similarly, when the filters on the air intake system were tested, there were no *L. monocytogenes* isolations. The authors specifically stated they were unable to demonstrate entry of *L. monocytogenes* into the plant via workers or fresh air intake.

When exudate from the raw meat received by the plant was tested, *L. monocytogenes* was isolated on 11 of 19 sample visits. Some of the subtypes detected on the raw meat-derived samples were indistinguishable from those isolated from the drains. Thus, raw meat was considered to be a likely source of the plant resident drain strains. In addition to the researcher samples, the FBO undertook a programme of final product testing. The FBO isolated *L. monocytogenes* from final product around 13 months after the plant opened. The contamination was traced back to a faulty piece of equipment, which was removed from the plant. The final product isolate, was strain B_3, the same strain that had colonised the post-cook area drains. The result is in broad agreement with the findings of Lunden et al (2003), who reported persistent *L. monocytogenes* were more likely to be isolated from final product, compared with non-persistent strains.

Although strain B_3 was also isolated from a water culvert that carried rooftop water away from the factory, there was no obvious vector from the factory to the culvert. Generally, the external environment samples did not contain *L. monocytogenes*. Berrang et al (2010) noted that some of the *L. monocytogenes* strains from the new plant were the same as those isolated from previous studies (Berrang et al 2005) in a different poultry CSM plant. Since there was no obvious connection between the two processing facilities, Berrang et al (2010) considered that contaminated raw materials from a common source might explain the observation.

4.1.11.1 *LISTERIA* SPP. AS AN INDICATOR FOR *L. MONOCYTOGENES*

In the early 1990s, Tomkin et al (1992) undertook a comprehensive programme of environmental sampling in 12 RTE meat processing plants. In total around 18,000 samples were taken over a two year period. Overall, 13% of the samples contained *Listeria* species, and 40% of those samples (i.e. 5% of the total samples) contained *L. monocytogenes*. A summary of the isolations on a per plant basis is provided as Table 9.

Table 9 The relationships between environmental samples testing positive for *Listeria* species and the presence of *L. monocytogenes* (reproduced from Tompkin 2002).

Plant identifier	No of positive Listeria samples	% of positive samples containing *L. monocytogenes*
1	115	96
2	90	71
3	128	62
4	328	57
5	237	54
6	204	47
7	46	41
8	85	38
9	90	34
10	219	27
11	241	23
12	318	5

The probability that a sample containing *Listeria* species would also contain *L. monocytogenes* varied between plants. Tomkins (2002) elaborated that the likelihoods however were stable between 1987 and 1991. Tomkins et al (1992) concluded that the significance of isolating *Listeria* in terms of risks for *L. monocytogenes* was plant-dependent, and a function of the unique ecologies of each plant. For Plant 1 (Table 9) the isolation of *Listeria* spp. was an excellent predictor for *L. monocytogenes*, whereas for Plant 12 (Table 9), the relationship was too poor to be of benefit.

4.1.11.2 SUMMARY AND CONCLUSIONS FROM PROCESSING ENVIRONMENTS

In summary, the review of the literature relating to CSM processing plant contamination by *L. monocytogenes* has determined that *L. monocytogenes* can persist in CSM plant environments for extended periods of several years. Raw meat entering CSM plants is likely to be the original source of plant persistent *L. monocytogenes*. Plant persistent strains are those most likely to be isolated from final products. Persistent strains can be isolated from drains, the plant infrastructure, worker's hands (with the worker being contaminated rather than colonised) and processing equipment, including equipment in the high care areas of plants. Equipment that has surface damage or that has not been designed to be cleaned easily is of particular concern.

Vacuum tumbling raw meat in refrigerated brine can allow the growth of *L. monocytogenes* naturally present on raw meat to more than six logs/g over the course of a 12-hour treatment. Such large inoculations of *L. monocytogenes* have the potential to survive cooking to 68.4^{o}C but not to 72.6^{o}C. Quantitative testing of CSM for *L. monocytogenes* can result in reports of less than the detection limit of the test method, but the same sample can test positive for *L. monocytogenes* after enrichment, indicating contamination of cooked CSM with very low numbers of *L. monocytogenes* rather than eradication. For effective thermal processing, the rate of heating of the meat is important. Slow heating can allow *L. monocytogenes* sufficient time to upregulate stress-response genes, which help protect cells against environmental stresses such as heat.

Surveys of commercial product chillers in Greece and the UK determined there was not widespread contamination by *L. monocytogenes* (NB: the UK study used an older test methodology considered appropriate at the time). A survey of plant technical managers believed packaging machines, conveyor belts and slicing machines are problematic equipment, likely to harbour *L. monocytogenes*. Their opinions are supported by evidence that knock out tables, work surfaces and food contact surfaces on processing equipment can become contaminated.

Cleaning and sanitation of the plant environment and equipment, particularly chilled vacuum tumblers, product conveyors and slicing equipment is key to preventing *L. monocytogenes* contamination of finished product. There is evidence that *L. monocytogenes* can become more resistant to sanitising chemicals over time. *L. monocytogenes* cells can grow in combination with other cells as biofilms, which have resulted in reduced cellular exposure to sanitisers. Biofilm growth may be a factor driving

increased sanitiser resistance. It is a good practice to change periodically sanitising compounds, in order to help prevent the emergence of sanitiser-resistant *L. monocytogenes*.

4.2 DELICATESSENS AND SME RETAILERS

Slicers are commonly used in the delicatessens and it has been known for many years that slicing blades can transfer *L. monocytogenes* to and from delicatessen foods (Gilbert and Maurer, 1968). Although reported as a potential hazard for more than 20 years, Humphrey and Worthington (1990) reported *L. monocytogenes* isolations from four of the 32 (13%) slicer blades that were sampled in UK butcher shops in the late 1980s. Slicer blade contamination, according to Hudson and Mott (1993a), can occur from the outside of the packing (i.e. the casing) if it is not removed prior to slicing. More generally, Chen et al (2014) discussed that slicers are used intermittently throughout a working day and are typically not cleaned after every use. Important points made by Chen et al (2014) was that slicers are exposed to potentially contaminated foods, have large contact areas between the food and the equipment, and most crucially, slicer surfaces are at ambient temperatures that support microbiological growth.

Worsfold and Griffith (2001) undertook an assessment of cleaning regimes and hygiene standards in butchers' shops in three local authorities (Kent, Avon and Somerset) in the UK. The assessments were by detection of ATP rather than specific microbiological testing. The conclusions of the work was that there was significant variation in the amounts of contamination both within and between surfaces in butchers' stores. Food and hand contact surfaces could become heavily soiled during food production and service. Worsfold and Griffith (2001) observed that staff undertook raw and cooked product handling throughout the day, which could lead to hands and food contact surfaces becoming contaminated. Contamination could then be transferred to dedicated cooked product equipment and surfaces such as chiller door handles, tap handles, soap dispensers, raw meat cleaning cloths and the buttons of telephones and cash tills. Observations carried out when businesses were busy determined that the risk of cross-contamination was increased if fewer staff were available for service and the hand washing facilities were not located at the front of the store (Worsford and Griffiths, 2001). An important point made by the authors was that the degree of soiling tended to be underestimated when only a visual assessment was made. The authors noted that visual assessment however was the method most commonly used by the store workers and enforcement personnel. A summary of the

degree of contamination in the stores is provided as Table 10. Worsfold and Griffith (2001) reported there was a significant benefit for the interim cleaning of slicer blades. On average, during use, slicer blades were contaminated with around 34,000 RLU (relative luminance units). An interim clean reduced the average RLU on blades by 88% to around 3800. As part of the conclusions of their studies, Worsford and Griffiths (2001) devised a list of best practices to reduce cross contamination in butcher's shops. These recommendations are listed as Table 11 and Table 12.

Table 10 A summary of the cleanliness of surfaces in UK butcher shops
(Reproduced from Worsfold and Griffith, 2001)

Surface	Visible heavy soil	Visible light soil	Visibly clean	ATP assessment (% pass defined as <500 RLU)
Hot tap wash hand basin	16	18	66	4
Wash hand basin	10	10	80	ND
Chiller interior	30	10	60	ND
Chiller door handle	5	5	90	4
Cooked meat surface in use	5	15	80	ND
Cooked meat scale pan in use	5	50	45	4
Meat slicer in use	10	75	15	4
Vacuum packer	10	30	60	8
Wiping cloths	5	15	80	0
Till keys	35	30	35	0
Butcher's apron	40	40	20	0

ND is not determined

Table 11 Recommendations to improve cleaning and disinfection schedules in butchers' shops (Reproduced from Worsford and Griffith, 2001)

Cleaning and disinfection schedules
Segregate cleaning activities.
Protect packaging and product during cleaning activities
Identify the frequency and timing of cleaning and disinfection
Include instructions for dismantling equipment
Include hand contact sites in the cleaning schedule
Identify who is responsible for checking cleanliness
Monitor efficacy of cleaning by visual inspection of difficult to clean areas
Use rapid hygiene test kits to verify cleaning

Table 12 Recommendations relating to the use of cleaning materials and equipment to improve hygiene in butchers' shops
(Reproduced from Worsford and Griffith, 2001)

Cleaning materials and equipment
Use colour coded cloths where possible
Keep cleaning cloths separate at all stages of use including their cleaning and disinfection
Use separate buckets for wash and rinse waters
Use disposable cloths or paper for cooked meat surfaces
Use chemical dispensers so that recommended dilutions can be prepared
Use food grade sanitizers on cooked product surfaces and hand contact surfaces
Apply sanitizers with hand sprayers to fixed high-risk surfaces food and hand surfaces both during interim and final cleaning procedures.
Use paper towels for drying high risk surfaces
Use adequate sized sinks for washing equipment
Use brushes for scrubbing equipment and surfaces
Use correct water temperature for cleaning
Ensure equipment is adequately dismantled to allow for proper cleaning
Use correct contact times for cleaning chemicals
Use fresh dilutions of cleaning chemicals
Use compatible detergents and sanitizers
Rinse to remove detergent or sanitizer depending on type
Hand washing facilities
Provide taps that do not require hand operation
Supply water of constant temperature to wash hand basins
Site wash hand basins close to the service area
Use paper towels for hand drying
Handling cooked ready-to-eat products
Avoid bare hand contact
Use separate packaging for cooked products
Use disposable aprons during the setting up periods to reduce contamination of the protective clothing

The Food Safety Authority of Ireland (FSAI, 2011) undertook a baseline survey of *Listeria* contamination of slicing equipment in retail and catering premises between September and December 2009. The survey examined more than 2500 swabs taken from 665 meat-slicing machines in the Republic of Ireland. Swabs were rubbed over the blade, meat holder, back plate and product collection table, where the sliced product was deposited. Overall, 0.7% (19/2,664) of the test samples yielded a *Listeria* species. Of these, 0.26% (7/2,664) were *L. monocytogenes*. *L. monocytogenes* was isolated from a slicer back plate (n=2), product collecting table (n=1), meat holder (n=2), and a rotary blade of the cooked meat slicer (n=2). Although supplementary information was collected as a strategy for the identification of risk factors, the low number of detections confounded robust statistical comparison.

Although samples from 55 different models of meat slicer were tested, there was no significant correlation between make of meat slicer and presence of *Listeria* species. Furthermore, there was no correlation between the location swabbed and the presence of *Listeria* species. A general conclusion from the FSAI was that the prevalence of *Listeria* on slicers in Ireland was quite low, although widespread issues relating to the frequency and effectiveness of cleaning and sanitation of slicers were identified. In contrast to the FSAI conclusions, slicing of cooked meat at butchers and delicatessens was determined by risk assessment to be a key factor in the post-processing contamination of CSM. Shop sliced CSM was 1.7 times more likely than pre-packed products to be the cause of fatal listeriosis compared with pre-packaged deli meats (Endrikat et al, 2010).

4.2.1 SURVEYS OF CSM CONTACT SURFACES OUTSIDE OF THE EU

An investigation of surfaces in a delicatessen in New Zealand by Hudson and Mott (1993a) reported *L. monocytogenes* in two of the 60 samples (3.3%) collected and tested. The positive tests were for samples taken from a CSM slicer and a knife used to cut retail portions of CSM (Hudson and Mott, 1993a).

4.2.2 STORE EMPLOYEES

Worsfold and Griffith (2001) observed that staff undertook raw and cooked product handling throughout the day, which could lead to hands and food contact surfaces becoming contaminated.

Gibson et al (2013) undertook observation of employee behaviour and determined the mechanisms and degrees of cross contamination in a mock retail delicatessen in the USA. The work measured cross contamination by quantitative determination of a fluorescent marker compound concentration rather than *L. monocytogenes*. Twenty one participants were recruited to the study, provided with training from an experienced industry professional, and then given a series of tasks to perform (Table 13).

Table 13 Typical delicatessen worker tasks undertaken by mock delicatessen participants

Sequential task
Go to refrigerator
Remove meat labelled “A”
Unwrap meat and save plastic wrap
Place meat on carriage tray of slicer
Turn power switch to on
Adjust slicer knob to setting 2
Slice and dispense five pieces of meat
Turn power switch to off and release meat grip
Place meat into a plastic bag labelled “A”
Re-wrap meat “A” and return to refrigerator
Repeat for meats “B” and “C”

In the Gibson study, meat “A” was evenly coated with 5µm melamine copolymer resin beads that fluoresced under ultraviolet light. Swabs soaked in ethanol were used to recover the beads from employee hands, personal protective equipment, various sections of the slicer as shown in Figure 13 and sliced and whole product.

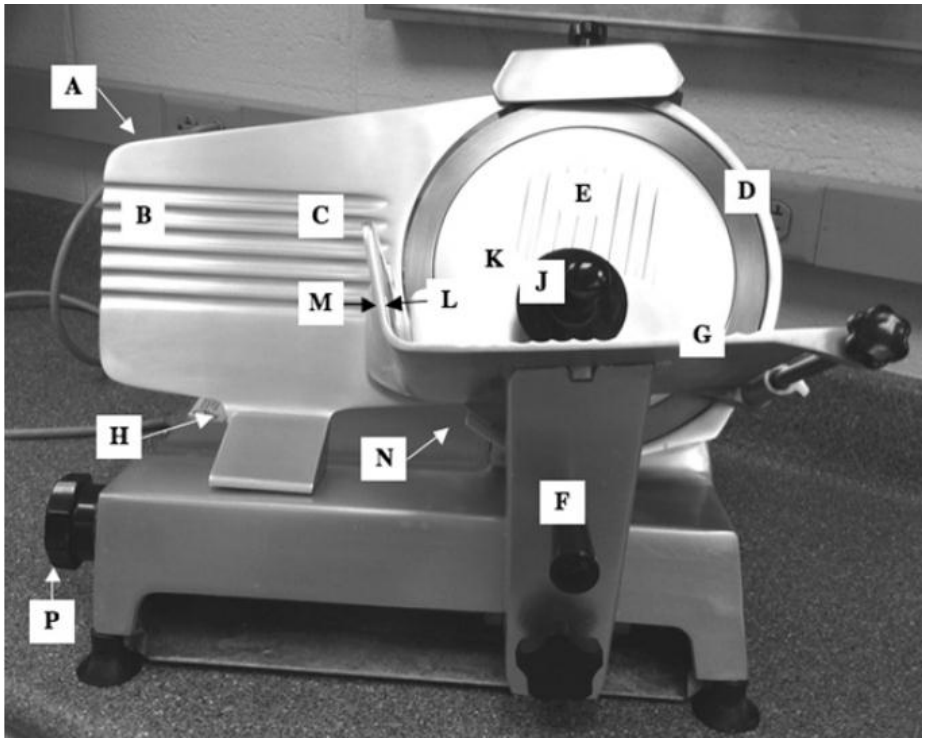


Figure 13 Surface areas swabbed on a delicatessen slicer. Reproduced from Gibson et al 2013

A, back of slicer; B, back plate (left); C, back plate (right); D, blade; E, blade guard; F, carriage handle; G, carriage tray; H, collection area; J, meat grip; K, reverse meat grip; L, inside side wall of carriage tray; M, outside side wall of carriage tray; N, side wall of collection area; P, slicer index knob.

In addition to the quantitative determination of spread of fluorescent compound (FC), the study participants were video recorded as they performed their tasks. The video footage was scored for hand contact events by four separate researchers. The participants were not informed as to the study purpose until after they had completed their tasks.

The results of the distribution of fluorescent compound are summarised as Figure 14. There was a large variation in the recovery of FC from surfaces. However, it was apparent that high concentrations of FC were recovered from the slicer carriage tray, sliced meat collection area, meat grip and the worker's gloves. In combination, these sites contributed to more than 40% of the average total FC collected. Despite the large recovery variation, these four surfaces had significantly higher ($p < 0.05$) concentrations of FC when compared with the 12 other surfaces (Figure 14). The outside wall of the carriage tray also contributed higher amounts to the total concentration of the FC, although the authors confess the area was not swabbed after slicer use for every participant.

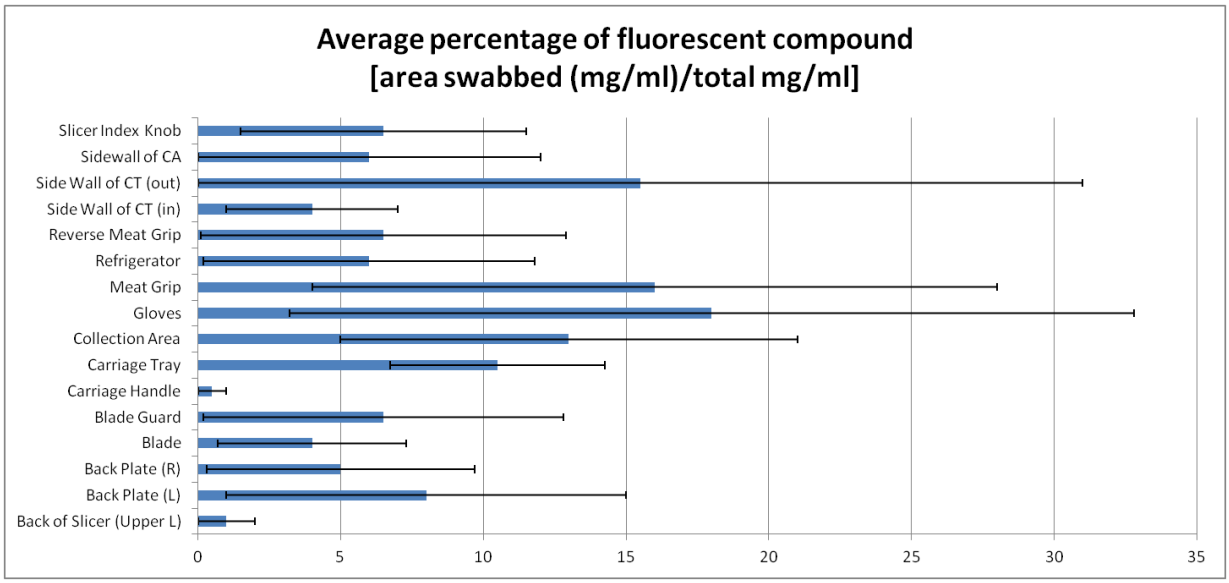


Figure 14 Average percent concentration of fluorescent compound collected by surface swabbing for all participants. Error bars are standard deviations. L is left; R is right; CT is carriage tray; CA is collection area. Reproduced from Gibson et al 2013.

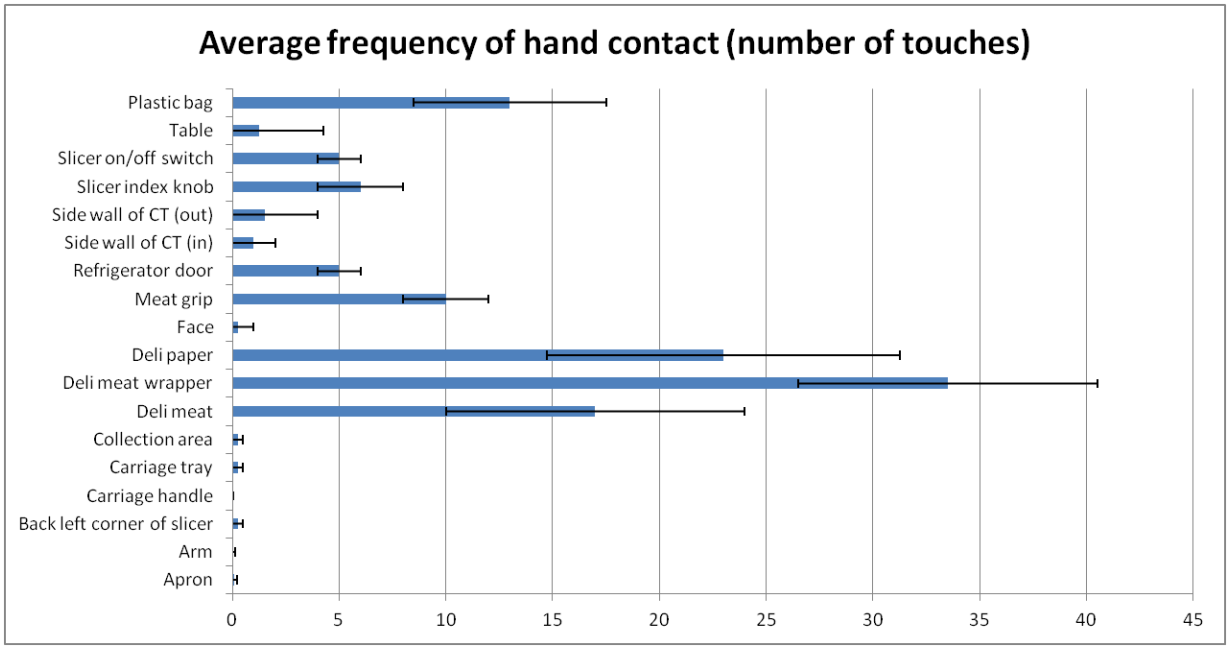


Figure 15 Average hand contact frequency by surface for all participants. Error bars are standard deviations. CT is carriage tray. Reproduced from Gibson et al 2013.

The analyses of the video footage revealed that, on average, each of the participants made 118 hand contacts when undertaking the task of slicing three meats. The average number of hand contacts grouped by surface for all participants is reproduced as Figure 15. The highest contact surfaces were the meats, meat wrappers and the sliced meat paper wraps. These surfaces had statistically significantly higher contacts compared with the 16 other surfaces. The number of contact events for the plastic bags, slicer meat grip, slicer index knob, and slicer on/off switch were also significantly higher than the remainder of the surfaces. The seven surfaces with elevated contact event counts received 92% of all hand contact events on average per participant. The results of the Gibson et al (2013) study are broadly reinforced by more recent studies undertaken by Chen et al (2014).

The observations of the Gibson study and the work of Worsfold and Griffith (2001) are important because they identify some of the mechanisms that spread contamination such as worker hands, gloves and any surfaces that are contacted by hands and gloves. The authors consider that knowledge of these mechanisms allows for a strategy of targeted cleaning and sanitation to contamination hot spots. Furthermore, advising delicatessen workers of the locations of contamination hot spots may modify worker behaviour resulting in more frequent glove changes or hand washing thereby lowering the incidence of cross contamination.

5 FOMITE-MEDIATED TRANSFER OF *L. MONOCYTOGENES*

Fomites are inanimate objects, such as any surface found in a processing plant, or substance, such as clothing, furniture, or soap, that is capable of transmitting micro-organisms from one substrate to another. As noted above, slicer blades used in the production of sliced meats are significant examples of fomites, especially when biofilms of *L. monocytogenes* become established on them (Hoelzer et al 2012a).

Construction and refurbishment within a CSM plant was hypothesised as being a source of dust which contained *L. monocytogenes* (cited by De Roin et al 2003). The plant was responsible for a major listeriosis outbreak in 1998 in the USA (Mead et al 2006). The dust had contaminated CSM after cooking but before packing, consequently, De Roin et al (2003) studied the ability of dust-like particles to carry *L. monocytogenes* and contaminate frankfurters, bologna sausage, chopped ham, and deli-style roast beef. The study used sand as a model for dust and found that a five-strain cocktail of *L. monocytogenes* could survive desiccation for extended periods; 151 days at 10^{o}C and 88% relative humidity, 136 days at 10^{o}C and 0% relative humidity, 73 days at 22^{o}C and 40% relative humidity, and 82 days at 22^{o}C and 0% relative humidity. The study concluded that since plant processing environments were usually characterised by low temperatures and high humidities, dust particles could harbour *L. monocytogenes* and subsequently be able to contaminate RTE cold meat cuts for several months. Hence air handling systems, which filter air and remove dust are an important control measure.

A small study undertaken in Finland sampled and tested the tools, protective clothing, worker's hands and the processing environment for indicator bacteria and *L. monocytogenes.* Of the 71 samples tested, a single sample from a screwdriver tested positive for *L. monocytogenes* (Aarnisalo et al 2006).

Midelet et al (2006) studied the attachment of *L. monocytogenes* to typical processing plant surfaces; stainless steel, polyvinyl chloride (PVC) and two polyurethanes. The authors allowed biofilms to develop on the surfaces, also including some non-pathogenic bacteria in co-culture with the *L. monocytogenes*. The ability of the organisms in the biofilm to pass on to an applied solid surface (tryptone soy agar) modelled meat in contact with equipment surfaces. The system was further

developed by observing how two dilute cleaning agents, a detergent sanitiser containing sodium hydroxide and sodium hypochlorite, and a disinfectant based on glutaraldehyde and QAC affected the biofilms. Key findings were that pure cultures of *L. monocytogenes* did not effectively colonise the stainless steel. However, when *L. monocytogenes* were in co-culture with the non-pathogenic bacteria, differences in colonization of the substrates was significantly improved. Thus, *Listeria* biofilm production could be enhanced by co-culture as would undoubtedly happen in plants producing sliced meats.

Midelet et al (2006) also noted that sub-lethal concentrations of the cleaning agents resulted in the remaining populations adhering more strongly to the substrate surface, and having a higher resistance to the agent. The authors did, however, note that the disinfectant concentrations used were very low, compared to those used by commercial processors. Finally the lethal effect of the dilute cleaning agents on *L. monocytogenes* in mixed culture biofilms appeared to be dependent on the non-pathogen members of the biofilm, with some bacterial species providing more protection than others.

Thus, the studies of Midelet et al (2006) highlighted that the nature of the fomites will affect transfer of *L. monocytogenes*, as will the nature of the other organisms present in the biofilm. Finally, the procedures used to clean and sanitise surfaces must use appropriate concentrations of reagents, or the result could be a population of more adherent organisms with increased resistance to the cleaning agents.

Whilst investigating the transfer of *L. monocytogenes* from artificially contaminated product to work surfaces, Vorst et al (2006a) found that the back plate, table, guard, blade and collection areas of a commercial slicer became contaminated with *L. monocytogenes*. In addition, the study observed that *L. monocytogenes* could be isolated from up to 30 slices of uncontaminated product that was cut after slicing contaminated product, without cleaning the blade in-between. The authors noted that meats with high fat content caused a visible ring of fat on the slicer blade which "likely prolonged *L. monocytogenes* transfer". Similar visible rings did not form when blade were used to cut a lower fat product such as cooked turkey breast.

Vorst et al undertook electron microscopic examination of new and used slicer blades and concluded that slicer blades become pitted and scratched through routine use and cleaning. They suggested that worn blades can serve as sites for bacterial attachment and extended bacterial transfer during slicing (Vorst et al 2006a).

Continuing with their investigations that worn blades can serve as sites for *L. monocytogenes* attachment, subsequent studies by Vorst et al (2006b) used a weak, medium and strong biofilm producing cocktail of *L. monocytogenes* to determine the effect of stainless steel roughness on *L. monocytogenes* transfer to turkey breast and bologna during slicing. Knives made from two steels were used; grade 304 with a relatively rough surface and grade 316 stainless steel with a smoother surface. There was greater initial transfer of *L. monocytogenes,* and it was more persistent with grade 304 blades for all three meats studied (Vorst et al 2006b).

The kinetics of bacterial movement from CSM to fomites such as food contact surfaces, and back, are complex. However, one factor that enhanced transfer from a circular blade was the application of greater force to the cutting blade (Vorst et al 2006a). The finding conflicts with the later observations of Sheen et al. (2010; Sheen and Hwang 2011) who discussed the possibility that bacterial cells are killed on the edges of slicer blades because surface shear forces generate instantly lethal high temperatures during slicing. The greater forces applied by Vorst et al (2006a), would likely result in greater shear. Chen et al (2014) clarified that any shear-based mechanisms of lethality have yet to be proven.

Lin et al (2006) investigated the transfer of *L. monocytogenes* onto oven-roasted turkey and bologna during slicing using commercial-scale equipment. Blade contamination from 10^1 to 10^3 cfu/blade were investigated, and different sample sizes (an entire meat package versus 25 g content) were studied. *L. monocytogenes* detection efficiency (BAX-PCR versus enrichment culture) and the survival of *L. monocytogenes* during refrigerated storage were also determined. The number of *L. monocytogenes*-positive CSM samples increased with increasing initial blade contamination concentrations. For turkey and bologna respectively, 80 and 3 positive CSM samples were recorded using 10^3 cfu/blade, whereas only 12, 8, and 2 positive samples were observed for 10^1 cfu/blade. For the lowest contamination levels on the blades, *L. monocytogenes* detections were not achieved until after 60 days refrigerated storage of the CSM. Sampling an entire meat package (75-130g) was found to be significantly more effective

than analysis of a 25 g sample only for roast turkey (12.5 vs. 7.5% +ve samples, *P*<0.05). The use of the BAX-PCR significantly improved detection rates compared with enrichment culture, but only for roast turkey meat (*P*<0.05). The findings of Lin et al (2006) were in broad agreement with those reported by Vorst and colleagues (2006a, 2006b).

Keskinen et al (2008a) investigated the transfer of surface-dried *L. monocytogenes* during the slicing of roast turkey breast at higher concentrations of *L. monocytogenes* than those used above. Strong and weak biofilm-forming cocktails of *Listeria* strains were inoculated onto stainless steel blades (10^6 CFU/blade) under ambient conditions (22^{o}C and 78% relative humidity) and the meats were sliced six or 24 hours post-inoculation. Cells were applied with or without sub-lethal injury, inflicted by a cold-shock at 4^{o}C for 2 h or exposure to chlorine-at 100 ppm for 1 min. The strong biofilm-forming *Listeria* cocktails had a greater cumulative transfer (3.62 log cfu/30 slices) than weak biofilm-forming cocktails (3.12 log cfu/30 slices) in turkey. The number of *L. monocytogenes* cells transferred was typically 3.5 log cfu. The drying time significantly reduced the number of cells transferred (3.72 log cfu/30 turkey slices after 6 h drying compare with 3.01 log cfu/30 turkey slices after 24 h drying; P<0.05). Sub-lethal injury inflicted by low temperature also affected the transfer of *L. monocytogenes*. For sliced turkey, the cold-shock significantly (p<0.05) increased the transfer (3.69 log CFU), compared with an uninjured control (3.30 log cfu) and injury by chlorine exposure (3.12 log CFU).

Subsequently Keskinen et al (2008b) also showed that the numbers of *Listeria* transferred decreased as the number of slices increased. *L. monocytogenes* was quantifiable without enrichment or extended refrigerated storage for up to 16 slices of CSM, in broad agreement with the previous studies reviewed above (Vorst et al 2006a; Vorst et al 2006b).

As part of routine surveillance, Garrido et al (2009) deliberately collected two different freshly-sliced CSM samples from delicatessens (Table 18). The samples were typically a cooked ham and a cooked turkey that were sliced consecutively in the same slicer. Garrido undertook PFGE-based typing of all *L. monocytogenes* isolated from the paired samples and demonstrated that for six out of 11 establishments, there were identical *L. monocytogenes* pulsotypes in both products. The study findings add further evidence that delicatessen-slicing equipment can transmit *L. monocytogenes*.

Perez-Rodriguez et al (2007) studied the transfer of the pathogens *Staphylococcus aureus* and *Escherichia coli* O157:H7 from slicer blades to CSM. Twenty slices were studied, post inoculation of the slicing blade, and all were contaminated, despite less than 100 cfu/cm^2 being applied to the blade. The work demonstrated that the phenomenon of low levels of inoculum contaminating large numbers of slices is not restricted to *Listeria* spp.

As discussed previously (section 4.2.2), the number of contact events for slicer meat grip, slicer index knob, and slicer on/off switch were all counted by Gibson et al (2013). These slicer surfaces were contacted significantly higher than the remainder of the surfaces. The results of the Gibson et al (2013) study are broadly reinforced by more recent studies undertaken by Chen et al (2014), who also determined cross contamination between slicer blades and slicer food contact surfaces and the products being sliced.

In summary, broadly similar findings were reported by a number of researchers using a range of concentrations of strain cocktails of *L. monocytogenes* (Sheen 2008; Sheen and Hwang 2008). At 10^3 cfu/blade, the contamination transferred to slices of meat was sporadic and difficult to model. Based on the conclusions of five publications, it appeared that for low-level contaminations of around 10^3 cfu/blade, the transfer of *L. monocytogenes* was variable, although as many as 150 subsequent slices could be sporadically contaminated with low numbers of *L. monocytogenes*.

Slicer food contact surfaces can transfer *L. monocytogenes* both to and from the products being sliced.

6 OUTBREAKS OF LISTERIOSIS DUE TO *L. MONOCYTOGENES* IN CSM

Statutory testing of CSM and a zero tolerance to *L. monocytogenes* were introduced in the USA in 1989 as the US Food Safety Inspection Service (FSIS) response to a multi-state listeriosis outbreak with multiple fatalities (FSIS, 1989; FSIS, 2003; Olsen et al 2005; Gottlieb et al 2006). The 1988 outbreak was caused by consumption of contaminated frankfurters. Plant B was identified as the source of the previous frankfurter outbreak in 1988 and a turkey CSM outbreak in 2000. Although isolated more than a decade apart, the two outbreak strains were indistinguishable by PFGE typing. Olsen and colleagues (2005) discuss the strong possibility that the outbreak strain may have persisted in plant B for at least 12 years periodically and sporadically contaminating product. Despite this relatively early recognition of the dangers posed by *L. monocytogenes* many more outbreaks were to follow.

One of the first European reports of an outbreak of listeriosis occurred in France throughout 1992. Over the yearlong period that the outbreak persisted, there were 279 infections, 22 abortions and 63 deaths (Goulet et al., 1993). The outbreak was caused by *L. monocytogenes* serotype 4b phagetype 2389:3552:2425:1444:3274:2671:47:52:108:340, which was isolated from some patients. The French government undertook an extensive national investigation that included case control studies and extensive interviews with infected consumers. Despite the isolation of a specific phage type from affected patients, the size and duration of the outbreak meant it was possible that there was more than one source of *L. monocytogenes*. Consequently, the decision as to the likely source was based largely on the results of the patient interviews. After extensive statistical analysis, consumption of a product called 'langue du porc en gelee' (a cooked joint of pork packed in a bag containing cooking residue gelatin) was reported as the most likely source of the illness (Goulet et al, 1993). There have been other outbreaks caused by similar products and the same *L. monocytogenes* phage type in France. In 1999, ten cases of listeriosis were reported; two adults with an underlying medical condition and one infant died because of the infection. Improved diagnostic procedures allowed the contaminated food to be identified as "rillettes" (a ready to eat sliced meat with consistency similar to pâté). Rillette samples yielded *L. monocytogenes* of phage type 2389:3552:2425:1444:3274:2671:47:52:108:340 with macro-restriction patterns that matched the outbreak strain (de Volk et al. 2001). Also in 1999 and in France, 32 patients presented to physicians with listeriosis. Five adults, all with underlying medical conditions, died. The infection resulted in one spontaneous abortion and seven premature births. Four of the premature neonates died. The *L. monocytogenes* strain that caused the outbreak was again the

2389:3552:2425:1444:3274:2671:47:52:108:340 phage type. No isolations were made from food, but case control studies strongly implicated another jellied pork product as the likely source (de Volk et al. 2001).

Mead and colleagues (2006) reported what they believed to be the second largest outbreak of listeriosis in US history. In total, 108 people were infected by *L. monocytogenes*, with 14 fatalities (not including the deaths of four unborn children; CDC, 1999). The outbreak lasted from January 1998 to February 1999 with illnesses recorded in 24 US states before a product recall caused an abrupt end to the incident (Mead et al 2006). Initial case control interviews implicated frankfurter-style sausages and deli-style meats as likely sources of the outbreak. Investigation and laboratory testing of the brands of these foods consumed by patients focussed attention on a single company, Bil Mar Foods (a subsidiary of the Sara Lee Corporation). Extensive testing of products, including a part-consumed package of frankfurters from a patient's refrigerator narrowed the field further to a processing plant owned by Bil Mar. The plant manufactured both frankfurters and unsliced deli-style meat. During their investigations into the outbreak, Mead and colleagues undertook an extensive programme of isolate typing using PFGE. Those assiduous efforts significantly advanced our understanding of cross contamination between foods for *L. monocytogenes*.

For example, there was serological evidence that illness was caused by the consumption of one of two types of deli-style meat consumed by different family members. One of these meats was manufactured at processing plant A, whereas the other meat was not. However, both meats were bought from the same deli and sliced on the same machine. Mead et al (2006) considered that it was highly likely that cross contamination via the deli slicer had occurred. It was one of the first pieces of evidence that supported cross contamination from different CSM types via slicing blades as a significant enough issue to have caused human illness.

Mead et al (2006) discuss the possibility that equipment upgrades within the processing facility may have played an important role in triggering this outbreak. In July 1998, an old ceiling-mounted chiller unit was cut into smaller sections on site, removed and replaced with a new unit. The authors speculated that the outbreak strain had colonised the old refrigeration unit and that its removal led to increased contamination of production equipment, environmental surfaces, and the meats produced thereafter. A hypothesis by De Roin et al (2003) was that dust generated by the construction work was

the vector that spread the contamination. There is some evidence to support the Mead hypothesis, although there was insufficient routine testing undertaken by the plant operators to confirm that was absolutely the case. Overall, eight packages of frankfurters and seven packages of deli meat were made available to researchers for testing. *L. monocytogenes* serotype 4b was isolated from six packs of these products with a pulsotypes that were indistinguishable or closely related to the outbreak strain. Mead and colleagues were unable to isolate the outbreak strain from the factory environment because as soon as an issue was suspected, the plant management instigated extensive upgrades to basic cleaning and sanitation and plant environment samples were taken only after these hygiene improvements. After lasting for more than one year, the outbreak abruptly ended when 35 million pounds (roughly 15,800 tonnes) of product from processing plant A was recalled. There were a number of key conclusions from the Mead study. The first, based on the laboratory test results for numbers of *L. monocytogenes*, was that significant outbreaks could occur even when only low levels of *L. monocytogenes* contamination were detected in the food that caused the illness. Mead and colleagues also noted that a final decontamination stage after packing could help prevent similar large outbreaks in future.

Also in 1999, there was a separate small multistate outbreak in the United States that was caused by the consumption of deli meat. The report of the outbreak is short and provides little detail other than to record that there were two fatalities (CDC, 1999).

Between May and December 2000, there were 30 clinical isolations of *L. monocytogenes* with indistinguishable PFGE patterns isolated from 11 US states (Olsen et al 2005). There were four deaths and three miscarriages caused by the outbreak. A case-control study implicated sliced processed turkey purchased from delicatessens. A traceback investigation identified a processing plant (A) and a packing plant (B) which further processed and packed meat manufactured by plant A as the likely sources of the outbreak. Environmental swabs taken at plant A did not contain *L. monocytogenes*. However, as a precaution Plants A and B recalled 16 million pounds (7,250 tonnes) of processed meat.

An outbreak of febrile gastroenteritis occurred in Los Angeles, USA in the summer of 2001 (Frye et al 2002). Sixteen of the 44 people attending a commercially-catered party became ill with symptoms of one or more of fever, diarrhoea, headache and vomiting. After laboratory testing, six stool specimens from those experiencing illness were discovered to contain *L. monocytogenes*. Fifteen of the ill people

recalled having eaten cooked turkey sandwiches. Food that had been served at the party was examined and leftover cooked sliced turkey was tested and found to contain 10^9 CFU *L. monocytogenes*/g meat. All patient and food isolates were serotype 1/2a and had matching DNA fingerprints as determined by PFGE. However, when the outbreak pulsotype was checked against the Centres for Disease Control's (CDC's) PulseNet listserv there were no matches with outbreaks in other areas. The authors suggested that a single pack of cooked sliced turkey was contaminated and noted that the walk in refrigerator at the delicatessen where the CSM was bought was running at a temperature of 11-12^{o}C (Frye et al 2001). The authors noted that *L. monocytogenes* typically multiplies rapidly at temperatures above 3^{o}C (Wilkins et al 1972) and speculated that low numbers of *L. monocytogenes* had multiplied due to the ineffective refrigeration, leading to the outbreak.

A listeriosis outbreak affecting nine US states between June and October 2002 affected fifty-four case patients and caused eight deaths not including three pregnant women who lost unborn babies (Gottlieb et al 2006). The case control investigation revealed that the consumption of turkey deli meat was associated with the illness. Consequently, an investigation of several turkey processing plants was commenced. The outbreak strain was found in the environment of one processing plant and in turkey products obtained from a second plant. In combination, the processing plants recalled a mass of more than 13 million Kg of product, which ended the outbreak.

In late August 2008, the Canadian Food Inspection Agency and a Canadian CSM manufacturer called Maple Leaf Foods jointly issued a product recall for Sure Slice brand CSM, along with a warning that consumers should not eat any products in the extensive Sure Slice product range (Powell et al 2011). The warning was a response to a listeriosis outbreak that had commenced in May 2008. Maple Foods supplied hospitals and elderly care homes and the outbreak ultimately resulted in 22 deaths and at least 57 other infections where the vulnerable group patients survived (Weatherill, 2009). The outbreak was documented extensively because of a number of factors, including class action lawsuits against the Canadian Government and the CSM manufacturer. In addition, the outbreak was subsequently scrutinised by an independent investigator, Sheila Weatherill, supported by an expert advisory group because of a lack of confidence by the public in the government's ability to manage public health, driven largely by the high numbers of deaths. In total almost 200 batches of CSM were recalled.

The key events for the well-documented outbreak are shown as Table 14.

Table 14 Key events in the 2008 Canadian listeriosis outbreak (adapted from Weatherill, 2009)

Month (2008)	Event
February to July	Sporadic positive *Listeria* test results from the Maple Leaf processing plant environment
June 3	First human illness identified
June 17	First death linked to listeriosis
July 10	First two listeriosis cases in the outbreak identified through matched DNA fingerprints of patient isolates
July 18	Case control studies first identify Maple Leaf Foods as a possible source of contaminated food products
July 22	11 food samples from a Toronto long-term care home sent for microbiological testing
July 29	More than double the normal number of listeriosis cases reported by almost half of Ontario public health units
August 4	CSM samples from long-term care home test positive for *Listeria monocytogenes*
August 7	The Canadian Food Inspection Agency initiates a food safety investigation
August 12	DNA fingerprinting matches isolates from several patients in several provinces
August 13	Maple Leaf Foods advises distributors to hold certain products
August 16	The CFIA confirms *Listeria monocytogenes* fingerprint match between Maple Leaf Foods (Sure Slice) product isolates and patient isolates
August 24	Product recall of affected Sure Slice batches

A summary from the investigators report was that the most probable contamination source for the Maple Leaf products was "commercial meat slicers that, despite cleaning according to the manufacturer's instructions, had meat residue trapped deep inside the slicing mechanisms" (cited by Powell et al 2011). Although the source was identified with a high degree of confidence, the Weatherill report made 57 recommendations that were aimed at addressing systematic failures from processing plant and senior executives within the processor to laboratories and healthcare professionals within government. Mason (2009) summarises the most significant of these recommendations by identifying the shortcomings they were designed to prevent. The most significant failing, in terms of this review, was an inability of senior commercial staff within the processing plant to understand the warnings that repeated plant-environment isolations of *L. monocytogenes* were being made by plant technical staff. It was also found that several government agencies did not have clear practices describing how best to work together to prevent or minimise any foodborne disease outbreaks. Furthermore, a government microbiological test results reporting system commissioned in the spring of 2008 was not functioning

well enough to alert authorities to the possibility of an outbreak at an earlier stage. An important final key recommendation of the Weatherill report was that processing equipment manufacturers and processors needed to work together to establish practical, effective equipment cleaning protocols and hygienic equipment designs. Maple Leaf settled the class action suits from affected consumers for the final sum of $27 million Canadian.

Also in the summer of 2008, Pichler et al (2009) reported the first *L. monocytogenes*-associated gastroenteritis reported in Austria (for any food). The first case was an elderly patient who had been hospitalised for gastroenteritis with septicaemia caused by *L. monocytogenes*. In addition, within a few days, faecal samples provided by seven of 19 other patients complaining of gastroenteritis were also reported as containing *L. monocytogenes*. All of the patients had travelled to an un-named foreign city as part of the same package tour. All of the patient isolates were serovar 4b and had PFGE-determined fingerprints that were indistinguishable. Case control efforts revealed that 15 of those infected with *L. monocytogenes* had eaten from platters of mixed cold cuts (an American term synonymous with CSM) at a wine tavern during the trip. Of these patients, 12 (80%) developed febrile gastroenteritis within 48 hours. An epidemiological investigation determined that consumption of a platter of cold cuts at the wine tavern was the most likely source of the outbreak. Samples of jellied pork that had been prepared by the tavern owner were available for testing and found to contain *L. monocytogenes* at concentrations of up to four log cfu/g. PFGE fingerprinting showed the isolates from the pork were the same as those from the patients confirming the source of the outbreak.

7 PREVALENCE OF *L. MONOCYTOGENES* IN CSM

7.1 FINAL PRODUCT SAMPLING IN PROCESSING PLANTS

Published studies of final product sampling, prior to despatch, are less common compared with surveillance of retail product at point of sale. However, such sampling was undertaken on two occasions in a small CSM manufacturing plant in Greece (Samelis and Metaxopoulos, 1999). In total 64 samples were tested. A summary of the test results are shown as Table 15.

Table 15 Incidence of *Listeria* spp. and *L. monocytogenes* in final products at a Greek CSM plant (reproduced from Samelis and Metaxopoulos, 1999)

Product	Number of samples positive / No of samples tested	
	Listeria spp.	*L. monocytogenes*
VP-CSM		
Ham	4/6	1/6
Pork shoulder	2/6	0/6
Ham-like product	1/4	1/4
Smoked turkey breast	0/4	0/4
Bacon	0/4	0/4
Pariza	0/2	0/2
Mortadella	0/4	0/4
Non-sliced VP cooked meat		
Frankfurters	0/8	0/8
Country style sausage	4/10	1/10
Emulsion sausages	0/12	0/12

Levine et al (2001) reported the results of a Food Safety and Inspection Service (FSIS) survey in the United States which determined the prevalence of *L. monocytogenes* contaminating sliced ham and luncheon meats over a ten years period from 1990-1999. The researchers visited a range of meat plants in the United States and collected 2,287 samples that were tested for *L. monocytogenes.* Overall, 118 samples (5% of those tested) contained *L. monocytogenes*. No quantitative information relating to the numbers of *L. monocytogenes* contaminating products was reported.

Berzins et al (2007) sampled cold smoked sliced pork (which was not cooked) sourced from 15 meat plants in Latvia and Lithuania over a 15 month period in 2003-2004, testing for the presence of *L. monocytogenes*. In total, 312 meat packs were collected and tested five days before the manufacturer's stated end of shelf life and 120 (38%) contained *L. monocytogenes*. The process steps for the production of the cold smoked sliced pork were investigated at seven processing plants and a probabilistic model was generated to determine risk factors. Brining by injection was a significant predictor for a positive test result. In addition, a long cold-smoking time (>12 h) was also associated with positive detections of *L. monocytogenes*. The isolates obtained were genotyped by PFGE and a diverse range of pulsotypes was revealed. The researchers suggested the diversity was due to the contamination of the product occurring at different processing stages. However, some *L. monocytogenes* pulsotypes were present in plants at more than one sampling point, adding to the body of evidence that *L. monocytogenes* can persist in the plant environment and be transferred to other areas inside CSM plants.

UK industry unpublished data for RTE fully cooked foods including CSM primarily sold through major UK multiples provides a useful comparison. A shared dataset of more than 276,000 test results collected on either the day of production or at the end of shelf life provided by a variety of chilled food manufacturers between 2003-2012 records that *L. monocytogenes* was isolated from just under 0.6% of samples of RTE fully cooked foods using enrichment. The test results dataset did not include standardised sample descriptions and so it was not possible to separate the CSM test results from other ready to eat cooked foods.

7.2 AT POINT OF SALE

7.2.1 UK SURVEYS OF *L. MONOCYTOGENES* IN CSM

Over the last two decades in the UK, there have been a number of surveillance exercises undertaken that provide an overview of CSM contamination by *L. monocytogenes*. These surveys range from tightly focussed surveillance of food intended for consumption by patients, to extensive nationwide

surveillance of the entire UK. Patient food in a single London hospital was surveyed for *L. monocytogenes* contamination between 1989 and 1990 (Houang and Hurley, 1991). Of the 854 sliced meat samples examined, 9% contained *L. monocytogenes* whilst 2.29% of the 1465 non-meat samples surveyed contained *L. monocytogenes* over the same period. As a direct consequence of the findings, CSM was withdrawn from the patient menus at the hospital (Houang and Hurley, 1991).

A Welsh surveillance program undertaken between 1993 and 2005 (Meldrum et al, 2005 and Meldrum et al, 2006) was designed and applied to monitor the microbiological quality and safety of specific ready-to-eat products, including cold sliced meat. The surveillance focused detection on six pathogens (*Escherichia coli, Listeria* spp. (specifically *L. monocytogenes*), *Salmonella, Bacillus cereus, Staphylococcus aureus, Campylobacter, Clostridium perfringens*) in over 25 RTE food products. Fifteen thousand two hundred and twenty eight (15,228) RTE food samples were collected during the study period. The overall unsatisfactory (≥100 cfu/g) rate due to *Listeria* spp. was small (0.5%). *L. monocytogenes* was isolated from sliced beef and poultry (0.7%, 11/1589) and in unsliced, cooked poultry (0.6%, 6/1073). This Welsh surveillance (Meldrum et al, 2005 and Meldrum et al, 2006) was the first major coordinated food-sampling program in a large UK region.

Following the Welsh study, a UK-wide survey was undertaken by Gillespie et al (2000) and sampled cold sliced meats collected from 2,579 catered premises (public houses, hotels, cafes, restaurants) and residential homes during June and July 1998. Only five (0.1%) of the 3,494 samples examined, contained *L. monocytogenes*. A similarly low prevalence of *L. monocytogenes* in CSM was reported by a subsequent UK survey. Willis and Greenwood (2003) analysed the laboratory test results from 15 local authorities in Wessex over a 30-month period. The microbiological quality of a range of RTE foods considered typical for a family-shopping basket was assessed which included 493 samples of CSM. For the sliced meats, no samples of ham (n=192), beef (n=120), pork (n=51), chicken (n=57), lamb (n=19) or duck (n=3) contained *L. monocytogenes*. However, two samples of turkey contained *L. monocytogenes*. Furthermore, the prevalence of *Listeria* spp. at unacceptable levels was highest in sliced turkey (3.9%) and sliced pork products (3.9%). A separate national survey undertaken at roughly the same time as the Willis and Greenwood (2003) study collected a total of 2894 cold meat samples from 630 catering and 1658 retail outlets in the UK (Elson et al 2004). In 2.1% of samples (n=60), *L. monocytogenes* was detected in 25g, but present at less than 20 cfu/g. However, a single CSM sample contained between 10^4 and 10^5 CFU *L. monocytogenes* /g (Elson et al 2004).

A later British study by Sagoo et al (2007) collected 2,981 VP and MAP CSM samples from 52 local authorities in the period between September and November 2003 and analysed them at the end of their shelf life. Most of the samples were collected from supermarkets (61%; 1,822 samples) and convenience or corner shops (30%; 909 samples). Thirty-four laboratories tested the samples for *Listeria* species and a range other bacterial indicators and human pathogens. All *L. monocytogenes* isolates from samples that contained more than 10^2 cfu/g were characterised further by serotyping, phage typing, and amplified fragment length polymorphism (AFLP) typing. In total, 143 (4.8%) of the samples tested were found to contain *L. monocytogenes*, with 25 samples containing greater than 10^2 CFU/g. The AFLP-phage type 1/2-IX-NT was found in twelve (48%) of the positive samples. A single SME processor manufactured half of the *L. monocytogenes*-positive CSM samples. Eight of the 12 isolates from this manufacturer were of the 1/2-IX-NT type. The 1/2-IX-NT type was also isolated from four human patients suffering from listeriosis in 2003 (Sagoo et al 2004).

Sagoo et al (2007) also observed that *L. monocytogenes* was detected most frequently from beef samples 17/145 (11.7%), followed by tongue (8.4%), poultry (6.0%) and ham (5.5%). *L. monocytogenes* was detected also in four pork samples (9%) but the sample numbers collected were too low to be of robust statistical significance. Excluding supermarkets, the majority of premises did not label freshly sliced cooked meats with 'use by' information.

In terms of the overall general microbiological quality of CSM, Williamson et al (2007) determined there were differences between greengrocers, butchers and market stalls compared with supermarkets (Table 16). Sample collections from local grocers appear to have been under-represented. Based solely on elevated numbers of general indicators, the finding may be an indication of imperfect handling or storage in some retailer groups.

Table 16 Retailer classification (n=1127) and the likelihood of *L. monocytogenes* detection

Premises types	Butchers	Delicatessens	Local grocers	Market stalls	Supermarkets
Number of premises	390	207	71	184	275
L. monocytogenes detections*	24	16	1	26	15
L. monocytogenes percentage detections	6.15%	7.73%	1.41%	14.1%	5.45%

* *Listeria monocytogenes* detection in 25g on day of purchase

Little et al 2009 also undertook UK-wide surveillance. Between May 2006 and April 2007, 6,984 RTE foods were collected and tested for *L. monocytogenes*. The samples included sliced meats (2,168), hard cheese (1,242), sandwiches (1,088), butter (878), spreadable cheese (725), confectionery products containing cream (515) and probiotic drinks (368). Sandwiches had the highest *L. monocytogenes* prevalence (7%) followed by sliced meats (3.7% within shelf life, 4.2% at the end of shelf life). Only 0.4% of sandwiches, 0.7% of sliced meats within shelf life and 1% at the end of shelf life exceeded 100/g (EU food safety criteria limit). The study of Little et al (2009) emphasized that food business operators (FBOs) with staff who were trained in food hygiene and those that complied with hazard analysis and critical control point principles had product of satisfactory microbiological (including *Listeria*) quality. Also, the authors reaffirmed that effective control of *L. monocytogenes* in RTE foods (particularly CSM and sandwiches) is essential in order to minimise the risk of disease in humans.

The UK Food Standards Agency commissioned a survey to determine the prevalence of *L. monocytogenes* in CSM in 2007 (FSA-B18024). The primary focus of the survey was larger national retailers. Overall, 1,686 samples of CSM were randomly collected from 1,000 retail outlets across the UK and tested for *L. monocytogenes* and a range of bacterial indicators. *L. monocytogenes* was detected in 21 samples (1.53%) but all of the positive samples were below the 100 cfu/g limit.

In autumn 2008, all date codes of Samsono brand Ears and Tongue Roll imported into the UK and manufactured in Lithuania were recalled due to contamination with *L. monocytogenes* (FSA, 2008). Of the 18 samples tested, 17 exceeded the 100/g legal limit. The most heavily contaminated sample contained 2.8 million *L. monocytogenes*/g. The product originated from an EU-approved plant and carried a storage temperature recommendation of 0-6°C, which was lower than the legislated maximum of 8°C in England, Wales and Northern Ireland. The *ca*. 0.3 Kg packs therefore contained ~840 million *L. monocytogenes*.

More recently, a UK-wide retail survey of CSM was commissioned by the FSA as project FS241042, and completed in February 2013 (FSA, 2014). Sample collections were commenced in March 2012 and completed in February 2013. Overall, 1049 samples were collected and *L. monocytogenes* was isolated from 4% (n=40) of samples. None of the samples tested contained *L. monocytogenes* that exceeded the statutory limit of 100 cfu/g, although four samples were at that limit before the end of their shelf life.

The Madden survey was focussed on SME retailers and identified some issues with some refrigerated displays that are more fully discussed in section 10.1.

Although the single hospital study recorded a prevalence of 9%, overall, the majority of the wide-ranging surveys recorded low prevalences of a few percent (Table 17). The two national surveys with the highest prevalences had extenuating factors of testing CSM at the end of shelf life and selectively targeting vendors such as markets that would elevate *L. monocytogenes* detections compared with surveys that used random sample and vendor selection. A conclusion of the UK surveys reviewed, is that over the last 25 years there has been a low prevalence of *L. monocytogenes* in the CSM consumed in the UK generally. However, although the reported prevalence is low, for consumer safety, it is important that any refrigerated storage of CSM prior to retail purchase is sufficiently adequate to prevent the multiplication of *L. monocytogenes*.

7.2.2 SURVEILLANCE OF FERMENTED AND SPECIALITY COOKED SLICED MEATS

Gormley et al (2010) collected a total of 2359 ready-to-eat (RTE) specialty meat samples from 968 markets and specialty food shops between April 2008 and March 2009, in the UK. The samples were examined for a variety of indicator organism and human pathogens including *L. monocytogenes* and *Listeria spp.*. The types of meat sampled included chorizo, salami, pepperoni, bologna, prosciutto, Parma ham, pastrami, Serrano, bresaola and various dried meats such as biltong and jerky. Seven samples yielded *L. monocytogenes* (0.3%) although six of these seven (86%) contained potentially hazardous levels of *L. monocytogenes* greater than 10^2 CFU/g. The meats that exceeded the statutory limit were Portuguese bacon, Italian salami, three samples of ears and tongue roll from Lithuania and a sausage manufactured in Hungary (Gormley et al 2010).

Table 17 A summary of recent CSM surveillance for *L. monocytogenes* in the UK

Surveillance target	Surveillance year	Number of CSM samples tested	Percentage positive *Lm* detections (%)	No. of samples exceeding 100 CFU *Lm* /g	Notes	Reference
Single hospital	1989-1990	854	9.0	*NR	London Hospital	Houang and Hurley (1991)
Nationwide	1998	3494	0.1	NR		Gillespie et al (2000)
Wessex	1998-2000	493	0.4	NR		Willis and Greenwood (2003)
Nationwide	2002	2894	2.1	1		Elson et al 2004
Nationwide	2003	2981	4.8	25	End of shelf life analysis	Sagoo et al (2007)
NW Britain	2006-2007	1127	7.3	5	NW England and Flintshire. Survey may be skewed by high detections in markets	Williamson et al (2007)
Nationwide	2007	1686	1.5	0	Focussed on national retailers	UK-FSA (2011)
Nationwide	2008-2009	2359	0.3	6	Focussed on speciality meats (section 7.2.2)	Gormley et al (2010)
Nationwide	2012-2013	1049	4%	0	Four samples had 100 cfu/g before the end of shelf life	Madden et al (unpublished)

*NR is not reported

7.2.3 NON-UK EUROPEAN SURVEYS OF *L. MONOCYTOGENES* IN CSM

In Spain (Lafarga et al, 1994) sixty luncheon meats and 36 pâté samples were collected at retail and tested for the presence and numbers of *Listeria* spp.. Twenty one samples contained *L. monocytogenes*, nine contained *L. innocua* and the RTE meats yielded more *Listeria* spp., with 41.6% of samples positive, compared with pâté, where 13.9% of samples were positive. Eighty-three percent (83%) of positive samples had less than 10 cfu/g, which was below the legal limit of ≤100/g in Europe for RTE foods (EC 2073/2005). Based on the distribution of positive samples in shops, Lafarga et al (1994) suggested that the slicing machines were the source of cross-contamination for the contaminated RTE products.

In a German study (Krockel, 2000), *L. monocytogenes* was isolated from 5% (n=78) of RTE meat products after pasteurisation, slicing, packing and storage under refrigeration until one week after the indicated "best before" date on the package. In all four positive samples, the level of *L. monocytogenes* was below 100 cfu/g. Two positive samples were Bologna-type sausages, one was cooked turkey breast, and the last was cooked pork ham. Genetic typing of the isolates revealed that three samples contained a single distinct genotype that was different in each sample. The fourth sample contained two different genotypes. In the majority of tested products, the pH varied between pH 5.2 to 5.8, which favoured the growth of lactic acid bacteria (LAB) to high concentrations (>10^8 /g). The study of Krockel (2000) suggested that the consumption of refrigerated RTE meat one week after the "best before" date presented a low (but not zero) risk of food poisoning.

The Food Safety Authority of Ireland undertook 17 surveillance exercises for *Listeria* between 2001 and 2009, sampling a range different foodstuffs (O'Connor et al 2010). Those relevant to this report were; pre-packed sliced ham (n=618), sampled in 2003, fermented meat (n=757) in 2004, and loose cooked sliced ham (n=919) in 2005. For pre-packed ham, one sample (0.2%) contained *L. monocytogenes* whilst there were no detections from loose ham. Twenty samples of fermented meat (2.6%) contained *L. monocytogenes*. All of the positive samples contained *L. monocytogenes* below the statutory limit of 100 cfu/g. All of the reports are available at: http://www.fsai.ie/enforcement_audit/monitoring/micro_monitor_surveillance/national_programme.html

Similarly low prevalences were also reported by a CSM survey at retail (n=200) undertaken in Germany (Krokel, 2008). In total, 200 samples were purchased at retail. One hundred and fifty samples were tested immediately after purchase and no *L. monocytogenes* detections were reported. The remaining 50 samples were tested one week after their use-by-date. One *L. monocytogenes* isolation was made from the 50 stored meat samples. A previous study by the same group (Albert et al 2003) did not detect *L. monocytogenes* at numbers greater than 10^2 cfu/g in any of 108 organically produced raw fermented sausages and sliced cooked meat products sampled.

A Greek study to determine the prevalence of *Listeria* in CSM (Angelidis and Koutsoumanis 2006) collected 209 samples from July to October 2004 from 13 retail stores around the town of Thessaloniki.

The sampled stores represented every major Hellenic supermarket chain, and included CSM manufactured in Greece (n=136) and CSM imported from other EU member states (n=73). Using a commercially available enzyme-linked fluorescent assay (ELISA), 17 positive *L. monocytogenes* detections (8.1%) were reported. The samples which contained *L. monocytogenes* were bacon (12 detections from 49 samples; 24.5%), salami (3 detections from 30 samples; 10%), cooked ham (1 detection from 27 samples; 4.0%) and fermented ham (1 from 6; 16.7%). In all cases, the numbers of *L. monocytogenes* were estimated to be <10 cfu/g.

Also in Spain, Aguado et al (2001) collected 369 CSM samples from 55 retail outlets. Thirty four (9.2%) of CSM samples tested positive for *L. monocytogenes.* Although overall, these positive samples came from 18 retailers, 56% of the isolates were isolated from only five retailers. Random amplified polymorphic DNA (RAPD) allowed Aguado et al (2001) to establish that in each of those retailers, the same strain of *L. monocytogenes* could be present in different meat products from different commercial brands, manufactured on the same day, in the same processing plant. The authors considered a likely explanation for the widespread contamination on a single date to be a slicing machine that was either imperfectly cleaned, or not cleaned, before use with a different product for a different customer.

A more-recent Spanish study by Garrido et al (2009) collected 220 processing-plant sliced, VP delicatessen meat products and 200 delicatessen meat products that had been sliced in store from retail outlets in Navarra, Spain. The *L. monocytogenes* prevalences for the processing plant sliced and delicatessen-sliced products were 2.7% and 8.5% respectively, indicating possible handling issues for store-sliced delicatessen meat. Of the six positive results for plant-sliced meats, four contained *L. monocytogenes* at concentrations of less than 10 CFU/g while two contained between 100 and 1000 cfu/g. From the 17 store-sliced samples that contained *L. monocytogenes*, six contained less than 10 cfu/g; three contained 10-100 cfu/g; seven contained 100-1000 cfu/g and one sample contained over a 1000 CFU/ g of *L. monocytogenes*. Of the 23 total stains isolated from CSM, nine were serotype 1/2a, four were 1/2b, seven were 1/2c and three were 4c. A key finding of this study was that molecular characterization of isolates confirmed an apparent ability of *L. monocytogenes* to persist within manufacturing environments. The same pulsotype was repeatedly isolated from different batches of product off the same brand manufactured on different days, and from different products sliced in the same store. The authors concluded that there is a need to improve the hygiene, cleaning and

sanitation of slicing equipment. Their recommendation was for the establishment of effective equipment cleaning practices, and improved education for food workers.

Berzins et al (2009) sampled VP sliced meat products in Riga, Latvia from January to April 2005. The types of product sampled consisted of cold-smoked beef, dried beef, cold-smoked pork, cooked smoked ham, cooked smoked beef, liver pate and cooked smoked turkey. Samples were stored at 6^{o}C until 0 to 5 days before the end of their shelf life before testing for *L. monocytogenes.* Of 212 packages of product tested, 38 (18%) contained *L. monocytogenes.* The greatest *L. monocytogenes* prevalence was in cold smoked beef (31 positives from 54 collected samples; 57.4%) and cold smoked pork (five positives from 25 collected samples; 20%). There was no cooking stage for either of these meats. There were no positive *L. monocytogenes* detections for cooked smoked ham (n=44), cooked smoked beef (n=11) and cooked smoked turkey (n=5). Although very few of the positive samples exceeded the statutory limit of >100 *L. monocytogenes* cfu/g five samples of cold smoked beef, and one of dried beef, contained between 100-1000 CFU/g (Berzins et al 2009). The authors noted that for the positive sample types, there were no processing steps capable of eliminating *L. monocytogenes*, and hence no CCP.

More recent Spanish surveillance (Perez-Rodriguez et al, 2010) assessed meat handling practices and the microbiological quality of CSM during the slicing at retail. It was found that larger establishments (LE) had more hygienic handling practices than did the SME retailers. However, Perez-Rodriguez et al noted that the poorer handling practices observed in some SME businesses did not result in any significant differences in the microbiological qualities of the sliced cooked meat when compared with LE businesses. The prevalences of *L. monocytogenes* and *L. innocua* were 7.35% (5/68) and 8.82% (6/68), for large and small retailers respectively. Some businesses were assessed as having acceptably hygienic handling practices, although *L. monocytogenes* was still detected on CSM. Consequently, the authors hypothesised that any contamination was likely to be associated with not having dedicated slicers for different products at retail premises. Seasonality was a significant factor ($p<0.05$) for the presence of *L. monocytogenes* with all positive test results being returned during the warmest months.

In Belgium, Uyttendaele et al (1999) compared the prevalence of *L. monocytogenes* on CSM (n=3405) purchased unsliced, and sliced in store, from a large supermarket chain. The samples were bought once per month between January 1997 and December 1998 and *L. monocytogenes* was isolated using

selective media and confirmed by an antibody typing method. Unsliced cooked ham samples (n=1069) had a *L. monocytogenes* prevalence of 1.5% but for sliced cooked ham (n=879) the prevalence was 6.1%. For unsliced cooked loin (n=87) and sliced cooked loin (n=127) the figures were 3.4% and 10.2% respectively.

The significant increases in prevalence for sliced product reported by Uyttendaele et al (1999) are broadly supported by the later observation of Garrido et al 2009. Both authors believe that cross contamination can occur during in-store slicing, and that contaminated slicers are a significant potential source of *L. monocytogenes* on CSM.

A summary of recent non-UK EU surveillance is shown as Table 18. There was no standardisation of CSM sample types, samples were taken at different times of the year and the laboratory testing protocols were different for most of the studies. However, it is interesting to note that there was an apparent tendency for the warmer, southern European countries to have higher *L. monocytogenes* prevalences in CSM compared with the cooler northern countries (Table 18).

Table 18 A summary of recent CSM surveillance for *L. monocytogenes* in continental European surveys

Surveillance target	Surveillance year	Number of CSM samples tested	Percentage positive *Lm* detections (%)	No. of samples exceeding 100 CFU *Lm* /g	Notes	Reference
Belgium	1997-1998	3405	4.9	NR	Slicing meat caused significant increase in *lm* detection	Uyttendaele et al (1999)
Portugal	1998-2000	47	21	ND		Guerra et al (2001)
Spain	1999	369	9.2	NR	Work not intended as surveillance	Aguado et al (2001)
Ireland	2001-2009	2291	0.9	0		O'Connor et al (2010)
Germany	2003	108	NR	0	Surveillance of organic sliced ham only	Albert et al (2003)
Thessaloniki, Greece	2004	209	8.1	0		Angelidis and Koutsoumanis (2006)
Riga, Latvia	2005	212	18	6	Samples were stored at 6°C until between 0 and 5 day before use-by-date before testing	Berzins et al (2009)
Germany	2008	200	0.5	0	25% of samples were stored at 7°C until one week after use-by-date before testing	Krokel (2008)
Navarra, Spain	2003-2005	220	2.7	2	Processing plant sliced and VP	Garrido et al (2009)
		200	8.5	7	Delicatessen sliced	
Córdoba, Spain	2009	147	7.35	NR	Primary study focus was hygienic CSM handling not surveillance	Pérez-Rodríguez et al (2010)
26 EU member states plus Norway	2010-2012	3448	0.12	2	One sample from each batch was tested at end of shelf life.	EFSA (2013)

*NR: Not reported; VP: Vacuum packaged; ND: not determined.

7.2.4 SURVEILLANCE OF *L. MONOCYTOGENES* IN CSM OUTSIDE OF THE EU

Between 1992 and 2000, CSM surveillance was undertaken in Switzerland (Jemmi et al 2002) by veterinary staff at border patrol stations collecting samples which were transported to a single central laboratory for examination. The surveillance included both CSM imports as well as exports. Overall, 255 cooked- and cured-meat products and 132 cured- and dried-meat products were tested. The incidence of *L. monocytogenes* detections were 6% and 3% respectively. The study collected supplementary information relating to the samples and attempted unconditional fixed-effect logistic regression to identify the main hazards associated with the presence of *L. monocytogenes*. For CSM, the year of manufacture and the source processing plant were identified as weak predictors of risk.

A large US study collected almost 32,000 ready to eat food samples in Maryland and northern California over a two year period between 2000 and 2002 (Gombas et al 2003). The locations were chosen as sites in the CDC FoodNet where active surveillance for listeriosis was undertaken. Luncheon meats was one of the eight food categories sampled and in Maryland, 1.17% of the 2600 samples tested were contaminated with *L. monocytogenes*, whereas in California the prevalence was 0.61% for the same number of collected samples. However, it should be noted that 'luncheon meats' comprised bologna, poultry and ham. The authors noted 'Ham could include products made from pork or poultry (such as turkey) and could include regular, low-salt or low-sodium, low-fat, extra-lean, and fat-free varieties. Bologna could include products made from pork, beef, turkey, or mixtures of these meats and could include regular, low-salt or low-sodium, low-fat, and fat-free varieties.' This definition emphasised the need for care when comparing results of surveys in different countries.

No reason for the difference in prevalence of *L. monocytogenes* between the two US states was proposed by Gombas et al, but they noted that the prevalence of *L. monocytogenes* in factory packaged luncheon meats was 0.19% in Maryland but 0.55% in northern California. Again, no reason was proposed but the respective prevalences for 'in-store-packaged' samples were 4.2% and 0.70%. Thus, in-store handling apparently had no significant effect in California, but a significant effect in Maryland. The authors noted that their observation 'warrants further investigation'. From the studies noted above it could be postulated that hygiene standards, especially slicer cleaning, were higher in California than in Maryland. It is unfortunate that follow-up studies were not published to clarify the observations of Gombas et al.

In Argentina, Marzocca et al (2004) reported that 5.6% (5/90) of RTE VP cooked meats sampled in a large chain of supermarkets contained *L. monocytogenes*, as determined by enrichment of a 25g sample. Follow up work collected 115 environmental samples from CSM processing plants, which included processing lines, raw materials, tools, and refrigerators. Overall, 13.2% of the plant samples contained *L. monocytogenes* with 5% of isolations in the packaging areas, 6.7% in the meat processing lines. Additional sampling revealed *L. monocytogenes* isolations from 1.5% of retail packs.

In São Paulo, Brazil, Martins et al (2011) tested the prevalence of *L. monocytogenes* in retail cooked ham (n=136). The surveillance was in the last quarter of 2006 and during 2007. One (0.8%) cooked ham contained *L. monocytogenes,* all of the other samples had counts <10 cfu/g and were considered to be low risk for consumers.

El-Shenawy et al (2011) undertook surveillance of ready to eat food sold from street vending carts in Egypt. A wide range of foods were sampled which included shawarma (shaved multispecies meat that was spit-roasted), luncheon meat and basturma (cured beef). The publication does not make clear if the meat was sold hot or cold, but ten positive detections for *L. monocytogenes* were reported from CSM.

In New Zealand, researchers collected 104 pre-packaged ham samples at retail and tested for *L. monocytogenes* but determined presence in only one sample (Wong et al 2005). The presumptive *L. monocytogenes* was confirmed by immunoassay and the number of *L. monocytogenes* in the positive sample was estimated to be 50 cfu/g.

The New South Wales Food Authority (NSWFA, 2009) surveyed 154 packaged, sliced RTE meat products purchased from a range of retail stores including chain supermarkets, small grocers, farmers markets and retail butcher shops. The surveillance was undertaken exclusively in the area around Sydney and aimed to determine if local microbiological standards were being met. Six positive detections (3.9%) were made and the NSWFA instigated audits in those premises that had manufactured the CSM due to their failure to meet legal requirements.

A twenty-week longitudinal study undertaken by Mottin et al (2011) determined the numbers of a range of indicator bacteria and *Listeria* with speciation in pork luncheon meat samples sliced and packaged at three supermarkets in Porto Alegre, Brazil. By comparing these test results with those for

pre-packed CSM (n=54), the authors concluded that their results clearly demonstrated that bacteria were introduced into the luncheon meat during store slicing and packaging operations. *Listeria* spp. were found on the products sliced and packed at supermarkets at a prevalence of 20% overall (16/80). The prevalence of *L. monocytogenes* varied by store and ranged between 5% (1/20) to 20% (4/20). The store with the highest prevalence of *L. monocytogenes* also had total bacterial counts 2 logs higher than the other stores, suggesting poor hygiene controls.

It is apparent from the reported prevalences from continental European and global surveillance that it is not uncommon for *L. monocytogenes* to be isolated from CSM. However, it is unusual for *L. monocytogenes* numbers to exceed 100 cfu/g meat, even in warmer countries, which have a tendency towards higher CSM prevalences for *L. monocytogenes* compared with cooler climates (Table 19). Shelf lives given to CSM internationally vary widely and are generally significantly longer than those used in the UK. Extending shelf life without ensuring either absence of *L. monocytogenes* (e.g. by in-pack thermal processing) or using suppressive formulations presents greater opportunity for growth to high levels. See section 8.4.

Table 19 A summary of recent CSM surveillance for *L. monocytogenes* outside of the EU

Surveillance target	Surveillance year	Number of CSM samples tested	Percentage positive *Lm* detections (%)	Regional statutory limit for *Lm*	No. of samples exceeding regional limit	Notes	Reference
Switzerland	1992-2000	387	0.6	<100 CFU/g	0	Sampled CSM imported to and exported from Switzerland	Jemmi et al (2002)
MD and CA United States	2000-2002	9199	0.9	Zero tolerance	82	Luncheon meats subsection of a large RTE study	Gomas et al (2003)
Argentina	2002-2003	90	5.6	Absence in 25g	5		Marzocca et al (2004)
Auckland/Christchurch New Zealand	2003-2004	104	1.0	Zero tolerance	1		Wong et al (2005)
Edmunton, Canada	2005	100	3	Zero tolerance	NR		Bohaychuck et al (2006)
Sydney, Australia	2008	154	3.9	ND in 25g	6		NSW Food Authority (2009)
Porto Alegre, Brazil	2009	300	11.7	NR	NR	Pooled samples of 5 analysed.	Mottin et al (2011)
Egypt	2010	72	13.9	None	N/A	RTE CSM sold by street vendors	El-Shenawy et al (2011)

NR: Not reported; ND: Not detected; NA: Not applicable.

8 FATE OF *L. MONOCYTOGENES* CONTAMINATING CSM

8.1 STUDIES USING NATURAL CONTAMINATION

Bersot et al (2008) determined the fate of naturally occurring *L. monocytogenes* on VP sliced mortadella stored at 5°C. During the storage, it was observed that the numbers of *L. monocytogenes* increased from 0.40 MPN/g at day 0, to 71.6 MPN/g after 40 days storage (Bersot et al 2008), demonstrating an ability of *L. monocytogenes* to grow under refrigeration and in the absence of air.

Although not directly comparable with the results of Bersot (2008), donated UK industry data for RTE fully cooked foods (which included CSM) showed a small rise in the prevalence of *L. monocytogenes* over similar time periods. A comparison of 360,000 samples collected on the day of manufacture with 130,000 at end of shelf life between 2004 and 2011, showed respective prevalences after enrichment of 0.7% and 1%.

8.2 FATE OF *L. MONOCYTOGENES* ON CSM USING INOCULATED STRAINS

Glass and Doyle (1989) studied the growth of a five-strain mixture of *L. monocytogenes* inoculated onto a range of meat products including ham, bologna, sliced chicken, sliced turkey, fermented semi-dried sausage and cooked roast beef. The meats were surface inoculated at concentrations of either 200 or 10^5 cfu/g meat, stored at 4.4°C and periodically sampled over 12 weeks or until spoilage occurred. Numbers of *L. monocytogenes* increased on ham, chicken, turkey, bologna and some formulations of wiener sausages. *L. monocytogenes* was unable to grow, but did not decline on summer sausage, some wiener sausages formulations and roast beef. This study was one of the first to report lower pH preventing *L. monocytogenes* growth. The authors concluded that the cocktail of *L. monocytogenes* generally grew well on meats above pH 6.0 and poorly or not at all below pH 5.0. For the *L. monocytogenes* strains used, growth was most prolific on processed poultry products (Glass and Doyle 1989) which are known to have a higher pH than red meat products (Madden et al, unpublished).

Ingham and Tautorus (1991) studied the survival of *L. monocytogenes* on inoculated sterile slices (10^6 cfu/slice) of cooked uncured turkey loaf stored in VP at 3°C. *L. monocytogenes* populations decreased by 0.2 logs over 15 days at 4°C. Other human pathogenic and hygiene indicator bacteria were also studied. No significant ($P < 0.01$) difference between the survival of *Enterococcus faecalis* and *L. monocytogenes* was found suggesting that enterococci may serve as potential indicators of *L. monocytogenes* contamination of processed meats.

Michel et al (1991) also evaluated the survival of several potential human pathogens, including *C. perfringens*, *E. coli, S. typhimurium* and *L. monocytogenes*. The strains were inoculated onto the surfaces of precooked roast beef slices then stored in VP at 3°C for 70 days in the presence of the natural competing microflora. Only the populations of *C. perfringens* and *E. coli* decreased significantly during the storage period. *L. monocytogenes* remained present on slices for the duration of the experiment.

Hudson and Mott (1993b) inoculated a two-strain cocktail of *L. monocytogenes* onto samples of cooked beef incubated at 5°C or 10°C under aerobic or VP conditions. In summary, *L. monocytogenes* multiplied under all of the conditions assessed. As might be expected, the lag time was lower for the higher temperature.

In agreement with previous studies, Yu et al (1993) found that *L. monocytogenes* could multiply on ham at refrigeration temperatures and also determined the fate of sub-lethally injured *L. monocytogenes* inoculated on to CSM. Prior to inoculation of chopped ham, a three-strain cocktail of cultured cells of *L. monocytogenes* were either heat-injured (56°C, 30 min) or freeze-injured (-18°C, 14 d). The initial inoculation was roughly 350 cfu/g with storage at 5°C. There was no difference between the fates of freeze- and heat-treated cells. After one week, there was a single log reduction in *L. monocytogenes* numbers for both treatments. However, the *L. monocytogenes* cells then recovered and increased to 10^7 to 10^8 cfu/g after five weeks.

Duffy et al (1994) investigated the growth of a single strain of *L. monocytogenes* inoculated onto beef, pork, chicken and turkey CSM and stored at 0°C or 5°C. *L. monocytogenes* grew on all four meat

species. There was a greater lag time and reduced growth rate at 0°C on meat with a pH 5.9 and an a_w of 0.993 compared to 5°C storage, with a pH of 6.90 and an a_w of 0.960. Thus the intrinsic properties of the CSM, combined with the storage temperature, can limit the growth of *L. monocytogenes*.

Cooked hams have the cooking process as a CCP, but this is not the case for dry cured hams, referred to a 'country-style' in the US. Ng et al (1997) purchased whole dry cured hams from six manufacturers, sliced them and inoculated slices with one of four pathogens, including *L. monocytogenes*. Uninoculated slices were included as controls and the slices VP and stored at either 2°C or 25°C for up to 28 days. No growth of *L. monocytogenes* was seen but in hams from three processors, no significant reduction in numbers was seen at either temperature. Unfortunately a_w values were not reported, but pH values ranged from 6.0 to 6.4. It was noted that uninoculated control samples from two processors yielded *L. monocytogenes* during the study (i.e. the hams were contaminated naturally). Overall, the composition of dry cured hams appears to affect the survival of *L. monocytogenes*, but more detailed studies would be required to define the cure required for a safe product.

Chikthimmah and Knabel (2001) studied *L. monocytogenes* survival on Lebanon bologna sausage, a smoked and fermented ready to eat beef sausage with a relatively low pH of 4.4 - 4.6. A three-strain cocktail was used to inoculate commercially produced Lebanon bologna sausage slices to 6.7 log cfu/g. The sausage was VP and stored at either 3.6°C or 13°C. At the higher temperature, the population fell to undetectable levels in less than 10 days whilst it took up to 25 days at 3.6°C. The finding again demonstrated the susceptibility of *L. monocytogenes* to low pH.

Ingham et al (2004) used a cocktail of five *L. monocytogenes* strains to inoculate 15 retail RTE meat products selected on the basis that drying, fermentation, and/or smoking were used in their manufacture, e.g. summer sausage, pork rind, smoked cured beef and beef jerky. Strictly, the products are outside the range covered by this report. The study has been included to provide a complete overview of the literature and because some of the products were compositionally related to CSM. The initial *L. monocytogenes* concentrations varied between 3.1 to 4.4 log /slice (0.6 x 3.7 x 3.7 cm slices) and the products were stored under VP or in air at room temperature (21°C) or under refrigeration (5°C) for up to 11 weeks. Numbers of *L. monocytogenes* decreased for all products during storage. The lowest decrease, 0.8 log cfu/slice, was observed with smoked cured beef slices during storage at 5°C. The greatest decline observed was 3.3 log cfu/slice on a pork rind product stored for five weeks in air at

21°C. Hence all products would be safe with regard to *L. monocytogenes* provided it was not present in the final product.

Contrasting results were reported by Nufer et al, (2007); who studied the effects of storage temperature (3°C or 7°C) on two *L. monocytogenes* serotypes inoculated onto sliced bologna and stored under a MAP of 100% nitrogen for 28 days. Starting from an initial concentration of one cfu/g, *L. monocytogenes* strain 217 grew to 10^6 cfu/g during 28 days storage at 3°C. *L. monocytogenes* 22/3A remained below one cfu/g under the same conditions. When the experiment was repeated at 7°C, *L. monocytogenes* 217 multiplied to 10^6 cfu/g in 14 days, compared with only 10^4 cfu/g for *L. monocytogenes* 22/3A. Nufer and colleagues (2007) proposed that in light of their findings, different growth models should be used for different strains of *L. monocytogenes* when undertaking risk assessments for naturally contaminated food products.

Uyttendaele et al (2004) evaluated a challenge testing protocol designed to assess the ability of *L. monocytogenes* to grow on cooked ham (and pâté). Four strains were used and all showed an acid-adaptive response. Cold adapted strains appeared to gain a slight advantage from their pre-treatment when inoculated onto ham, and would thus reach unacceptable levels more quickly than a standard inoculum. The result suggests that plant adapted strains could grow more quickly than wild type strains, depending on the provenance of the latter. VP cooked ham stored at 7°C allowed the growth of *L. monocytogenes* to unacceptable levels within a few days, emphasising the need for this product to be properly cooked, and subsequent recontamination avoided.

The ability of a three-strain cocktail of *L. monocytogenes* to grow on cooked sliced ham and roasted sliced turkey was assessed by Burnett et al (2005) using inoculums of approximately 10^3 cfu/g. The CSM were stored aerobically to reflect retail handling and both cooked sliced ham and roasted sliced turkey supported the growth of *L. monocytogenes* at 5°C, 7°C and 10°C, hence cooking was again seen to be a CCP.

Garrido et al (2010a) reported that in Navarra, Spain; domestic refrigerator temperatures typically ranged between 0.6°C and 14.5°C. Using only a single strain of *L. monocytogenes* that was originally isolated from CSM, sliced cooked ham was inoculated at a concentration of <10 CFU/g and stored at

either 5°C or 9°C. After only 5 days of storage at both temperatures, the numbers of *L. monocytogenes* increased to greater than 100 cfu/g.

Acknowledging the significant body of literature that reports growth of *L. monocytogenes* on a wide range of CSM types, more recent studies have sometimes sought more novel experimental approaches to simply reporting that *L. monocytogenes* grows on CSM. Hwang and Sheen (2011) attempted to identify factors that influence rates of *L. monocytogenes* multiplication. They investigated if there was any influence of the natural microbiota present on CSM on *L. monocytogenes* growth. The studies involved laboratory culturing of a five-strain mixture of *L. monocytogenes* and inoculation onto sliced ham either alone or in combination with *Brochothrix* spp. (part of the natural spoilage microbiota of ham, and able to grow relatively rapidly at refrigeration temperatures). The inoculated CSMs were VP and stored (4, 6, 8, 10 or 12°C) for up to 12 weeks. The authors reported that there was a small, but significant, reduction of 7-8% in the growth rate of *L. monocytogenes* for the *Brochothrix*-treated meats at 4-8°C. There was however, no significant effect due to *Brochothrix* co-culture at the other temperatures assessed. Hwang and Sheen (2011) concluded that their study showed *L. monocytogenes* growth suppression caused by native microflora. However, the study used laboratory-cultured strains and it is unlikely that a reduction of 7-8% in *L. monocytogenes* growth would result in any meaningful impact on *L. monocytogenes* numbers in CSM offered at retail.

Garcia Diez and Patarata (2012) studied chouriço, which is manufactured from coarsely-minced pork and fat that has been seasoned with salt and variable ingredients such as paprika, garlic, and wine. The mixture is prepared and allowed to rest before being stuffed into natural casings before being allowed to dry at a low temperatures for 1 to 4 weeks. The sausage may be cold smoked prior to final drying. Diez and Patarata (2012) inoculated the sausage batter with *L. monocytogenes* and other food borne human pathogens prior to fermentation, with and without a starter culture. The sausage was tested after 7, 15, and 30 days of drying; and after 30 days of storage at 4°C under vacuum. In chouriço containing wine, the alcohol content and the low pH helped prevent the growth of *L. monocytogenes*. The drying step was found to be important for the elimination of pathogens, with *L. monocytogenes* populations falling during drying. Numbers of *L. monocytogenes* were reduced by approximately two logs after 15 days of drying and were undetectable after 30 days drying. The decline of the *L. monocytogenes* populations was expedited by the addition of a starter culture of *Lactobacilli sakei,* again demonstrating that reduced pH can help control *L. monocytogenes.*

Overall, there is a large volume of literature that has assessed the fate of *L. monocytogenes* either naturally present on CSM or *L. monocytogenes* inoculated onto CSM. This evidence supports a general conclusion that growth can routinely occur during extended storage of >5 days, even on CSM that have been cured and brined and even if the CSM is stored at temperatures as low as 5^{o}C.

In general, lower pH results in a longer lag phase before growth. However, if the pH is too low, some *L. monocytogenes* strains are unable to multiply and if the pH is lowered further, decline in the numbers of *L. monocytogenes* can occur. If the pH of the CSM matrix is below pH 4.2, most *L. monocytogenes* strains are unable to multiply, and the populations are highly likely to decline (Warriner and Namvar, 2009).

Furthermore, the fate and rate of multiplication of *L. monocytogenes* can be strain-specific that is in part a function of how strains reacts to the CSM environment. More specifically, the lag time before the commencement of growth is influenced by a number of factors including storage temperature and the physicochemical characteristics of the CSM matrix.

8.2.1 ENVIRONMENTALLY PREDISPOSED *L. MONOCYTOGENES*

8.2.1.1 TEMPERATURE ADAPTATION

Like all bacteria, the growth of *L. monocytogenes* in new environment can be influenced by the environmental conditions to which it has been previously exposed. The European Reference Laboratory for *Listeria monocytogenes* recognised adaptation as an important consideration in guidance notes for challenge testing in 2001, and after an update to the original guidance in 2005 (AFSSA 2005). The use of cold-adapted cultures was recommended as part of the protocol, as was the use of wild-type strains isolated from similar products to those to be tested. The approach was supported by the work of Uyttendaele et al (2003) who showed that pre-incubation of cultures at 7°C, as opposed to 30°C, resulted in shorter lag phases in model cooked meat substrates. Consequently, the cold adapted strains would reach unacceptable levels more quickly than non-adapted stains. Challenge tests-undertaken by Everis and Betts, 2013, compared the growth of strains of *L. monocytogenes* treated with the AFSSA (Agence Française de Securité Sanitare des Aliments) cold adaption procedure,

or grown at 30°C, and inoculated on to cooked ham, stored at 8°C for 7d. The growth rate of the cold-adapted strains was more than 3-fold greater than the non-adapted cultures.

Dykes, 2003, studied a wild-type (from a meat processing plant) strain and reference strain (ATCC 19113) of *L. monocytogenes* and showed that pre-incubation at 4°C decreased the lag time of both strains on VP bologna stored at 4°C. However the effect was more marked with the reference strain, indicating that studies should use more than one strain, as recommended by AFSSA (renamed ANSES).

Overall, the provenance of *L. monocytogenes* with regard to temperature (and other environmental parameters) can directly affect their ability to multiply on CSM, and hence impact on the incidence of listeriosis caused by the consumption of contaminated CSM.

8.2.1.2 PH ADAPTATION

The importance of pH and any influence on survival and multiplication of *L. monocytogenes* pre-exposed to low pH was investigated by a single group of researchers over several years (Formato et al 2007; Barampala-Davis et al 2008; Bylashov et al 2009). The value of the studies was that they all used a standardised cocktail of ten *L. monocytogenes* strains. All of the papers have the same general method, which either pre-exposes the *L. monocytogenes* cocktail to a growth media at pH 4.4 (acid adapted) or pH 6.4 (non-adapted control). The fate of the control and treatment *L. monocytogenes* cocktails were then evaluated typically for a variety of CSM types and environments such as a simulated human gastric model.

The fate of a 2 log cfu/g *L. monocytogenes* cocktail of the acid-adapted or control strains was determined after inoculation onto bologna slices that were VP and stored at 10^{o}C (Formatio et al 2007). After storage, the inoculated bologna was fed to a simulated gastric model. Acid adaptation of *L. monocytogenes* prior to product inoculation did not affect subsequent survival or growth on bologna or resistance to simulated gastric fluid with a pH of 1.0. The ability to survive the fluid in the gastric model increased with product age, the growth phase of the cells, and possibly age of the cells. Typically, *L. monocytogenes* multiplied to approximately 6 log CFU/cm^2 by the eight day of storage.

The fate of the same ten strain cocktail of *L. monocytogenes* used by Formatio et al (2007) was determined after placing into the same gastric model (Barmpalia–Davis et al 2008). Sliced bologna was inoculated with a ten strain cocktail of *L. monocytogenes* at an initial higher concentration of 4 to 5 log CFU/g CSM and stored it for 82 days at 4^{o}C. At the end of storage, the populations of *L. monocytogenes* increased on the bologna to 8.7 logs. Subsamples of the bologna were removed from storage at various points and fed into a physical model of a human gastric intestinal system. There were still live cells on the bologna when it was fed to the gastric model. These live *L. monocytogenes* cells multiplied inside the model system and the total number of *L. monocytogenes* after exposure to the gastric system was further increased (Barmapalia-Davis et al 2008).

The findings of these three publications are important for two reasons. Collectively, they provide further evidence that different meat types can influence the survival, multiplication or decline *L. monocytogenes*. The fate of *L. monocytogenes* contaminating CSM is dependent on the nature of the CSM. In particular, the lower pH of the fermented salami and pepperoni helped reduce the numbers of *L. monocytogenes* contaminating the meat during storage. In addition, the Barmapalia-Davis et al work also provided evidence that an environment contaminated with *L. monocytogenes* can have an influence on survival in a new environment if cells were to be transferred. Although there were lower numbers of *L. monocytogenes* transferred into the gastric system on the salami, the cells persisted in the low pH environment better than the cells transferred on the higher pH bologna. The Barmapalia-Davis et al study (2008) provided evidence that the low pH of the salami, whilst hostile to *L. monocytogenes*, predisposed the bacterial cells to better survival in the low pH model stomach.

8.3 MODELLING THE FATE OF *L. MONOCYTOGENES* IN CSM

The ComBase online predictive model (http://www.combase.cc) shows for a pH 6.7 meat product containing 1% NaCl inoculated with a single *L. monocytogenes* strain, numbers will double every 7.8 hours when stored at 8°C, and every 14.2 hours when stored at 5°C (Figure 16).

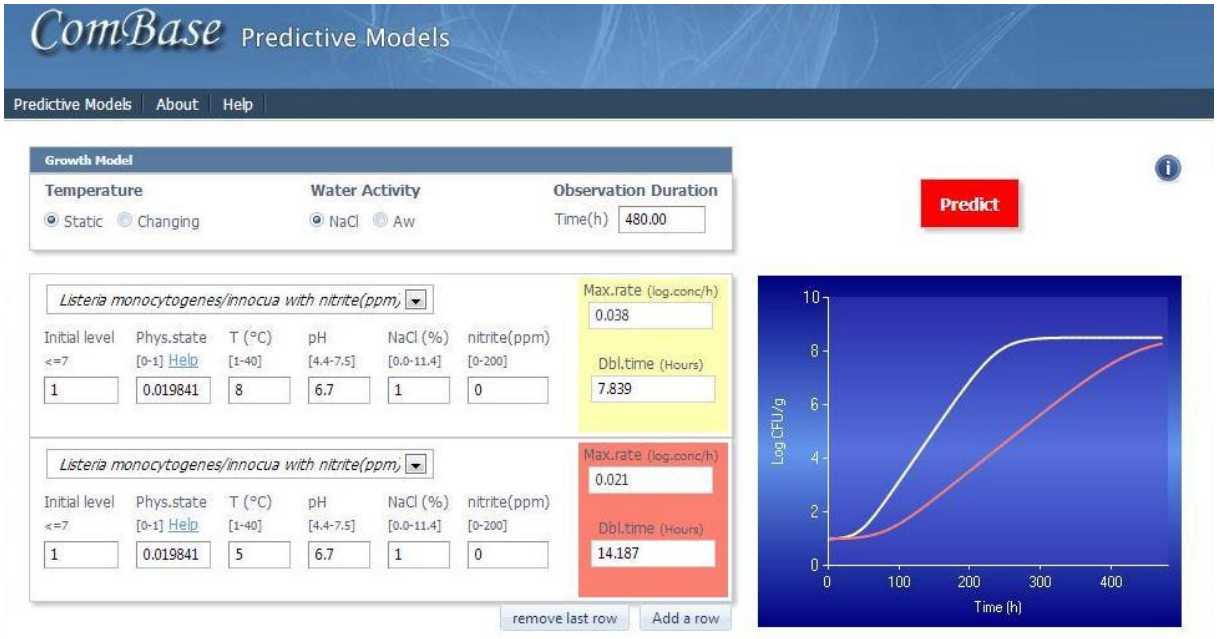


Figure 16 A ComBase model prediction of *L. monocytogenes* doubling time at 5°C and 8°C

Mataragas et al (2006) used predictive growth modelling to determine the growth limits and the kinetic behaviour of *L. monocytogenes* (strain NCTC 10527) in CSM. First, a growth/no-growth binary logistic model was used to determine the probability of growth as a function of temperature (0 to 10^{o}C) and water activity (0.88 to 0.98). At low inoculum concentration (10 cfu/g) the minimum temperature for growth was found to be 4^{o}C at a water activity (a_w) value of 0.98, and 8^{o}C when a_w was 0.96, respectively. At a higher inoculum concentration (10^4 cfu/g) the minimum temperatures for growth were 2^{o}C and 8^{o}C respectively as above for the same a_w values.

To describe the kinetic behaviour of *L. monocytogenes,* Mataragas et al (2006) used primary models that determined the kinetic growth parameters of maximum specific growth rate (μ_{max}), the length of time of the lag phase of growth (lag phase duration, LPD) and initial (N_0) and maximum (N_{max}) populations of cells[1]. In addition, secondary models were used to describe the effect of temperature (T) on the primary parameters. The initial *L. monocytogenes* concentration modelled was 1.5 log cfu/g and the storage temperatures were 4^{o}C, 8^{o}C, 12^{o}C, and 16^{o}C, respectively. The predicted kinetic parameters reported by Mataragas et al (2006) (Table 20) were validated by additional laboratory-based experiments. It is important to note that when the storage temperature was decreased from 16^{o}C to 4^{o}C, the specific growth rate (μ) of *L. monocytogenes* decreased from 0.99 to 0.08 per day and the lag phase increased from 0.85 to 5.52 days. The finding underscores the importance of effective temperature controls for *L. monocytogenes* and CSM.

The secondary models determined the functional dependences of temperature on the kinetic growth parameters as depicted by Figure 17.

$$(\mu_{max})^{0.5} = 0.058 \times (T + 1.03)$$

[1]When bacteria encounter a new environment, it takes them time to determine what nutrients are available to them. Once that information is available, the cells upregulate all the biochemical pathways that are required to make use of the nutrients in the new environment. Although the cells are busy, it looks like nothing is happening. Once the required pathways are up-regulated, the cells start growing and multiplying and the population of bacteria expands exponentially. Exponential expansion is casually referred to as log growth, the stage prior to that when the cells are adapting to the environment is called the lag phase. LPD is the length of time the cells are in lag phase. N_o is the initial number of cells and *N*max is the number of cells in the population when the end of the log phase is reached (i.e. when the cells exhaust all the nutrients that are available to them).

$$\left(1/exp(LPD)\right)^{0.5} = 0.06 \times (T + 0.66)$$

$$(N_{max})^{0.5} = 0.058 \times T^2 - 0.076 \times T + 2.298$$

Figure 17 Functional dependences of temperature on kinetic growth parameters determined by the secondary models (Mataragas et al 2006)

These predictive models can be used by the meat industry to develop food formulations and manufacturing practices that do not support the growth or maintenance of *L. monocytogenes.*

Table 20 Observed and predicted growth parameter values for *L. monocytogenes* in CSM (Mataragas et al 2006).

Temp (°C)	μmax (day^{-1})		LPD (days)		*N*max (log cfu g^{-1})		95% CI of predicted values					
	Obs	Prd	Obs	Prd	Obs	Prd	Lμ	Uμ	L LPD	U LPD	L *N*max	U *N*Max
Development												
4	0.08	0.09	5.52	5.53	4.33	4.37	0.04	0.15	12.48	3.31	3.84	4.93
8	0.30	0.27	3.05	3.31	4.46	4.32	0.22	0.34	4.60	2.37	3.97	4.68
12	0.54	0.57	2.33	1.96	4.97	5.11	0.49	0.66	2.78	1.28	4.74	5.51
16	0.99	0.98	0.85	0.98	7.04	6.97	0.81	1.16	1.96	0.21	6.30	7.67
Validation												
10	0.42	0.41	3.58	2.57	4.56	4.60	0.34	0.48	3.48	1.85	4.28	4.94

Obs, observed value; Prd, predicted value; L, lower limit; U, upper limit; CI, confidence interval; μ, specific growth rate constant.

The same authors (Mataragas et al, 2007) used time-temperature profiles for retail (n=31) and home (n=34) refrigerators, together with growth models for LAB and *L. monocytogenes* to determine the shelf life of cooked cured meat products. A probabilistic model to calculate the percentage of a product failure at or before a specific time was used in order to estimate the product "use by" date. Both storage temperature and temperature variability were the most important factors for the duration of shelf life, whilst the initial microbial load had a smaller effect. For example, a 3°C temperature reduction from 5°C to 2°C would increase shelf life by up to 19 days. The same temperature decrease from 10°C to 7°C, would only extend shelf life by 6.5 days.

Koutsoumanis et al (2007) developed a probabilistic model to evaluate the compliance of RTE foods with EU safety criteria for *L. monocytogenes* (i.e. a maximum allowable concentration of 100 cfu/g or ml). The Monte Carlo model was applied for 160 RTE sliced meat products (e.g. bresaola, chicken breast, coppa, ham (cooked and fermented) etc.) sampled from the Greek market and the predicted results were presented in the form of three main outcomes. The outcomes were cumulative probability of growth, the percentage of packages able to support growth and the percentage of packages with more than 100 cfu/g at the end of the shelf life. Table 21 represents a snapshot of the main outcomes from the Koutsoumanis et al (2007) study. An overall conclusion of the Koutsoumanis study was that *"compliance of RTE foods with the new safety criteria should not be considered a parameter with a discrete and binary outcome because it depends on factors such as product characteristics, storage temperature, and initial contamination level, which exhibit considerable variability even among different packages of the same RTE product. Rather, compliance should be expressed and therefore regulated in a more probabilistic fashion."*

Table 21 A snapshot of the characteristics and contamination predictions for sliced RTE meat products in the Hellenic retail market (adapted from Koutsoumanis et al 2007)

Product			*n*	pH	a_w	Shelf Life (Days)	Predicted % of packages	
No.	Name	Manufacturer					Able to support growth	With >100 CFU/g at the end of shelf life (Contaminated)
1	Bresaola	V	1	6.37	0.930	98	6.4	9.5
2	Bresaola	V	2	6.75	0.924	98	0.1	3.3
3	Chicken breast	XV	1	5.98	0.968	36	86.0	82.4
4	Chicken breast	XV	2	5.57	0.974	36	85.9	66.0
5	Chicken breast	XV	3	5.52	0.965	36	74.8	41.5
6	Chicken breast	XV	4	5.76	0.966	36	81.7	67.6
7	Coppa	IV	1	6.28	0.921	-[a]	2.1	-
8	Coppa	V	1	6.04	0.925	97	7.7	11.0
9	Coppa	V	2	6.11	0.905	98	0.1	3.3
10	Ham (Cooked)	IV	1	6.37	0.965	-	76.6	-
11	Ham (Cooked)	IV	2	6.04	0.983	-	95.9	-
12	Ham (Cooked)	IV	3	5.52	0.975	-	85.6	-
13	Ham (Cooked)	IV	4	6.10	0.984	-	95.8	-
14	Ham (Cooked)	IV	5	6.19	0.988	-	97.4	-

Pal et al (2008b) used primary predictive models and growth data to determine a selection of the fastest growing strains of *L. monocytogenes* to be recommended for future use in challenge studies on delicatessen meat and poultry food matrices. No single strain was found to be consistently fastest in all media (tryptic soy broth and slurries prepared from cooked uncured sliced turkey breasts). However, a

range of ribotypes were recommended for further growth studies, based on their characteristic short lag time, fast growth rate or otherwise short time to multiply 100 fold.

The same authors (Pal et al, 2008a) used three of the recommended ribotypes (DUP-1044A, DUP1038B and DUP-1030A) and evaluated growth dynamics in RTE sliced uncured turkey breast and cured ham, under typical storage conditions. Treatment conditions included high pressure treatment (400 MPa for 15 min) and a formulation containing 2.0% (w/v) potassium lactate and 0.2% (w/v) sodium diacetate (PL/SD). The Baranyi model was used to estimate lag times and growth rates for samples VP stored at 4^{o}C, 8^{o}C, or 12^{o}C for several weeks. In samples without antimicrobials (i.e. high pressure only and controls) changes in temperature determined changes in the growth rates (GR) of *L. monocytogenes* (e.g. a ten-fold increase in GR from 4^{o}C to 8^{o}C and a two-fold increase from 8^{o}C to 12^{o}C, respectively). The antimicrobial treatment (PL/SD) inhibited the growth of *Listeria* only at 4^{o}C. A significant growth in *L. monocytogenes* levels (>100 fold, $P<0.05$), within the typical product shelf life of 60 to 90 days, was observed for storage at 8^{o}C and 12^{o}C.

A quantitative microbiological risk assessment (QMRA) model was developed by Mataragas et al (2010) to estimate the fate of *L. monocytogenes* in sliced cooked, cured ham-like meat products. The model was focused on the exposure assessment and the risk characterisation stages included the cooking and slicing steps of processing and storage, distribution and consumption. The input data for the variables were taken from the literature and expert opinion. The main purpose of the study was to identify factors that influenced the risk of Listeriosis and then to evaluate the effectiveness of putative interventions or mitigation strategies. The study targeted high-risk populations (immunocompromised including elderly, infants and pregnant women). The model predictions were that the average *L. monocytogenes* dose per serving of CSM was 2.42 log cfu, and that the average was highly variable (95% CIs between -0.34 to 6.65 log cfu). Furthermore, in agreement with some reported outbreaks (Section 6) low prevalence and high doses were predicted to be responsible for most cases of listeriosis. Sensitivity analysis (Figure 18) predicted that: at-home fridge temperature, retail temperature, initial *Listeria* population at retail, serving size consumed and storage time at both home and retail had the greatest influence on the number of listeriosis cases per year. Sharp changes in the number of listeriosis cases were also predicted when the values of the above parameters rose above certain thresholds (e.g. >22 days storage time at retail, >5 days storage time at home, >7^{o}C retail temperature or >9^{o}C home fridge temperature). The predicted listeriosis cases per year (average 155 cases, 90%CI 0.0004 to 692) in elderly people were compared with the 94 recorded cases in the EU (EFSA, 2008) to

verify the reliability of the model. The Mataragas et al (2010) study provided a working example that explained how risk managers could use the risk assessment results to underpin food safety policy. However, further work is required to adapt models like the one developed by Mataragas et al (2010) to provide a "user-friendly" interface in order to become a useful tool for the CSM industry.

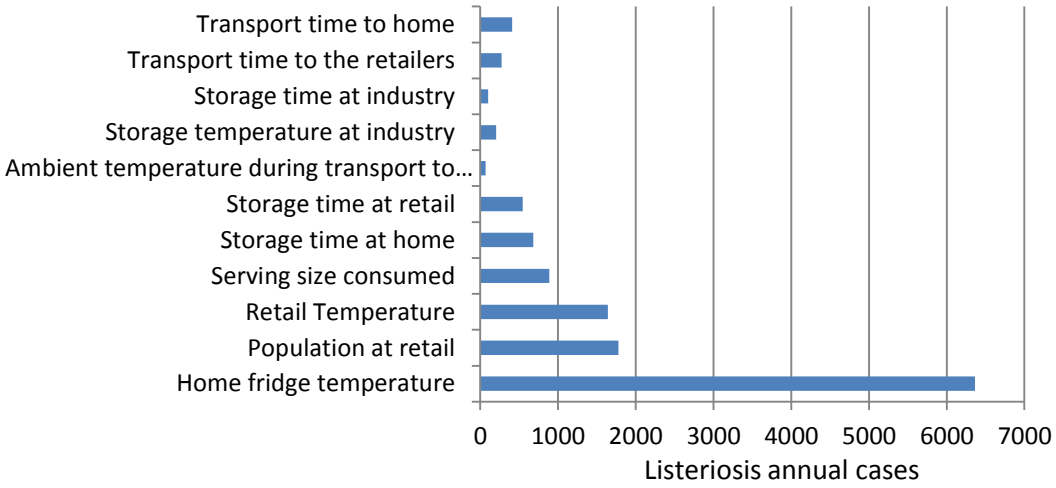


Figure 18 A sensitivity analyses, which predicts the factors associated with predicted listeriosis cases from sliced cooked cured ham. (Adapted from Mataragas et al (2010).

Pradhan et al, 2010 also developed a QMRA that considered two types of deli meats (ham and turkey). The model purpose was to estimate the relative risks of listeriosis-associated deaths attributable to *L. monocytogenes* contamination of the meats. The Pradhan et al (2010) model predicted that the use of growth inhibitors (e.g. lactate and diacetate), in products contaminated during the manufacturing process, would reduce human listeriosis deaths linked to ham and turkey by 2.8- and 9-fold, respectively. The reduction would be 1.9 and 2.8-fold, respectively for products contaminated at retail.

In broad agreement with the Mataragas model, sensitivity analysis predicted that storage temperature was the most important factor associated with listeriosis in deli ham and turkey. The study also suggested that reducing the storage temperature at home to below 7^{o}C would greatly reduce the risk of human listeriosis deaths (62% and 79% reduction in cases, for the consumption of pre-packed and retail-sliced ham, respectively, when compared with a baseline scenario of a storage temperature >10^{o}C).

8.4 SHELF LIFE DETERMINATION

A comparison of the fate of a three-strain mixture of *L. monocytogenes* using the AFSSA protocol (AFSSA, 2008) with a more "industry standard" challenge protocol concluded there were no significant differences between the two protocols (Everis and Betts, 2013). For the products assessed, AFSSA advise the inoculation of three batches of product at concentrations below 100 CFU/g meat, with chilled pre-incubation of the culture and storage for 7 d at 8^{o}C followed by 14 d at 12^{o}C. The comparison protocol offered as a commercial service by the researchers used a single batch of product, an inoculum level of between 100 and 1000 CFU/g and cultures grown overnight at 30^{o}C with a storage regime of 21 d at 8^{o}C. The isolates used for the comparison were from chicken, a meat factory environment and a patient with meningitis as a consequence of consuming contaminated cheese (NCTC 11994). The researchers concluded that the growth of *L. monocytogenes* for both protocols over 21 days at 8^{o}C was similar.

Peck et al (2006) summarised shelf lives for delicatessen meat, finding them to range from 15-28 days for UK cured pre-packed RTE delicatessen meats sold at retail (Table 22). By comparison, shelf lives of products manufactured outside of the EU were 14-84 days for a range of cured and non-cured CSM (Table 23). The variation was largely due to lack of agreed shelf life determination protocols internationally.

Table 22 Shelf lives of UK pre-packed multiple retailer delicatessen meat (NaCl, Shelf Life, CCPs)

Product	Atmosphere	% Salt	Shelf Life (d)	Heat process	Preservative
Honey cured ham	MAP + O_2 scavenger	1.0	15-25	>70°C/2 min	Sodium nitrite
Smoked ham	MAP + O_2 scavenger	2.3	15-25	>70°C/2 min	Sodium nitrite
Cooked ham	VP	2.1	28	>72°C/2 min	Sodium nitrite
Turkey ham	MAP	1.0	15-25	>70°C/2 min	Sodium nitrite
Cured sliced meat	MAP	2.3	21	72°C/2 min	Sodium nitrite
Cured cooked sliced meat	VP	2.3	23	>72°C/2 min	Sodium nitrite

Derived from Peck et al (2006)

Table 23 Examples of Non-UK Pre-packed Multiple Retailer Deli Meat (NaCl, Shelf life, Processes)

Product	% Salt	Shelf Life	Heat process	Preservative	Country
MAP honey roast ham		4 weeks		Sodium nitrite	Australia
MAP hickory smoked ham		4 weeks		Sodium nitrite	USA
Cured sliced meat MAP	2.1	4-8 weeks		Sodium nitrite	Italy
Cured sliced meat VP	2.0	2-3 weeks	>70°C/2 min	Sodium nitrite	Finland
Hot smoked game VP	1.5-1.6	14 days	>70°C/2 min		Finland
MAP cooked meat		75-84 days			USA
VP cooked pork shoulder		6 weeks		Sodium nitrite	USA
MAP Cooked turkey		5 weeks			Australia
VP Cooked chicken		>3 weeks			Spain

Derived from Peck et al (2006)

Marklinder (2004) found that consumers' opinions in Sweden relating to the length of time that products could be stored varied, depending whether the packs were opened or not (Table 24). There were 52 observations for ham unopened, and 101 observations for ham opened.

Table 24 Swedish consumers' stated domestic storage times for opened or unopened packs of ham

Storage time	Recipient responses to perceived safe domestic storage times for ham in Sweden (% respondents that agreed a safe storage interval)*	
	Opened	Unopened
1 day	2	0
2 days	5	0
3-4 days	32	2
5-6 days	12	0
1 week	25	12
2 weeks	2	6
3 weeks	0	0
On 'Best before' date*	7	54
Judgement	13	15
Other/no opinion	2	12

*Product was ham with an expiry date of ~3 weeks. Note that these products would be expected to be labelled 'use by' rather than 'best before'. (Adapted from Marklinder et al., 2004).

9 INTERVENTIONS FOR THE CONTROL OF *L. MONOCYTOGENES* ON CSM

9.1 THERMAL PROCESSING

The effect of an in-package thermal pasteurisation of turkey bologna for 10s at 85°C in a water bath was assessed by McCormick et al (2003). The treatment caused a greater than six logs reduction to *L. monocytogenes* inoculated onto the meat. Decimal reduction times for lower temperatures were 124s and 16.2s for 65°C and 61°C respectively. Similar encouraging results have been reported by other authors undertaking similar studies. Muriana et al (2002) reported that immersing deli meats in water at 90-96 °C for two minutes would reduce *L. monocytogenes* numbers by more than two logs. Later work by Juneja (2003) using beef is summarised as Table 25.

Table 25 Decimal reduction times for *L. monocytogenes* on beef slices (adapted from Juneja, 2003)

Temperature (°C)	Decimal reduction time (s)
60	4.67
65	0.72
71.1	0.17
73.9	0.04

The effectiveness of *L. monocytogenes* inactivation during in-pack pasteurisation depends on a number of factors including pack volume and the roughness of the product surface (Muriana et al 2002, Murphy et al 2003b)

Enns et al (2007) used a single strain of *Listeria innocua* that was 1.3 times more heat resistant than *L. monocytogenes* to assess a two-step cooking method as an intervention for *L. monocytogenes*. The work was undertaken using pilot scale equipment under commercial processing conditions and so a non-pathogenic strain of *Listeria* was used as a surrogate for *L. monocytogenes*. The effect of grilling raw sliced chicken breast that had been marinaded in a solution of *L. innocua* was determined. In addition, the effect of a post-packing hot water pasteurisation was investigated. Water at 72°C was sprayed onto the packed meat for 10 minutes (Enns et al 2007). The initial searing of the product produced a two to three log decrease to the numbers of *L. innocua*. The hot water pasteurisation

caused a further two log decrease in *L. innocua*, and the authors concluded that these steps would have to include additional control measures to achieve the desired 7 log reduction in *L. monocytogenes* (Enns et al 2007). There some issues with the Enns et al (2007) study that would prevent commercial adoption. The first was that the meat used for the study was initially raw, and the grilling and pasteurisation treatments did not fully denature the protein (i.e. the authors report the centre of the meat was still raw). The second consideration is that the heat caused significant water loss of around 10% from the meat, which would have considerable commercial implications. Finally, the chicken breast was sliced more thickly than is common for CSM in the UK, and thus the experiments were not a good model for commercial practices.

Ha and colleagues (2012) investigated a novel method aimed at improving the heating of cooked sliced ham using infrared emitting lamps. The study used an aluminium chamber and positioned near infrared (NIR) emitting lamps above and below meat samples that had been inoculated with a nine-strain cocktail of *S. Typhimurium* (n=3)*, E. coli* O157:H7 (n=3) and *L. monocytogenes* (n=3). To enable direct comparison with conventional heating, the study replaced the NIR lamps with electric resistive lamps. Numbers of each of the potential human pathogens were determined after each of the different treatment methods. A four-log reduction to the numbers of all three pathogens could be obtained on the surface using the NIR after 50 seconds. To achieve a similar reduction with the electric resistive lamps, a 180 second treatment time was required. Furthermore, the NIR uniformly reduced the numbers of *L. monocytogenes* on the ham surface as well as inside the meat. In contrast, conventional convective cooking showed that *L. monocytogenes* numbers inside the ham were roughly 2-log higher compared with surface slices after 180 seconds of cooking (Ha et al 2012). There was no significant difference in colour between the NIR treatment and control ham slices (Ha et al 2012). Although interesting, the results of the study indicate that NIR is better at generating heat which penetrates inwards from the meat surface within a few minutes. The purpose of the study was not clear however. From a practical viewpoint, the cooking of CSM typically lasts several hours and a consideration is the prevention of significant amounts of water being removed from the meat. The water loss for NIR used as a substitute for traditional baking was not determined. The authors did suggest that their experimental set-up could easily be scaled-up and ham slices could be fed along a conveyor belt and exposed banks of NIR lamps prior to packing as a way of sanitising the meat. Although a promising intervention, no indication was given regarding likely additional costs and any organoleptic alterations compared with a traditional process.

The UK's chilled prepared food industry has since 1989 applied processes equivalent to a minimum of 70°C for 2 minutes to deliver six decimal reductions to *L. monocytogenes* populations with an additional safety margin (Chilled Food Association, 1989). The thermal process is derived from two similar scientific studies (Gaze et al 1989. Mackey et al 1990).

A table of lethal rates at different temperatures is published by CFA (Table 26) in its manufacturing guidelines to aid process implementation (CFA, 2006). The guidance states "These data are based on laboratory studies and are supplied as an example of the necessary process to achieve a six-log reduction of *Listeria monocytogenes*, the most heat-resistant vegetative pathogen of significance in chilled foods. As a consequence, all other vegetative pathogens, such as *Staphylococcus aureus*, *Campylobacter*, *E. coli* and *Salmonella*, will also be heat-inactivated (i.e. at least a six-log reduction)."

Table 26 Lethal rates for *L. monocytogenes* (reproduced from CFA, 2006)

Temperature (°C)	Time (min, sec)	Lethal Rate
60	43'29"	0.046
61	31'44"	0.063
62	23'16"	0.086
63	17'06"	0.117
64	12'40"	0.158
65	9'18"	0.215
66	6'49"	0.293
67	5'01"	0.398
68	3'42"	0.541
69	2'43"	0.736
70	2'00"	1.000
71	1'28"	1.359
72	1'05"	1.848
73	0'48"	2.512
74	0'35"	3.415
75	0'26"	4.642
76	0'19"	6.310
77	0'14"	8.577
78	0'10"	11.659
79	0'06"	15.849
80	0'05"	21.544
81	0'04"	29.286
82	0'03"	39.810
83	0'02"	54.116
84	0'02"	73.564
85	0'01"	100.000

9.2 GAMMA IRRADIATION

Sommers et al (2002a) and Sommers et al (2002b) report that ionizing radiation from a gamma source can pasteurise RTE CSM. Using a five-strain cocktail of outbreak strains of *L. monocytogenes* inoculated onto bologna; up to five logs of *L. monocytogenes* could be removed using a three-kGy dose. Roughly, each one kGy dose of radiation applied caused a 1.5 reduction to the numbers of *L. monocytogenes* on the CSM. As part of their studies, Sommers et al (2002a, 2002b) investigated the impact of glucose concentration within the bologna on *L. monocytogenes* kill. In brief, cooking meat which contains dextrose, results in the production of antioxidants that may interfere with activity of ionizing radiation. Beef bologna was manufactured with dextrose concentrations of 0, 2, 4, 6, and 8% (w/w). There was no significant impact on *L. monocytogenes* kill between the different sugar concentrations. However, the authors noted that lipid oxidation increased significantly in irradiated bologna. There were also other organoleptic consequences for irradiating the CSM. The ionizing radiation caused a loss of redness, and promoted a loss of brightness. Although legal in the EU (subject to appropriate labelling), off odours caused by lipid oxidation are a significant barrier to the adoption of irradiation as an intervention for *L. monocytogenes* on CSM.

9.3 APPLICATION OF ELECTRON BEAMS TO CONTROL *L. MONOCYTOGENES*

Irradiation was also shown to effectively control strains of *L. monocytogenes* on beef bologna slices (and frankfurters, which are beyond the scope of this report) (Sommers et al 2002a; Sommers et al 2002b). Furthermore, the same group showed that the common anti-oxidants dextrose and sodium erythorbate did not have a detrimental effect on the control of *L. monocytogenes* (Sommers et al 2002a; Sommers et al 2002b). Compared to cheese slices that require 1.5 kGy to produce a 5 log reduction in *L. monocytogenes* a total of 3 kGy was required to produce a 5 log reduction in Turkey slices (Sommers and Boyd, 2005).

When turkey ham or breast roll inoculated with a five strain cocktail of *L. monocytogenes* was treated with 2.5 KGy of radiation using an electron beam, a reduction of 4-5 log cfu/g to the numbers of *L. monocytogenes* was observed by Zhu et al (2008). The study also assessed any effect of applying one

of five antimicrobial treatments: 0.1% potassium benzoate, 2% sodium lactate, 0.1% potassium benzoate plus 2% sodium lactate, 2% sodium lactate plus 0.1% sodium diacetate and a combination of 0.1% potassium benzoate, 2% sodium lactate, and 0.1% sodium diacetate. Compared with irradiation only, none of the treatments showed any significant influence on the numbers of *L. monocytogenes*. Overall, the Zhu et al (2008) study and related later work (Zhu et al 2009), showed that low doses of irradiation (1.0 to 2.5 kGy) effectively reduced the number of *L. monocytogenes* and natural flora on CSM. However, the *L. monocytogenes* and other natural flora that survived irradiation took advantage of the cleared niche and the lack of competition resulted in rapid multiplication during a subsequent 28-d storage period at 4°C. Zhu and colleagues believe that "additional hurdles are needed to ensure microbial safety after low-dose irradiation".

The inactivation kinetics for a single strain of *L. monocytogenes* and a single strain of *L. innocua* exposed to E-beam radiation were determined for VP dry-cured ham. Initial inoculations were almost nine-log cfu/g. Broadly, a dose of radiation equivalent to one kGy caused around a two-logs reduction to both strains of *Listeria*. *L. innocua* was slightly more susceptible to the radiation compared with *L. monocytogenes*. The USDA has a zero-tolerance policy for *L. monocytogenes* in RTE meat products, which Hoz et al (2008) interpret to mean less than a single *L. monocytogenes* cell in a 25g sample or a food safety objective of less than four cfu/100 g (-1.39 log cfu/100 g). Using the worst case decimal reduction value of *L. innocua*, Hoz et al (2008) calculate an application of 1.12 kGy was required to reach the USDA food safety objective. Dry-cured hams treated with one and two kGy had negligible sensory modifications for texture, appearance, odour, and flavour (Hoz et al 2008). The methods section of the Hoz study states that "… after irradiation, samples were stored at 4^{o}C until use", which we interpret to mean that no long term refrigerated storage to determine the fate of sub-lethally injured *L. monocytogenes* was undertaken.

Cabeza et al (2010) evaluated electron beam irradiation as a potential control measure for *L. monocytogenes* on cooked ham. A dose of irradiation (two kGy or three kGy), was applied to inoculated VP ham. The initial population of the single strain of *L. monocytogenes* applied to the meat was 10^{7} – 10^{8} cfu/g. The application of a two-kGy dose caused a reduction to the *L. monocytogenes* population to 2.53 log cfu/g. The application of a three-kGy dose decreased the population by over seven-logs and no subsequent *L. monocytogenes* growth was detected (Cabeza et al 2010). Although effective, the application of a three-kGy dose of irradiation caused detrimental effects on the sensory quality of the product (Cabeza et al 2010). In particular, Cabeza et al (2010) report an increase in the

sensory properties of the ham associated with spoilage, which was a likely consequence of lipid oxidation by the irradiation. The authors conclude that an E-beam dose of two kGy guarantees the microbiological safety of the product along its shelf life, even if a noticeable temperature (10^{o}C) abuse were to occur during storage. However, the early manifestation of off-flavours would mean that product shelf life would be reduced significantly.

Song et al (2011) also assessed the impact of a two-kGy dose of electron beam radiation to sliced ham inoculated with two strains of *L. monocytogenes*. Song et al (2011) observed a 2.58 log cfu/g reduction in *L. monocytogenes* numbers. With the same dose, Cabeza et al (2010) reported a 4.5 log reduction. Any potential reason for the 100-fold difference was not acknowledged or discussed by Song et al (2011). Song et al (2011) made no apparent assessment of any organoleptic changes in the ham.

In summary, electron beam radiation can cause significant reductions to *L. monocytogenes* contaminating CSM. The current issues are that a low dose of radiation does not kill all of the bacteria on the surface of the CSM. The partly cleared niche allows those cells not killed by the radiation to multiply rapidly, even at refrigeration temperatures. If the higher doses of radiation required for total bacterial kill are applied, organoleptic changes to the meat occur. As the worst case, these changes include lipid oxidation, which is indistinguishable from prematurely spoiled CSM.

9.4 PLASMA

Plasma is electrically energised matter in a gaseous state. Typically, plasmas are a natural state of matter that occurs mostly at high temperatures. However, referred to colloquially as cool plasmas, there are now technologies available for ambient temperature generation of plasmas at atmospheric pressure (Song et al 2009). Plasmas cause bacterial cells to rupture (Kim et al 2011). Song et al (2009) determined the effect of applying cool plasma to ham contaminated with a three-strain cocktail of *L. monocytogenes*. Using 150 W for plasma generation, a 120-second cool plasma exposure reduced the numbers of *L. monocytogenes* on ham from an initial 8.9 log cfu/g down to 7.2 log cfu/g; a reduction of 1.7 log cfu/g. A similar treatment applied to sliced cheese caused a greater than seven logs reduction. The authors concluded that the effect of atmospheric pressure plasma was strongly

dependent on the type of food (Song et al 2009). Although the inoculations were much higher than would be expected in recently manufactured CSM, based on the Song et al (2009) study only, it is unlikely that a meaningful impact would be made were plasma to be routinely used for CSM decontamination. Furthermore, there are legal barriers preventing the use of cool plasma as a CSM decontaminant in the EU currently.

Kim et al (2011) also assessed cool plasma as an intervention for *L. monocytogenes*. Contaminated slices of bacon (6 mm, 1g rectangles) were inoculated with 10 ml (10^9 cfu/ml) of *L. monocytogenes* (strain KCTC 3596). The samples were then sealed in plastic petri dishes and incubated at 10^oC for 1 h to facilitate bacterial attachment. The samples were subjected to the plasma treatment for 60 or 90 seconds. Three levels of input power (75, 100 and 125 W) were used to generate the plasma in either a helium or an oxygen/helium mixture at a flow rate of 10 litres/min. Statistically significant microbial reduction was achieved using the helium/oxygen mixture only. The Kim et al (2011) study used a lower exposure time and less power to generate the plasma compared with the Song et al (2009) study. However, in contrast to the findings of Song et al (2009), the initial *L. monocytogenes* count of 7-8 log cfu/g was significantly reduced ($P<0.05$) to 5.8 log cfu/g after plasma treatment for 90 s at 125 W (Kim et al 2011).

A second study by the same research group (Lee et al 2011) tested the efficiency of cool plasma for the inactivation of *L. monocytogenes* and aerobic bacteria in chicken and ham. He, N_2 (both 7 L/min) and He + O_2 and N_2 + O_2 mixtures (both 0.07 L/min) were used to generate the plasma jets. *L. monocytogenes* was inoculated on either agar media (average concentration of 7.59 log cfu/ml) or slices of cooked chicken breast (~6.3 log cfu/g) and ham (~6.6 log cfu/g). The plasma jets were applied for two minutes. The numbers of *L monocytogenes* on the agar plates was significantly ($P<0.05$) reduced for all of the gases assessed for plasma generation. The reductions in counts were 0.87 log cfu/g for He, 4.19(N_2), 4.26(He + O_2) and 7.59 (N_2 + O_2), respectively. Significant ($P<0.05$) reductions in counts were also observed for *L monocytogenes* in chicken breast (by 1.37 log /g for He and by 4.73 log /g for the N_2 + O_2 gas mixture, respectively). Similar results were obtained in ham where the reduction in counts were by 1.94 log /g for He and 6.52 log /g for the N_2 + O_2 gas mixture. The plasma generated by the N_2 + O_2 gas mixture was the most effective inhibitor of *L. monocytogenes* and aerobic bacteria, where the numbers remained below that level of detection (<100 cfu/g) after storage at 10^oC for seven days.

Unpublished results from work undertaken in the UK have indicated that cool plasma has a potential to become an effective method for the decontamination of metal food contact surfaces after further development (Chris Dodd, Mike Hutchison pers. comm.)

The work completed by Song et al (2009), Kim et al (2011) and Lee et al (2011) have reported conflicting results. Kim et al (2011) and Lee et al (2011) are reports from the same research group that demonstrated a potential for cool plasma generated at atmospheric pressure as a non-thermal cold pasteurization method for raw meat and CSM. The plasma was observed to reduce and inhibit the growth of pathogens on RTE CSM and to extend shelf life. In contrast, Song et al (2009) report small reductions to *L. monocytogenes* populations on ham. Currently, there is a lack of information on the effectiveness of cool plasma to enable a reliable assessment to be made. Additional research is needed to further assess and optimise the application of this relatively new method.

9.5 ULTRA VIOLET LIGHT

CSM inoculated with 10^7 cfu/g of a single strain of *L. monocytogenes* was subjected to a range of doses UV-C from 0 to 8000 J/m^2 by Chun et al (2009). None of the treatments were able to completely remove all of the *L. monocytogenes*. At the highest dose of UV-C, numbers of *L. monocytogenes* were reduced from 7.01 to 4.27 log cfu/g. After 9 days storage at 4^{o}C *L .monocytogenes* populations showed a small decline to 3.91 log cfu/g compared with the post treatment numbers (Chun et al 2009). No sensory data was reported for the intervention. However, high doses of UV-C for other foods, including meats, have been previously reported to cause rapid lipid oxidation and rancidity. A general drawback with all light-based treatments is that shading by components and a lack of penetration into foods are serious limitations to effective decontamination.

9.6 HIGH-PRESSURE

The use of high pressure processing (HPP) as an intervention for *L. monocytogenes* on sliced cooked ham was assessed by Aymerich et al (2005) and Jofré et al (2008, 2009). These studies from a single

Spanish research group both used the same three-strain cocktail of *L. monocytogenes* as an inoculant for cooked sliced ham. The main focus of the Aymerich et al (2005) was to compare a variety of interventions such as nisin (section 9.9.14) and lactate (section 9.9.7) with or without the application of a relatively low pressure of 400 MPa for 10 min at 17^{o}C. A control for the study that used only the pressure treatment showed a significant reduction of viable *L. monocytogenes* and no growth for 42 days at either 1^{o}C or 6^{o}C. However, Aymerich et al (2005) found that during prolonged storage, the *L. monocytogenes* recovered; was able to multiply, and the populations increased to 10^{6} CFU/g after 84 days at 6^{o}C.

Another Spanish study (Morales et al, 2006) assessed HPP (450 MPa for 10 min at 12^{o}C) as an intervention to reduce the levels of inoculated *L. monocytogenes* (type Scott A) on two types of dry-cured hams (Iberian and Serrano). The initial *L. monocytogenes* inoculum was six log cfu/g and the samples were VP and held at 4^{o}C for 20 h, before the HPP treatment. During this short time period declines in the *L. monocytogenes* levels of 0.44 cfu/g and 0.51 cfu/g were observed in Iberian and Serrano ham, respectively. Immediately following the HPP treatment the *L. monocytogenes* levels dropped by 1.50 log cfu/g in Iberian ham and 1.16 log /g in Serrano ham. After one week of storage at either 4^{o}C or 8^{o}C the levels were further reduced by 0.89 log cfu/g in the Iberian ham and 2.09 cfu/g in Serrano hams, respectively. The decline continued and was significant ($P<0.05$) only in the Iberian ham at up to sixty days of storage at 4^{o}C. There were no significant ($P>0.05$) changes in the levels of *L. monocytogenes* in Serrano ham until the end of the storage period at either 4^{o}C or 8^{o}C, and the situation was similar in the Iberian ham stored at 8^{o}C. No changes to the sensory characteristics for both types of hams were observed after applying HPP.

Koseki et al (2007) designed a multifactorial experiment involving four parameters (pressure applied, time of pressure-holding, duration of storage at 10^{o}C and inoculum level) to estimate the fate of *L. monocytogenes* in sliced cooked ham, during storage following HPP treatment. A pressure-resistant strain of *L. monocytogenes* (ATCC 19117) was chosen and recovery / no recovery (<100 /g) scores were used as outcomes in a logistic model, to estimate the minimum pressure necessary for effective HPP treatment. The model of Koseki et al (2007) was used to determine process conditions and shelf life and predicted the risk of *L. monocytogenes* recovery during storage. It was predicted that HPP at 400 MPa for 20 min resulted in a 50 days recovery time of *L. monocytogenes* to return to its initial inoculum of three log cfu/g. Similar results were obtained at 500 MPa for 5.2 min and 550 MPa for 1.7 min, respectively. Furthermore, HPP with a 20-min pressure-holding time at 450, 500, 550, and 600 MPa

with a higher initial inoculum (5 log /g) resulted in recovery times of 9.5, 33.4, 57.1, and 82.2 days, respectively.

As part of a larger study mostly focussed on bacteriocins, Jofré et al (2007) undertook pressure treatment of cooked, sliced ham as a control treatment. The pressure treatment moderately reduced *L. monocytogenes* counts initially. However, *L. monocytogenes* populations gradually increased to more than six-log cfu/g at the end of three-month storage. In comparison, numbers of *L. monocytogenes* in controls that were not pressure treated exceeded eight-log cfu/g at the end of storage.

Later work by Jofré et al (2009) inoculated CSM ham, dry cured ham and beef loin samples with a variety of microorganisms that included two strains of *L. monocytogenes* at a concentration of 3.5 logs cfu/g. The inoculated samples were VP in plastic bags with oxygen and water vapour permeabilities and subjected to high-pressure treatment of 600 MPa for six minutes at 31^{o}C. The pressure-treated CSM and controls were stored at 4^{o}C and sampled at 0, 30, 60 and 120 days after inoculation. *L. monocytogenes* populations in the non-pressure treated controls increased to more than seven-log cfu/g by day 60, although there was a decline to around six-log cfu/g by day 120. The concentrations of *L. monocytogenes* immediately after the pressure treatment remained similar to the untreated controls (Jofré et al 2009). However, at day 60, there was a decline in both ham samples to two-log cfu/g and to three-log cfu/g for the beef samples. At the end of the 120 days, *L. monocytogenes* populations in all the pressure-treated samples were below three-log cfu/g. Thus, the Jofré et al (2009) study concluded pressure treatment can prevent *L. monocytogenes* multiplication during refrigerated storage, a finding in keeping with their previous studies (Jofré et al 2007, 2008).

Also in Spain Marcos et al (2008b) used the synergetic effect of HPP (400MPa for 10min at 17 degree C) and natural antimicrobials (enterocins or lactate–diacetate) to assess the growth of *Listeria monocytogenes* in sliced cooked ham during refrigerated storage (1^{o}C and 6^{o}C). Three independent sample lots were prepared during production of ham: a control (lot C), a lot containing 2400AU/g of enterocins (lot E), and a lot containing 1.4% potassium lactate and 0.1% sodium diacetate (lot L–D). *L. monocytogenes* (CTC1010, CTC1011, CTC1034) was inoculated (10^4 cfu/g) at the slicing stage and the samples were VP. Half of the samples were non-pressurized and half were subjected to HPP. The samples were tested at days 0 (after packaging), 1 (after HPP), 21, 42, 63, and 84 during storage. A cold

chain break of 24 h was simulated during storage at day 40 or 60, when the samples were kept at room temperature.

In the non-pressurised samples, only the L-D treatment inhibited the growth of *L. monocytogenes* during the 84 days of storage at both 1°C and 6°C, irrespective of the 24 h increase in temperature at day 40 or 60. In the lot C and lot E samples stored at 6°C, *L. monocytogenes* had grown to more than eight-log /g after 21 days. During storage at 1°C in both the lot C and lot E samples, there was no growth until day 60, when the counts rose to a maximum (7.5 log /g) facilitated by the 24 h cold chain break.

Combining HPP and antimicrobials significantly reduced ($P<0.05$) the levels of *L. monocytogenes* immediately after the HPP treatment (day1) by 2.5 to 3.4 log /g for both storage temperatures. There was a steady growth of *Listeria* counts in lot C samples stored at 1°C, which attained four-log cfu/g after 84 days and the growth was not affected by the cold chain break at 60 days. The *L. monocytogenes* levels in lot E stabilised at 1.90 log cfu/g for up to 42 days and then decreased (below 1 log cfu/g) after the cold chain break at 60 days. The products treated by HPP and stored at 6°C had *L. monocytogenes* levels between 2.1–2.6 log cfu/g at day 21, with no significant ($P>0.05$) differences between the lots. The counts increased in controls to a maximum of 7.5 log cfu/g, after the cold chain break at 40 days. This break did not significantly ($P>0.05$) affect the counts in the lot L-D up to 63 days, but induced an increase to 7.0 log cfu/g in lot E. The results of the study (Marcos et al 2008b) showed that a treatment consisting of pressurization, storage at 1°C and addition of enterocins accomplished the EU safety criterion (<100 /g) even when high initial *L. monocytogenes* contamination levels of 10^4 cfu/g were applied.

Further evidence that high-pressure treatment can cause significant reductions to numbers of *L. monocytogenes* was provided by another Spanish study by Hereu et al (2012). The application of a high-pressure treatment on two dry cured hams decreased the numbers of a single inoculated strain of *L. monocytogenes* by roughly three-log units immediately after treatment, with further declines to almost undetectable levels at the end of a two months storage at 8°C. Although Hereu et al (2012) determined that the application of high pressure caused greater *L. monocytogenes* death and was more reliable than adding nisin. However, it was felt that there was improved protection when both hurdles to *L. monocytogenes* survival were used in combination.

Liu et al (2012) also assessed the combined effect of HPP and enterocin (LM-2) on the shelf life of RTE VP, sliced, cooked ham. HPP at 400 MPa in combination with enterocin extended the shelf life to 70 days, when the concentration of enterocin was 256 AU/g. A shelf life of 90 days was obtained when the concentration of enterocin was 2560 AU/g. Freshness, assessed by TVB-N (total volatile basic nitrogen), was not affected when compared with the control samples ($P > 0.05$). HPP at 400 MPa in combination with enterocin (256 AU/g) reduced *L. monocytogenes* (ATCC 35152) in challenge tests (10^4 /g initial inoculum) to undetectable levels for up to 20 days. When the enterocin concentration was increased to 2560 AU/g, *L. monocytogenes* was not detected for up to 30 days. The reported observations of Liu et al (2012) confirm the earlier findings of Marcos et al (2008b) that enterocin in combination with HPP has a significant potential as an intervention for *L. monocytogenes*.

Stollewerk et al 2012 have also reported that high-pressure treatment is an effective intervention for *L. monocytogenes*. The Stollewerk et al (2012) compared the decline of *L. monocytogenes* in pressure-treated ham manufactured with either KCl and potassium nitrite as the curing agents or the traditional NaCl and sodium nitrite. The pressure-treated potassium-cured ham had detectable quantities of *L. monocytogenes* until 56 days of storage at 4^oC compared with only 14 days of storage at 4^oC for pressure-treated ham manufactured using sodium salt (Stollewerk et al 2012).

In the USA, Myers et al (2013) evaluated HPP (600 MPa for 3 min) using a five-strain cocktail of *L. monocytogenes* that was inoculated (10^3 cfu/g) onto either RTE ham or turkey slices. These slices were then VP and stored at 4.4^oC for up to 182 days. For both products, counts of *L. monocytogenes* reduced by 3.85–4.35 log cfu/g, immediately after treatment - irrespective of the level of salt concentration (1.8% and 2.4%) and curing status (cured or uncured). Uncured-high salt ham samples and the uncured-low salt turkey samples permitted *L. monocytogenes* growth to levels of up to 2.4 log cfu/g over the duration of the study. However for the other CSM types, the *L. monocytogenes* concentration then remained relatively constant at low levels of -0.1 to 0.6 log cfu/g until the end of study period. Controls that were inoculated initially at low levels (one-log cfu/g) and not treated by HPP permitted growth to between five and eight log cfu/g following 28 days of storage. The study also determined that sodium nitrite treatment (0 or 200 ppm) had no inhibitory effect on the growth of *Listeria* spp.

Although there are a number of microbiologically comprehensive studies describing the effects of *L. monocytogenes* exposure to HPP, few studies have undertaken work that described any physical or organoleptic changes to CSM after high pressure treatment. Morales et al (2006) determined that there were no sensory changes to dry cured ham after HPP (450 MPa). In contrast, Campus et al (2008) concluded that high-pressure treatment (300MPa) on dry cured loin affected quality and colour, but that VP could minimize the alterations. Fulladosa et al (2009) used a trained tasting panel and concluded that a high-pressure treatment of 600 MPa had a significant effect on both CSM flavour and texture as well as on the overall slice appearance.

In summary, it is apparent that pressure treatment of CSM can, depending on the pressure applied, either cause significant reductions in the numbers of *L. monocytogenes*, or help prevent the multiplication of *L. monocytogenes*. Thus, the technology shows promise as a workable intervention. There were three publications that assessed organoleptic changes as a consequence of the application of high pressure. One study considered there to be no change, another, that the changes were minor (Campus et al 2008), and the final report considered significant organoleptic change (Fulladosa et al 2009). The three studies used different pressures and the study with the highest pressure (Fulladosa et al 2009) concluded unacceptable sensory change. Although HPP shows promise as an intervention for *L. monocytogenes*, it is likely that more work is required before an opinion could be made regarding consumer acceptability of HPP treated CSM products.

9.7 PULSED LIGHT

Hierro et al (2011) used a single strain of *L. monocytogenes* inoculated onto ham or bologna slices to determine the effect of pulsed light. *L. monocytogenes* was applied to the CSM at a concentration of seven-log cfu/cm^2. Pulsed light intensities of 0.7, 2.1, 4.2 and 8.4 J/cm^2 were applied to the CSM. A pulsed light treatment of 8.4 J/cm^2 reduced *L. monocytogenes* by 1.78 cfu/cm^2 in cooked ham and by 1.11 cfu/cm^2 in bologna. For the ham, a trained tasting panel were unable to differentiate between light-treated and non-treated control samples. However, for bologna, the panel was able to detect changes in the flavour of the CSM for light-treatments above 2.1 J/cm^2. An additional benefit of the pulsed light treatment for ham was a 30 d increase in shelf life. There was no shelf life extension for the bologna sausage. Furthermore, a pulsed light dose of 2.1 J/cm^2 caused only a 0.7 log cfu/cm^2 reduction to the numbers of *L. monocytogenes* (Hierro et al 2011). The same group of researchers

(Hierro et al 2012) investigated pulsed light as an *L. monocytogenes* decontaminant for beef slices. The results were similar for the bologna. A one-log reduction was observed when the highest dose of 8.4 J/cm^2 of pulsed light was applied. However, there were negative changes to the flavour and texture of the beef reported.

Pulsed light was assessed for its effectiveness as an anti-listerial treatment for the surfaces of salchichón (dry cured pork sausage) and dry cured pork loin (Ganan et al 2013). *L. monocytogenes* was applied to the salchichón at roughly four log cfu/g and to the loin at 4.75 log cfu/g. A range of light doses were applied to meats, and increasing the dose decreased the numbers of *L. monocytogenes*. The highest dose of 11.9 J/cm2 caused a 1.5 log cfu/g reduction to the numbers of *L. monocytogenes*. For the salchichón, small differences in the colour of the treated CSM were observed. A trained taste panel failed to detect any changes in the organoleptic properties of the salchichón either immediately after treatment or during 30 days refrigerated storage. Although panellists perceived changes in the sensory quality of loin immediately after pressure treatment, these differences disappeared during refrigerated storage.

All of the studies summarised in this section have been published from the same research group. The group consider pulsed light to be a credible method for increasing the safety of RTE dry cured meat products, although they acknowledge there may be ineffective *L. monocytogenes* kill for some CSM such as bologna. In addition, the application of pulsed light can cause colour and texture changes to some CSM such as beef slices. Pulsed light reduces, but does not eliminate *L. monocytogenes* populations.

9.8 MODIFIED ATMOSPHERE PACKAGING

Modified atmosphere packaging (MAP) is a widely used storage technique in the food industry (Arvanitoyannis and Stratakos 2012). Kramer and Baumgart (1992) used storage in a CO_2 atmosphere to inhibit the growth of *L. monocytogenes* in sliced frankfurter-type sausages. The effect of various concentrations of CO_2 (20, 30, 50 and 80% v/v) was tested at three storage temperatures (4^{o}C, 7^{o}C and 10^{o}C). An 80% concentration of CO_2 stopped the growth of *L. monocytogenes* at all storage temperatures. At lower CO_2 concentrations (50% v/v) growth was inhibited for the first three weeks of storage at 4^{o}C. Storage at 7^{o}C and 10^{o}C resulted in *L. monocytogenes* growth of 1.4 log fold and three log fold, respectively, during the 21 day storage period. The experiments of Kramer and Baumgart (1992) showed that the inhibitory effect of carbon dioxide on *L. monocytogenes* became less effective as the concentration of gas decreased.

Although Kramer and Baumgart (1992) pursued a promising approach, there are issues when using CO_2 at high concentration as a MAP. The central issues is that CO_2 is highly soluble in water. In addition, the solubility of CO_2 increases as temperature decreases (Gill, 1988). If too much CO_2 is absorbed into water in the meat, there is a risk of pack collapse. In addition, there is a chance that the dissolved gas will increase the volume of any free water in the meat, leading to excessive drip (Gill, 1988). These mechanisms are key considerations for MAP containing high concentrations of CO_2 and packs that are likely to be stored at lower temperatures. Peck et al (2006) reported gas mixtures and shelf lives recommended by MAP equipment manufacturers for cooked meats (Table 27). Although manufactures recommend CO_2 concentrations of around 40%, interviews carried out as part of this study indicated that 30% are considered the maximum attainable concentration for use in retail meat MAPs. A danger of pack collapse is what caps the CO_2 concentration in the MAPs used by CSM manufacturers for retail packaging in the UK (section 12.3.6).

Table 27 Generic international equipment manufacturers' recommended shelf lives, temperatures and gas mixtures for MAP cooked cured meat

Generic shelf lives	Temperature (°C)	Generic gas mixtures
Cooked cured meat 3-7 weeks Poultry 1-3 weeks	0°C to +3°C	Retail: 40% CO_2, 60% N_2 Bulk: 50% CO_2, 50% N_2

9.9 CHEMICAL INTERVENTIONS

9.9.1 ACETIC ACID

Dipping bologna sausage for 1 minute in 2.5% or 5% acetic acid was found to inhibit the growth of a ten strain cocktail of *L. monocytogenes* for 90 days when storage was at 4°C (Samelis et al 2001). Similarly, the observations of Geornaras et al (2004) revealed that immersion of bolgna sausage and ham in 2.5% acetic acid for 2 minutes could generate a four-log reduction to the numbers of a ten-strain cocktail of *L. monocytogenes* when stored at 10°C for 48 days compared with untreated controls. However, the organic acid tainted the meat and the acid treatment did not score as well as the controls when an untrained panel undertook a blind sensory evaluation (Geornaras et al 2004).

Although not strictly within the definition of CSM used for this study, a reduction in number of *L. monocytogenes* in beef jerky during drying was reported when the jerky was dipped in a 5% solution of acetic acid for 10 minutes compared with an undipped control (Yoon et al 2006). However, the drying was at 60°C and so both the treatment and control showed large reductions to the numbers of *L. monocytogenes*.

Acid resistance was evaluated by comparing the effect of exposure of *L. monocytogenes* cells to acid and neutral pH environments after growth on the surface of ham and turkey slices or in homogenates of these products (Skandamis et al 2012). Control strains were cultured in broth. Three *L. monocytogenes* strains were assessed individually by the study. Growth of *L. monocytogenes* on meat product slices was markedly slower than in homogenates. Pathogen reductions following exposure to pH 1.5, after 10 and 27 days of storage were strain-dependent and in the ranges of 0.5–2.5, 1.3–4.5 and 4.0–7.6 log units for cells grown on product slices and in 1:4 and 1:9 homogenates, respectively. Compared with broth controls, the study demonstrated that *L. monocytogenes* cells growing on food surfaces or in viscous matrices had a higher resistance to lethal acid conditions than cells growing in liquid broth substrates. (Skandamis et al 2012) believed that part of the reason for enhanced survival on food was that CSM has a large surface area and the surface of the CSM provided an increased buffering capacity thereby helping to protect *L.* monocytogenes cells from acid.

9.9.2 SODIUM ACETATE

To determine any the effect of a variety of CSM additives including sodium acetate, Wederquist et al (1994) inoculated turkey bologna slices with a seven-strain cocktail of *L. monocytogenes*. After inoculation, the slices were VP and stored at 4^{o}C for up to 98 days. Over the 98 day storage period, the slices treated with 0.5 % sodium acetate showed a single log increase in *L. monocytogenes* numbers compared with a five-log increase in the *L. monocytogenes* populations on the controls (Wederquist et al 1994). There was no organoleptic evaluation of the treatment.

9.9.3 SODIUM DIACETATE

When applied to frankfurters, a 0.25% sodium diacetate (SD) solution inhibited the growth of a ten-strain mixture of *L. monocytogenes* at 4^{o}C for 50 days (Bedie et al 2001). However, the same concentration of SD applied to bologna caused no significant changes to the growth of *L. monocytogenes* compared with untreated controls (Barmapalia 2005). The concentration of SD

required to inhibit the growth of a ten-strain cocktail of *L. monocytogenes* on bologna sausage at 4^{o}C was 5% and there was no inhibition if only a 2.5% solution of SD was applied (Samelis et al 2001).

9.9.4 SODIUM NITRITE

Duffy et al (1994) reported that increased lag and reduced growth was observed when 3µM sodium nitrite was added to meat. Compared with an untreated control, the nitrite doubled the time taken for a three-log increase to the numbers of *L. monocytogenes*. Furthermore, the effectiveness of the nitrite was increased significantly by the addition of 0.042% (w/w) sodium ascorbate. In the absence of nitrite, ascorbate had no detectable effect on growth (Duffy et al 1994). The key findings of the early work undertaken by Duffy was that *L. monocytogenes* multiplication was impeded as nitrite concentration increased. Furthermore, nitrite-mediated growth impedance was enhanced by ascorbate. A general conclusion from Duffy and colleagues (1994) was that the addition of nitrite extended lag times and prevented or impeded *L. monocytogenes* multiplication.

9.9.5 TRI-SODIUM PHOSPHATE

The immersion of cooked meat in tri-sodium phosphate solution and the impact to bacterial numbers on the meat was investigated by Dickson et al (1994). The study looked at the potential human pathogens *Salmonella* and *E. coli* as well as *L. monocytogenes*. The Gram-negative pathogens were assessed as more sensitive than *L. monocytogenes* to the treatment, but up to 1.5 log CFU/g reductions to the populations of the single strain of *L. monocytogenes* used by the study were observed for contaminated beef adipose tissue (Dickson et al 1994).

9.9.6 LACTIC ACID

Samelis et al (2001) undertook initial evaluations of the effectiveness of lactic acid as an intervention for *L. monocytogenes* contamination of CSM. Bologna was inoculated with a ten-strain cocktail of

L. monocytogenes at a concentration of between 10^2 and 10^3 cfu/g. Dipping in a 5% (w/v), solution of lactic acid was for one minute. The CSM were VP and stored refrigerated at 4°C for up to 120 days. Numbers of *L. monocytogenes* in the untreated controls exceeded seven-log cfu/g. The treated bologna showed no significant increase in the numbers of *L. monocytogenes* until 90 days. Beyond 90 days, uninhibited *L. monocytogenes* growth was observed (Samelis et al 2001).

Georgnaras et al (2004) also undertook and assessment of lactic acid to populations of a ten-strain cocktail of *L. monocytogenes* contaminating sliced bologna sausage or sliced ham. The CSM was immersed into a 2.5% (w/v) lactic acid solution for 2 minutes. Compared with untreated controls, the treated sliced ham showed a four-log reduction to the numbers of *L. monocytogenes* over 48 days storage at 10°C. However, a shorter dip for 1 minute in a 2.5 % solution of lactic acid did not significantly retard or reduce growth of *L. monocytogenes* on bologna sausage when stored at 4°C over 120 days. However, although effective, lactic acid-treated samples of bologna were not assessed favourably by sensory evaluation tests undertaken by an untrained testing panel (Georgnaras et al 2004).

9.9.6.1 LACTIC ACID IN COMBINATION WITH OTHER ADDITIVES AND TREATMENTS

Juncher et al (2000) investigated 2% lactate in combination with 0.5% acetate and 2% lactate in combination with 0.25% glucono-delta-lactone (GdL) Both combinations were observed to be able to prevent the growth of *L. monocytogenes* on sliced saveloys manufactured with either 60 ppm or 150 ppm nitrite (Juncher et al 2000). A mixture of five *L. monocytogenes* strains (three environmental and two product isolates) were inoculated on both sides of 20 g saveloy slices (10 cfu/g). The slices were packed in modified atmosphere (80% N_2/20% CO_2) using a film with low oxygen transmission rate (0.45 $cm^3/m^2/atm/24h$) and stored at 5 or 10 degrees C for up to 4 weeks. Significant *L. monocytogenes* growth ($P<0.05$) was observed only on slices manufactured with 60 ppm nitrite stored at 5°C and treated with the lactate-GdL combination, but the final count levels at 28 days were still below 10 cfu/g. Although the addition of chemicals did not negatively affect the colour or the lipid oxidation rate during storage at 5°C, the treatment did not score as well as the untreated controls in the sensory evaluation tests performed by a trained panel (Juncher et al 2000).

9.9.7 SODIUM LACTATE

Sodium Lactate (E325) is approved for food use in the European Union. However, schedules 1-3 of 95/2/EC only currently permit E325 to be used for the adjustment of the pH of baby food, as a preservative for canned and bottled fruit and vegetables, and bread.

Miller et al (1994) inoculated cooked, quartered beef top rounds with various bacterial strains, including *L. monocytogenes*, and tested for any inhibitory effect of sodium lactate (1 to 4%), for samples stored under temperature challenge (10^{o}C) for up to 4 weeks. Limited proliferation of *L. monocytogenes* was obtained for samples treated with 3 and 4% sodium lactate in comparison with control samples.

Wederquist et al (1994) undertook early studies describing the effect of sodium lactate (SL) on *L. monocytogenes*. A seven-strain cocktail of *L. monocytogenes* was used to inoculate turkey bologna at between 2 and 2.75 log cfu/g before applying a 2% (w/v) solution of sodium lactate. The CSM was then VP and stored refrigerated at 4^{o}C. The lactate-treated bologna showed a two-log increase in *L. monocytogenes* numbers compared with a six-log unit rise for control untreated slices over a 98 days storage (Wederquist et al 1994). The authors concluded that sodium lactate was a viable treatment for significantly retarding *L. monocytogenes* growth on turkey bologna. However, in the EU, additive E325 is currently not allowed for use with CSM.

When a solution of 3% SL was applied to frankfurters, there was significant inhibition of the growth of a ten-strain mixture of *L. monocytogenes* at 4^{o}C for 70 days (Bedie et al 2001). However, when the same concentration of SL was applied to sliced bologna, all the tested additives showed no significant differences to the growth rates of *L. monocytogenes* compared with untreated controls (Barmapalia 2005; Samelis 2001).

A general study by Cegielska-Radziejewska and Pikul (2004) assessed the effect of 1% or 2% SL included into unsmoked, cooked, medium-comminuted poultry meat sausage produced in a commercial poultry processing plant. The sausage was sliced after cooking and packed in nitrogen or air and the numbers

of total aerobic psychrotrophs (bacteria that can grow at refrigeration temperatures) and lactic acid bacteria naturally present in the sausage were followed over storage at 6^{o}C. The study concluded that sliced poultry sausage treated with 2% sodium lactate and packaged in nitrogen had a seven-fold increase in shelf life when compared to untreated controls packed in air. Extensive sensory and physicochemical assessments of the consequences of including the SL were undertaken. After compensating for a slight increased saltiness by reducing the amount of NaCl included into the sausage, there were no significant differences to the meat colour, odour or taste because of the SL treatments (Cegielska-Radziejewska and Pikul 2004). In contrast to the finding of no significant organoleptic differences for poultry sausage, a more recent study by (Shafit and Williams 2010) reported that there were off-flavours in turkey steaks treated with SL.

The opinion of Boles et al (2007) is that the type of sliced meat product, the storage conditions and the water content may influence the efficacy of an additive. Using sliced dried beef jerky (which does not meet the CSM definition for this study) Boles et al (2007) found no effect of SL to the survival of *L. monocytogenes* compared with untreated controls. The rate of natural decline of *L. monocytogenes* applied to beef jerky stored under vacuum at 21^{o}C over a six week period was not significantly influenced by SL (Boles et al 2007). A number of authors have indicated there were differences between the activities of some additives when applied to different CSM types (Bedie et al 2001, Barmpalia et al 2005, Thompson et al 2008). Taken collectively, the weight of evidence indicates a product-by-product approach to the assessment of additives is prudent.

9.9.7.1 SODIUM LACTATE IN COMBINATION WITH OTHER ADDITIVES

Blom et al (1997) used a three-strain mixture of *L. monocytogenes* that were rifampicin-resistant. Two strains were originally isolated from sausage and one from a knife blade at a meat processing plant. The purpose of the Blom et al (1997) study was to determine the effect of propylparaben, sodium lactate and sodium acetate on sliced servelat sausage and cooked ham. Although propylparaben was able to inhibit *L .monocytogenes* in liquid growth media, no significant influence on numbers of *L. monocytogenes* was observed when it was applied to sliced servelat and cooked ham (Blom et al 1997). However, a solution composed of 2.5% sodium lactate in combination with 0.25% sodium acetate was able to inhibit growth of *L. monocytogenes* on sliced cervelat and cooked ham when stored

at 4°C for five weeks. For sliced servelat, the inhibition was maintained when the storage was at 9°C. However, for cooked ham at 9°C the inhibition was achieved for a shorter period of only 2-3 weeks. An *ad hoc* consumer acceptance trial was undertaken using 171 members of the public in a local supermarket. There was no strong preference for treated and untreated CSM and no influence for age or gender. Blom et al (1997) therefore concluded there was no organoleptic change to CSM that would prevent adoption of the lactate-acetate treatment. Blom et al (1997) concluded from their findings that broth-based inhibition studies might not be an appropriate model for food borne pathogens. The authors also reiterated advice provided by other studies that strict control of temperature during production and storage was very important for the effective control of *L. monocytogenes*.

Calicioglu et al (2002) determined that modifying traditional marinades with food grade SL, acetic acid and Tween 20 improved the effectiveness of the beef jerky drying process. Consequently, initial reductions to the numbers of a five-strain cocktail of *L. monocytogenes* showed a roughly two logs improvement over a 10 hour drying at 60°C compared with unmodified marinaded, dried controls. Follow up work by Calicioglu et al (2003) using acid adapted *L. monocytogenes* strains demonstrated that the modified marinades resulted in significantly lower numbers of surviving *L. monocytogenes* after 42 days of storage at 25°C. After 60 days however, bacterial populations were reduced to the same numbers in both modified and non-modified traditional marinades regardless of whether the *L. monocytogenes* were acid adapted or non-adapted (Calicioglu et al 2003). Neither of the Calicioglu studies undertook sensory work to determine if there were changes to the palatability of the product after treatment with the modified marinades.

Barmpalia et al (2005) studied the effects of sodium lactate (SL), sodium diacetate (SD) and glucono-delta-lactone (GDL) either individually or in combinations on a cocktail of *L. monocytogenes* inoculated onto bologna sausage slices. The ten *L. monocytogenes* strains used represented different isolates from food and clinical sources. The slices were inoculated at a concentration of 10^2-10^3 cfu *L. monocytogenes*/cm^2. Storage was VP and at either 4°C (good refrigeration) or 10°C (mild refrigeration-abuse temperatures). In control samples that lacked any chemicals, the numbers of *L. monocytogenes* increased to around 10^8 cfu/cm^2 after 10 - 20 days at 4°C and, and to the same concentration after 8-12 days storage at 10°C. Modelling of the data collected by the study that expressed *L. monocytogenes* growth as a function of time, indicated that the treatment that resulted in the lowest maximum specific growth rate (μ_{max}) was the combination of 1.8% SL and 0.25% SD. All of the chemical combinations slowed rather than prevented the growth of *L. monocytogenes*. Barmpalia

et al (2005) discussed the results of their study that were in disagreement with those reported by Samelis et al. (2002). The Samelis et al (2002) work indicated that the combination of 1.8% SL and 0.25% SD provided similar inhibition of *L. monocytogenes* as 1.8% SL and 0.25% GDL. Barmpalia et al (2005) suggested that although both products had the same basic formulation, the texture of the sliced product (bologna) may have supported bacterial growth better than frankfurters.

Lloyd et al 2009 determined the impact of incorporating SL (2% w/w) into pre-cooked turkey loaf batter and a post-cook dip of cooked meat in either SL(3.6%) /SD (0.25%) or SL(3.6%) /PL(3.6%) /SD (3.6%) on the growth of *L. monocytogenes*. In brief, none of the treatments were significantly different to each other, and all showed an extended lag phase compared with a water treated control. The lag phase was extended to 56 days when the experiment was ended. A potential criticism of the Lloyd et al (2009) study was the lack of organoleptic assessment of the organic acid treated product.

Follow on work by the same researchers (Lloyd et al 2010) reported the effect of incorporation of SL (2% w/w) and SD (0.25% w/w) into precooked ham loaf batter followed by a PL (3.6%) or SL (3.6%)/PL (3.6%)/SD (0.25%) combination dip. In summary, none of the treatments were significantly different to each other between zero and 42 days. The SL/PL/SD combination showed an extended lag of *L. monocytogenes* growth of up to 56 days, when the experiment was ended, compared with a water control. As before, no organoleptic assessment of the organic acid treated products was undertaken.

A number of studies have made the same basic comment that product texture (Skandamis et al 2012) or cut and uncut surfaces (Singh et al 2005) might have an impact on *L. monocytogenes* growth or the efficacy of interventions. One proposed mechanism for the differences is that rougher surfaces have a larger surface area that may help buffer the effects of acidic conditions (Skandamis et al 2012).

Carroll et al (2007) used a three-strain cocktail of streptomycin-resistant *L. monocytogenes* to study the effects of various marinade additives on the survival of *L. monocytogenes* in turkey deli loaves. The marinades contained either sodium tripolyphosphate (0.45%), sodium lactate (3%), sodium diacetate (0.25%), sodium citrate (SC, 0.75%) and sodium lactate (3%) in combination with sodium diacetate (0.25%). The addition of SL alone did not significantly affect the lag phase of an initial 10^3 cfu/g population of *L. monocytogenes* but SD, SC, and the SL/SD combination marinades all extended the lag phase of *L. monocytogenes* by up to 21 days. By the last sampling at day 74 growth of at least one log unit was detected in all treated samples (Carroll et al 2007). However, a trained taste panel detected

flavour differences for the SD, SC, and the SL/SD combination marinades, although these differences were described as minimal and thought not likely to be detected by most consumers (Carroll et al 2007).

Thompson et al (2008) used a combination of 1.875% sodium lactate and 0.125% sodium diacetate applied to pork bologna and turkey roll contaminated with *L. monocytogenes*. The treatment could completely inhibit the growth of a five-strain cocktail of *L. monocytogenes* on VP turkey roll stored at 3^{o}C over a 12 weeks period. For pork bologna, a small increase of less than one log unit was observed when the CSM was treated in the same fashion over a 12 weeks period. As part of the study, Thompson et al (2008) undertook consumer assessments using untrained members of the public. There was no difference between the treated and untreated samples in terms of sensory characteristics.

9.9.7.2 TEMPERATURE, SODIUM LACTATE AND SODIUM DIACETATE

Having established that SL and SD can prevent or inhibit the multiplication of *L. monocytogenes* when applied to bologna slices, Grosulescu et al (2011) investigated if temperature had any influence on the inhibition. It is not surprising that at 56.3^{o}C, the decimal reduction times (DRT, the time taken for a 90% reduction in a bacterial population) were longer than the DRTs at 60^{o}C in the absence of SD and SL. Using experimental data created as part of the study, the authors created a model to determine how concentrations of SL and SD and temperature influence *L. monocytogenes* kill. The findings reported by Grosulescu et al (2011), are the outputs from the model rather than experimental results. Of the three factors studied, temperature was the best at decreasing the survival of *L. monocytogenes* with observed DRT ranging from 2.8 min at 60^{o}C to 24.61 min at 56.3^{o}C (Grosulescu et al 2011). At 56.3^{o}C, the predicted DRT increased from 33.96 to 47.82 min with an increase in SL concentration from 0% to 4.8%. Thus, the model predicted that addition of SL showed a protective effect and at higher temperatures made the organism more heat resistant. The predicted DRT for *L. monocytogenes* at 56.3^{o}C decreased from 33.96 to 28.87 minutes, with an increase in SD concentration from 0% to 0.1875%. Thus, at low concentrations, SD increased *L. monocytogenes* decline. However, higher concentrations of SD at higher temperatures made the organism more heat resistant. A combination of

SL and SD was also effective at lower temperatures. However, higher levels of SD at higher temperatures again resulted in *L. monocytogenes* that was more heat resistant.

9.9.8 POTASSIUM LACTATE

Aymerich et al (2005) reported that VP cooked ham prepared by the addition of potassium lactate (PL) to the uncooked batter was able to inhibit a three-strain cocktail of *L. monocytogenes* for 84 days at 1^{o}C. However, over the same time-span at 6^{o}C, a four-log increase in *L. monocytogenes* growth was observed (Aymerich et al 2005). The best intervention was achieved if the PL was applied using a high-pressure treatment and the cooked ham was stored at 1^{o}C. Under such conditions, no increase in *L. monocytogenes* was detected after as long as 84 days storage using either two or four log cfu/g as the initial bacterial concentration. The high-pressure method of application of the PL is an anti-listerial intervention in its own right and so it is difficult to assess whether the inhibition is a consequence of PL or high-pressure (or both).

As part of an assessment of bacteriocins as *L. monocytogenes* inhibitors, the same researchers evaluated PL as an inhibitor of *L. monocytogenes* (Jofré et al 2007). The main difference between the Aymerich et al (2005) study and the Jofré et al, (2007) study was that the PL was applied using interleavers (thin pieces of plastic used to separate individual slices of CSM) in the later work. Over 90 days storage at 4^{o}C, a four-log increase in the numbers of *L. monocytogenes* was observed for cooked ham. Jofré et al (2007) concluded that PL applied using interleavers was not an effective inhibition treatment for *L. monocytogenes*.

Fulladosa et al (2009) undertook sensory evaluation of dry-cured hams treated with PL. The evaluations concluded that PL did not have any significant impact to the colour, flavour or texture of the hams (Fulladosa et al 2009).

9.9.8.1 POTASSIUM LACTATE IN COMBINATION WITH OTHER ADDITIVES

Ready to eat commercial turkey breast with PL (1.5% w/w) and SD (0.05% w/w) included into the uncooked batter was inoculated with a ten-strain cocktail of *L. monocytogenes* after cooking and slicing (Lianou et al 2007a). Two contamination/storage scenarios were considered. A plant-contamination scenario where the slices were initially inoculated with one to two-log cfu/cm^2 with VP and 4^{o}C storage. The packs were opened for testing at 5, 15, 25 and 50 days, then were left for 12 days under aerobic conditions at 7^{o}C to simulate home storage. During the home storage, packs were tested every 3rd day. In a retail or home-contamination scenario the original product (VP and *Listeria* free) was sliced after storage at 4^{o}C for the same time intervals detailed above (5, 15, 25 and 50 days) and inoculated as above (one to two log cfu/cm^2), simulating contamination at slicing/handling either at retail or home. The slices were packed in delicatessen bags and were stored and tested for 12 days as for the first scenario (every 3rd day). Significantly ($P<0.05$) lower *L. monocytogenes* population levels were obtained during the 12 day storage for the lactate-diacetate combination compared to controls for both contamination/storage scenarios. For the "plant-contamination" scenario *L. monocytogenes* populations in the controls reached 7.3-7.7 log /cm^2 either during storage under vacuum (for packs opened after 25 days) or during the 12 days aerobic storage (packs opened after 5 or 15 days). In the packs treated with the lactate-diacetate mixture the *L. monocytogenes* population attained maximum levels (2.9-6.8 log /cm^2) after 50 days of VP storage. For the "retail/home-contamination" scenario the *L. monocytogenes* multiplies to a level of 2.3 log cfu/cm^2.

The same research team (Lianou et al 2007b) obtained analogous results for *L. monocytogenes* contaminating commercially-manufactured ham using similar plant-contamination and retail/home contamination scenarios. Both studies of Lianou et al (2007a,b) demonstrated the potential use of potassium lactate-sodium diacetate mixtures to reduce the growth of *L. monocytogenes* in ready to eat chicken breast or ham contaminated either before the packaging step at the processing plant or during retail/home handling.

The effect of potassium lactate and sodium diacetate combined with modified atmosphere (MAP) packaging was evaluated in trials involving pork chops and sliced cured ham inoculated with *L. monocytogenes* (Michaelsen et al, 2006). The hypothesis was that the high concentration of CO_2 (99.5 to 100%) would increase the effectiveness of the antibacterial treatment. *L. monocytogenes* was inhibited for 28 days on ham samples treated with PL-SD when stored at 4^{o}C in either VP or MAP

conditions. However, the VP PL-SD combination was the only one that extended the inhibitory effect after 4 weeks of storage. The use of temperature challenge (storage at 10^{o}C) produced no inhibitory difference between samples (both pork chops and ham) treated with either PL-SD and MAP or MAP or PLSD alone. Therefore Michaelsen et al (2006) concluded that MAP using high concentrations of CO_2 did not improve the inhibitory effect of PL-SD on pathogens.

In an Australian study (Mellefont and Ross, 2007) used both PURASAL-HiPure P (containing 58 to 62% potassium lactate) and PURASAL-Opti.Form PD 4 (a blend of 54.5 to 57.5% potassium lactate and 3.7 to 4.3% sodium diacetate) to inhibit the growth of *L. monocytogenes* in modified atmosphere (30% CO2 and 70% N2) packaged sliced ham. The authors observed a listeriostatic rather than listericidal effect on *L. monocytogenes* challenged samples, as no growth was observed for seven weeks for both treatments, at either ideal storage temperature of 4^{o}C or abusive temperature conditions of 8^{o}C. The initial inoculation level (one-log cfu/g or three-log cfu/g) had practically no effect on the *L. monocytogenes* multiplication rates.

As part of the studies discussed in section 9.9.7.1, Lloyd et al 2009 also determined the impact of incorporating PL (2% w/w) into pre-cooked turkey loaf batter with a post-cook dip cooked meat in either SL (3.6%)/SD (0.35%) or SL (3.6%)/PL (3.6%)/SD (3.6%) on the growth of *L. monocytogenes.* All of the treatments showed an extended lag phase compared with a water treated control. There was no significant *L. monocytogenes* growth for up to 56 days, which was when the experiment ended. As mentioned previously, a potential criticism of the Lloyd et al (2009) study was the lack of organoleptic assessment of the organic acid treated product.

The same researchers (Lloyd et al 2010) also reported the effect of incorporation of PL (2% w/w) and SD (0.25% w/w) into precooked ham loaf batter followed by a PL (3.6%) or SL (3.6%)/PL (3.6%)/SD (0.25%) combination dip. The study is very similar to the 2009 work with the principle difference the species of meat treated. In summary, and as reported previously for turkey loaf, none of the treatments were significantly different to each other between zero and 42 days. The SL/PL/SD combination showed an extended lag of *L. monocytogenes* growth of up to 56 days, when the experiment was ended, compared with a water control. As before, no organoleptic assessment of the organic acid treated products was undertaken.

9.9.9 SODIUM LEVULINATE

In the United States, food flavouring and other additives that have been used historically in food processes are allowed for use without dose effect and response testing on the grounds that they are generally-regarded-as-safe (GRAS; Code of Federal Regulations, 2010).

Levulinic acid (4-oxopentanoic acid) is GRAS and used as a flavouring (Thompson et al 2008). Thompson et al (2008) assessed the ability of sodium levulinate to prevent the growth of a five-strain cocktail of *L. monocytogenes* that was inoculated onto pork bologna sausage and turkey roll. Sodium levulinate added to a final concentration of 3% (w/w) was able to completely inhibit the growth of *L. monocytogenes* on pork bologna and turkey roll when *L. monocytogenes* was inoculated at a concentration of 10^3 cfu/cm^2 and the CSM was VP and stored at 3°C for 12 weeks. Controls, which did not contain any sodium levulinate, saw a four log increase to numbers of *L. monocytogenes* under the same storage conditions. A sensory evaluation of sodium levulinate-treated CSM compared with untreated equivalents determined no significant difference between the two CSM types based on the opinions of 132 turkey roll consumers and 112 bologna sausage consumers.

9.9.10 POTASSIUM SORBATE

Wederquist et al (1994) inoculated turkey bologna slices with a seven-strain cocktail of *L. monocytogenes* and treated the CSM with 0.26% potassium sorbate (PS). After VP and refrigerated storage at 4°C for 98 days, a four-log increase in growth was observed, compared with a six-log increase in the untreated controls. Thus based on the findings of Wederquist et al (1994), it is apparent that PS can mildly inhibit the multiplication of *L. monocytogenes*.

Samelis et al (2001) also investigated the effect of immersing bologna sausage in PS. In the Samelis (2001) study, a stronger concentration of 5% (w/v) PS was used. The bologna was contaminated with a ten-strain cocktail of *L. monocytogenes* with VP and storage at 4°C for 120 days. Growth of the

L. monocytogenes was observed and the authors concluded that PS was not and effective intervention for *L. monocytogenes*.

9.9.10.1 POTASSIUM SORBATE IN COMBINATION WITH OTHER ADDITIVES

Islam et al (2002) assessed the effectiveness of 15 to 25% concentrations of sodium benzoate, propionate or diacetate and potassium sorbate to reduce populations or inhibit growth of *L. monocytogenes* on chicken luncheon meat. Slices were sprayed with 0.2 ml solutions of preservatives before inoculation using a five-strain cocktail of *L. monocytogenes* (five-log cfu/ml). The effect on numbers of *L. monocytogenes* was estimated at three temperatures (4^{o}C, 13^{o}C, or 22^{o}C) after 0, 3, 7, 10 or 14 days of storage. Sodium benzoate and diacetate had the highest instant effect on *L. monocytogenes* concentration (0.78 to 1.32 log cfu/g reduction at day 0). All the preservatives decreased the concentration of *L. monocytogenes* (1.5 to 3 log cfu/g reduction compared with the levels in controls) after 14 days at 4^{o}C. Storage at 13^{o}C for 14 days was effective only for the highest concentration (25%) of all used preservatives, whereas at 22^{o}C only sodium diacetate was effective for seven days or longer (1 to 3.96 log /g reduction compared to the levels in controls).

Glass et al (2007a) determined the effect of PS (0.05% w/w) in combination with sodium benzoate (SB, 0.05% w/w) as an intervention for *L. monocytogenes* on cooked, sliced ham and uncured cooked sliced turkey. The PS and SB were incorporated into the meat batter prior to cooking. The *L. monocytogenes* applied was a five-strain cocktail comprising two isolates from salami and one each from a clinical isolation, goat's cheese and milk. Packages of meat were inoculated at a *L. monocytogenes* concentration of four-log cfu/package before VP in gas impermeable pouches and storage at 4^{o}C for 13 weeks. The addition of PS and SB to the uncured turkey meat initially slowed the pathogen growth rate compared with the untreated control. However, by six weeks the populations of *L. monocytogenes* on the turkey had increased by at least five logs. In contrast, adding PS and SB to cured bologna prevented the growth of *L. monocytogenes* during the 13-week storage period at 4^{o}C, compared with a more than a three-log increase in listerial populations in the untreated control bologna. In addition to providing further evidence that it is prudent to assess interventions on a product-by-product basis, Glass et al (2007a) have demonstrated meaningful inhibition of *L. monocytogenes* by combining PS and SB.

When using smoked composite ham, the authors reported that for all treatments, including the control, there were no significant changes to the *L. monocytogenes* populations during the first two weeks of incubation at 4°C. Over a further 12 weeks storage at 4°C, the 0.1% (w/w) benzoate, 0.2% (w/w) propionate, 0.3% (w/w) sorbate, and the combination of 1.6% (w/w) lactate with 0.1% (w/w) diacetate treatments allowed significant increases to the *L. monocytogenes* populations.

9.9.11 POTASSIUM BENZOATE

As part of a larger study of chemical interventions for the control of *L. monocytogenes*, Samelis et al (2001) evaluated dipping *L. monocytogenes*-contaminated bologna for 1 minute in a 5% (w/v) potassium benzoate (PB) solution. Over a 120 days period at 4°C and under VP, there were no significant changes to the initial *L. monocytogenes* population of 10^2 CFU/cm^2.

Broadly similar findings were reported by a later study (Georgnaras et al 2005). Georgnaras et al (2005) also reported that immersion of bologna sausage or ham in a 5% (w/v) solution of PB for two minutes caused a four-log reduction in the numbers of a ten-strain cocktail of *L. monocytogenes* compared with undipped controls. The inhibition occurred during storage for 48 days at 10°C. However, PB treatments were not assessed positively when compared with untreated controls in sensory evaluation tests by an untrained panel (Georgnaras et al 2004).

9.9.12 PROPIONIC ACID IN IN COMBINATION WITH OTHER ADDITIVES

Glass et al 2013 undertook work to determine the fate of *L. monocytogenes* when propionic acid-based ingredients were used in the manufacture of cured turkey with a high moisture content, which was stored at 4 or 7°C. Five formulations of sliced, cured (120 ppm of sodium nitrite) sliced turkey were assessed, which were a 3.2% dilution from a blend of SL (56% v/v) and SD (4% v/v); a 0.3, 0.4, or 0.5% of a liquid propionate solution buffered with sodium hydroxide; or a 0.4% sodium propionate (SP) and sodium benzoate (SB) blend. The turkey was inoculated with a five strain cocktail of laboratory cultured *L. monocytogenes*, vacuum-sealed and stored at 4 or 7°C for up to twelve weeks. A control

with no antimicrobial added supported growth, with a population increase noted within four weeks of storage at 4°C. Although growth was observed at six weeks for the SL-SD treatment, all of the treatments that contained liquid propionate or propionate-benzoate limited *L. monocytogenes* growth. An increase of one log during 9 weeks storage at 4°C was observed for the SP and SP-SB treatments. *L. monocytogenes* grew more rapidly when storage was at 7°C, the authors reported that relative inhibition was similar at the elevated temperature when compared with 4°C. The authors concluded that propionate-based ingredients can beneficially extend the lag phase of *L. monocytogenes* on sliced, high-moisture, cured turkey. However, no sensory evaluation of the treated meats was undertaken.

9.9.13 CETYLPYRIDINIUM CHLORIDE

Singh et al (2005) studied the effect of a 1% solution of cetylpyridinium chloride (CPC), a quaternary ammonium compound, on *L. monocytogenes* contaminating sliced cooked beef. The USDA and FDA have approved CPC for use with poultry products based on a 'generally regarded as safe' (GRAS) history as an active ingredient in some mouthwashes (Singh et al 2005). Two types of beef slices were used by the study: a sliced surface and an exterior surface (i.e. the meat surface in contact with the casing during cooking operations). Singh et al (2005) used a five-strain cocktail of *L. monocytogenes* for inoculation of the meat slices, which were individually VP. In comparison to other chemical additives, the experiments on sliced roast beef surfaces were relatively short with the last data point obtained after six weeks. There were significant decreases in the numbers of *L. monocytogenes* inoculated onto the surfaces of sliced meat at concentrations of 10^4 and 10^7 cfu /g, followed by storage at either 0°C or 4°C when compared with untreated controls. There was an effect reported for slice side (Singh et al 2005). A greater than one-log increase in *L. monocytogenes* numbers was detected after four weeks on treated the exterior surfaces (i.e. the edge of the slice originally in contact with the casing during cooking) when stored at 4°C. Singh et al (2005) believe that differences in surface characteristics are the reason for the different findings but do not discuss what these characteristics were. The CPC treatments did not have any significant effect on meat colour assessed using an objective measure, but treated meats had reduced firmness. The authors described the reduced firmness as relatively minor. The treated samples were not subjected to any further sensory evaluation.

9.9.13.1 CETYLPYRIDINIUM CHLORIDE IN COMBINATION WITH OTHER ADDITIVES

Lim et al (2007) investigated the effects of CPC (0.5%), acidified sodium chlorite (ASC) (0.12%) individually and in combination (0.25% CPC-0.06% ASC) on several pathogens (*E. coli* O157, *L. monocytogenes* and *S. aureus*) inoculated onto sliced roast beef followed by storage for 10 days at 4^{o}C. The antimicrobial agents were either sprayed on the surface of the beef or applied to an absorbent pad onto which, the meat was placed. The CPC and CPC-ASC antimicrobial agents were the most efficient in reducing the levels of all pathogens. CPC sprayed on beef surface reduced *L. monocytogenes* and *S. aureus* to undetectable levels 2 hours after application, and the counts were also significantly ($P<0.05$) reduced for *E. coli* O157. Further, the spray treatment with CPC-ASC mixture significantly reduced ($P<0.05$) the levels of all pathogens at the end of day 0, by 4.07 (*E. coli* O157), 6.37(*L. monocytogenes*) and 4.32(*S. aureus*) log cfu/cm^2, respectively. Using ASC alone was effective only for reducing *E. coli* O157, where the bacterial counts were decreased by 6.09 log /cm^2 after 10 days of storage. Sensory evaluations revealed that the CPC treatment can extend the shelf-life of the product without impairing its quality, producing only a slight discoloration.

9.9.14 NISIN AND OTHER BACTERIOCINS

Jacobsen et al (2003) tested four different methods of preservation using the bacteriocin producing *Leuconostoc carnosum* (*Leuc. Carnosum*) 4010 or partially purified leucocins 4010 solutions to inhibit the growth of *L. monocytogenes* in sliced saveloy. A five-strain mixture of *L. monocytogenes* (10 cfu/g) was used to inoculate the sliced or peeled saveloy before adding *Leuc. carnosum* or leucocins 4010.

In the first method, the batter was inoculated with *Leuc. Carnosum* (10^6 /g), stuffed in the casing and incubated for 18 h at 15^{o}C or 20^{o}C, before heat treatment. In a second approach purified leucocins 4010 were added to batter (20 ml/kg) before stuffing and heat treatment. Alternately, to both sides of the 20 g slices were added 200 microlitres of partially purified leucocins 4010 after heat treatment.

The third method added 100 microlitres of *Leuc. Carnosum* cells suspensions (10^8 /ml) to both sides of each slice. The fourth method used jet disinfectors (nozzles) to apply lyophilized *Leuc. Carnosum* (10^8 /ml) whilst slicing (2mm slices) and after heat treatment. The four methods were evaluated for different periods of storage lasting from one day to four weeks. The methods using live cells of *Leuc. Carnosum* were the most effective as they inhibited the growth of *L. monocytogenes* in cooked, sliced and gas packed saveloy stored at 5°C and 10°C. The fourth method using nozzles was the most efficient as *L. monocytogenes* never exceeded 10 /g during 4 weeks of storage at 10 degrees C. By comparison *L. monocytogenes* grew to circa 10^7 /g in the control samples.

Mattila et al (2003) studied the effect of the bacteriocin pediocin AcH that had been reabsorbed into heat-killed *Lactobacillus plantarum*. Free pediocin AcH was bound to cells that originally secreted it by adjusting the pH of the sausage to pH 6.0. The Mattila study used a single strain of *L. monocytogenes* that was inoculated onto sliced cooked sausage before storage at 6°C for 21 days. The untreated control sausages maintained a concentration of 2.7 log cfu/g throughout the storage period. Samples treated with a final concentration of 10^4 heat-killed, bacteriocin-bound *Lactobacillus plantarum* cells per gram showed a decreased in number of *L. monocytogenes* from 2.7 log cfu/g at day 0 to undetectable levels (less than two log cfu/g) at sampling day six, using a quantitative testing method. However, enrichment testing indicated that *L. monocytogenes* was still present albeit at very low levels. The authors concluded that the treatment was not sufficient to kill all *L. monocytogenes* but might be beneficial especially combined with other interventions that could also injure *L. monocytogenes* contaminating CSM products (Mattila et al 2003).

Samelis et al (2005) evaluated nisin as a growth inhibitor of a ten-strain cocktail of *L. monocytogenes* inoculated onto bologna sausage VP and stored at 4°C for 120 days. Prior to packing, the bologna was immersed in a solution of 5000 IU/ml of nisin. Compared with the undipped controls, the nisin treatment slowed *L. monocytogenes* growth. Nevertheless, a two-log increase in *L. monocytogenes* was observed after as little as 20 days (Samelis et al 2005). Based on the observations of Samelis et al (2005), nisin does not appear to be an effective intervention for *L. monocytogenes* multiplication.

Geonaras et al (2005) also assessed nisin (Nisaplin brand, which contains 2.5% nisin) and also did not observe any significant influence to the growth rate of a ten-strain cocktail of *L. monocytogenes* applied to bologna sausage or ham when the CSM was dipped into a 0.5% solution of bacteriocin.

Ruiz et al (2010) also evaluated the anti-listerial properties of nisin on RTE VP diced turkey ham inoculated with *L. monocytogenes*. Storage of the treated ham was at 4°C for 63 days. As might be expected, the antimicrobial effectiveness of nisin increased as concentration increased from 0.2% to 0.5% (w/w). All of the nisin treatments resulted in four log reductions ($P<0.05$) in *L. monocytogenes* when compared with the positive control on the day of treatment. Four log reductions were observed also on day seven for 0.4% nisin treatment and days seven and 14 for 0.5% nisin treatment, compared with the untreated control. For the 0.5% nisin treatment, *Listeria monocytogenes* counts decreased from 4.97 log cfu/g on the day of nisin application, and remained less than two-log cfu/g throughout the 63 days of storage. Thus, in contrast to the Samelis et al (2005) and Geonaras et al (2005), Ruiz et al (2010) evaluated nisin favourably as an *L. monocytogenes* intervention.

Hereu et al (2012) observed a one-log reduction from an initial *L. monocytogenes* concentration of seven-log cfu/g using a single ham isolate of *L. monocytogenes* for inoculation. The nisin was applied to the surface of dry cured ham. After two months of storage at 8°C, the *L. monocytogenes* was still detectable at concentrations of three-log cfu/g.

From the above studies it is apparent that nisin either inhibits *L. monocytogenes* growth or causes reductions to *L. monocytogenes* populations on cured meat products such as turkey ham or sliced ham. However, on reconstituted processed meats such as bologna, there is no significant influence on *L. monocytogenes* populations. As has been stated previously, it is important that any *L. monocytogenes* interventions be assessed on a product-by-product basis.

9.9.14.1 NISIN IN COMBINATION WITH OTHER ADDITIVES OR TREATMENTS

Although Geonaras et al (2005) did not observe any reduction in the growth rate of *L. monocytogenes* using a 0.5% solution of nisaplin, more encouraging results were obtained when the bacteriocin was combined with either acetic acid, lactic acid or potassium benzoate. *L. monocytogenes* was not detected after 48 days storage at 10°C. No detection was a two to three log reduction in *L. monocytogenes* populations compared with using the acids or potassium benzoate individually. The

sensory characteristics of the bologna were however, assessed as inferior to untreated product by an untrained panel of consumers (Georgnaras et al 2005). The authors were optimistic that the treatments could be fine-tuned further to a point where there was a beneficial antimicrobial effect without significant organoleptic change to the product. However, there have been no additional publications describing further developments.

Samelis et al (2005) also evaluated nisin combined with organic acids and salts as *L. monocytogenes* growth inhibitors for *L. monocytogenes*-contaminated bologna. The most effective treatments were reported as nisin in combination with: 5% lactic acid, 5% SD, 5% acetic acid and 3% PB, lesser effects were noted with 3% PS and SA (Samelis et al 2005). Samelis et al (2005) concluded the best choice of organic acid or salt for use with nisin for bologna dipping would be SD at a concentration of 3% (w/v).

Jofré et al (2007) investigated the efficacy of a range of bacteriocins from different sources as control interventions for *L. monocytogenes*. The bacteriocins used were enterocins A and B, sakacin K and nisin. The bacteriocins were incorporated into interleavers (thin plastic sheets, which are used to keep slices of meat separated), and their effectiveness against *L. monocytogenes* inoculated onto sliced, cooked ham. In addition to an application of bacteriocin, bacteriocins in combination with either SL or high-pressure application at 400 MPa for 10 minutes at 17^{o}C were assessed. For the non-pressurized samples, nisin plus SL was the most effective treatment, inhibiting *L. monocytogenes* growth for 30 days at 6°C. After three months, the nisin and SL treatment contained *L. monocytogenes* numbers that were 1.9 log cfu/g lower than the control. For the other bacteriocin treatments without pressure, *L. monocytogenes* did not exhibit a lag phase and grew progressively to a final count of eight-log cfu/g. After pressure application, the ham slices that included nisin, sakacin, and enterocins had reductions to *L. monocytogenes* populations of four-log cfu/g. At the end of storage, *L. monocytogenes* levels in the pressure-treated bacteriocin-containing batches were the lowest, with counts below 1.51 log cfu/g.

9.9.15 BACTERIOPHAGES

Vermeiren et al (2006) tested the lytic activity of the P100 phage against three *L. monocytogenes* strains at 30°C and at 7°C. The assessments were undertaken in broth, on cooked chicken fillet and on cooked ham. Preliminary broth experiments revealed that each of the three *L. monocytogenes* strains

were susceptible to the action of bacteriophage P100 at both of the assessed temperatures. In the first application test on a VP cooked poultry product, the presence of phage P100 caused a reduced *L. monocytogenes* count of more than three-log cfu/g compared with the untreated control after 21 days at 7^{o}C. When industrially-prepared cooked ham was assessed, P100 at a concentration of 1×10^{7} pfu/cm^{2} had no significant affect compared with the no phage control until two weeks. After two weeks, the phage reduced the growing population of *L. monocytogenes*. At the end of the 21 days storage, the phage treated CSMs were around 3.5 log cfu/g lower than the untreated controls for all three *L. monocytogenes* strains assessed. Thus, the P100 treatment was assessed as effective for the *L. monocytogenes* strains that were examined.

Guenther et al (2009) used two strains of *L. monocytogenes* as contaminants of cooked turkey breast at a concentration of 10^{3} cfu/g. A virulent phage was also added at a concentration of 3×10^{8} pfu/g. Guenther et al (2009) observed a 1.5 log reduction in the numbers of *L. monocytogenes* compared with non-phage controls after storage at 6^{o}C for 6 days. Guenther et al (2009) suggested that the uneven surface and complex matrix of foods such as cooked turkey breast might serve to shield the pathogen from the phages, resulting in reduced contact and leading to lower infection efficacy.

9.9.15.1 BACTERIOPHAGES IN COMBINATION WITH OTHER ADDITIVES OR TREATMENTS

Immobilization of the phage particles by food was also suggested by Holk and Berg (2009) as an explanation for poor *L. monocytogenes* population reductions. In addition to *L. monocytogenes* bacteriophage, Holk and Berg (2009) also assessed the effect of adding cultures of *Lactobacillus sakei* as a competitive exclusion bacterium. By combining both bacteriophage and *L. sakei,* Holk and Berg (2009) were able to achieve 100-fold reductions to the populations of a two-strain, cold-adapted cocktail of *L. monocytogenes* applied to cooked sliced ham compared with the use of phages alone. The Holk and Berg (2009) results also indicated better anti-listerial activity for both the phage and *L. sakei* treatments at lower storage temperatures (4^{o}C) compared with higher storage temperatures (8^{o}C and 10^{o}C).

9.9.16 CONTROL USING PACKAGING FILMS CONTAINING CHEMICAL ADDITIVES

Scannell et al (2000) evaluated the immobilisation of either nisin or another bacteriocin called lacticin 3137 into cellulose based packaging paper inserts and plastic films. Lacticin 3137 was adsorbed and showed anti-listerial activity on packaging paper but showed no activity on plastic film. In contrast, nisin adsorbed and was active on both materials (Scannell et al 2000). On sliced ham stored at 4^{o}C, the adsorbed nisin produced a decrease of one-log unit in *L. innocua* populations (Scannell et al 2000).

In the USA, McCormick et al (2005) investigated the antimicrobial effect of wheat gluten films containing nisin on *L. monocytogenes*, which was inoculated onto turkey bologna slices with VP and storage at 4^{o}C. The wheat films were powdered (7% w/w) with Nisaplin (containing 2.5% nisin) and had an initial activity of 6.21 × 10^4 IU/g. The films were aseptically applied to slices, which contained a 10^8 cfu/g initial *L. monocytogenes* load. Single or supplementary water bath thermal treatments (pasteurization) were also performed onto the VP inoculated slices, at either 65^{o}C for 81 seconds or 60^{o}C for 6.75 minutes, respectively. Three treatment scenarios were attempted: (i) in-package pasteurised bologna to no pasteurised bologna (no wheat gluten films were applied), (ii) in-package pasteurised bologna to no pasteurised bologna (both treated with nisin-containing wheat gluten films in contact with the meat) and (iii) in-packaged pasteurised bologna treated with or without nisin-containing wheat gluten films. The samples were tested by plating at day 0 and weekly for up to eight weeks. McCormick et al (2005) found that the pasteurisation alone reduced the population of *L. monocytogenes* by 3.8- to 7.0-log cfu/g on day 0 and the remaining population fluctuated between 1.2- and 3.8-log cfu/g during the 8 weeks of storage. Gluten films containing nisin, without pasteurisation, only reduced the *L. monocytogenes* loads by one-log cfu/g during the first four weeks after treatment, followed by an increase during the last 4 weeks of storage. The treatment was much more efficient when pasteurisation was used (2.75-log cfu/g reduction during the whole storage period). The findings of McCormick et al (2005) emphasised the potential use of nisin containing films combined with heat treatment and an intervention to control *L. monocytogenes* in CSM. However, the effect of spoilage microorganisms and sensory evaluation tests were not considered in their study.

Limjaroen et al (2005) used polyvinylidene chloride (PVDC) films containing sorbic acid (0%, 1.5%, and 3.0% w/v) to inhibit the growth of *L. monocytogenes* in beef bologna. Slices of beef bologna were surface inoculated with *L. monocytogenes* at 10^3 or 10^5 cfu/g and the sorbic acid-containing films were

placed between the slices. After 28 days of storage at 4°C the level of *L. monocytogenes* in beef bologna treated with sorbic acid-containing films (either 1.5% or 3.0% w/v) decreased significantly ($P<0.05$) and it was 4.4 logs lower than that in the untreated controls. Common spoilage organisms were also inhibited on the beef. The findings of Limjaroen et al (2005) demonstrated that sorbic acid-containing films may be useful in enhancing the safety and shelf-life of RTE delicatessen products.

Cagri et al (2006) manufactured edible thin films of whey protein that contained p-aminobenzoic acid (PABA) and/or sorbic acid. The films were evaluated as wrappings to reduce the numbers of *L. monocytogenes* in packaged foods. PABA and sorbic acid were used at concentrations between 0.5% and 1.0%. A summary of the project results are shown as Table 28.

Table 28 *L. monocytogenes* population reductions on bologna and summer sausage after wrapping in antimicrobial-impregnated whey films after 21 days storage at 4°C (reproduced from Cagri et al 2006)

Treatment	Concentration (% w/w)	Reduction in *L. monocytogenes* numbers compared with an unwrapped control (log cfu/g)	
		Bologna	Summer sausage
PABA	0.75	1.5	2.1
	1.0	2.2	1.9
Sorbic acid	0.75	3.0	2.2
	1.0	3.4	2.5
PABA:Sorbic acid	0.5:0.5	2.8	3.0

Wrapping bologna in whey film containing 1% sorbic acid was reported by Cagri et al (2006) as the most effective treatment for bologna, and the PABA:sorbate combination was the most effective treatment for *L. monocytogenes*.

Cooksey (2005) reported having reviewed antimicrobial packaging. Identified by Cooksey, those packaging materials of most relevance to the current study are reported by Franklin et al (2004). The study used a five-strain cocktail of *L. monocytogenes* applied to the surface of hot dogs at a concentration of five-log cfu/g. The hot dogs were packed in film coated with methyl cellulose and hydroxypropyl methyl cellulose solutions containing 10 000, 7500, 2500 or 156 IU/ml nisin or no nisin (control). The higher concentrations of nisin were able to keep the *L. monocytogenes* populations below a detectable level of 2.9 log cfu/package for their study. More specifically, packaging films

coated with 10,000 and 7,500 IU/ml nisin significantly decreased *L. monocytogenes* populations on the surface of hot dogs by greater than two-log cfu per package throughout the 60 days study. Control samples and samples containing 156 IU/ml of nisin did not inhibit *L. monocytogenes* and concentrations of nine-log cfu/ package were achieved for both after 60 days' storage.

Alginate, zein and polyvinyl alcohol (PVA) biodegradable films containing enterocin (200 and 2000 AU/cm^2) were assessed as control measures for *L. monocytogenes* in sliced-cooked ham that was either air or VP (Marcos et al 2007). Control air packing and storage at 6^{o}C showed a significant increase ($P<0.05$) to the numbers of *L. monocytogenes* from 10^4 cfu/g to 10^7 cfu/g in 8 days. The addition of enterocins resulted in significantly ($P<0.05$) lower counts than those in controls, during the entire storage period (eight days) but growth of up to one log was observed for both zein and PVA. Air-packaging with alginate films containing 2000 AU/cm^2 of enterocins effectively controlled *L. monocytogenes* (there was no growth) for eight days. In the control VP samples stored at 6^{o}C *L. monocytogenes* grew from 10^4 cfu/g to 10^8 cfu/g in 29 days. The use of enterocins significantly ($P<0.05$) lowered the counts in all samples compared with controls, but growth was stopped for 15 days only when the alginate films contained 2000 AU/cm^2 of enterocins. The conclusion of Marcos et al (2007) study was that biodegradable films containing enterocins can improve the safety of sliced cooked ham by delaying and reducing the growth of *L. monocytogenes*.

As follow up work to their previous study (Marcos et al 2008a) assessed the effect of cumulative treatments of biodegradable alginate films containing enterocins (2000 AU/cm^2) and HPP (400 MPa for 10 min), as control interventions to prevent the growth of *L. monocytogenes* in sliced-cooked ham. The results showed that the pressurisation was more effective in controlling *L. monocytogenes* at 6^{o}C than the antimicrobial packaging. In combination enterocins and HPP caused a reduction to the levels of *L. monocytogenes* from four log cfu/g to 0.6 log cfu/g and maintained these low counts for up to 22 days. When the storage temperature was lower (1^{o}C), low counts were maintained for up to 60 days. However, the authors (Marcos et al 2008a) emphasised that commercial and home refrigerator temperatures may run at higher temperatures. Therefore, the application of additional technologies, such as HPP and antimicrobial packaging, would assure the safety of contaminated ready to eat foods during shelf life.

Bacteriocins incorporated into packaging films as anti-listerial interventions have been evaluated by Santiago-Silva et al (2008). The bacteriocin pediocin originally isolated from *Pediococcus* spp., was incorporated into cellulose acetate films. The films were able to inhibit the growth of *L. innocua* (a surrogate for *L. monocytogenes*) on cooked sliced ham stored at 12^{o}C for 15 days (Santiago-Silva et al 2008). Compared with ham wrapped in untreated control film, there was a two-log reduction in *L. monocytogenes* numbers for the treatment. When the authors attempted to increase the concentration of pediocin in the film, the film surfaces became rough because at high concentrations there are solubility problems with pediocin that cause pediocin granules rather than molecules to be dispersed into the film matrix.

Barbiroli et al 2012 incorporated lysozyme and lactoferrin into packaging as a strategy for the control of food-borne pathogens. Lysozyme and lactoferrin were incorporated into paper pulp and carboxymethyl cellulose fibres and paper was manufactured. Encouraging results were obtained when the antimicrobial-immobilised paper was added to broth with cultures of *Listeria innocua* and *Escherichia coli.* The treated paper increased the lag time for growth of the pathogens and decreased the final count in broth by at least one-log unit. Evaluations were then made of practical applications of the treated paper. Barbiroli et al 2012 concluded that lysozyme and lactoferrin incorporated paper could decrease the total aerobic count on thin veal slices by one-log unit as compared to controls without paper after 48 h at 4 ºC (Barbiroli et al 2012).

Theinsathid et al (2012) examined the use of environmentally sustainable packaging films manufactured from poly lactic acid and lauric arginate. Lauric arginate is an antimicrobial compound derived from lauric acid, L-arginine, and ethanol. Lauric arginate is described by the authors as naturally-occurring and by WHO (2009) as GRAS. An initial assessment using agar diffusion demonstrated zones of inhibition in *L. monocytogenes* lawns (Theinsathid et al 2012). On cooked, sliced ham inoculated with a single strain of *L. monocytogenes* a four-log reduction after a single day's storage at 4^{o}C was obtained using poly lactic acid films coupled with 2.6% (w/w) lauric arginate. Although refrigerated storage caused some loss of transparency of the film, it was otherwise unchanged mechanically (Theinsathid et al 2012). In order to fully evaluate the intervention, further work is required to determine the effect of prolonged storage on *L. monocytogenes* populations and the integrity of the film.

Hereu et al (2012) determined that nisin applied to packaging films was less effective compared with nisin applied directly to the surface of dry-cured ham. Thus, it is difficult to justify the use of nisin-

coated films when superior inhibition of *L. monocytogenes* was obtained by direct application of the bacteriocin.

Later work by Marcos et al (2013) evaluated a combination of polyvinyl alcohol (PVOH) films containing nisin (450 AU/cm^2) and HPP (600 MPa, 5 min, 12^oC) as a method for the inactivation of *L. monocytogenes* inoculated ($5x10^5$ cfu/g) onto sliced fermented sausages with no added sodium salt. The numbers of *L. monocytogenes* in the HPP and film treatment after 90 days of VP storage (7 days at 4^oC and the rest at 12^oC) was 4.1 log cfu/g. However, the result was not significantly different ($P>0.05$) from that obtained by using only PVOH films containing nisin. The lack of effect of HPP on *L monocytogenes* was attributed to a protective effect exerted by the low water activity of the product and its lactate content. However, the findings of Marcos et al (2013) proved a potential antilisterial effect of PVOH film packaging in combination with nisin, to efficiently reduce the levels of *L monocytogenes* in sliced fermented sausages.

9.9.17 ESSENTIAL OILS AND PLANT EXTRACTS

Hao et al (1997) assessed the inhibition of *L. monocytogenes* on cooked beef sirloin strips after exposure to nine plant extracts. The extracts used by the study were angelica root, banana puree, bay leaf, caraway seed, carrot root, clove extract, marjoram, pimento leaf and thyme. Only the clove extract (Eugenol) had any noticeable effect to a high-level *L. monocytogenes* initial inoculum (10^5 cfu/g) on cooked beef strips. However, the difference between the clove extract and the control was one-log cfu/g. When a low inoculum (10 cfu/g) was used and the strips incubated at 15^oC for seven days there was a significant difference for the clove extract compared with the control, but the *L. monocytogenes* population on the clove-treated meat had still grown to 3.77 log cfu/g.

Distillates from horseradish pulp that contained 90% allyl isothiocyanate and 9% 2-phenethyl isothiocyanate were found to inhibit the growth of *L. monocytogenes* in a lab-based model system (Ward et al 1998). However, when an assessment of inhibition was undertaken on inoculated cooked beef slices, five times the quantity of distillate was required to prevent *L. monocytogenes* growth. The large quantity of distillate required meant that use of distillate as an intervention was impractical (Ward et al 1998).

In Canada, Oussalah et al (2007) determined the antimicrobial effect of alginate-based films containing essential oils (oregano, cinnamon, winter savory) on *L. monocytogenes* present in bologna and ham. The alginate films had been immersed in 2% or 20% solutions of $CaCl_2$ and contained 1% (w/v) essential oils. The bologna or ham slices (n=300) were inoculated with *L. monocytogenes* at a concentration of three log cfu/cm^2, the films were aseptically applied to the meat surfaces before storage for five days at 4^oC in petri dishes. All three essential oils on alginate films penetrated with 20% $CaCl_2$ were effective in reducing *L. monocytogenes* below the detection level (<10 cfu/ml) on bologna slices after five days of storage. The treatments did not work well in ham containing *L. monocytogenes*, where the pathogen was highly resistant. Although the work of Oussalah et al (2007) shows the potential of the essential oils on alginate films as pathogenic growth inhibitors on bologna and ham, the long term storage effects on the bacterial growth and any impact to the quality of these products have yet to be determined.

Zhang et al (2009) also assessed the anti-listerial effects of plant extracts. The range of plant extracts evaluated consisted of clove, oregano, rosemary, pepper, nutmeg, liquorice, turmeric, aniseed, cassia bark, fennel, prickly ash, round cardamom, dahurian angelica root and angelica. Of the extracts the most inhibitory were those from clove, rosemary and liquorice (Zhang et al 2009). A 1:1 mixture of rosemary and liquorice was observed to cause reductions to the numbers of *L. monocytogenes* on VP cooked ham. After 28-days of refrigerated storage, the *L. monocytogenes* population decreased by 2.5, 2.6 and 3.0 logs when sprayed with 2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml rosemary and liquorice mixture respectively, (Zhang et al 2009). Both of the plants have strong flavours, but no assessment was made of any taint introduced by the treatment.

9.9.18 BACTERIAL ANTAGONISTS

A desire for reduced salt contents and less chemical preservation of sliced meat products has led researchers to look at biologically-based alternatives and the development of bio-preservation. The main group of bacteria thought to potentially be useful for the purpose of bio-preservation are the lactic acid bacteria (LAB) since they are often the dominant component of the microbiota of both VP

meat products and LAB are able to inhibit undesirable bacteria such as *L. monocytogenes* (Alves et al 2006).

Bredholt et al (1999) obtained indigenous suppressive lactic acid bacteria from samples of cooked sliced ham and servelat from VP and gas packed packages that had been challenged with a three strain rifampicin resistant cocktail of *L. monocytogenes*, which had failed to grow (Blom et al 1997). The five strains of LAB when added to cooked sliced VP ham at concentrations of 10^4 and 10^6 cfu/g were able to inhibit the growth of *L. monocytogenes* at up to four weeks at 8^{o}C. Bredholt et al (1999) assessed each of the five inhibitory strains for any sensory consequences. A professional taste panel found that VP hams individually inoculated with each LAB strain and stored at 8^{o}C for 21 days were of acceptable quality. Further work identified one of the strains as *Lactobacillus sakei* (Bredholt et al 1999). The *L. sakei* stain was used in a follow-on study by Bredholt et al (2001). *L. sakei* was sprayed onto cooked ham and servelat sausage under industrial processing conditions and found to inhibit the growth of a three stain cocktail of *L. monocytogenes* when the meat products were stored at 4^{o}C and 8^{o}C for 28 days. Consumer preference panels did not show any negative effects for the application of *L. sakei* to meat products (Bredholt et al 2001).

Other studies have screened Lactic acid bacteria for inhibition to *L monocytogenes.* Budde et al (2003) isolated 72,000 bacterial colonies originating from 48 different VP meat products that included ham, salami, cooked loin and smoked bacon. A total of 153 strains producing antibacterial products were obtained from 46% of the examined packages. The predominant inhibitory strain was identified as *Leuconostoc carnosum.* When the *L. carnosum* was added at a concentration of $1.2x10^5$ or $6.3x10^6$ cfu/g to VP meat sausage it reduced the population of *L. monocytogenes* by at least three-log units when the meat was stored at 5^{o}C for 28 days (Budde et a 2003).

Jacobsen et al (2003) also studied the inhibition of *L. monocytogenes* using a bacteriocin-producing strain of *L. carnosum*. The study assessed pork saveloy slices produced in a laboratory using a commercial product process. Inhibition was assessed using a five strain mixture of *L. monocytogenes*. Four different methods of application of *L. carnosum* were used, including addition to uncooked sausage batter before cooking, addition of isolated crude bacteriocins to uncooked batter before cooking, application of *L. carnosum* by pipette to both sides of the meat slices after heat treatment and finally spray application of *L. carnosum*. Populations of *L. monocytogenes* in the control CSM slices had

increased by six-log cfu/g. In the best two treatments, the addition of live *L. carnosum* to the surface of cooked sausage slices by pipette or jet application caused the numbers of *L. monocytogenes* to stay unchanged at both 5°C and 10°C over the 28 days duration of the experiment. The best *L. monocytogenes* inhibition was obtained using the jet nozzle application method.

Sensory evaluation of cooked sliced ham showed that *L. carnosum* did not cause significant organoleptic change compared with the controls. Jacobsen et al (2003) also indicated that unpublished data showed a further 38 treated commercial products were undistinguishable from untreated controls by sensory testing.

Alves et al (2006) assessed the impact of *Lactobacillus sakei* to the growth of two strains of *L. monocytogenes*. *L. monocytogenes* serotype 4b and *L. monocytogenes* serotype 1/2a were inoculated onto sliced ham, VP and stored for up to 10 days at 8°C. Both *L. monocytogenes* strains were inhibited by the *L. sakei*. Inhibition by *Lactobacillus* spp. is attributed often to bacteriocin secretion. However, the inhibitory effect caused by *L. sakei* was not due to bacteriocin secretion because the same level of inhibition was seen in both bacteriocin-producing and non-producing strains.

Kaban et al (2010) determined the effect of two strains of *Lactobacillus sakei* (Lb 706 (bacteriocin positive) and Lb 706-B (bacteriocin negative)) on the growth of *L. monocytogenes* in bologna sausages contaminated during slicing with bacterial concentrations that varied between three and four log cfu/g. The slices were VP or stored in modified atmosphere (50% CO_2/50% N_2) for up to six weeks at 4°C. Growth at low levels (up to one log cfu/g) was observed only for the treatment with bacteriocin negative *Lactobacillus sakei* strain under VP. The observations of Kaban et al (2010) demonstrated a synergistic inhibitory effect for CO_2 (in modified atmosphere) in combination with *Lactobacillus sakei* on the growth of *L. monocytogenes*.

9.9.19 A SUMMARY OF CHEMICAL INTERVENTIONS

In summary, there were a number of beneficial interventions for *L. monocytogenes* on CSM identified. A common omission from many of these studies was that sensory evaluation of treatments was not undertaken and so further work is required before a full assessment can be made. The beneficial interventions could be classified into two distinct groups. The first group caused a reduction in the numbers of *L. monocytogenes* possibly with a significant delay before the commencement of exponential growth. In this group were included immersion in lactic or acetic acid. The second category slowed the rate of exponential growth and included treatment with bacteriocins such as nisin. The identified anti-listerial interventions requiring further organoleptic sensory assessment included sodium acetate, cetylpyridinium chloride and the use of essential oils with strong flavours. One potential intervention with significant benefit was identified. Sodium levulinate added to a final concentration of 3% (w/w) was able to completely inhibit the growth of *L. monocytogenes* in pork bologna and turkey roll for 12 weeks with no detectable organoleptic change. Also identified was treatment with sodium diacetate on its own or in combination with sodium lactate and sodium citrate, which extended the lag phase of *L. monocytogenes* by up to 21 days, with only minor organoleptic changes. Other interventions such as immersion in acetic or lactic acid, sodium lactate and sodium diacetate in combination, and potassium benzoate applied to contaminated CSM caused reductions in the numbers of *L. monocytogenes* of up to four logs or extended the lag before exponential growth for 80-90 days, although with unacceptable sensory changes. Although a number of active films envisaged for use as food wraps have been shown to have benefit in the laboratory, there has been no attempt to develop these further into commercial products. A particular barrier to the adoption of many potentially beneficial interventions is the restrictions on additives in Regulation 1333/2008. Sodium levulinate for example is not an approved EU additive and cannot be uses in foods within EU member states. All additives used in cooked meat must comply with the provisions of food category 8.2.2 in Annex II of Regulation 1333/2008. EU approval is required for new additives and new uses of already authorised additives.

9.10 THE IMPACT OF TRAINING GIVEN FOR CLEANING AND OPERATING OF MEAT SLICERS

Neal (2013) assessed the relative effectiveness of three different methods for training employees in the effective cleaning and sanitising (C&S) of delicatessen slicers. The instruction methods were: written instruction, demonstration and written instruction with demonstration. A control group were included that had no instruction. Instruction method effectiveness was measured by the time taken to complete cleaning and sanitation, an ATP-based bioluminescence measurement of sanitation effectiveness and participant survey. Written instruction in combination with demonstration was assessed as the best method of training because the time taken to undertake C&S increased, the ATP readings were lower (indicating effective sanitation) and the method was perceived to be effective by the participating employees.

9.11 USE OF DIFFERENT GLOVES TO HANDLE CONTAMINATED MEAT AND SLICED HAM

Lianou and Sofos (2007) reviewed the mechanisms that spread *L. monocytogenes* in food processing and retailing environments. The authors summarise that food workers practices such as wearing gloves for extended periods of time and washing hands less frequently when wearing gloves compared with handling foods with bare hands promotes the spread of *L. monocytogenes* contamination (Lianou and Sofos 2007).

Perez-Rodriguez et al (2006) developed a risk management statistical model to rank different cross-contamination scenarios during the processing of sliced ham. The model allowed the effect of various 'what if' scenarios to be evaluated. Although the approach was novel, some of the scenarios that were assessed were unlikely. For example, using the same gloves to handle contaminated meat before handling the final CSM product was ranked as the highest risk cross-contamination practice for *L. monocytogenes*. It is unlikely that such a scenario would happen in any size of CSM plant in the UK. Perhaps not surprisingly, the model predicted that changing into a fresh pair of gloves after handling contaminated meat presented the lowest risk for *L. monocytogenes* transfer onto final product (Perez-Rodriguez et al 2006). An interesting finding from the study was that handling food with bare hands and not washing before handling final product was only slightly less risky compared with washing hands before handling the final product (Perez-Rodriguez et al 2006). Although models such as the one

constructed by Perez-Rodriguez can be useful tools for the assessment of risky processing practices, it should be kept in mind that they are only simulations of real world events and that models are heavily dependent on accurate information. Perez-Rodriguez confesses a significant lack of knowledge regarding *L. monocytogenes* transfer coefficients, although the authors considered that such a lack of basic knowledge provided their model with an "important uncertainty component".

9.12 PREDICTION OF THE FATE OF *L. MONOCYTOGENES* IN CSM

A study by Garrido and colleagues (2010) constructed a model to assess the risk of listeriosis from consuming contaminated smoked fish or sliced cooked ham in the Navarra region of Spain. The authors compiled the results from previous studies relating to *L. monocytogenes* prevalence in smoked fish and sliced cooked ham (Garrdio et al 2009), along with information relating to serving size, numbers of people likely to consume the food, storage duration and temperature and included the information in their model. The final output was an estimation of the annual number of Listeriosis cases and the impact to these cases were some of the source data to change. Interestingly, the consumption of cooked ham was found to be responsible for the most of the cases, with two cases annually per 100,000 of the population being predicted. A key finding of the model was that maintaining a storage temperature for the product from producer to fork at 4^{o}C gave the largest decreases to the risk of illness (Garrido et al 2010).

10 THE RETAILING OF CSM

10.1 STORAGE TEMPERATURES

The UK chilled prepared foods market is dominated by a few multiple retailers with their own brands. Generally, for retailer own-label chilled prepared foods, distribution of products to their Regional Distribution Centres (RDCs) will be done at a temperature not greater than 5^{o}C. RDCs supply product directly to retail stores. In the UK, chill temperature was specified as 8^{o}C maximum in England, Wales and NI (Anonymous, 2006). In Scotland, there is no stipulated temperature, but chilled food is required to be stored in a refrigerator, or refrigerating chamber, or a cool ventilated place. Historical Public Health England (PHE; previously called HPA, Health Protection Agency) surveys of product temperatures at retail have identified generally good compliance with the 8^{o}C limit, but tend not to report values for temperatures that extend beyond the limit (Table 29).

Table 29 UK-determined retail product temperatures (Peck et al 2006)

Product	Storage temperature (°C)	% Samples held at temperature	Number of samples	Outlet types	Reference
Cold meat and pate	≤8	95	3,766	Supermarkets, butchers, delis, market stalls, other retail	Elson et al. (2004)
	>8	5	215		
	Total		**3,981**		
VP and MAP cooked RTE meats at the end of shelf life	<5	71	2,030	Supermarkets, corner shops, butchers, greengrocer, delis, market stalls, farm shops	Sagoo et al. (2006)
	>5-8	24	685		
	>8	6	160		
	Total		**2,875**		
Overall totals	≤8	94	5,796		
	>8	6	375		
	Total		**6,171**		

More recently in 2012-2013, routine surveillance of *L. monocytogenes* in CSM sold from SME retailers in the UK also collected information on typical product temperatures (FSA project FS241042). In total 1049 CSM samples were collected from across the UK. A summary of the findings are provided as Table 30. Overall, roughly one third of the samples collected were stored above 8°C in the chillers of SME retailers, with two thirds of samples stored over 5°C. Fifteen percent of the CSM samples were stored at ≥10°C, and the highest storage temperature recorded was 19°C.

Table 30 A summary of the pack temperatures of CSM sold at retail from SME stores in the UK between April 2012 and February 2013.

Storage temperature range (°C)	Percentage* samples held at temperature (%)	Number of samples
<5°C	27.5%	288
≥5 to <8°C	39.4%	413
≥ 8°C	33.2%	348

*NB: Rounding to three significant figures may mean the percentages do not total exactly 100%. (Hutchison and Madden, unpublished)

The potential issues with some SME chillers became apparent at the early stages of FS241042, and so were an area of particular focus for the project. Initial observations suggested that open-faced display chillers tended to have higher temperatures than chillers with doors or open-faced displays fitted with plastic slats. An *ad hoc* comparison of 50 randomly selected CSM sample temperatures was undertaken. The temperatures of samples purchased from chillers with doors (n=25) were compared against those bought from open-faced chillers (n=25). The mean sample temperatures were 5.8°C from the chillers with doors and 11.4°C from the open-faced displays. The difference was significant (t-test, P<0.02; Hutchison and Madden, unpublished).

For the FS241042 survey, temperatures were measured using infrared thermometers (Fluke models 63 and 68). In total, samplers in different regions used six infrared units and similarly elevated temperatures were observed for all of the devices used. The temperature of some samples was measured using two or three thermometers, with no significant differences observed between different devices (Hutchison and Madden, unpublished).

In addition, a radiometric (creation of a record of all of the temperatures in a thermograph simultaneously) thermal imaging camera was used to record the temperatures inside some of the chiller cabinets from which samples were taken. Figure 19 shows typical temperatures for chilled foods inside a typical open-faced chiller unit.

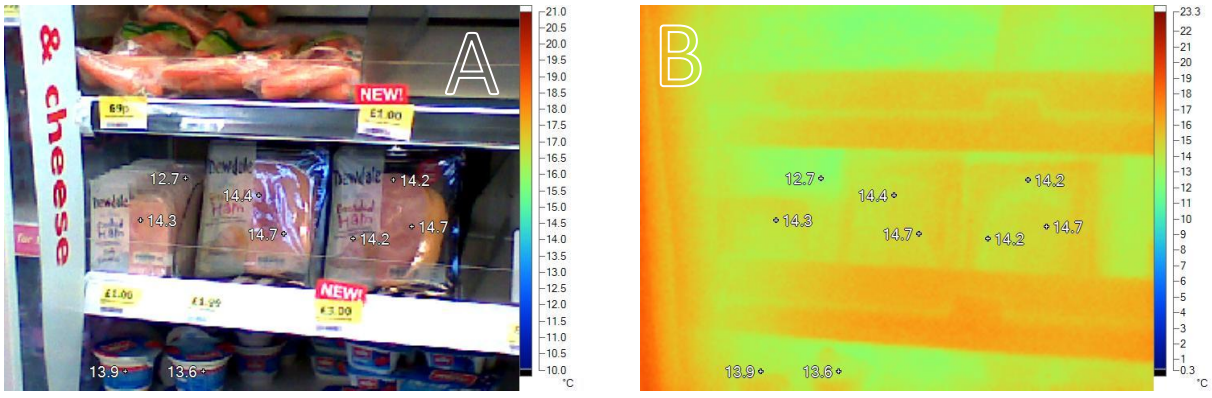


Figure 19 Typical temperatures inside a chiller cabinet situated inside an SME retail store. Image A is a normal photograph overlaid with the pack temperatures (°C). Image B is the corresponding thermograph, again overlaid with pack temperatures (°C).

The thermal imaging of chiller contents inadvertently provided some evidence as to one of the reasons for elevated temperatures in open-faced chillers. Figure 20 shows typical temperatures of product inside an open-faced chiller and the temperature of the fluorescent tube inside the display unit to be 30.9°C. Other thermographs of the tubes in different chillers showed typically tube temperatures between 15°C and 35°C. It was considered likely that in some open-faced chillers, the fluorescent tubes were a heat source that was contributing to the elevated CSM sample temperatures.

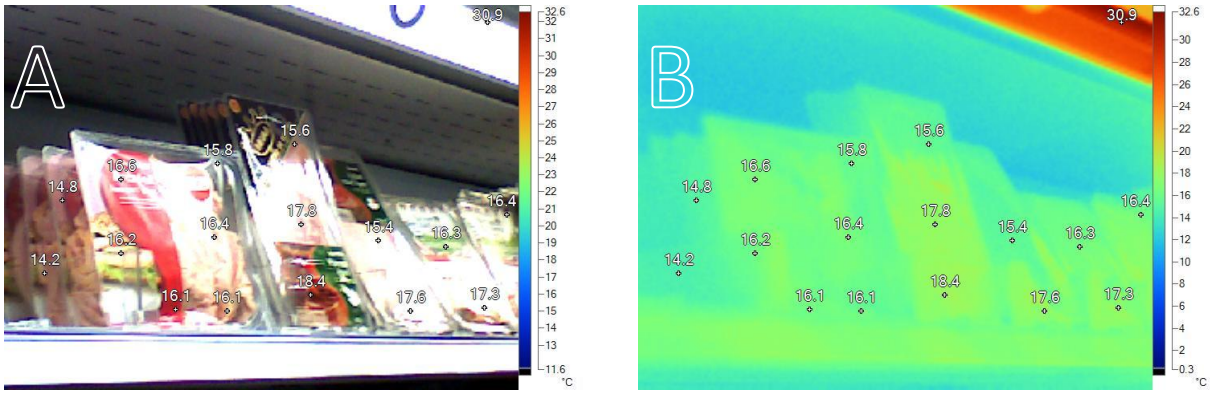


Figure 20 Typical temperatures inside a chiller cabinet situated inside an SME retail store. Image A is a normal photograph overlaid with the pack temperatures (°C) and the temperature of the fluorescent tube. Image B is the corresponding thermograph, again overlaid with pack temperatures (°C) and the temperature of the fluorescent tube.

Limited investigations with a chiller manufacturer and discussions with some shop workers revealed the following information relating to open faced chillers. When chiller units are supplied from the manufacturer, they are equiped with custom fluorescent tubes. The custom tubes are designed so that they generate less heat than standard tubes and have a perspex sheath which helps contain any heat that is generated. In addition, and in contrast to standard fluorescent tubes,the custom tubes provide bright light at low temperatures. In the three stores that were asked, the shopkeepers said they had replaced the chiller tubes with standard tubes. Furthermore, one store owner was aware that standard tubes were brighter at higher temperatures and said he had turned the temperature on the chiller unit up to have the chiller contents more brightly illuminated.

A number of points were concluded from the *ad hoc* investigations undertaken for FS241042. These were that fitting open-faced chillers with plastic slats helps to reduce the chiller temperature.

Furthermore, standard fluorescent tubes should not be used as replacements for cool-operating fluorescent tubes because they are sources of heat inside the chiller units. Light emitting diode (LED)-based replacements for fluorescent tubes are not significant sources of heat and do not suffer from reduced illumination intensity at refrigeration temperatures.

10.2 USE-BY DATE COMPREHENSION

Improving consumer understanding of and compliance with "use by" dates would likely contribute positively to consumer protection especially amongst older people (McIlveen and Semple 2002). Several surveys have found that only around half of UK respondents correctly identify the "use by" date as an indicator of food safety (e.g. TNS, 2008; FSA, 2011; WRAP, 2011). In one study, 83% of respondents correctly matched the statement "It is not safe to eat food after this date" with the "use by" date (Wrap, 2008). A different study (WRAP, 2010) showed that, when asked what they would do with food that was a day past its "use by" date, only 15% of respondents gave the correct answer – "It could be unsafe and should be thrown away". Forty-eight per cent selected "It depends on the food type" and 25% selected "The food could be unsafe to eat but they would test it and use their judgement".

10.3 UK DOMESTIC REFRIGERATOR TEMPERATURES

A summary of commercial refrigeration temperatures is provided in section 4.1.6. In general, the temperature at which a refrigerator operates is critical for the safe storage of chilled food. A recommendation made in 1991 in the UK concerning the microbiological safety of foods advised that the maximum temperatures in domestic refrigerators should not exceed 5^{o}C (Richmond, 1991). Since no follow up work was undertaken, it is not clear whether the target temperature was ever achieved. The Food Standards Agency and NHS recommend less than 5^{o}C as the operating temperature for domestic refrigerators (NHS, 2012).

Surveys of consumer storage and handling of refrigerated foods indicate that performances are similar throughout the world. In the last major public UK survey (Evans et al, 1991) results showed the mean

temperature over 7 days (evaluated from top, middle and bottom sensors) ranged from -1°C to 11°C. The overall mean air temperature for all the refrigerators in the survey was 6°C, with 70% of refrigerators operating at average temperatures above 5°C (Figure 21).

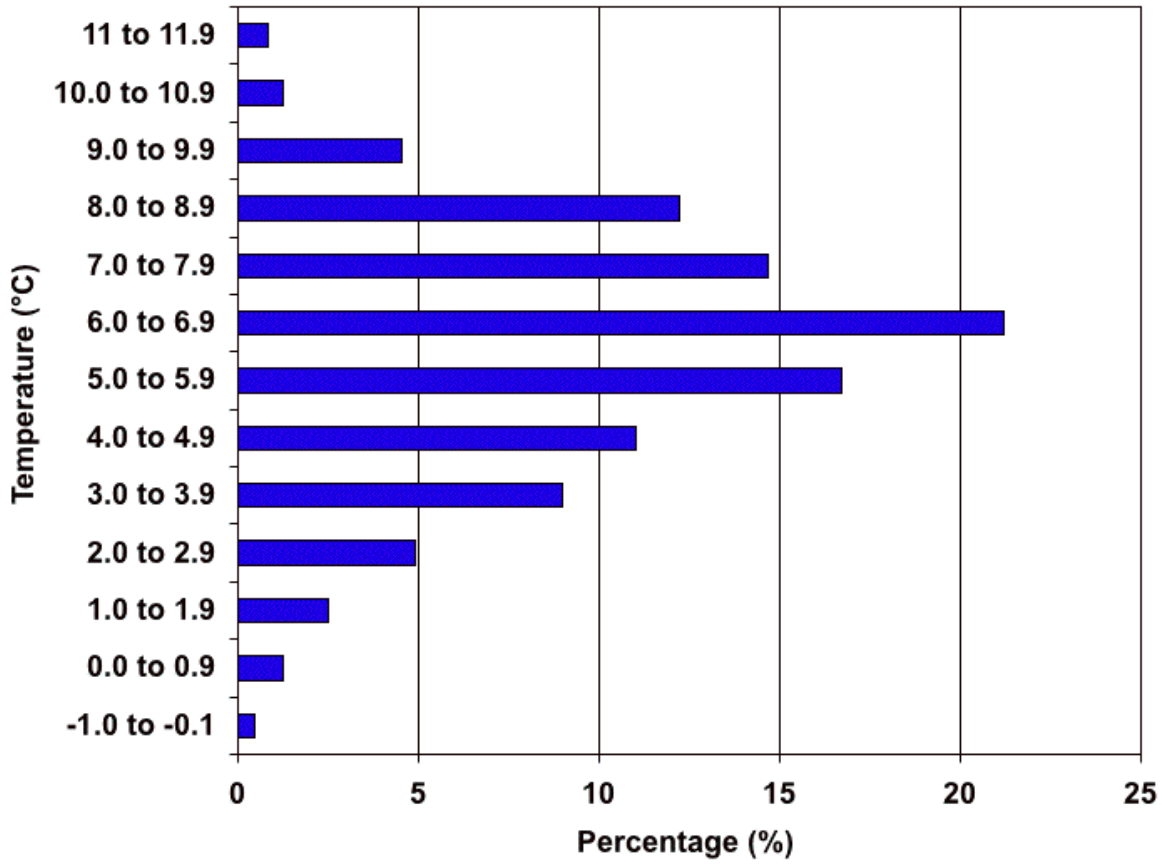


Figure 21 Mean temperatures for all refrigerators in a UK survey (Evans et al., 1991)

The percentage time spent between certain temperatures was calculated for all of the domestic refrigerators assessed by the study. The greatest proportion of time (80%) was spent between 3.0°C and 8.9°C. Approximately 28% of the time the internal temperatures were less than 5°C, 35% of the time the range was 5.0-6.9°C, 28% of the time was 7.0-8.9°C, and 9% of the time was spent above 9.0°C. Only four refrigerators (2%) in the whole survey operated below 5°C during all the monitoring period, and 33% of refrigerators spent all their time above 5°C.

This historic information shows a significant number of UK domestic fridges did not maintain food at a low enough temperature to prevent significant *L. monocytogenes* multiplication.

10.4 CONTINENTAL EUROPEAN AND INTERNATIONAL DOMESTIC REFRIGERATION TEMPERATURES

There is a wide range of temperatures experienced between the northern and southern regions of Europe, which varies with season. Although ambient temperatures would be expected to influence refrigeration temperatures and confound comparisons, a review of all European studies relating to domestic refrigeration has been compiled (Nauta et al., 2003). The study showed that overall, the average air temperature in EU refrigerators was 6.64°C (Nauta et al., 2003).

Published after the Nauta review, a Swedish survey of fridge temperatures was undertaken by Marklinder and colleagues, which reported in 2004. The study collected and analysed data on the home storage and handling practices of chilled foods, including sliced cooked ham. There were no trends identified for refrigeration practices when supplementary characteristics (e.g., sex, age, education, age of the refrigerator) of the respondents were analysed. A key finding of the work was that roughly 20% of food items that required chilling were stored at temperatures higher than 10^{o}C. Also, although most participants knew broadly what the temperature inside their fridge should be, less than a quarter knew the actual temperature. Mean food temperatures were found not to be related to the age or type of refrigerator in the study.

Garrido et al (2010a) reported that in Navarra, Spain domestic refrigerator temperatures typically ranged between 0.6^{o}C and 14.5^{o}C.

Awareness of the correct refrigerator temperature has been reported to be variable, but according to a review of data published internationally over the 30 years studies show remarkable similarities in the performance of domestic fridges, in consumer attitudes and handling of chilled foods (James et al, 2007). The James et al (2007) review found in addition that the performance of refrigerators remained remarkably unchanged throughout the world over that period.

A study undertaken in the Republic of Ireland determined that 22% of consumers were aware of the correct refrigerator temperature (Kennedy et al., 2005). Whilst a Swedish survey (Marklinder et al., 2004) found a much better level of awareness amongst its survey group, with 85% of respondents

knowing the recommended refrigeration temperature (in this case 8°C). However, the Marklinder et al (2004) survey found 83-94% were at >5°C, 22-44% were at >8°C, and 5-19% were at >10°C. 40% of food storage temperatures exceeded the maximum recommended temperature for the food being stored. Marklinder et al. (2004) also found that mean food temperatures were not related to the age or type of refrigerator in Sweden. Only 25% knew, or regularly measured, the temperature of their refrigerator. In the Irish study, 23% of those asked had a refrigerator thermometer.

A survey of 2001/2002 found that 47% of yoghurt samples in French domestic refrigerators were at >6°C, and more than 75% of meat product samples were at >4°C (Cemagref/ANIA, 2004). In addition, 5% of domestic refrigerators were operating at >10°C. An earlier survey of domestic refrigerators in France revealed that in one in four households the average temperature of the refrigerator was above 8°C, and only 11% were at the recommended temperature of <4°C (Derens et al. 2001). Also in France, a web-based survey of 809 respondents determined that only 37% ensured that the temperature in their refrigerator was 4°C or below (Lagendijk et al., 2008).

A US Government report (CFSAN/FSIS, 2001) reported that 73% of domestic refrigerators were found to be at ≤5°C and 4% at >8.3°C. Jol et al. (2005) report that while major manufacturers and retailers operate a constant and effective cold chain, surveys in the US have revealed that 20% of domestic and commercial refrigerators operate at a temperature of >10°C.

An Australian telephone survey (Jay et al., 1999) found only 16% of respondents knew the temperature of their refrigerator. A 2004 New Zealand survey of domestic refrigerator temperatures found that 16 out of 53 fridges tested (30%) were operating above 5°C (NZ Foodsafe Partnership, 2004). Twenty six (49%) showed temperatures ranging from 5°C-7°C. Four of the 53 fridges (7%) had average air temperatures above 7°C, and the warmest average air temperature recorded was 9.9°C. The lowest recorded temperature at any one time was -2.5°C. Almost 72% of the fridges surveyed recorded higher temperatures on the top shelf than on the bottom shelf. Of all the fridges surveyed, 23 (43%) had average air temperatures between 1°C and 5°C.

While an increasing number of refrigerators are sold with a single point temperature display, Laguerre et al. (2002) found that the temperature measured using a thermometer does not represent the “true operating conditions of the refrigerator”. Indeed temperatures in refrigerators are not static. Various studies such as Koutsoumanis and Taoukis (2005) note major temperature variations throughout a refrigerator.

10.5 CONSUMER BEHAVIOURS

Cates et al (2006) studied the consumer awareness in the United States of *Listeria* and investigated how cold meats and frankfurters were stored once purchased from retail outlets. Cates et al reported that only 44% of respondents were aware of *Listeria* compared to 94% for *E. coli* and *Salmonella*. The domestic storage of frankfurters as defined by the USDA guidelines was assessed as part of the study. Overall, 783 respondents reported correct storage compared with 104 who stored incorrectly. For delicatessen meats, 551 of respondents stored correctly compared with 310 that did not.

11 THE COMMENTS AND OPINIONS OF ENVIRONMENTAL HEALTH OFFICERS REGARDING CSM AND *L. MONOCYTOGENES*

A questionnaire (Appendix 3) was prepared to gather comments and opinions from environmental health officers (EHOs) regarding *L. monocytogenes* and CSM. The questionnaire was divided into two sections. One section asked for responses from the viewpoint of the EHO and their opinion of their own knowledge and their enforcement colleagues. The second section asked the EHO for their opinions of the technical knowledge and operating practices of the FBOs they visited as part of their professional duties. The phrasing of the questions were edited by a consultant social scientist (Dr Stephanie Chambers, University of Dundee) with a limited knowledge of microbiology and cooked meat processing; to ensure the questions were clear, unambiguous and not leading.

The structure and content of the questionnaire was made after consideration of previously identified key areas of good practice for meat processing (FAO, 1991). In addition, the form included questions intended to gather information on aspects of processing such as cleaning and sanitising practices and their frequency in order to identify potentially questionable hygienic practices. Finally, the EHOs were asked about the determination of shelf life of CSM and how the consequent expiry dates of products were determined in the FBOs that they visited.

The questionnaire was hosted online and the web site required authentication before providing access to the questions to ensure all the responses were legitimate. An *en masse* emailing was sent by the FSA to the Heads of Service at all of the Local Authorities in the United Kingdom. The emails contained the login details and the site URL as an invite for EHOs to respond to the questionnaire. In addition to the online questionnaire, informal (i.e. not structured from the questionnaire) face-to-face discussions were undertaken with three practicing EHOs. The face-to-face discussions were focussed on hazards and concerns encountered in the field, how EHO sampling frequencies were determined and how CSM shelf life was defined. In total, feedback was received from 93 EHOs and a significant volume of information and specific comment was generated. The responses were grouped into those focusing on EHO perceptions of their own requirements and areas of concerns, and those relating to EHO perceptions of FBO requirements and concerns. To ease presentation and discussions, the responses to

each of the online questions have been grouped into related key food safety principle areas, for the EHO-focused questions shown in Table 31.

The responses were further analysed by each of the following topics to identify priorities within each group:

- Basic food safety principles
- HACCP-related
- Personnel Issues
- Supply Chain
- Regulatory

A summary of the numbers of responses recorded on a question-by-question basis and the mean response score was used as an indicator of the relative importance of these topics. Table 32 is a ranked summary for FBOs requirements as per EHOs. In addition, the information in Table 32 is ranked in order of importance, where the responses received from EHOs indicated that additional training or guidance could be of benefit to food business operators (FBOs). Table 33 also shows, in ranked order, the key areas where the responses received from EHOs where they felt they could benefit from further information or training. It is interesting to note that there were differences in what the EHOs perceived the FBOs required compared with what the EHOs felt they required. The need for guidance/support for FBOs was scored more highly than for EHOs, indicating an overall EHO perception that they were better informed than FBOs.

Table 31 EHO form questions grouped by key food safety principles

Subject area	Corresponding questionnaire question
Key food safety principles	
Control of key hazard organisms	2
Monitoring salt use and packing vacuum/gas mix	3
Changing product formulation	4
Water/ brine quality	15
Plant cleaning and sanitising	16
Post process handling	19
HACCP-related	
HACCP principles	1
Plant physical separation of processes	12
Plant 'high care'	13
Plant 'high risk'	14
Monitoring of critical control points	18
Personnel issues	
Food handler basic training	6
Return to work procedures after illness	7
Supply chain	
Raw materials sourcing	9
Raw materials acceptance	10
Shelf life determinations with regard to EC 2073/2005	20
Regulatory	
Understanding of 2073/2005 EC	22-23
L. monocytogenes ISO test method (or validated equivalent)	24-25

Table 32 A ranked summary of EHO perceived FBO requirements and areas of concern

Question	Mean score	Number of responses	Ranking
Shelf life determinations	4.65	62	1
Plant cleaning and sanitation	4.50	58	2
Monitoring salt, nitrite etc	4.37	62	3
Control of key hazard organisms	4.31	62	4
Plant high risk area	4.31	62	4
Post process handling	4.24	63	6
Food handler basic training	4.18	62	7
Plant high care area	4.05	61	8
HACCP principles	3.94	63	9
Water and brine quality	3.69	59	10
Plant physical separation	3.57	63	11
Process monitoring	3.49	63	12
Changing product formulation	3.48	62	13
Raw materials acceptance	3.45	62	14
Return to work procedures	3.03	61	15
Raw materials sourcing	2.94	62	16
Knowledge of shelf life consequences	2.23	62	17
Knowledge of ISO 18593	2.08	60	18
Understanding of EC 2075/2003	2.08	63	18
Use ISO test method or validated alternative	2.00	62	20

Table 33 A summary of EHO perception of their own requirements for training and additional information, and areas of concern

Question	Mean score	Number of responses	Ranking
Shelf life determinations	4.41	88	1
Plant cleaning and sanitation	4.05	81	2
Monitoring salt, nitrite etc	4.04	89	3
Control of key hazard organisms	4.02	89	4
Post process handling	4.01	88	5
Changing product formulation	3.82	89	6
Food handler basic training	3.78	87	7
Plant high risk area	3.78	87	7
Plant high risk area	3.74	86	9
Water and brine quality	3.64	84	10
Raw materials acceptance	3.46	87	11
Plant physical separation	3.17	88	12
Process monitoring	3.17	89	12
Raw materials sourcing	3.02	88	14
HACCP principles	2.96	89	15
Understanding of EC 2075/2003	2.91	86	16
Knowledge of shelf life consequences	2.75	85	17
Return to work procedures after illness.	2.70	87	18
Knowledge of ISO 18593	2.59	85	19
Use ISO test method or validate alternative	2.31	85	20

There were also differences in the numbers of responses made for each question. A paired t-test comparing the numbers of responses for each question for EHOs and FBOs showed the difference in responses was significant ($P<0.01$). Overall, a significantly larger number of EHOs responded to the EHO-focussed questions and the 'preferred format for additional information' section compared with the FBO questions.

11.1 KEY FOOD SAFETY PRINCIPLES

It was clear that EHOs were keen to enhance their own understanding of basic food safety principles because this topic area accounted for five out of the six highest scored responses overall, see Table 34. The reason for the interest was not immediately clear, which may indicate EHOs felt that they lacked knowledge of core areas of longstanding hygiene legislation, and microbiology requirements.

Table 34 Ranked EHOs' perceived information requirements regarding food safety principles for EHOs

Basic Food Safety Principles	Mean Score	Rank by Topic	Overall Rank
Shelf life determinations	4.41	1	1
Monitoring salt/other parameters	4.37	2	2
Plant cleaning sanitisation	4.05	3	3
Control of key hazard organisms	4.02	4	4
Post-process handling	4.01	5	5
Changing product formulation	3.82	6	6
Water/ brine quality	3.64	7	10

Guidance for EHOs on shelf life determination was seen to be the highest priority issue, with a mean score of 4.41 out of 5, being only 0.04 points ahead of the next highest ranking topic (monitoring salt/other process parameters).

EHO comments were captured through an open-ended question on general shelf life issues, to which 35 EHOs responded from both the EHO and FBO needs perspectives. Table 35 is a summary of selected responses, with the duplications removed. The comments corroborated a clear desire for further guidance, with only two acknowledging that such guidance already existed and one stating that it was '*not specific enough*'. The responses indicated that raising the awareness of existing guidance was required. In addition, some of the responses indicated that some areas of existing guidance would benefit from a review and partial rewrite to make aspects of the guidance clearer, and to provide more targeted, rather than general, advice.

Table 35 Selected EHO comments (unedited) on their own needs in relation to shelf life issues. The topics listed are those for which a need for information was expressed.

Selected EHO comment relating to shelf life issues from an EHO viewpoint
Determining suitable product durability dates - taking into consideration product composition/ cook date / slicing date / vacuum packing date / dispatch date. FBOs of micro and SMEs tend not to get advice from food research organisations or do product life modelling/testing - as it is expensive. Could this be made more accessible and affordable?
Product shelf lives are currently well defined in other guidance documents and regulations
End product testing and compliance with EC 2073/2005
What in practice is shelf life validation? If there is any doubt advice should be it can't be done.
Shelf life determination (required) particularly for small medium businesses, product characteristic testing
Understanding of frequency of sampling that is likely to provide due diligence defence, a reasonable minimum. This often takes a shot in the dark approach.
Additional guidance for less experienced officers too, e.g. Products likely to support *Listeria* growth regarding key profiles such as pH salt aw etc.

EHOs felt there was a need for guidance on monitoring salt and other parameters and the perception was that this was almost as important as shelf life guidance (mean score 4.37). Information on salt monitoring and other process parameters was ranked third overall. Comments from EHOs indicated that guidance on the measurement of pH and a_w in particular would be helpful to them to help guide SME FBOs on appropriate manufacturing practices.

It is the opinion of the project team that such advice should include an indication of the types of measurements needed (e.g. % NaCl and/or % sugar, laboratory testing), the types of laboratories to be used, the role of accreditation and a guide to results interpretation.

EHOs' felt that plant cleaning and sanitisation guidance would also be beneficial and this area of hygiene was the next highest ranked (mean score 4.05). This was a key recommendation from the first Pennington Group Report in 1997. FSA have funded previous work to address these types of gaps, which includes advice on cleaning and sanitisation in the Agency's *E. coli* cross-contamination guidance, although based on the responses collected, effective cleaning and sanitation remains an important information and training gap for EHOs and some FBOs. We are aware of the development of *L. monocytogenes* guidance for SMEs that is being progressed by FSA at the time of writing. It is understood the guidance will include a section to cover this key topic.

EHOs' comments in relation to their own needs for support on plant cleaning are shown in Table 36. Thirty-two EHOs commented on their own and FBOs' perceived needs.

Table 36 Selected EHO comments relating to their perceived needs in relation to plant cleaning

Selected EHO comment relating to plant cleaning and sanitation from an EHO viewpoint
Proper cleaning techniques for individual machines including how they come apart. I have particular problems with the effective cleaning of vacuum packers and slicing machines
Businesses need to get into the mind-set that you clean until visibly clean then apply a sanitiser. Too many just clean until visibly clean and some think a tap water rinse is fine then they can apply a sanitiser.
A greater and more simplified understanding of the techniques and materials used to break down biofilms and environments in which listeria may survive and multiply
Detailed product performance information and how mixing information (if applicable) links in with this. Specific usage instructions along with contact times of the sanitiser/disinfectant and whether any post cleaning disinfection rinsing is required or not.
The use of hoses in high risk areas.
Clear guidance on what is expected in terms of separation especially in terms of the smaller operators who may not have the space or resources
How to achieve high standards in a small production area of a high street butcher.
Upper level cleaning, ceilings and walls
Most places don't have the room to segregate operations. Cleaning is hugely important for effective *Listeria* control. A good number of businesses have attitude if it's visibly clean that's good enough.
Physical separation and cleaning procedures are considered pre-requisites for all businesses
There is plenty of information already available - the key is determining the attitude of the 'plant' operator and workers and their behaviours which are not simply addressed by writing additional procedures and practices - what is of more use is how the 'plant' hygiene is validated then verified.

For the EHO perceptions of what was required to assist FBOs, five out of the six highest ranked topics related to basic food safety principles, indicating EHOs' perception of there being fundamental deficiencies in some FBOs' food safety assurance knowledge (Table 37). It is worthwhile to note that core hygiene legislation and microbiological criteria for foods and food processing environments have been in place for nearly a decade in the EU.

Table 37 EHO responses regarding FBO perceived information requirements relating to basic food safety principles

Basic Food Safety Principles	Mean Score	Rank by Topic	Overall Rank
Shelf life determinations	4.65	1	1
Plant cleaning and sanitisation	4.50	2	2
Monitoring salt/other parameters	4.37	3	3
Control of key hazard organisms	4.31	4	4
Post-process handling	4.24	5	6
Water/ brine quality	3.69	6	10
Changing product formulation	3.48	7	13

Guidance for FBOs on shelf life determination was the highest priority, with a score of 4.65 out of a potential maximum of five.

Guidance on the determination of shelf life in relation to *L. monocytogenes* for SMEs and enforcers is freely available (CFA 2010). However, based on the comments and question score responses, it appears that either there is low awareness of the availability of this material or the guidance lacks sufficient detail for SMEs.

As before, EHOs' comments were collected through an open-ended question for general shelf life issues. There were 35 EHO responses from both the EHO and FBO needs perspectives. The comments in relation to FBO perceived requirements agreed on a clear need for further guidance as practices were reported that are at odds with consumer protection and, in some cases, statutory obligations (Table 38; with comments restating already-made points not reported).

Table 38 Selected EHO comments on FBOs' perceived needs in relation to shelf life issues

Selected EHO comment relating to shelf life from an FBO viewpoint
Shelf life is not clearly understood and guidance is vague. Determination of shelf life is often not done, and FBOs rely on vague guidance or old practices.
Don't think any small businesses have done any microbiology for shelf life. The best you can hope for is a bit of product gets held until it goes off (look / smell) and estimate from that. Some businesses don't give a shelf life, and some give a very short life of a few days as a precaution. Not much of that is specific to *Listeria*.
Majority of plants monitor cooking temperatures. I think most small businesses would welcome clear instructions on how to determine a safe shelf life. Lab test costs are an issue for small shops that make their own sliced meats

Poor info on shelf lives not aware of any business that has done anything specifically for *Listeria*.
Lack of awareness of FBOs about shelf life and the fact that vac packing and refrigeration does not stop survival and growth of *Listeria*.
There is a common practice within the industry to produce cooked sliced meat (particularly turkey) with extensive shelf life that cannot be justified based on HACCP principles or good scientific evidence. Industry tends to rely on the history of 'no known events' associated with the product rather than applying good scientific evidence to determine shelf life.
FBOs have limited understanding of how different bacteria react to differing environments. They do not understand water activity, pH etc and they do not have the resources to do product testing. As this testing can only be recommended by the EHO it is a constant battle to get FBOs to determine shelf lives safely rather than going to the supermarket and looking at similar products and copying them. Ireland's guides on shelf life determination- Guidance Note 18 is quite useful
Determining suitable product durability dates - taking into consideration product composition/ cook date / slicing date / vacuum packing date / dispatch date. FBOs of micro and SMEs tend not to get advice from food research organisations or do product life modelling/testing - as it is expensive. Could this be made more accessible and affordable?
Many small businesses take advice from manufacturers of packaging equipment and are told that foods will have e.g. A 10 day shelf life in MAP and they do not understand that the microbiological quality of the food as it is packed and the physical properties of that food are the factors that influence shelf life.
Most of my businesses are small, but they produce hams which are distributed to local hospitals and schools. I know for a fact that they do not do any sampling and second guess the use by date that they give the food.
No-one knows how to do shelf life properly.
The importance of actually establishing product shelf lives
Small businesses struggle with determining shelf life and rely on advice and direction from EHO. Practical guidance on this subject for the small business is essential.
Guidance already exists - and yet the understanding of supervisors and staff may be lacking - those running such high risk businesses should have to demonstrate understanding prior to manufacture - rather than afterwards as at present Product shelf life testing is very expensive so for a small producer the tendency would be to use historical data rather than their own analysis
Cooking is the only control that is generally understood and is assumed to be the only one necessary by FBOs and EHOs
Shelf life is an important factor which is not very well understood by food business operators currently
The way in which shelf life is determined is not understood by FBOs. It is believed that copying shelf life periods from reputable brands is adequate.
Additional guidance for less experienced officers too, e.g. Products likely to support *Listeria* growth regarding key profiles such as pH salt aw etc.
A lot of businesses give prolonged shelf life (e.g. > 3 days) and don't have a clue how to validate the shelf life they wish to give.
Shelf lives given often seem long and not always scientifically verified.
The cooking process is considered to be well understood. There is other guidance on the determination of shelf life with regards to *Listeria* mono.
Where Listeria spp is identified through monitoring there seems to be little understanding that L. monocytogenes

is also likely to be present and grow. Long shelf life and rewrapping is not clearly understood as a contributory factor. Lot of guidance e.g. Irish guide/SANCO but not in a very user friendly format for SMEs.

The next highest ranking topic was plant cleaning and sanitisation guidance for FBOs with a mean score of 4.50. As discussed previously, despite regulations that are almost 10 years old and the importance of effective sanitation both in terms of food safety and product shelf life, the topic is perceived as an important information and training gap for EHOs and SME FBOs.

EHOs' comments in relation to FBO needs for support on plant cleaning and sanitisation are shown in Table 39. There were 32 EHOs comments relating to their own and FBOs' perceived needs, which indicated a strong consensus of a requirement for detailed practical guidance.

Table 39 Selected EHO comments relating to FBO requirements for plant cleaning and sanitation

Selected EHO comment relating to cleaning and sanitation from an FBO viewpoint
Cleaning needs to be targeted to Listeria - more than obvious food contact surfaces, airborne transmission common source for Listeria on product.
Cleaning needs to be customised to what's being cleaned and whether the surface will touch food. Equipment needs to be disassembled for proper cleaning and sanitation.
Again, recognising where listeria can survive and proliferate, drains, condensers, door seals, rusty surfaces etc and how subsequent product contamination can occur
Cleaning is hugely important for effective Listeria control. A good number of businesses have attitude if it's visibly clean that's good enough.
The importance of design in minimising Listeria (total separation between raw and ready to eat processes).
Anything that relates to small premises e.g. Shop and preparation area.
Cleaning difficult to access areas
Cleaning effectively to remove listeria from the processing environment
Education and updates on new information, air pressures in high care, need to keep hoses off floors, should be complete segregation of low care and high care, examples of correct materials for example wall cladding, avoidance of ponding/condensation from ceilings, flow of drains, avoidance of corrosion/rust
Physical separation and cleaning procedures are considered pre-requisite for all businesses
Again, if receiving area requirements appear too technical any advice will be a waste of time.
High care and high risk only recently on the radar due to BRC requirements
Greater understanding of validation and verification of hygiene controls rather than relying on assumptions

Drainage flows, design of floor drain covers, condensation control. Evaporator cleaning, defrost drainage pipework routing.
Footwear change and avoiding hosing.
The drainage systems including the siting of drains, flows, the falls etc. It is felt that there is a reasonable understanding of the requirements for separation although these have scored highly because of their importance.
The importance of prerequisites in supporting the FSM system

11.2 HACCP-RELATED

A high risk area (HRA) is required for the manufacture of RTE fully cooked chilled foods such as CSM, whereas a high care area (HCA) is necessary for the manufacture of RTE chilled foods containing cooked and uncooked components. 'Cooking' is defined by the BRC as 70°C for a minimum of two minutes at the coolest part of the food. We noted that a number of EHOs made reference to HCA in relation to RTE CSM, which could be interpreted as an indication of a lack of understanding by EHOs of hygiene terminology or specific gaps in their training.

Delivering plant High Risk (or High Care) regimes were the most highly ranked concern in HACCP-related topics. High risk areas obtained a mean score of 3.87, which ranked as the seventh priority overall. High care areas were a close second at a mean score of 3.74 with a ranking of ninth overall (Table 40). In combination, these results and the associated EHO comments indicated that the production area design and the plant layouts were topics where EHOs felt additional information would be beneficial.

Table 40 EHO comments regarding their perceived needs in relation to HACCP and plant layout

HACCP-related	Mean Score	Rank by Topic	Overall Rank
Plant high risk	3.87	1	7
Plant high care	3.74	2	9
Plant physical separation	3.17	3	12
Process monitoring	3.17	3	12
HACCP principles	2.96	5	15

Plant physical separation was ranked 12 by EHOs. Based on the comments made, there were three reasons for the poor ranking. Some EHOs considered that compartmentalisation was an aspect of HCA and HRA, which is a reasonable stance to adopt. Some EHOs however did not consider separation of process stages to be important, although there is evidence in the literature that physical separation is beneficial (Lunden et al 2003). Finally, a few EHOs acknowledged the practical issues that SME FBOs faced, particularly where space and funds were limited (Table 41). In such businesses, some EHOs considered that compartmentalisation was not important because it could never be implemented in practice.

Issues arising with improving the understanding and application of HACCP principles were ranked 15 overall, with a low mean score (2.96), indicating that EHOs perceived that they had little need for practical HACCP implementation support.

Fifty-two EHOs responded to the open question on difficulties in enforcing high risk regimes (Table 41). Many referred to the Microbiological Criteria for Foodstuffs Regulation 2073/2005 as being an area warranting support. The establishment of shelf life was also spontaneously referred to frequently, further substantiating the EHO concerns discussed previously (section 11.1) and shelf life issues being ranked highest overall.

Table 41 Selected EHO comments on the difficulties experienced when enforcing in high risk processing areas

Selected EHO comments relating to high risk and general processing areas
Butchers shops producing CSM, due to risks of cross contamination
Total physical separation of equipment and staff from raw to post-cook
Guidance on sampling under 2073/2005 is extremely poor, particularly for SMEs.
Not all premises have space to implement High Risk Area
Its news to most premises they should be testing for shelf life and plant environment also
There is a knowledge gap on implementation of 2073/2005
2073/2005 understanding depends on business size. Larger operators know about it and do what they should, smaller ones claim not to know, but probably do and are highly resistant to paying for any lab testing
Food businesses such as cooked meat packers sometimes consider it is acceptable for food with less than 100cfu/g LM to be distributed as they don't consider they have manufactured the food, our view is that LM should be absent in 25g unless they can demonstrate that LM cannot grow above 100cfu/g during shelf life. It needs to be made clear that a business that cuts and wraps or simply rewraps cooked meats is included by the terms "by the food business operator that produced it in regulation 2073/2005.

What should a LA do if Listeria is found to be 'present' in a routine sample, but no levels are provided as they are not routinely checked. Obviously another sample will be taken to get a count, but what happens in the meantime?
Attitude of EHOs to Listeria control does not encourage FBOs to consider it
Frequency of testing is left up to FBOs, it should be defined by regulation
Cooling, vacuum packing, shelf life determination
Good practice in low care, segregation of staff, good cleaning of common areas e.g. tea rooms/hand rails/lockers/loading bays. Ability to have an effective sampling programme of routine samples and FBO response to failures, lack of effective documented maintenance programmes
Effective cleaning of complex food equipment, and effective hygiene monitoring procedures, in particular for hand hygiene.
CSM has good awareness, and adhere to stringent controls. Smaller retailers have procedures in place but do not carry out verification sampling. In small businesses emphasis is on time / temp controls
Cleaning underneath equipment such as slicers
When a business is well managed and very clean with good hygiene operating practice and they still have a persistent issue with Listeria.
Structure including drainage, management of water in the premises during production and after cleaning. Staff awareness of bacteria. Commercial pressures to extend shelf life. Disjointed supply / cold chain, often small manufactures do not control the whole cold chain to customer, this can lead to temperature abuse that is very hard to identify.
The enforcing of separation of low and high risk areas especially employees
The problem is often that the cooked meat production is a small part of the overall business. So it is therefore difficult to enforce absolute separation of cooked and raw. For example, they may be cooking hams in a boiler in the raw meat preparation room and cooling them in a shared chiller. It would therefore be impossible to achieve the same standards as would be expected in a purpose built factory.
Small independent butchers producing small amount of cooked meat
We had a problem with the floor and internal drains a few years ago, Listeria was present. New cleaning equipment bought, new cleaning methods introduced and drain sealed off, and negative results were obtained again.
As small business they have difficulty keeping up with all the new or revised information and what seems to be changing priorities from us, the industry and the public.
Small producers, ethnic issues
Vac packed goods, opening packs, splitting meats and re vac packing
The above will be a no go area for most FBOs. Advice should simply be - you must obtain advice from your EHO. EHOs need advice on these issues that is pragmatic.
Shelf life in SMEs
The shelf life testing. What to do if you find L mono when there should be absence. Micro testing - sampling programme etc considering the size of the business.
Shelf life determination. Cleaning practices of new business.

We do not have any CSM manufacturers - but do have sandwich manufacturers who use CSM. This leads to further complications when determining shelf life etc as the sandwich manufacturer may want to extend the shelf life of an ingredient.
Lack of physical segregation of work spaces and staff in SMEs
Older premises with poor separation, cleaning/disinfection problems caused by poor design/construction. Verification of cleaning disinfection processes and personal hygiene procedures are difficult

11.3 PERSONNEL ISSUES

Guidance for EHOs on the basic training of food handlers was ranked seventh overall and was the priority personnel issue for EHOs, receiving a mean score of 3.78 (Table 42). Compared with the corresponding score for FBOs, the EHO score was lower (Table 43). Section 4.1.10 demonstrates the importance of employee hygiene, which was reflected by the EHOs ranking of sixth from an FBO viewpoint. A number of comments (n=36) were made relating to employee illness and return to work procedures. These aspects were not highly ranked by EHOs and the reason was that there were already adequate provision of government (FSA 2009) guidance in this area. Such guidance was felt to be distributed widely in both EHO and FBO communities.

Table 42 EHO perceived requirements to personnel issues from an EHO viewpoint

Comments relating to personnel issues	Mean score	Rank by topic	Overall rank
Food handler basic training	3.78	1	7
Return to work procedures after illness	2.70	2	18

Table 43 EHO perceived requirements to personnel issues from an FBO viewpoint

Comments relating to personnel issues	Mean Score	Rank by Topic	Overall Rank
Food handler basic training	4.18	1	6
Return to work procedures after illness	3.03	2	15

A number of EHOs made specific comment that they considered worker hygiene to be an issue in some of the premises that they visited. The two areas that were highlighted were inadequate hand washing,

and the importance of clean clothing use. It was not clear if inadequate hand washing meant infrequent washing, or poor hand washing technique (e.g. not using hot water and soap). Inadequate hand washing by some food handlers is not confined to the CSM sector. Previous work, funded by the FSA, and involving some of the authors of this report, generated publicity materials that show the effect of various hand washing methods, as assessed by pressing hands onto agar plates. Graphic materials with minimal wording appear popular in the raw meat and fresh produce sectors and could be recycled for distribution in the CSM industry. One EHO commented there was adequate information already available, but which was not used by SME FBOs. On a related topic, some EHO raised concerns that there was a low awareness amongst some food handlers that minimal handling was the best practice. More generally, a number of EHOs noted there was poor understanding of cross contamination in processing plants. The clothing issues identified by EHOs included FBO employees wearing outside clothing in process areas and not removing personal protective equipment (PPE) such as aprons when handling waste or using lavatories. Clothing can act as a fomite for *L. monocytogenes,* which relates to the concerns of poor understanding of cross contamination made by some EHOs for some businesses. The inadequacy of 8°C as a control for *L. monocytogenes* was noted by two EHOs.

Table 44 Selected EHO comments on issues related to food handlers from an EHO perspective

Selected comments
DH already have guidance on return to work which FBOs should already be aware of. Shelf life is often poorly understood with no validation/micro examination carried out. Frequency of sampling guidance is also poor for both FBOs and food safety officers
There is information in the public domain regarding fitness to work. I'm not sure how much more would be required to cover listeria specifically
If there was a concern, most businesses would just ask the employee for a note from the doc to say they were fit for work.
There is existing info on return to work
Training/advise on the human impact of listeriosis. Individual food handlers' role in the transmission of Listeria e.g. clothing change and hand washing.
The different exclusion times for different bacteria. E.g. E.coli requires, negative stool samples not just 48 hours clear. Food handlers don't fully understand that it is their responsibility to inform their employer.
I think most food handlers have little awareness of specific information about Listeria but rather have a general knowledge of contamination and growth factors of common pathogens, food handlers that have attended level 3 and level 4 hygiene training should have better awareness. Perhaps lower level training could cover this better.
General environmental conditions for survival, maintenance issues, the food handlers role in the control and the importance which can contribute to the problem e.g. use of high power pressure hoses, importance of good accurate record keeping. Reporting problems to FBO and local authorities
Hand washing. Use of gloves.

Personal hygiene, particular hand washing and the cleaning and wearing of clean clothing is still poorly managed. Ensuring that aprons are removed when handling any waste, visiting the toilet and leaving the food production area. Understanding that returning to work after the 48 hrs guide does not mean that you are no longer shedding harmful bacteria.
Sources and control of listeria contamination.
Cross contamination cleaning and disinfection.
Temperature control as this needs to be lower than 8 degrees. General attitude of food handlers to keeping clean and food not handled more than necessary
How Listeria may enter premises and controls
Importance of a disciplined approach in limiting cross-contamination
The concept of vacuum packing itself and inherent dangers
Appreciation of the sources and consequences of L. monocytogenes
Symptomless carrier status

11.4 SUPPLY CHAIN

Raw material issues were perceived to be of a moderate to low priority for EHOs and FBOs (Table 45, Table 46). Most businesses undertake some assessment of raw materials, particularly meats on arrival. Basic visual and temperature assessments are widespread practices (section 12.3.1). However, the relatively high mean scores given to 'Raw Materials Acceptance Criteria' by EHOs (3.46) and for FBOs (3.45) indicated a need to support EHOs and FBOs in this area, possibly by providing guidance on the development of specifications, and the provision of example specifications.

Table 45 EHOs' perceived requirements in relation to supply chain issues from an EHO viewpoint

Supply Chain	Mean Score	Rank by Topic	Overall Rank
Raw materials acceptance criteria	3.46	1	10
Raw materials sourcing criteria	3.02	2	14

Table 46 EHO perceived requirements in relation to supply chain issues from an FBO viewpoint

Supply Chain	Mean Score	Rank by Topic	Overall Rank
Raw materials sourcing criteria	2.94	2	16
Raw materials acceptance criteria	3.45	1	14

Thirty-eight EHOs made some comment on raw materials issues, with the majority expressing a view stating that the importance of raw material control was not clearly understood by some FBOs (Table 47). Raw materials are a primary source of plant resident *L. monocytogenes* and it would assist in the exclusion of *L. monocytogenes* from processing environments if FBOs were informed of this fact, and treated raw meat as a potential source of *L. monocytogenes* which could contaminate the processing environment. On a related matter, several EHOs reported that there was no testing of raw materials for *L. monocytogenes* undertaken by any of the businesses they visited. A range of knowledge was revealed in this section of the questionnaire. A number of EHOs confessed to having a limited understanding of *L. monocytogenes*, particularly in relation to raw meats. One EHO confided a limited knowledge, but felt (correctly) it would be difficult to reliably decontaminate raw meat if it were contaminated with *L. monocytogenes*. Another EHO felt any contamination issue could be solved by a listericidal treatment, although the treatment details were not provided. A few EHOs flagged that potentially-contaminated packing materials for raw materials (such as cardboard boxes) were not treated as potentially hazardous by some businesses and consequently could remain in processing areas for extended periods of time.

Table 47 EHO comments regarding raw materials and CSM processing

Comment
Again, I have limited knowledge of the organism but I would imagine it would be fairly difficult to remove the organism completely from the raw material.
If the meat has a listericidal treatment there is no reason for the HR areas to have biofilms of Listeria. Sources, contamination, control and staff practices need to be examined and controlled to prevent the contamination.
Most small businesses don't realise contaminated raw materials can be spread around the plant.
Temperature is typically checked to make sure a batch of temperature abused raw meat doesn't get through - if the meat was chilled down again, temperature only check wouldn't protect against that.
Most places have some checks in place to make sure they don't get lumbered with a poor batch of raw meat
How to effectively remove resident populations of Listeria from the working environment, in particular, high risk areas such as slicing and packing. The concept and effect on the growth and survival of listeria of the process known as 'deep chilling' which extends the period between the end of cooking but before slicing and packing, often for periods as long as 28 days which adds a significant potential risk to the growth of listeria if it becomes contaminated prior to this point.
The need for alternating cleaning chemicals so that Listeria doesn't become used to the same one. Shock cleaning.
Listeria status of raw meats, temperature abuse evidence
I don't think that there is enough training available to EHOs to help us to help businesses. We have to rely on reading information and to be honest there is so much that we have to read, there aren't enough hours in the day

to do it.
Checks are made to make sure the meat arrives chilled and has no odours. Whether meat is a source of Listeria is not checked by micro testing by any FBO which I visit.
Are butchers part of the CSM consideration, as I have never seen this particular point raised in their HACCP?
The delivery check process, where raw meat is placed, the lack of thorough cleaning down of surfaces where meat is checked in, understanding the contamination risks on the outside of boxes and packaging. Frequency on cleaning internal chiller shelving, removing of cardboard from storage areas. Personal hygiene of staff.
For FBOs advice needs to be really simple and easy. E.g. Your supplies may be a source. However, you are responsible for stopping spread which you will do by 1, 2, 3.
Again, small scale producers are not likely to have info on Listeria status of the meat. Info about physical attributes that suggest an issue would be important and explanation that preventing Listeria coming into the premises can decrease the risk post-cooking contamination.
There is an assumption that raw materials are beyond the control of small scale manufacturers
The location of specific sources need to be identified and the probability of certain categories of meat being contaminated need to be understood.
Use of 'authorised' known suppliers and need for basic traceability records. Controls re: environmental controls.
Raw material path from delivery to storage through the plant. Removal of any contaminated packaging.
Entire food chain controls for Listeria better understood in dedicated plants than in smaller producers/retailers.
Auditing of suppliers and advice specifications and what to look for would be helpful . Not easy to do positive release on raw product and is there testing of the raw meat in slaughter houses or cutting plants
It is considered that there is limited understanding of the long term residency of L mono in CSM plants.
Controls over clean and dirty areas and possible routes for contamination

11.5 REGULATORY ISSUES

Additional support for regulatory issues was ranked lowest overall from both the EHO and FBO perspectives (Table 48, Table 49). Potentially, there were two reasons for the low priority. On the one hand, most EHOs are likely to have a good working knowledge of food sector regulations, and therefore would not perceive there to be much need for additional assistance on regulatory matters. From a FBO perspective however, there was an apparent assumption by EHOs that if their higher priority concerns such as employee hygiene, cross contamination and effective environmental cleaning were addressed, that would result in *de facto* compliance. Overall, most EHOs did not consider that the *L. monocytogenes* laboratory testing method was an important consideration. Although EC 2073/2005 specifically states a test method (ISO 18593), the consensus of the EHOs interviewed verbally was they

would be satisfied if the SME FBOs they visited did any sort of *L. monocytogenes* testing at all, and that the laboratory protocol was a minor consideration.

Table 48 EHO requirements for regulatory issues

Regulatory issue	Mean Score	Rank by Topic	Overall Rank
EHO understanding 2073/2005	2.91	1	16
EHO know shelf life testing consequence	2.75	2	17
EHO Knowledge of ISO 18593	2.59	3	19
EHO Lm ISO test method (or validated equivalent) knowledge	2.31	4	20

The open-ended question asking for concerns about environmental and food sampling from an EHOs' perspective gained the second highest response rate (n=63), indicating the EHOs considered these topics worthy enough to merit consideration for guidance, although there were higher priorities.

Table 49 EHO perceived FBO requirements for regulatory issues

Regulatory issues	Mean Score	Rank by Topic	Overall Rank
FBO knowledge of shelf life testing consequence	2.23	1	17
FBO understanding of 2073/2005	2.08	2	18 (equal)
FBO Knowledge of ISO 18593	2.08	2	18 (equal)
FBO Lm ISO test method (or validated equivalent) knowledge	2.00	4	20

EHOs perceived regulatory aspects of *L. monocytogenes* as being the lowest priority for FBOs. This could be because EHOs perceive their role at least in part as being the providers of regulatory information or they do not have high expectations of FBOs in this regard. However, more than two thirds of the responses (n=63) of EHOs recorded a comment to the open question on issues with environmental and food sampling. In common with the EHO viewpoint, the content of numerous comments indicates a high level of concern regarding this topic for FBOs, although other areas have been assessed as a higher priority (Table 50). The majority of comments indicated that sampling frequencies are largely set by the FSA's code of practice (CoP) and risk assessments of potential vulnerabilities in the intended consumers.

A range of EHO responses were submitted relating to sample collections. It was clear that in some areas of the country, very little environmental sampling was undertaken, whilst in others, the LA aimed to visit all FBOs in their region and undertake sample collections at least once per year. In some regions, premises are assessed for risk on the basis of their historical performance and new business are visited frequently to ensure appropriate and hygienic practices are adopted. In some cases EHOs decided whether to take samples during the inspection if there was cause for concern. The types of samples that were collected were variable. ATP swabs were the most popular, and had an advantage of immediate results. A number of EHOs used ATP swabbing to demonstrate hygiene issues to FBOs during their inspection. Sponge swabs, drumstick swabs and cloths were also mentioned. There was little information supplied from the EHOs describing the laboratory tests that were undertaken. However, typical testing regimes were determined during the FBO interviews (section 13.1.1.3). Several EHOs commented that the numbers of samples tested in their LA had been reduced, or sampling ceased, over recent years in response to reduced budgets. Another commented that sampling was limited specifically because of a lack of funding.

Table 50 Selected EHO comments on environmental and food sampling

EHO comment
In line with national programme and regional programme, resources permitting
We carry out regular sampling of cooked sliced meat for microbiological examination and undertake Biotrace swabs during inspection for hygiene indication and the effectiveness of cleaning techniques.
We sample cooked meats from all our butchers at least once a year
Food and swabs when visit was not totally satisfactory
Environmental sampling ATP swabs only, unless an area of particular concern/investigation. Sampling RTE foods included each year in sampling plan, L. mono included in examination request.
Food samples and swabs of surfaces
Swabs from areas that are not visibly clean to show bacteria present
Swabs and 50g samples of food
Swabs and food for micro testing with most frequent visits to the premises with issues
Period swabbing of food contact surfaces - chopping boards, knives, slicers at least once per year. Test food samples too.
Micro swabs and ATP swabs at least once per year for well managed businesses. More frequent for newly opened businesses, and those with issues
Quite a lot and we do specific swabbing and investigation if we find Listeria in a product

Mostly none
Limited due to lack of resources, tends to be reactive or as part of an agreed county wide project, local project or reactive work, or where we feel that food items need sampling in order to verify what an FBO is telling us during an inspection.
Swabs, ATP, final product
None that I am aware of.
Drain swabs and food contact surfaces
If a business has a problem we would carry out product and environmental sampling, we recently took numerous samples over a few weeks with a local business that was having listeria (and other) problems. These have now been resolved. But we do not have the resources to carry out routine sampling; this is the responsibility of the business.
Finished product testing normally from the final consumer e.g. retail shelf
Manufacturers are subject to at least yearly sampling which may include environmental swabbing
I very rarely do sampling. I have done some end product food samples and Spongicle/cotton bud type swab samples of slicing equipment and surfaces. I have also taken cloths and swabbed food handlers' hands. I've swabbed wash hand basins and taps. I've taken water samples. This wasn't specifically for Listeria though.
Sampling programmes targeting specific foods
Programmed sampling. My main cooked sliced meat people are an approved premises, and they sample weekly. (have own lab) they look for lots of different bacteria, including Listeria.
Group, regional and national studies and occasionally our own projects.
Environmental, water, food and end of shelf life.
Routine sampling monthly, survey sampling, responsive or complaint sampling
None due to resources being cut
Cooked meat manufacturing environment
Regional sampling coordinated by PHE. Approved premises sampling.
General lack of awareness
Food sampling of approved and high risk premises. Environmental swab sampling as part of investigation of poor food sampling results
Plenty of food sampling, not so much environmental sampling
Take part in regional and national studies
We carry out sampling from our shellfish areas and locally produced dairy products; our sampling has been significantly reduced in recent years because of budget restrictions.
Food samples obtained every 12-18 months from each manufacturer.
Minimal routine food sampling, mostly limited to dairy sector
It varies but we sample high risk food such as cooked sliced meat and other RTE regularly
Regular sampling of ready to eats foods manufactured locally. Swabbing and enhanced sampling when Listeria

found, participation in local national surveys for presence of Listeria
None at present
Routine sampling of high risk RTE foods, including at end of shelf life
Sampling approximately monthly from any new premises that have just come to light as well as ongoing annual samples from existing businesses.
Investigation of Listeria spp and L. monocytogenes isolations in cooked meats by environmental swabbing and food sampling (test incoming unwrapped cooked meats and finished products) some routine sampling at retail point of sale of prewrapped products from local producers.
None - should do, but resources issue!
They would be included as part of our annual sampling programme and would be sampled twice per year. Only food samples are currently taken. Environmental sampling tends to be reactive to problems.
Environmental swabbing, end product micro testing

Sixty-four EHOs, the highest response rate for any of the open questions, commented on how they decided inspection frequencies and samples collections. The majority of EHOs responded that they used the Food Law Code of Practice and its risk basis for inspections (Table 51). Sample collections tended to be decided either based on inspections that caused concern, on the basis that all premises visited were routinely sampled, or as a response to a complaint, depending on the LA.

Table 51 Selected EHOs' comments regarding the reasons for sample collection

EHO comment
Inspections are based on Code of Practice and sampling according to sampling plan
Inspection - risk rating. Sampling - all local producers are part of our annual sampling plan.
Inspections based on score from risk rating scheme. Sampling can be either as part of a survey or inspection-linked where any particular concerns.
Planned programmes
Pre-planned schedule, aim to visit all businesses at least once per year
Scheduled visits and response to complaints
Target the businesses with hygiene issues
Set schedule created based on risk determined previous inspection performance If something dangerous is found, extra visits put in to manage the business back to safety.
Follow schedule
According to risk rating frequency, officer knowledge and routine sampling results

Inspections are calculated in accordance with the risk rating scheme in the food law Code of Practice. sampling is not carried out unless there are exceptional reasons such as a significant risk
Annex 5 risk rating / Local and national sampling programmes / Specific concerns identified during inspections
Risk rating, conditions found at inspection, track record of business
Risk and intelligence about problems.
No data for this.
Based on risk
FSA risk based scoring. Sampling schedules / budgets formed annually. Will respond with sampling if required following a complaint or visit or other concerns.
Routine inspection carried out via the risk rating and sampling determined with FBO and consulting the Lab
Inspections are based on the code of practice and the risk rating attached to the premises. Most of the cooked sliced meat premises I visit are independent butchers who produced cooked meats for a local market. Sampling would be carried out if the inspections or any complaints suggested that there may be a problem. We would not do any random sampling. We also participate in national surveys.
Annual sampling plan and in response to NI wide and UK wide surveys
Local sampling programme and in line with Liaison Group surveys
CoP scores, risk factors
Inspections are based upon the cop Annex 5 and sampling is according to our LA sampling Annual sampling plan
Risk, complaint, most sampling is led by officer/team input.
Programmed by risk and activities of business
High risk inspections when due on risk assessment
Professional judgement based upon risk and practical matters relating to ease/difficulty.
Inspections determined by food law cop, sampling on an ad hoc basis
Risk based inspection programme, weekly routine sampling and response to failures in premises in our area
Inspections undertaken in accordance with the risk rating as directed by the Code of Practice. Samples taken as part of routine sampling plan.
Risk based inspections as per FSA CoP. All locally produced high risk products are sampled quarterly to verify compliance with EC Reg 2073/2005

Some two thirds of EHOs (n=59) responded to the open question asking when (on what basis) they decided to take enforcement action (Table 52). Around 50% of the respondents mentioned adherence to LA 'enforcement policy' as the basis for action (Table 52). A number of EHOs commented that enforcement was generally the last stage in a chain of events that typically gave the FBO ample opportunity to correct the issues of concern. A strong theme from more than half of the EHOs was that both FBO attitude and a history of previous inspection issues were also a factor in any decision to

prosecute. In particular, FBOs that reverted to unsafe practices as soon as the inspection frequencies were relaxed, were more likely to be prosecuted. The EHOs also commented that the severity of the transgression, assessed by the risk to public health, was also a factor when deciding to prosecute. Finally, and related to the already-mentioned FBO attitudes, how the transgression occurred was also considered before any decision to prosecute. An FBO who had deliberately untaken hazardous practices with knowledge of the potential consequences was more likely to be prosecuted compared to a FBO that was unlucky enough to have a chance incident that could not reasonably have been foreseen.

Table 52 EHO comments relating to decisions to take enforcement action

EHO comment
If CCP failure where risk to health, or unsatisfactory sample results received.
A graduated approach from informal to formal action
Results of inspection, sample results and complaints - refer to enforcement policy
Enforcement policy dictates general approach.
Enforcement policy
Degree of danger to public heath
The law dictates if prosecution
Risk to health with consideration of previous history and non-compliances
Repeated infringements and businesses that let things slide as soon as visits stop.
When business don't do what's needed to correct their operations. If businesses lapse after being warned and told how to rectify.
Enforcement policy. Try and obtain compliance informally first
Where there are circumstances that create a significant risk to food safety and depend on the individual situation. The level and type of enforcement action is wide and varied and ranges from verbal advice, written advice and warnings to withdrawal of approval
Sampling?- if an informal sample fails, we would sample again, but formally, and may consider enforcement action if formal sample fails, and there are other factors in place re food safety. We have a graduated enforcement policy.
Enforcement policy
Health risk, previous compliance / advice provided , public interest , enforcement policy, confidence in management
Refer to [county] Public Health and Protection enforcement policy.
Based on severity of non-compliance, attitude of management and willingness to learn, and prior offences

committed/general compliance record.
Enforcement policy, seriousness of the offence, risk to public health, history of compliance, graduated approach to enforcement, willingness of the FBO to put matters right etc.
Enforcement policy, attitude of the company, past history and severity of incident
When inspection reveal consistent issues or a complaint raises concerns
If a business has a record of non-compliance or there is a serious offence witnessed. We have to follow an enforcement policy which requires a graduated approach where businesses are given assistance to comply. Having said that, if the offence is very serious then immediate action would be required to protect public safety.
Degree of risk and proven complaints
Haven't had to take any, my approved premises works with me and does everything I ask.
By following the Council's enforcement policy.
Liaise with PHE lab and FSA Incidents team
Where there are failings at CCPs
Based on risk
Upon risk and severity; along with confidence in the FBO, and previous compliance.
In house policy, management and legal discussion, seriousness of issue,
I consider premises history, level of risk, level of understanding of risk, impact on population, nature of food safety breaches
Graduated approach & risk based.
Normal risk based approach
Focused on adverse results received
Public health risk
Inadequate validation or verification of HCCP following inspection either routine or inspection following unsatisfactory results
Based upon public health risk
Internal enforcement policy
In accordance with the code of practice and our enforcement policy.
Risk based and graduated approach
Enforcement policy guides
Enforcement policy
Based on enforcement policy and factors highlighted e.g. Public health, previous compliance etc.
According to our enforcement policy, decided in accordance with the code of practice and other national enforcement guidelines.
Assessment is made following complaint investigation, sample failures and inspections. The resultant action taken is proportionate to the risk and will follow the department’s enforcement policy.

Consider history, willingness to: assist in investigation; comply; to make changes. Judgement on wilful non-compliance v isolated incident which was not reasonably foreseeable.
Dependent on level of risk and scope.
We use an enforcement protocol
Risk based as per FSA Guidance and our enforcement policies
EHO autonomy
According to analysis and discussion with labs and our own enforcement policy
When there is an evident concern of unacceptable risk or lack of HACCP based procedures that would address Lm as a hazard.
According to enforcement policy, staged approach unless imminent / serious risk.
Following inspection and or sample results
Usually provide advice and re-sample to see it if is an ongoing problem with subsequent batch. If significant Unsatisfactory result or further batches affected may request voluntary removal of the item from sale until procedures improve.
Would consider LA enforcement policy approach, previous history, the circumstances of the incident, whether HACCP systems were in place an followed. The quality of the evidence including expert evidence
Case by case, enforcement policy, discussion with Principal EHO
Enforcement action taken in line with our enforcement policies. A graduated approach is taken. The history of the premises influences enforcement action.
After consultation with my Manager and the business
As per Council Enforcement Policy. Risk to public taken into account. Compliance history of business also considered. Severity of risk and remedial measures that are required. Food Law Code of Practice and guidance consulted as well as relevant EC regulations.
Risk to health, non-compliance with regulations

11.6 EHO CONCLUSIONS

All of the top six ranked topics for each audience (EHOs and FBOs) relate to the control of *L. monocytogenes* and are therefore covered by longstanding legal requirements for compliance with 852/2004 or 2073/2005 (Table 53). The finding that EHOs want more information on such issues is surprising because it may demonstrate a persistent deficiency in current SME FBO competencies. If that is the case, it is an issue that should be urgently addressed. The finding was discussed informally with a single EHO, who considered that compliance with 852/2004 and 2073/2005 was important. The opinion of the EHO was that there is a lot of information and it is scattered widely. The request for

more information was as likely to be driven by the desire to have reliable information available in a single place, as it was to be an indication of training or knowledge gaps for EHOs. The EHO questioned also felt that because there is a statutory obligation for 852/2004 and 2073/2005 compliance, the information requests may have been driven by EHOs' desire to make sure they are enforcing the law correctly and proportionately.

Guidance on shelf life determination was identified as the principal priority for both EHOs and FBOs. Guidance for FBOs scored higher than that for EHOs. However, based on the EHO comments, it is clear that some FBOs and some EHOs might not be as informed as they would like to be on shelf life determination. The recommendation is that guidance on shelf life determination be reviewed and redrafted to make it clearer and the revised advice should be promoted to EHOs, possibly via an FSA communication to EHOs.

Despite the fact that guidance on plant cleaning and sanitisation for both audiences ranked as the second most important topic area provision of similar guidance was a key recommendation from the first Pennington Group Report in 1997. However, EHOs felt it was still an area where additional assistance would be beneficial. It is acknowledged that the FSA has funded work to improve understanding in this area and has included advice on cleaning and sanitisation in its *E. coli* cross-contamination guidance. However, from an EHO perspective, it remains an important information and training gap for some EHOs and some SME FBOs.

Guidance on monitoring salt, and other process or product parameters, was ranked third for both audiences, again scoring higher for FBOs. The reasons for the request are not clear. FSA study FS 425012 (Thomas et al 2012) reported a perception by some EHOs that *L. monocytogenes* could be controlled by high salt concentrations in cold smoked fish. Whilst that is true for some other pathogens such as *Clostridium spp.*, *L. monocytogenes* is exceptionally halotolerant (salt tolerant). At 4^{o}C *L. monocytogenes* can grow in the presence of 13% w/w NaCl (Thomas et al 2012) which would render a product organoleptically unacceptable. Irrespective of the underlying reason, guidance on the measurement of pH, and a_w in particular, was felt to be required by EHOs to enable them to guide SME FBOs. It is recommended that this should include an indication of the types of measurements needed (e.g. % NaCl and/or % sugar, laboratory testing), the types of laboratories to be used and the interpretation of results.

One practical application of laboratory testing is that it can be used to help with the control of key hazard organisms. That topic was ranked fourth for each audience, with manufacturer-focused information on plant high-risk areas scoring equally for FBOs. Guidance on hazard organism control should set out parameters for the growth and survival of *L. monocytogenes*, and could potentially include the use of predictive modelling (e.g. www.combase.cc) to assess growth potential. One EHO commented that this was over-complex for FBOs. If that is the case, EHOs could encourage industry to undertake laboratory testing and make use of that information as inputs to predictive models for *L. monocytogenes* growth. Similar practices are required for full compliance with 2073/2005 unless it is known that *L. monocytogenes* is inhibited by the product formulation. Cost was identified as a key barrier to microbiological testing. Some SME businesses are reluctant to pay for testing themselves and are reliant on EHO sample collection and testing, which is typically infrequent, and may only occur once per year. Reduced LA budgets have resulted in a reduction or the suspension of microbiological testing by some EHOs.

Table 53 Top six priorities for identified for EHOs and FBOs

Priority	EHOs' perception of their own requirements (mean score)	EHO perception of FBOs' requirements (mean score)
1	Shelf life determination (4.41)	Shelf life determination (4.65)
2	Plant cleaning and sanitisation (4.05)	Plant cleaning and sanitation (4.50)
3	Monitoring salt/other parameters (4.04)	Monitoring salt/other parameters (4.37)
4	Control of key hazard organisms (4.02)	Control of key hazard organisms (4.31)
5	Post-process handling (4.01)	Plant high risk (4.31)
6	Changing product formulation (3.82)	Post-process handling (4.24)

The open-ended question asking for concerns about environmental and food sampling from an EHOs' perspective gained the highest response rate, indicating that this is also considered to be an important issue where EHOs felt they would benefit from additional advice.

Many EHOs referred unprompted to the Microbiological Criteria for Foodstuffs Regulation 2073/2005 as being an area warranting support. It was noted the interest was related to product shelf life establishment in particular with regard to *L. monocytogenes*. Raising awareness of existing and possibly revised guidance would allow this requirement to be addressed.

Basic training of food handlers was ranked seventh overall and was the priority personnel issue, receiving a mean score of 3.78. This was lower than the score given in terms of FBO requirements (4.18) but in that case, its ranking of sixth indicates its relative importance for both enforcers and businesses.

The relatively high mean score given to 'Raw Materials Acceptance Criteria' (3.46) was indicative of a need to support EHOs in this area, possibly by providing example specifications suitable for distribution to SMEs.

11.7 PREFERRED FORMAT FOR GUIDANCE OR TRAINING MATERIALS

The responses to the question asking for the preferred format for any guidance or training materials are shown as Table 54. Most of the EHOs asked had a preference for training courses or workshops closely followed by written online guidance. A comparison of the response scores revealed the two options were equally favoured (t-test; P=0.78).

Table 54 Ranked responses showing the preferred format for the identified required guidance

Format of guidance	Mean score	Number of responses	Ranking
Guidance training course or workshop	4.20	90	1
Guidance web written	4.15	89	2
Guidance web interactive	3.78	89	3
Guidance booklet pamphlet	3.42	85	4

The approach given the highest ranking by EHOs was the provision of courses and/or workshops (mean score 4.20), reflecting the need for concerted action on the reported knowledge and training gaps.

Web-based written guidance was ranked a close second (mean score 4.15), suggesting again that FBOs needed detailed information to help them understand and effectively manage *L. monocytogenes*. Web-

based interactive guidance received ~10% fewer points than either of these options (3.78) and booklets/pamphlets were viewed as being least acceptable to FBOs.

The FSA will undertake a series of EHO training workshops around the UK in 2014 and these could go some way in helping address the identified information requirements. We note that many of the issues identified in the current study are similar to those identified previously by other FSA industry practice reviews (FS425012). With that in mind, there may be merit in the supply of general information covering RTE generally rather than, for example, CSM and cold smoked fish individually. There is also a need to ensure that environmental health degree courses and continuing professional development (CPD) activities adequately cover the identified information shortfalls so that all EHOs are cognisant of *L. monocytogenes* control and the statutory requirements.

12 VISITS TO COMMERCIAL PREMISES INVOLVED IN THE MANUFACTURE AND RETAILING OF CSM

12.1 OPERATIONS SUMMARY OF THE PREMISES VISITED

As part of this study a number of visits to commercial processors and retail premises that sliced cooked meats in store were made between March 2013 and September 2013. The aim of the visits was for the researchers to understand industry issues and concerns regarding *L. monocytogenes* in CSM, and to properly understand how CSM was manufactured and sold. In order to gain a representative overview of the industry, a range of premises with diverse products and throughputs were visited. An overview of the participating businesses for CSM manufacture is provided as Table 55, and the retail premises are summarised as Table 63 and Table 64.

Table 55 The numbers of workers employed and a brief description of the products processed at the CSM manufacturers visited as part of the current study

Plant identifier	Number of employees (at the site visited)	Outline description of operations
A	22	Small manufacturer with all operations undertaken from single site. Undertook all processing stages from raw ham through to sliced product. Sold directly to SME retailers and also to trade suppliers. The company only made ham and ham-derivative products (e.g. crumbed ham, peppered ham). All products were shipped in VP. Annual turnover was roughly £5 million.
B	230	A major manufacturer with multiple manufacturing sites. Sold directly to major retailers (labelled as the retailer brand) and also manufactured own branded products on behalf of other CSM retailers. Undertook all operations beginning with raw meat primals. A wide range of red meat products were manufactured including premium (e.g. Wiltshire ham), derivative (e.g. peppered ham) and economy hams (e.g. spiced, chopped, extruded ham), sliced beef, and sliced fermented sausages (e.g. salami and pepperoni). Almost all products were shipped in MAP, with a fraction of a percentage point of the total throughput being VP. VP or MAP was based on customer preferences.
C	200	A major manufacturer with multiple manufacturing sites. Sold directly to a different set of major retailers to plant B. As for plant B, the retailer products were packaged in retailer branded materials. Also manufactured product for a number of own brand CSM manufacturers and subsidiary companies owned by plant C's parent company. Undertook all operations starting with raw meat. Principal products were poultry and poultry derivatives including premium chicken breast, economy chicken roll, and flavoured sliced poultry meat (e.g. barbeque or spicy Mexican chicken slices). All products were shipped in MAP.
D	2500	A major manufacturer with multiple manufacturing sites. Manufactured own brand products and products for retailers in retailer packaging. Plant D also supplied two major sandwich manufacturers. Plant D manufactured a wide range of chicken and turkey products including premium products such as sliced turkey and chicken breasts and economy products such as chicken roll. Plant D also manufactured flavoured sliced poultry meat. All operations were undertaken commencing with raw meat. All packaging was MAP.
E	15	A small manufacturer of own brand CSM. Business did not undertake any cooking of meat, but bought in pre-cooked unsliced products for slicing and VP. The business was a butcher that was located in an SME symbol mini-supermarket-type outlet (~15m x ~50m) that was owned by the butcher. There was a wide range of products sold that included roast beef, roast chicken, roast turkey, corned beef, garlic sausage and sliced fermented sausages. All products were VP.
F	8	A small manufacturer of own brand CSM located on a family owned pig farm. Although eight people worked on the site; exclusively, the farmer and his wife undertook the CSM operations. Business did not

		undertake any cooking of meat, but bought in deboned, Wiltshire cured pre-cooked hams (only). Ham was sliced and VP for sale to local SME shops or cling film wrapped in pre-weighed quantities for sale at farmer's markets. Packaging was branded with the farm name.
G	12	A small manufacturer of cured, sliced, boiled ham only. All operations were undertaken commencing with raw hams. The cooking method was novel because it was the only operation where the hams were extruded into casings, which were placed into cook bags and boiled. Packing was VP. Sold mostly into wholesale meat supply companies for further sale in SME retailers and butchers.
H	34	Small manufacturer with all operations undertaken from a single site. Undertook all processing stages from raw ham through to sliced product, except curing (ham was purchased pre-cured). The product was sold directly to SME retailers. The company made few varieties of hams, including honey roast and country style. All products were shipped VP.
I	5	Small supermarket producing cooked meat on the same site. Curing time was kept to a minimum; the product was boiled in a cooking bag, chilled, VP and sold sliced in the front shop.
J	11	Small family business producing a variety of smoked and un-smoked hams. All operations were undertaken on a single site. The products were not sliced but sold whole under VP that were couriered to a variety of customers across UK.
K	7	Small butcher producing on site a range of CSM including roast ham, chicken and beef. The business owned pig farms, which supplied the raw meat. The ham was cooked without curing in oven (convection and steam) and sold sliced and VP in the butcher shop.
L	450	Major manufacturer with one manufacturing site, supplying a variety of cooked meats, prepared and packed in a multitude of ways. The products were supplied as wholesale directly to a series of major retailers and part were exported. Undertook operations starting either with raw or frozen meat, sourced from local and import suppliers. Frozen meat was always defrosted at below 5^{o}C. Dry salt and brine injection was used for curing and steam oven cooking temperature was monitored by a computerised system. The products sliced products were either VP of MAP (30% CO_2 and 70% N_2).
M	56	Medium to large manufacturer of ham, chicken, turkey and beef CSM. Raw meat was cured/brined on site as appropriate. All cooking was in mother/cook bags inside steam ovens. Cooked product was sliced and VP in 200g retail packs. Sales were to wholesalers for further sale to SME retailers.

12.2 PREMISES TYPES

Some businesses did not conveniently classify as either a CSM manufacturer or a CSM retailer. Typically, these small operations bought in pre-cooked meats for slicing and sale. For the purposes of this study, if the sliced meat was offered for sale at more than one retail premises, the business was classified as a CSM manufacturer. If the CSM was offered for sale only in the premises where it was sliced, the business was considered a retailer.

12.3 PRACTICES OBSERVED IN CSM MANUFACTURING PLANTS

As part of this study, visits were made to CSM processing plants operating under commercial conditions. The plants visited covered a range of manufactured products and manufacturing throughputs. The products that were observed during manufacture are shown in Table 56.

Table 56 Products manufactured by the CSM premises visited

Meat species	Products manufactured by plants visited
Cattle	Roast beef, peppered roast beef, sliced corned beef from cans manufactured in South America
Pig	Sliced, cured ham (baked, boiled, steam-oven cooked, Wiltshire cured) uncured ham. Sliced, coated ham (peppered, breaded, honey roasted) Sliced, spiced chopped ham (using soluble myosin/actin)
Chicken	Sliced chicken (roasted) Sliced chicken breast Sliced coated chicken breast (Spicy Mexican, barbeque) Chicken roll (usingsoluble myosin/actin)
Turkey	Roast turkey breast Coated turkey breast Turkey roll (usingsoluble myosin/actin)

As will be discussed below (Table 57) there were a number of practices conducive to the control of *L. monocytogenes* noted during packaging in the larger manufacturing facilities. In addition, visits to different CSM manufacturers gave an overview of the entire industry, which allowed a number of common issues and themes to emerge.

In general, it was noted that many of the issues raised as knowledge gap concerns from the EHO interviews could be addressed by simple summaries of peer reviewed information and the in-depth technical knowledge of the type noted in Table 57. During the processing plant visits to the larger manufacturers, it was clearly stated that one of the purposes of the visits was to identify good manufacturing practices that could be used as the basis of good practice guidance for smaller processors. The majority of the plants visited had no objections because SME were not perceived to be a threat to the larger processor's market share. One or two technical managers stated however that they felt some of their strategies were innovative enough to be considered trade secrets. Therefore, a concern raised that such trade secrets should not be made widely available. However, there was also widespread acknowledgement that the interests of the industry would be best served by the avoidance of a *L. monocytogenes* outbreak from CSM. Were a best practices guide to be created, it might be prudent to ask the permission of organisation that created the innovation. As part of that process, it should also make clear that multiple plants were visited and nothing that was actually unique was observed. Overall, there were very similar control strategies for things like no water use in high risk during processing and double wrapped packaging.

12.3.1 RAW MATERIALS RECEPTION

The standard operating procedures and checklists for raw meat received into the plant were obtained from several FBOs. A collation of the good practices identified from these documents and those observed during the factory visits are provided as Table 57.

Table 57 Good practices observed for raw materials arriving at CSM plants

Identified good practice	Reason for practice
Check date of kill and butchery dates clearly identified on the raw meat and within a plant specific cut off.	Interval typically 3-4 days after kill to reduce likelihood of spoilage and *L. monocytogenes* growth. In addition, fresh meat cure is more effective and nitrite inhibits *L. monocytogenes*.
Meat was covered during transit.	Prevention of contamination (physical and microbiological).
Adequate chilled air circulation around the meat.	Did the air circulate to the centre of the pallet to reduce the likelihood of microbial growth, including a possibly *L. monocytogenes* arriving into plant.
Temperatures taken at the centre of a pallet/batch.	Centre is where temperature will typically be highest and so the practice helps ensure meat had not spoiled and that there was minimal *L. monocytogenes* growth as a vector into a plant.
Check to ensure no physical evidence of temperature abuse.	No excessive blood drip from product, white fat rather than yellow, no sour odours to ensure meat not spoiled and no *L. monocytogenes* growth as a vector into a plant.
Check frozen meat for absence of ice crystals	Meat that has ice crystals was only partly frozen or was re-frozen after a temperature abuse e.g. as a result of a power break
Controlled defrost for frozen meat at below 5^{o}C by tumbling at low pressure to thaw by reverse sublimation (solid to gas without a water phase)	Defrosting avoiding temperature abuse for long periods and hence minimising bacterial growth
Cleaning and sanitising of re-useable transport crates inside the factory, in dedicated washing units with dedicated storage spaces	Avoid cross contamination of future batches and along the processing chain
Widespread use of liners for re-useable dolavs	Part of a combined strategy to prevent cross contamination between batches of meat
Use of one-time packing for raw meat	

12.3.2 BRINING AND CURING

For ham, successful brining is important because nitrite and nitrate can inhibit the growth of *L. monocytogenes* (section 2.3.2.1). In order to ensure that brining had proceeded as intended,

some plants undertook verifications along broadly similar lines. In the opinion of the project team, the most valuable of these checks in terms of *L. monocytogenes* risks are listed in Table 58.

Table 58 Good practices observed for brining and curing

Identified good practice	Reason for practice
Brines clear with no undissolved ingredients	Nitrite helps prevent *L. monocytogenes* growth. If curing salts are not properly dissolved, lowered nitrite concentration in meat.
Defined order for the addition of brine ingredients	If ingredients were added in the wrong order, total dissolution would not be achieved.
Brine temperature check	Typically 5°C or less to help ensure no microbiological growth prior to contact with the meat. Brine penetration of meat is reduced at below 3°C.
Brine age check	Four days was considered to be the oldest that could be used to help ensure no microbiological growth prior to contact with meat. We noted that most plants mixed fresh batches of brine daily.
Brine laboratory check	In the larger processors, each brine batch was checked by titration to ensure correct nitrite concentration. Nitrite helps prevent *L. monocytogenes* growth and additionally the tests were undertaken to ensure the legal upper limits on permitted concentrations in products were not exceeded.
For injected meats, a check that brine needles were cleaned according to schedule	Blocked needles will minimise cure of the meat. Prevention of *L. monocytogenes* contamination of meat. Prevention of *L. monocytogenes* biofilm formation on food contact surfaces.
For tumbled meats, a check that tumbler/ vacuum tumbler was cleaned according to schedule	Prevention of *L. monocytogenes* contamination of meat. Prevention of *L. monocytogenes* biofilm formation on food contact surfaces. Prevention of *L. monocytogenes* growth during extended tumbling.
Use adequate draining during dry curing / periodic waste removal from processing area / adequate height between meat and floor (e.g. >30 cm)	Prevention of *L. monocytogenes* cross contamination from different joints, floors and drains.
Use of a separate dry space for storage of salt and spices/cooking ingredients; using of colour coded containers for salt and spices	Prevention of contamination from factory environment and plant workers

Brines were considered to be a key strategy area by a number of manufacturers. It was widely regarded that the nitrite in brine could slow *L. monocytogenes* growth and thus care was taken when mixing brines. In a number of plants, laminated (i.e. cleanable) recipe cards listing the

ingredients and required masses were provided to employees. There were simple checks in some plants such as all of the required assembled ingredients were required to be weighed and to be in agreement with the total mass stipulated on the ingredients card. It was felt in some plants that having employees sign off on each batch of brine they mixed helped reduce the incidence of brine mixing errors because it made individual employees accountable for any mistakes.

In at least one larger manufacturer, brine composition was felt to be so important that there was computer-assisted checking of brine ingredients prior to mixing. In brief, an operator entered the type of brine into a computer and the system would not release ingredients into the mixer unless the ingredient masses and water volume inside the mixer matched those held in the ingredients database.

12.3.3 PLANT PHYSICAL SEGREGATION, PLANT HIGH CARE AND PLANT HIGH RISK

Segregation of ready to eat (RTE) and non-RTE foods and equipment was widely recognised as important for reducing the potential for microbiological cross contamination. Recognition of the importance of segregation has given rise to the concepts of compartmentalisation, high care areas (HCA) and high-risk areas (HRA) in food processing premises (Lunden et al 2003).

A HCA is broadly defined as an area designed to a high standard of hygiene where practices relating to personnel, ingredients, equipment and environment are actively managed to minimise the microbiological contamination of a RTE or ready-to-reheat product that contains uncooked ingredients. If best practices are followed, cold smoked and other uncooked meats should be produced in a HCA, separated from the raw materials and final packaged product.

A HRA is defined as areas designed to high standards of hygiene where practices relating to personnel, ingredients, equipment and environment are managed to minimise microbiological contamination of a ready-to-eat or ready-to-reheat product comprising only cooked ingredients. Good manufacturing practice (GMP) dictates that CSM should be manufactured in a HRA, again separated from raw materials and final packaged product. Table 59 presents a list of observed good practices with regards plant segregation and staff movement restrictions observed (predominantly in the large processing plants).

Table 59 Good practices observed for plant segregation

Identified good practice	Reason for practice
Existence of physical separation between: • raw product receiving area from the rest of the plant • product preparation area from rest of the plant • cooking area from rest of the plant • packing area from rest of the plant • Final product chiller and high care and packing areas	Avoidance of cross contamination of the final product from potential *Listeria* sources (raw product, personnel, environment, packing material)
Existence of a formally-defined high care area for cooked product	Avoidance of cross contamination of the final product from potential *Listeria* sources (raw product, personnel, environment)
Existence of staff movement restrictions between the "clean" and the "dirty end"(e.g. where there are raw uncooked materials) of the process. Change of overalls, PPE and hand-washing before moving between different areas.	The avoidance of cross contamination to the final product from potential *Listeria* sources coming from the raw product side of production and transferred via staff.
PPE of different colours for different processing areas	Make inappropriate movements visibly obvious
PPE of different colours for different workers	Make staff working inappropriately visibly obvious (e.g. cleaning staff operating CSM equipment and thereby contaminating control panels)
An elevated floor height of around 1.5 metres in HR compared with the rest of the plant	The floor helped enforce controlled movements of staff, products and equipment. For example packed product was dropped from conveyors into dolavs (large plastic crates) and could not return to the high risk area.
Automatic disinfectant boot scrubbers at the start of a long (~25m) corridor that was physically separated but exited into high risk so that boots had dried (i.e. contained little or no water) before they reached the high risk area.	Little or no water in high risk helps prevent the establishment of *L. monocytogenes*
Use of visible or physical barriers	A number of FBOs felt a physical barrier such as a bench where employees left their shoes on one side and put work boots on, on the other side of the barrier was helpful in reminding employees they had entered a controlled area. One manufacturer had installed an array of bright bluish fluorescent tubes (non-UV) at the entrance to the HRA as a visible reminder of the area's status

12.3.4 THERMAL PROCESSING

RTE CSM should be produced in HRA, which are physically segregated production areas with dedicated staff, equipment and utensils, in which particularly high standards of hygiene are maintained and only fully cooked (i.e. a minimum of 70°C for at least 2 minutes; Gaze et al, 1998) foods and ingredients are handled. In all of the plants visited, there was some form of verification that thermal processing had been achieved successfully. Most producers used either steam or convection ovens (or both), with digitised temperature controls. In smaller processors, boiled ham contained in mother bags (i.e. cook bags) was observed being processed in vats of near-boiling water. In contrast the most sophisticated of the convection ovens made a per batch electronic record of the cooking profiles. Profiles were typically stored for 12 months. The most sophisticated ovens had temperature controls that could be monitored and changed in real time, and these ovens could compensate for problems by increasing temperatures or cook times. A strategy observed in a number of plants was to keep as much equipment as possible on the raw meat side of the plant. For that reason, all of the computer monitoring equipment was kept on the raw meat side, but could be viewed from the HRA through windows. HRA staff could therefore manually check effective thermal processing had occurred before opening the oven doors onto the HRA.

A number of CSM manufacturers that did not cook meat in a bag commented that detritus could drop off the meat during cooking. The issue was evident in rack-cooked coated meat (e.g. barbeque chicken breast; Figure 22) and also in meats cooked in continuous flow ovens. In general, there was a strong opinion amongst technical staff that this material was an issue because it was a potential nutrient source for *L. monocytogenes* and consequently it was removed either continuously or on a frequent basis. In at least one plant, the material was removed from the oven floor using a dry squeegee before the rack was removed from the oven to minimise the transfer of organic material onto the rack wheels.

Some smaller manufacturers cooked the meat in water baths at 80-85°C and monitored the water temperature with thermocouple probes. Although these baths were covered, a number of the bath-

based processors had some issues with condensation in the areas around the baths and such moisture could facilitate the growth of *L. monocytogenes*.

Figure 22 Manual temperature checking of thermally-processed meats using a temperature probe

In addition to the automated temperature checks noted in the larger businesses, all of the CSM manufacturers that were visited also undertook manual backup checking of cooking effectiveness (Figure 22) using temperature probes (typically thermocouples). At one plant, imperfectly cooked product was considered to be such a hazard to the HR area that a physical barrier had been installed: as product exited a continuous flow oven, the core temperature was verified. If the temperature was below target (72^{o}C), then employees hit a 'panic button' that simultaneously stopped the line and also rapidly raised a barrier that physically blocked the access of the imperfectly-cooked product to the post-cook area of the plant. The screen resembled those installed in banks to rapidly shield staff in an emergency.

12.3.5 POST-COOK COOLING

Lunden et al (2003) reported that in one of the plants they investigated, an area between the ovens and the post-cook chillers was contaminated frequently with several *L. monocytogenes* pulsotypes. In some commercial processing plants visited, the project team also observed that such areas were prone to condensation because the air in the oven was moist – either as a consequence of water evaporation from un-bagged products, or due to the use of steam to minimise product water (weight) loss. After cooking, ovens were opened on the HC side of the plant, releasing the moist air into the area between the ovens and the blast chillers. As the warm moist air from the oven mixed with the cooler air near the blast chiller, water condensed onto the walls and ceilings. Three plants had recognised this issue, and the technical managers were convinced that if the problem with the ceiling condensation in the passage between the cookers and blast chillers was not addressed, a consequence would be an increase in *L. monocytogenes* contamination of cooked product. In two plants, the condensation was removed using a squeegee. In one plant, the squeegee had a hollow handle and a series of vents cut into the assembly that held the rubber blade. When the squeegee was used, the ceiling water was directed through the vents and collected inside the handle. Although a simple adaptation, the squeegee helped keep the floors in the area free from water. In another plant, an automated moist trap at the ovens/ chillers interface was in operation when the doors were opened.

The majority of processors used blast chillers for cooling the cooked meat and one major manufacturer had developed a novel process that helped minimise the adverse effects of the condensation in the blast chiller. The chiller had been adapted to provide the air flow as shown on Figure 23. Chilled air (-25^{o}C) was blasted from the chill unit and it mixed with the moist air predominantly in the region shown in red. Although condensation formed on the ceiling above the red arrow shown on Figure 23, there was little or no condensation on other internal chiller surfaces. The FBO undertook hourly checks to remove ceiling condensation and did not store any product underneath the red arrow area.

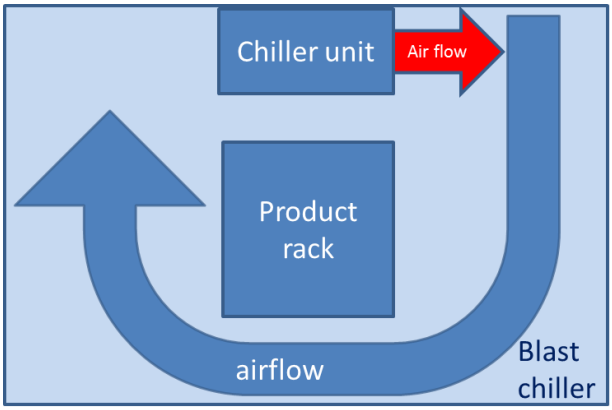


Figure 23 A schematic diagrams showing the airflow in a modified blast chiller that resulted in reduced condensation

There were also cases where rotary fans or water showers were used to cool meat whilst at two plants, the meat was left to cool without assistance to ambient temperature. Only three processors did not undertake some form of monitoring of the rates of cooling of final product. Minimum cooling rates were between 0.05 to 2°C per min whilst the fastest rates (25°C/min) were generated using liquid nitrogen tunnels. Where the cooling rate of the cooked meat was monitored, there were typically performance targets. Product cooling rates were dependent on the product and also the formulation, e.g. one large producer cooled products containing nitrate from 70°C to 5°C in 12.5 hours, whereas the products containing no nitrate were cooled in 10.5 hours. A different manufacturer cooled product with no added nitrate in under two hours, emphasising that the absence of the inhibitory effects of the nitrate had been noted, and was being compensated for by the rapid chilling. From discussions with technical staff it appeared that cooling rates were dictated by a number of factors, including plant throughput, the chilling method and considerations such as the number of available blast chillers.

In general, it was considered bad practice to cool post-cook meat using water showers. In those plants where the practice was observed, it undoubtedly led to excess water on the surfaces of the floors in the HC areas. In one plant, which had insufficient space to implement segregation for pre and post-cook activities, ovens that were equipped with two access doors had only one useable entrance. At that plant post-cook, cooled product was wheeled through water lying on the floors, into the product cooler. The technical manager at the plant reported not-infrequent *L. monocytogenes* isolations from final product and the HC environment.

12.3.6 SLICING AND PACKAGING

In a number of plants, a specific good practice relating to packaging was observed. Typically, packaging materials were stored in dry, clean rooms, separated from the processing areas but accessible from HRAs. It was common in plants for pre-formed plastic packaging to be received into a low care area of the plant from the packing supplier wrapped in a double layer of polythene. When required to be delivered into the HRA, the packaging was passed down a sliding ramp through a hatch. As the packaging slid down the ramp, the outer layer of polythene was removed such that the packaging arrived in the HRA wrapped in the inner layer of polythene with the original, potentially-contaminated, outer layer of polythene retained in the low care area.

Typically, due to lack of space, some smaller producers did not have a separate room for the storage of packing materials, and cooking ingredients. In some of these plants the storage spaces for packing materials, although dry, were in inappropriate places e.g. above a fridge, in the product storage area and next to the processing hall. In one plant, packaging was stored inside a post packing product chiller, with the justification that the packaging would be returned to the same place after it had been filled with product. Usually, washing hands before handling and a visual check were the only procedures used to ensure that the packaging was not contaminated.

In the majority of the smaller plants, slicing equipment either placed a set number of slices of meat directly onto a conveyor belt, or deposited the sliced meat onto the landing area of the slicer. The meat was taken from these areas by workers wearing gloves and placed into packaging, weighed to ensure a minimum weight, before the packaging was sealed.

In contrast, in the larger manufacturers, sliced meat was not handled by workers, partly as a hygienic precaution and also because high end automated slicers are equipped with laser-based equipment designed to produce slices of uniform equal weight within very small errors (<0.05g slice). In two of the larger manufacturers, there were two large reels (roughly 2m diameter) of plastic film of different thickness attached to the packer. The packing line was able to automatically draw the films into the packing machine and heat and form the thicker film into the base of a CSM pack. Either

twelve or sixteen packs were formed at one time. Sliced meat was deposited directly into the newly formed packs, a modified atmosphere was applied, and the thinner film automatically sealed to the top of the package. Another larger processor used the same general approach but used an automated feed of pre-formed packaging rather than forming the package at the time of use. The different approaches were driven by pack volume; smaller packs (e.g. 200g retail packs) could be readily formed on demand, but larger pack sizes (e.g. >500g for domestic use or >1kg for catering outlets) tended to take longer to make and hence be pre-formed and automatically fed into the packer.

In the very largest throughput plants, biofilm build up on slicer blades was not considered an important issue as blades were cleaned and sanitised using validated procedures. In addition, the slicer blades were sharpened every night and only had working lives of two to three weeks before they were replaced. Thus, the establishment of *L. monocytogenes* biofilms on blades was considered unlikely.

Labels identifying the product type, batch and use by dates were normally applied to the packages after sealing, and once the packs had been removed from the high care area. A number of manufacturers considered it was not essential that the labels were applied in the HRA and to bring labels into that area of the plant would open a potential route for *L. monocytogenes* contamination.

12.3.7 MODIFIED ATMOSPHERE PACKAGING

During the study, three types of CSM packaging were encountered. VP was the most common at smaller throughput units whilst at high throughput plants MAP predominated. The composition of the MAPs was consistent across different manufacturers and consisted of 30% CO_2, balance N_2 (possibly with a low (<1%) concentration of O_2). Ham can discolour under MAP if O_2 concentrations are too high and the product is exposed to strong lights. As was discussed previously (section 9.8). The maximum CO_2 concentration was limited by consideration of pack collapse. In addition to discolouration, O_2 concentration was kept low by a desire to minimise lipid oxidation and consequent rancid odours.

L. monocytogenes has a variety of metabolic pathways and shunts that allow it to utilise a variety of carbon sources. Respiration, the use of oxygen as a terminal electron acceptor, maximises the energy gained by the bacteria from such metabolism. However, L. monocytogenes also has pathways which allow the use of nitrate and nitrite as terminal electron acceptors for metabolic processes (Lungu et al 2009). The ability to use alternatives to oxygen as terminal electron acceptors explains why *L. monocytogenes* can out-compete fermentative organisms in VP and why MAPs are unlikely to prevent the multiplication of *L. monocytogenes*.

12.3.8 CLEANING DURING PROCESSING

12.3.8.1 DRY CLEANING

In the majority of the larger throughput plants, no water was used for cleaning during production in the HRAs. In a single larger-throughput plant, a strategy of using minimal water (e.g. damp cloths and hand-held trigger sprays) was in place. Any detritus that was required to be cleaned from a floor during production was collected using a dry squeegee. In addition, industrial vacuum cleaners were also observed in use in both smaller and larger production units. A common, strictly enforced practice, was to never disturb drains in the HRA. Thus, dry squeegees were not used to push detritus into drains during processing.

Furthermore, a number of larger throughput plants used a similar strategy with respect to slicer cleaning during operations. In one plant, there were two sets of slicers for each of the two packing lines in the HRA. A slicer was used for not longer than two hours of processing before being replaced with a freshly cleaned and sanitised slicer. Slicers could be changed more frequently than every two hours if there was a change of product type or flavour (e.g. barbeque sliced chicken breast manufacture was replaced with peppered beef manufacture). In general, complete cleaning and sanitation of the slicers took approximately 40-50 minutes and thus a dedicated cleaning team was required to service the two cutting lines. Larger processors normally had validated cleaning and

sanitising procedures and the effectiveness of cleaning during processing monitored routinely by microbiological or ATP testing. In the smaller businesses, it was less likely interim cleaning effectiveness during processing would be checked with some businesses undertaking no checking of interim cleaning at all.

Typically, a slicer blade/set of blades (Figure 24) could be cleaned and sanitised in around 15 minutes. The good practice of removing equipment or parts of equipment to physically separate rooms that were formed inside the high risk areas was witnessed in several plants. It was a particularly common practice for slicers in high risk to be stripped down and dry cleaned (or cleaned with a minimum of water) *in situ*, with the slicer blades being fully cleaned and sanitised (using water *ad libitum*) in a small room situated inside the high risk area. In other plants, the entire slicer units were removed from the HR area for cleaning. A number of the cleaning rooms were observed to have elevated (~20-30cm) thresholds as a strategy of containing any water used for cleaning inside the cleaning room. At least one plant had built custom cleaning rooms to their HR area as an extension. The location of the extension had been decided such that a drain spur could be added that joined the main drains above the area where the drain water exited the HRA.

Figure 24 Blade types commonly found in UK CSM plants.

A was an array of blades that were agitated quickly to effect cutting, B was a pair of scimitar blades that revolved to cut product into chunky pieces. A further example of a typical slicer is shown as Figure 11.

12.3.8.2 WET CLEANING

In the majority of plants visited, cleaning undertaken in the middle of shifts avoided the use of water in order to avoid splashing *Listeria* from the drains and floors. In those plants where wet cleaning occurred, it tended to be confined to equipment rather than the plant environment. Only two plants used hoses during mid-shift cleaning. A common practice, observed at these two plants, was to have staggered breaks for staff. One group of staff would leave the production area to eat, whilst the remaining staff cleaned the equipment. On their return, the first group cleaned their colleagues equipment, whilst they departed for their break. Based on scientific reports, and the experiences of the larger companies (verified by their comprehensive testing regimes) it was apparent that wet cleaning during processing was a significant risk factor with regard to *Listeria* contamination of final product. Consequently, processors should be advised against wet cleaning during production. Further evidence on the adverse effects of wet cleaning can be found in the prevalence rate for *Listeria* experienced by smaller processors with abundant water in HRAs.

12.3.9 CLEANING AND SANITATION AT THE END OF A DAY'S PROCESSING

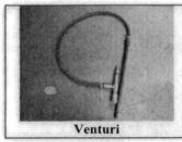
In the UK, the cleaning and sanitation of commercial meat processing plants is dominated by Holchem Laboratories and Ecolab. All of the larger processors visited used products and application systems from one of these companies. Included in the purchase of cleaning chemicals, is access to specialist technical assistance and independent auditing of plant cleaning during unsociable hours by Holchem/Ecolab staff. There are differences in the terminologies used by the two companies. Ecolab refers to a surfactant based soil remover as a cleaner, and a chemical designed to destroy microorganisms as a sanitiser. Holchem refers to a surfactant based soil remover as a sanitiser, and a chemical designed to destroy microorganisms as a disinfectant. For the purpose of clarity within the report, the cleaner/sanitiser nomenclature will be used. As part of this study, a former technologist and auditor from one of the chemical companies was interviewed to provide an overview of cleaning and sanitising across the larger processors, from the viewpoint of someone highly experienced in the practical aspects of cleaning and sanitation of CSM processing.

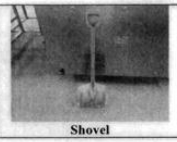
All of the large processors, had prepared high-quality cleaning instructions, which were made readily available to cleaning staff (Figure 25). Typically, the instructions were laminated so they could be cleaned and used as a guide in the presence of water during the cleaning process itself. Typically, there was a cleaning SOP for every single piece of equipment in each plant. Each SOP explained clearly what equipment and chemicals were required to complete the task and provided step-by-step instructions for cleaning (Figure 25). There was specific reference to difficult and problematic areas on the machines. These problematic areas had been identified over the course of several years and were considered to be very important knowledge by the FBOs because they ensured targeted cleaning for potential *L. monocytogenes* niches. Faulty and damaged equipment have been cited many times as the cause of an *L. monocytogenes* isolation. Consequently, the cleaning SOPs included instructions to report any damaged or missing parts to cleaning supervisors. Finally, there were illustrations of key areas to be checked on each machine to ensure visible cleanliness had been achieved. Several CSM manufacturers had adopted a similar approach. For those plants with photographic illustrations of problematic areas, the consensus was the illustrations had improved cleaning effectiveness.

In the larger throughput CSM processors, cleaning effectiveness was verified by a rolling programme of pre-production sampling and subsequent microbiological testing as described in section 12.3.9.2.

PROCEDURE: HCHY10

Webber Slicers


Venturi


Shovel

CHEMICAL LIST:

Chemical Type	Chemical Name	Percentage	Hazchem Symbol	Identification Colour
DETERGENT	MAXIFOAM PLUS	3-5%	CORROSIVE	CAUSTIC
DETERGENT	CHLOROFOAM PLUS	3-5%	CORROSIVE	CHLORINATED
DISINFECTANT	HOLQUAT	1-2%	Irritant	DISINFECTANT
DISINFECTANT	TERMINOL	1-2%	Irritant	DISINFECTANT
DISINFECTANT	SANITISING WIPES	NEAT		DISINFECTANT

CLEANING PROCEDURE:

1. Manually remove and debris.
2. Wipe the control panel using Sanitising wipes, and then ensure all electric panels are covered with food grade plastic bags before any cleaning starts.
3. Using a medium pressure hose pre wash all the components on the log table making sure to remove any belts and all loose pieces that may have been dismantled. Special attention to be taken inside belts that have been dismantled.
4. Rinse gross debris from under side of the blade on the stand whilst in its blade cover. Remove blade cover and rinse topside of blade. Replace blade cover firmly. (Safety Gloves must be worn)
5. Clean log table including the undersides.
6. Clean all areas of the slicer paying particular attention to the internals of the slicer dropping or raising the hatch of orbital area.
7. Clean transfer conveyor making sure the tension release on the conveyor is disengaged and the belt is lifted so the underside can be cleaned if possible, otherwise, clean transfer conveyor moving it by hand.
8. Paying special attention to the rollers on the underside of the conveyor
9. Clean up all debris from the underside of the transfer conveyor and slicer and rinse floor
10. Foam up all areas that have been pre washed including the undersides.
11. Green Pad all areas that have been foamed and paying attention to the underside and the food contact surface.
12. Green Pad carefully the underside of the removed blade whilst in its blade cover. Remove blade cover and carefully green pad the top surface of the blade. Replace blade cover

firmly. (Safety Gloves must be worn)
13. Leave foam on for the minimum of 20 minutes to achieve the correct contact time
14. Rinse off foam on all areas
15. Disinfect all areas of the slicer and transfer conveyor paying attention to food contact surfaces and the internals of the slicer
16. Remove plastic bag from control panel and wipe it down using Sanitising Wipes and then blade floor area with squeegee.

OTHER INFORMATION:

1. Any missing/damaged parts or equipment must be reported to the team leader / line manager.
2. After use, green pad to be taken out of high care and returned to team leader/line manager.
3. Avoid direct contact with air vents of the slicer that are on the back panel of the slicer.

KEY INSPECTION POINTS

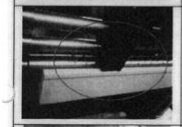
	Ensure blade guard internals are thoroughly clean and free from gross debris.		Ensure all surfaces of blade guard are thoroughly clean and free from gross debris.
	Ensure door guards are thoroughly clean and free from gross debris. Check all harbourage areas to ensure a thorough clean.		Check undersides of machine. Look at any ledges and ensure that ledges are thoroughly cleaned.
	Check the die and ensure all areas are thoroughly clean and free from entrapped debris.		Below the housing of the blade are, check underside of machine. Check any holes are free from gross debris and are thoroughly clean.
	Check all bolt holes and bold heads are thoroughly clean and free from contamination.		Main door guards are to be thoroughly clean both externally and internally. Check all edges and harbourage areas.
	Ensure all belts are thoroughly clean and free from gross debris. Check all rollers are thoroughly clean.		Check the log gripper and ensure all areas are thoroughly clean. Check all nooks and crannies for entrapped meats.

Figure 25 A photocopy of a typical cleaning SOP of the type found in larger-throughput processors

12.3.9.1 CLEANING STAFF

Cleaning staff in the larger businesses could be either plant employees or the employees of a contracted cleaning company. In one large CSM manufacturer, all of the equipment was cleaned by the workers who worked on the actual machines, at the end of their shift. This policy was preferred to the use of dedicated cleaning teams (i.e. external contractors) as the workers knew the equipment better (i.e. possible traps for meat residuals or biofilm formation) and they were periodically trained onsite on hygiene, cleaning and sanitation matters. This manufacturer also had an onsite training scheme for new staff, which was considered by the plant to be equivalent to a college-level food processing qualification (e.g. a HND). Lower throughput CSM processors tended to use cleaning chemicals sources from smaller chemical manufacturers, who typically did not supply technical assistance. In the smaller plants visited plant employees, exclusively, undertook cleaning. In the smallest plants, frequently it was CSM manufacture staff that undertook end of shift cleaning.

In all of the businesses visited, cleaning was undertaken according to well-established procedures that were widespread across the entire food processing sectors. In addition, all plants visited had equipment washing and cleaning SOPs that were undertaken outside of the processing area. Commonly, gross detritus was removed from equipment and the floors using either a squeegee or a hose. A variety of hose pressures, including pressure washers were in use. One small CSM producer used an industrial wet hoover to remove the detritus during the day. The same hoover was used for the end of day cleaning to remove the detritus and the cleaning solution. For slicing equipment, it was common for the guards and blades to be removed for end of processing cleaning. A foaming degreasing chemical was applied, that commonly contained chlorine. However, a variety of cleaners used were seen to be neutral pH, acidic or alkaline. Although alkaline detergents are recommended by most chemical manufacturers for the effective removal of fat and protein soil, the pH of the cleaning chemicals was decided by the preference of the plant technical managers in the majority of plants. In some plants or for some equipment, difficult to clean areas or pieces of equipment were scrubbed with brushes or scouring pads. Other less problematic areas were simply hosed after the application of the cleaning agent, to remove dissolved soil and detergent. Once cleaned, a sanitiser was applied to the surfaces to disinfect them. Sanitisers were rinsed off after a defined contact time, or left to drain naturally to maximise contact time as appropriate for the chemical used.

The following issues, which applied to some extent to both smaller and larger manufacturers, were identified as potentially problematic.

1. Wet surfaces. Prior to the application of the degreaser, the surfaces in some plants were washed down to remove gross detritus. After the wash, the surfaces were coated with sheets or beads of water and the cleaner was applied to the wet surfaces, hence diluting the cleaner. The same dilution effect was apparent when the sanitiser was applied after the cleaning chemical was hosed off. The issue is of particular concern for conveyor belts of the type formed from small pieces of interlocking plastic, because the spaces in between the individual links can retain water.
2. Over time there has been a tendency for some larger CSM manufacturers with multiple production sites to close their lower throughput premises and concentrate production in a smaller number, possibly even one, of production sites. At the retained sites, increased throughputs are achieved by increasing rates on production lines or longer processing shifts. Increased production is a risk factor for final product contamination by *L. monocytogenes* (Tompkins et al 2002). Furthermore, it has been shown that longer processing shifts reduce the time available for effective cleaning and sanitation, which is essential for effective *L. monocytogenes* control (Samelis and Metaxopoulos 1999). In a number of plants, approximately four hours was available for cleaning between each day of production. However, in at least one plant, there was near continuous production, with removal of equipment from the high care area for periodic cleaning, but cleaning of the plant environment only took place at the weekend. An unusually intensive programme of environmental testing supported the cleaning practices at the plant. A recent FSA retail survey (FS241042, 2013), found that food manufactured in this 'cleaned-weekly' plant had significantly better than average microbiological quality based on indicator bacteria and prevalence/enumeration of *L. monocytogenes*.
3. Related to point two, the floors in some premises were seldom dry except when no processing was undertaken such as during holidays and over weekends.
4. The cleaning and sanitation of chillers can be problematic because the chillers always contain product (i.e. the chillers are never empty), which restricts the frequency and type of cleaning that can be undertaken. During the visits, there was evidence of overfilled raw meat chillers in some of the smaller processing businesses. Over-stocked chillers can allow

cross contamination between raw meats during chilled storage, allowing more opportunity for *L. monocytogenes* to become resident in the plant environment.

5. In the businesses visited, it was rare for the drains to be fitted with slow-dissolving blocks of sanitising chemicals. When present the blocks were only fitted in HRA. Drain cleaning usually happened at the end of day, in some of the larger premises, and weekly in other larger processors but only monthly, if at all, in the smaller ones. Without drain blocks, the risk of splashing *Listeria* out from drains and onto the food contact surfaces existed (Berrang and Joseph, 2013a, b) if there was even low pressure hose usage in the processing area.
6. Although SOPs for washing and cleaning outside the processing areas existed for all premises visited, there was no evidence that these SOPs resulted in *Listeria* free food contact surfaces, in some plants, because sampling and testing of post clean surfaces was not undertaken. It should be noted that staff from two of the larger manufacturers were very firmly of the opinion that training their own employees gave better results. However, given the lack of supporting evidence, it could not be determined whether cleaning undertaken by plant employees, or the employees of a contracted cleaning company, gave the best result.

12.3.9.2 VERIFICATION OF EFFECTIVE CLEANING AND SANITATION

Regulation EC 2073/2005 states that "Food business operators manufacturing ready-to-eat foods [are required] to sample the processing areas and equipment for *Listeria monocytogenes* as part of their sampling scheme." Hence sampling for *L. monocytogenes* in the environments used to manufacture ready-to-eat foods should be undertaken to meet this requirement.

The larger throughput CSM manufacturers visited during this study undertook periodic testing to check cleaning and sanitation effectiveness. At one plant, more than 150 environmental swabs were tested for *L. monocytogenes* each week in order to satisfy the supply requirements of multiple customers. However, such an intensive regime was atypical and for most of the larger plants visited, checks were undertaken on a weekly basis, or following a four week plan, between the end of cleaning and the start of the next day's processing. Most processors collected a large number of

samples on a single day, which was varied each week although two large throughput manufacturers collected a small number of samples every processing day.

Sample collection either used diluent-soaked drumstick-style swabs, or sponge soaked in diluent. None of the plants visited used a dry swab after the wet swab to soak up residual diluent. Testing tended to be for *L. monocytogenes* (or in one case *Listeria* spp.) and also for microbiological indicator(s) organisms. Typically, the indicators were one or more of; total aerobic mesophiles, *E. coli*, coliforms or *Enterobacteriaceae*. Plants typically had a rolling weekly schedule of different locations to be sampled, which had been refined over several years. The schedules were repeated after six, eight, nine or twelve weeks dependent on the plant. Most of the technical managers who were asked were able to state the locations inside their plant that were difficult to clean effectively, and could occasionally be contaminated with *L. monocytogenes*. The sampling schedules were designed specifically to include the problematic areas (Table 60; Table 61). On finding *L. monocytogenes* positive product samples the cleaning and testing routines were intensified (i.e. repeated deep cleans) in these problematic areas, until negative results (for both product and food contact surfaces) were obtained for a defined time period (e.g. one month). Table 60 shows a typical sampling plan donated from one larger CSM manufacturer.

The sample locations listed in Table 60 can be grouped into three main areas, which could be applied equally to all of the larger throughput CSM manufacturers. The first were locations or objects that were likely to be touched by workers' hands, such as door handles and control panels. One technical manager believed workers were a primary source of *L. monocytogenes* and stated he could routinely isolate *L. monocytogenes* from touched surfaces each month, even when the other samples taken did not contain *L. monocytogenes*. The second area consisted of wet areas; floors, gullies (hollows in concrete floors that collected water and directed it to drains) and the drains themselves. These areas routinely held water and were only infrequently completely dry. The final group of sample was areas such as door seals or ceiling squeegees, which were also places that accumulated water.

Table 60 Weekly sample collection locations in the post-cook area of a high-throughput CSM plant in the UK

Week	Sampling locations
1	Packing feed conveyor x6, Knockout tables x3, Cooker door handles x10, Blast chiller exit door handles x10, Blast chill floor x10, End of line drain
2	Pack line log balances x8, Cooker door handles, Chiller door seals x10, Drains in knockout table room x4, Blast chiller entrance door handles x10, Blast chiller drains x10
3	Main packing exit conveyor x6, Reject packing exit conveyor x6, Cooker door handles x10, Final product chill door handles, Ceiling squeegee for area between chillers and cookers, Drain in area between chillers and cookers, Drains in knockout table room x4
4	Packing line on/off switch x6, Packing line control panel x6, Cooker door handles x10, Random log transport racks x10, Ceiling squeegee for area between chillers and cookers, Staff changing area floor, Random areas of high risk area floor x10, , Drains in knockout table room x4, Blast chiller floors x10
5	Knife blades (x6) as a pooled sample, breading area crumb bin, breading area gelatin bin, breading area draining rack, Cooker door handles x10, Final product chill door handles, Ceiling squeegee for area between chillers and cookers, High risk area floor squeegee, Blast chiller floors x10, Drain gully in front of cooker high risk exit at cookers 14-11, Random areas of the dry ingredient store #1 floor x5
6	Packing feed conveyor x6, Packing conveyor log grip x6, Cleaner anti cut gloves as a pooled sample, Blast chiller exit handles x10, High risk area brushes and squeegees x5, A new pair of randomly-selected disposable gloves from a dispenser x5
7	Drain gully in front of cooker high risk exit at cookers 10-8, Random areas of the dry ingredient store #1 floor x5, Blast chiller entrance handles x10, Blast chiller entrance seals x10, Boots in high risk area entrance x10
8	Blast chiller exit handles x10, Blast chiller exit seals x10, High risk area brushes and squeegees x5, Blast chiller floors x10, Drain gully in front of cooker high risk exit at cookers 7-4
9	Blast chiller evaporator coils pooled x5, temperature probes x5, on/off switches on temperature probes x5, Cooker door handles x10, Cooker door seals x10, Drain gully in front of cooker high risk exit at cookers 3-1, High risk area drains x10, A used pair of randomly-selected disposable gloves from a bin x5

Given that sampling schedules had been refined to focus on sampling locations that had a history of *L. monocytogenes* isolations, it was considered likely these types of locations could potentially be primary sources of plant environment contamination by *L. monocytogenes*.

In those plants examined, only the larger-throughput manufacturers routinely undertook sampling and testing specifically for *Listeria* spp..

The unpublished data from major chilled food manufacturers UK monitoring procedures was made available for study. In 2012, based on more than 38,000 test results for food contact surfaces, *L. monocytogenes* was detected in approximately 0.4% of swabs. In comparison, *L. monocytogenes* was detected in 1.6% of 50,000 swabs used to sample non-food contact surfaces (e.g. floor drains).

In addition to testing specifically for *L. monocytogenes*, adenosine triphosphate (ATP) sampling and general microbiological testing of food contact surfaces is also commonly undertaken by major, and some smaller, manufacturers. ATP testing is a rapid and fairly cheap method for verifying general cleaning efficacy prior to the commencement of a day's manufacture. ATP is present in the viable cells of all living organisms and its presence is indicative of ineffective cleaning and sanitation procedures. Although rapid, inexpensive and simple-to-use, ATP testing was not used by the majority of smaller manufacturers and neither was general microbiological testing.

12.3.9.2.1 LABORATORY TESTING OF PLANT ENVIRONMENT SAMPLES

All of the plants that were visited, which undertook the testing of environmental swabs, did so according to EC 2073/2005. For plant environment testing, the regulation stipulated that environmental samples should be collected according to ISO 18593:2004, with ISO 11290-1 or -2 used for laboratory testing. Due to the costs of the latter analyses, it may be appropriate for an abbreviated, cheaper test method to be used for environmental samples. The ISO method could be followed until the enrichment stage using modified Fraser Broth but tubes that do not turn black could be discarded as negative and not streaked onto Oxford or PALCAM for further study. Tompkin (2002) advises a similar strategy, because it allowed greater surveillance of the plant environment if the same outlay as for the full testing regime was maintained. The EC 2073/2005 regulation does allow for the use of alternative laboratory testing methodologies, as long as they are validated and authorised by the competent authority. Currently, there does not appear to be any validation of an

abbreviated testing method. In addition, a cheaper analytical method for *L. monocytogenes* may also help allay the testing cost concerns of the smaller processors.

12.3.10 *L. MONOCYTOGENES* ISOLATIONS IN CSM PLANTS IN THE UK

In those plants that tested environments for *L. monocytogenes*, most of the technical managers had clear ideas where *L. monocytogenes* could occasionally be isolated. A summary of these areas is provided as Table 61.

Table 61 A compilation of likely areas from which *L. monocytogenes* could be isolated in high risk areas of CSM plants

Location of *L. monocytogenes* isolation (in no particular order)
Conveyor belts, including hollow rollers and between the links of belts with spaces between links
Control panels for equipment in HR
Door handles – in particular to ovens, post-cook blast chillers and also product chillers
Basins and tap handles
Gel release button on alcohol dispenser
Areas around liquid nitrogen pipes and tunnels where condensation was created
Ceilings that are prone to condensation
Door seals such as those on chiller doors where where moisture can collect
Slicing equipment – blades and FCS
Employee hands/gloves
Drains and drain covers

12.3.11 GENERAL CONSIDERATIONS

Technical staff at several CSM manufacturing plants recognised that cooking was a critical control point for *L. monocytogenes*. If appropriate checks were made to ensure that the cooking stage of processing had proceeded as expected, then the product could be considered to be free of *L. monocytogenes* at that point. All of the CSM processors visited undertook checks to ensure

effective thermal processing. The clear strategy, in at least five UK plants, was to prevent any recontamination of the product after thermal processing. Generally, this goal which achieved by one or more of the following strategies:

1. The installation of alcohol gel dispensers at key points in the high risk area of the plant. These installations included all entrances to all product chillers and areas near equipment control panels. Before entry to the chiller, employees were asked to decontaminate their hands by applying the gel. The FBO operated an intensive final product monitoring programme for *L. monocytogenes* and also microbiological indicator organisms. Both the technical and laboratory managers reported that there had been noticeable reductions in both *L. monocytogenes* isolations from final products and environmental isolations as a consequence of establishing the use of alcohol gel on entry to the chillers. The activities of the FBO go some way to confirming the hypothesis made in section 12.3.9.2 that worker's hands were fomites for *L. monocytogenes* under commercial processing conditions. In one plant, novel door handles of the type shown in Figure 26 were in use at key locations. In order to open the door, the worker was required to squeeze the handle. The squeezing deposited a volume of alcohol gel directly onto the fingers of the worker.

Figure 26 A door handle that dispensed alcohol onto workers' hands

2. In addition to the gel dispensers, most of which were installed several years ago; two FBOs had more recently installed PPE dispensing stations at key places in the plant. Gloves, hairnets, polythene sleeves, alcohol gel and earplugs were available to workers *ad libitum*. The technical staff at the plant believed that if line workers had easy access to PPE, they were more likely to change their gloves after an inappropriate contact than if they had to expend effort to find a new pair.

3. Like many food manufacturers in the UK, several plants filtered the air pumped into the high care area of its plant through high efficiency particulate air (HEPA) filters and the air handling system maintained a positive air pressure so that when an external door in high risk was opened, air only flowed in one direction, i.e. out of the high care area. Although there is little evidence that airborne *L. monocytogenes* are a significant problem in dry food processing plants during general production (Autio et al 1999), the technical staff in some plants noticed that after the air handling systems were installed the floor, and other surfaces in the high risk area of the plant, dried more rapidly after cleaning. At least one FBO considered that completely dry floors and surfaces at the commencement of processing was beneficial for the control of *L. monocytogenes*. Furthermore, heat application in chillers as a procedure for drying reduces *L. monocytogenes* isolations (Eglezos and Dykes, 2011). Vogel et al, (2010) determined the reduction of *L. monocytogenes* on stainless steel under desiccation conditions and showed that the kinetics of the decline were complex, and influenced by salt and the presence of organic materials. An initial *L. monocytogenes* population of 10^8 fell by two to five logs over a ten-day period, and significant numbers could persist for at least three months. The study also concluded there was no difference in the survival abilities of *L. monocytogenes* strains that had been resident in a cold smoked fish plant, compared with non-resident *L. monocytogenes* isolates.

   Considered in combination, it seems likely that drying reduces, numbers of *L. monocytogenes* in processing environments but does not eliminate them. Furthermore, *L. monocytogenes* can become established initially in normally wet areas of the plant (Tompkin, 2002), and be distributed from that source. A similar viewpoint was expressed by a number of technical managers working in high throughput plants, and appeared to be the

basis for the strategy of keeping the high risk section as free as possible from water during processing (section 12.3.8).

4. Two of the CSM manufacturers visited worked on the principal that longer processing times increased the risk of *L. monocytogenes* contamination of product. Therefore, rapid processing and packing was desirable. To minimise processing times only small poultry breasts, or breast sections were used (300-400g). These were placed on a conveyor in the low risk section of the plant, injection brined and tumbling was not necessary because of the small masses of meat. The meat entered a continuous flow oven, and was cooked for approximately 20 minutes in one plant, and 22 minutes in the other. The exit from the oven was into the HRA where the meat was cooled in a liquid nitrogen tunnel prior to the product being sliced and then packed into MAP (30% CO_2, balance N_2). At one plant, the time from loading the uncooked breasts onto the conveyor until packaging was 36 minutes, and the cooked meat spent less than 12 min in high care area. At the other plant, the entire process took approximately two minutes longer. Despite the use of liquid nitrogen tunnels, which are considered by some technical staff to be areas likely to harbour *L. monocytogenes*, the staff in both plants indicated that their microbiological analyses showed that the rapidly manufactured product had fewer *L. monocytogenes* issues than conventional product.

12.3.12 RESPONSE TO AN ENVIRONMENTAL *L. MONOCYTOGENES* ISOLATION

For those plants that tested the environment for *L. monocytogenes*, the response to an isolation was universally to rapidly initiate deep cleaning of the plant, with the goal of eradicating *L. monocytogenes*. It is a strategy that is widely reported as effective in the peer-reviewed literature. A number of technical managers took the time to make clear that routine cleaning overnight in their plant was thorough and that it was fit for purpose, but deep cleaning was even more thorough with specific rather than general targets. Deep cleaning typically involved changing the cleaning method, and in some cases, the response was dependent on the location of the isolation. Examples of deep clean responses included:

- Rental and use of a steam cleaner in plant drains, ceilings and on those surfaces able to withstand high temperatures
- Changing the active agent in the cleaning/sanitising chemicals

- Using chemicals at stronger concentrations than recommended by the manufacturer (after checking there was no hazard to staff)
- Prolonged surface scrubbing times
- Removal of sensitive equipment from the high risk area for specialist cleaning and then the application or fogging of the entire factory environment (or affected part of the factory) with liquid or aerosolised peracetic acid (a highly corrosive mix of ethanoic acid and peroxide. N.B. A number of technical managers commented that the corrosive effects of peracetic acid on metal and stainless steel meant the treatment could be used only infrequently because it damaged the plant infrastructure a little more each time it was deployed)
- Stripping down equipment that was usually not stripped down for cleaning. For example, an isolation from a slicer in one plant resulted in the physical removal of the slicer from high risk, removal of all the slicer steel panels, removal of wire looms and electronics from inside the unit and unscrewing every single screw in the slicer for individual cleaning of the screw threads (a previous site of an *L. monocytogenes* isolation). The greased mechanisms inside the slicer were disassembled, degreased, cleaned and sanitised before re-assembly and re-greasing. There is evidence in the literature that grease with antimicrobial additives such as sodium benzoate is of benefit (Tompkin 2002; Lunden et al 2003), although no FBOs specifically mentioned the use of such greases.

After a deep clean, intensive sampling and microbiological testing throughout the plant, concentrated in the area of the original isolation was carried out typically to confirm the *L. monocytogenes* had been removed.

After an *L. monocytogenes* isolation and deep clean, several FBOs instigated observation of staff in an attempt to identify if there were any lapses that breached HR biosecurity. If staff were identified as lapsing into potentially risky practices, they were informed why their actions were risky and reminded of the importance good hygienic practices. A number of large throughput retailers have their own educational/induction material for staff that described what the CSM process stages were and what was important for good hygienic manufacture. Dependent on the nature of the infraction, a frequent corrective action was for staff to be asked to re-read such material.

There were differences in large throughput CSM manufacturer responses to non-*L. monocytogenes Listeria* isolations. Some manufacturers took the view that it wasn't *L. monocytogenes* and so they were not in breach of any laws and so took little or no further action. Others took the view that it wasn't *L. monocytogenes*, but it could have been and used the isolation as a reason to review instructions to cleaning staff, arrange third party cleaning assessment audits as well as arranging for additional cleaning to control the contamination. The literature suggests that the reliability of *Listeria* spp.. as an indicator for an *L. monocytogenes* isolation is plant dependent (Tompkin et al 1992, Table 9). In those plants that did not undertake further actions when non-*L. monocytogenes* isolations were made, a summary of the Canadian Maple Foods outbreak (section 6; Table 14) was provided. With an intention to be helpful, the summary was supplied so it could be passed on to senior managers for information. The independent inquiry into the Maple foods listeriosis outbreak implicated uninformed decision making by senior managers as a contributory factor.

12.4 DETERMINATION OF SHELF LIFE BY CSM MANUFACTURERS

12.4.1 SME MANUFACTURERS

During the visits to SME CSM manufacturing plants, it was apparent that, typically, the smaller manufacturers had undertaken no shelf life determinations for *L. monocytogenes*. However, the majority of SMEs shipped CSM products that had an expiry date on the package. When asked how the date had been determined, one technical manager stated that she had inherited the product expiry dates from the previous post holder and the original origins of the dates were unclear. In the remaining SME plants, there was a similar historical basis for product expiry dates. In essence the dates used were justified by a previous history of several years (and in some cases several decades) of using the same shelf lives without apparent incident. In addition, a small proportion of SME CSM manufacturers had, and continued to set, expiry dates from an organoleptic basis. Furthermore, some smaller manufacturers had used shelf lives supplied by EHOs. Although some EHOs reported that SME CSM manufacturers set shelf life by copying those from similar products displayed by larger retailers, none of the business visited used that approach to shelf life.

For the organoleptic approach, a few packs of some product batches were typically retained under refrigeration conditions and the smell, taste and appearance of the product assessed at the end of

shelf life. Some SME manufacturers were not aware there was a legal requirement for them to ensure there was <100 cfu/g *L. monocytogenes* on their CSM at the end of shelf life. When asked by telephone, two SME processors that knew about the quantitative test limit at the end of shelf life, were not aware that an absence in 25g product before it left the processing plant could be substituted as a cheaper alternative.

When asked further about product shelf life determinations, the majority of SME plant staff acknowledged they would not be confident designing a trial to determine an expiry date based on *L. monocytogenes* growth during refrigerated storage. Furthermore, at some SME businesses there was little or no awareness of the relative merits and pitfalls of using either naturally contaminated product or laboratory-cultured *L. monocytogenes* as an inoculant for shelf life trials. There were three main exceptions, at the SME level, where a shelf life determination had been carried out using a local contract microbiology laboratory. For one of the processors, a 10 day shelf life was used for their CSM range of products, although the laboratory recommended 14 days. The plant owner said that he preferred to "play it safe" by using the shorter shelf life.

Some of the SME CSM manufacturers visited were aware of EC 2073/2005 and the need to ensure <100 cfu/g *L. monocytogenes* on CSM at the end of shelf life. At two plants, the response was to apply a comparatively short shelf life of 7-10 d. One CSM manufacturer stated the company would like to reduce the shelf life further but that there was conflict between the economic and technical strands of the business. The CSM manufacturer's customers were continuously pushing for longer shelf lives to maximise the amount of time product could be offered for retail sale. When asked why the company did not undertake challenge testing or establish a history of *L. monocytogenes* numbers at the end of shelf life and use that information to support a shelf life extension, a common response was the cost of testing. One manufacturer had previously enquired about challenge testing of their products. The cost for a basic challenge for the assessment of a single product was over two thousand pounds. Enquiries undertaken as part of this study determined that two-to-three thousand pounds per product was a typical testing cost. One processor considered there were insufficient funds within the business to undertake challenge testing for the six products processed by the plant. Typically, in the UK, an *L. monocytogenes* enumeration undertaken by a contract lab with strict compliance (e.g. replicate plating) to the ISO-11290-2 protocol costs between £15 and £20 per sample for regular scheduled testing of multiple samples. Single or infrequent multiple sample tests will cost more. In addition, a number of laboratories charge additionally to confirm *Listeria* spp.. and speciate. Insufficient resources and a lack of the technical knowledge required to

design an inexpensive determination were common reasons that prevented the establishment of typical *L. monocytogenes* numbers at the end of shelf life.

A summary of the SME visits with regard to shelf life is that there was a limited awareness of the adoption of EC 2073/2005 (and the various corrigenda) and the obligations that it brought to CSM manufacturers by some businesses. Of the nine SME FBOs asked, seven (78%) of SME FBOs had concerns relating to the costs of laboratory testing for *L. monocytogenes*. Furthermore, there were technical knowledge gaps that were significant barriers to SME manufacturers reliably determining an appropriate shelf life for *L. monocytogenes*. After being informed of a statutory requirement to undertake shelf life testing, the majority of the SME CSM manufacturers thought that easy-to-follow, systematic guidance for shelf life determination would be of significant benefit to them.

12.4.2 LARGER THROUGHPUT MANUFACTURERS

In contrast, major retailers and CSM manufacturers have (mostly) independently developed their own protocols for the determination of shelf life of CSM (Table 62). One CSM manufacturer supplied two major retailers that had identical *L. monocytogenes* shelf life protocols. It was unclear if that was the result of collaboration or just coincidence. In addition, there were CSM manufacturers that supplied the same retailer but used different shelf life protocols for the same or similar products. The issue was investigated, including discussions with the company microbiologists for the retailers in question.

An example of how shelf life assessment operates in the UK is best illustrated by the case of Retailer B. Retailer B stipulated that its suppliers must undertake shelf life/temperature abuse testing using "product samples for shelf life testing [that] must be stored at the right temperature and the right times to reflect the supply chain". Retailer B further stipulated that for a depot, effective chilling should be 1^{o}C and that 12^{o}C can be used to simulate the temperature abuse of sensitive products. For storage/retail display in retail stores, effective temperatures are 2-4^{o}C for meat, 4-6^{o}C for other chilled products. Finally, to simulate consumer purchase and storage, CSM should be abused at 8-10^{o}C for the last two days of life. Within that rough framework of temperatures, CSM suppliers to Retailer B are free to develop their own shelf life protocols. The CSM manufacturer that had the same protocol for Retailer A and Retailer B developed a single protocol that met the supplier criteria

for both retailers. There were minor differences between CSM manufacturers' shelf life protocols to the same retailer because of the fairly flexible abuse criteria typically stipulated by most retailers.

As part of the CSM manufacturer interviews, several technical managers mentioned that *L. monocytogenes* shelf life testing was a significant cost to industry. The main issue is illustrated by Table 62. Larger CSM manufacturers that sold own brand product had their company protocol for shelf life with regard to *L. monocytogenes*. If the manufacturer supplied one or more retailers, then these retailers also had their own protocols (or outlines of how to simulate abuse). The retailer supply criteria that were identified were all similar; with minor differences in abuse temperatures and how far into shelf life, the simulated abuse occurred. Three different CSM manufacturers felt it would be exceptionally beneficial to the UK CSM industry if a standardised, temperature-abuse protocol for CSM and *L. monocytogenes* was developed by the FSA in their capacity as a regulator. It was hoped that the Agency would have sufficient authority to influence the British Retail Consortium and larger retailers into adopting the standard protocol. A single protocol would ease the laboratory testing costs because it would mean the same shelf life determination results could be reported to multiple retailers.

When retailers were asked if they would accept a BRC/FSA-endorsed abuse protocol, the response from a trade body and two retailer microbiologists was that the protocols were derived to mimic their distribution and retail chains. Several retailers made the point that the store chillers had slightly different temperatures for different products and information of the type provided in Table 62 was general guidance. It was possible that CSM suppliers could be asked to use a modified protocol at the discretion of the retailer technologist. One retailer reported that some of their luxury products were delivered directly to stores by an artisan CSM manufacture that was using a product-specific protocol that was entirely different to the protocol designed for the standard distribution chain. Furthermore, retail display chillers in major retail stores can be set at slightly different temperatures for different products, which may also result in a customised temperature abuse protocol for a specific product.

Table 62 A summary of major retailer and larger CSM manufacturer shelf life determination protocols. The retailer protocols shown are typical of those created by CSM manufacturers to comply with retailer supply criteria. There were minor protocol variations between different manufacturers supplying the same retailer.

Business operator	Product shelf life	Initial storage temperatures and duration	First temperature change(s) and durations	Second temperature change(s) and duration	Notes
CSM manufacturer A	Products with shelf life ≤20 d	3°C ± 1°C for 30% of the shelf life	Move product to 5°C storage for 50% of shelf life	Move product to 8°C storage for last 20% of shelf life	Packs opened to remove MAP 2 days before testing
CSM manufacturer A	Products with shelf life >20 d	3°C ± 1°C for 20% of the shelf life	Move product to 5°C storage for 50% of shelf life	Move product to 8°C storage for last 30% of shelf life	Packs opened to remove MAP 2 days before testing
CSM manufacturer B	Products with shelf life >14 d	2°C for 50% of shelf life	4°C for 30% of shelf life	6-8°C for last 20% of shelf life	
CSM manufacturer B	Products with shelf life ≤14 d	2°C for 60% of shelf life	4°C for 30% of shelf life	6-8°C for last 10% of shelf life	
CSM manufacturer C	All products	4°C ± 1°C for 40% of shelf life	10°C ± 1°C for 2 hours or 20°C ± 1°C for 1 hour depending on season	6°C ± 1°C until expiry	Seasonal temperatures are a simulation for transit temperature to consumer home. Testing 4d, 2d, 0d before expiry
CSM manufacturer D					
Retailer A	Products with shelf life 7-20 d	3°C ± 1°C for shelf life duration minus 7 d	5°C ± 1°C for 5 d	Move product to 8°C storage for the longer of 20% of shelf life or 2 d	8°C final storage also has product specific criteria for whether 2d or 20% is used
Retailer A	Products with shelf life >20 d	3°C ± 1°C for shelf life duration minus 7 d	5°C ± 1°C for 5 d	Move product to 8°C storage for the last 30% of shelf life	
Retailer B	Products with shelf life 7-20 d	3°C ± 1°C for shelf life duration minus 7 d	5°C ± 1°C for 5 d	Move product to 8°C storage for the longer of 20% of shelf life or 2 d	8°C final storage also has product specific criteria for whether 2d or 20% is used

Retailer B	Products with shelf life >20 d	3°C ± 1°C for shelf life duration minus 7 d	5°C ± 1°C for 5 d	Move product to 8°C storage for the last 30% of shelf life	
Retailer C	Products with shelf life 7-25 d	3°C ± 1°C	Ambient storage at 22°C for 2 h	Move product to 8°C storage for last 20% of shelf life	Before ambient storage there is an instruction to simulate distribution and retail by storage at 0-5°C
Retailer C	Products with shelf life >25 d	3°C ± 1°C	Ambient storage at 22°C for 2 h	Move product to 8°C storage for last 30% of shelf life	
Retailer D	Products with shelf life 7-14 d	3-4°C ± 1°C for 24 h	Ambient storage at 21°C for 2 h	7°C ± 1°C for remainder of shelf life	The ambient storage is to mimic customer purchase and transport home
Retailer D	Products with shelf life >14 d	3-4°C ± 1°C for 24 h	Ambient storage at 21°C for 2 h	7°C for remainder of shelf life	The ambient storage is to mimic customer purchase and transport home
Retailer E	Up to 20 d	4°C ± 1°C for 40% of shelf life	Ambient storage at 22°C for 2 h	8°C storage for the remainder of shelf life	
Retailer E	>20 d	4°C ± 1°C for 50% of shelf life	Ambient storage at 22°C for 2 h	8°C storage for the remainder of shelf life	

Table 62 illustrated that most CSM manufacturers and retailers describe the shelf life protocols in terms such as "30% of the shelf life". An important point that should be made clear is that all major CSM manufacturers that were visited based their product shelf lives on historical microbiological test results and, for new products, the historical data for similar products (similar with regard to CSM type, pH, water activity and protein/brine content). However, in contrast to SME CSM manufacturers, the historical information included *L. monocytogenes* laboratory test results. For the new products, microbiological testing would be undertaken to show whether the shelf life that was determined for a similar product was appropriate for the new product. As a historical test results database was built up over time, the initially set shelf life would be revised as appropriate.

Mathematical modelling as a method for the determination of the shelf life for a new product was not encountered in any of the premises visited.

In all of the shelf life protocols provided, there was a common approach of retaining a few packs of product from randomly selected batches and storing them, initially under appropriate refrigeration conditions. After a protocol-specific time, the products were typically subjected to a simulated temperature abuse. As discussed above, the time until the commencement of simulated abuse could be a percentage of the product shelf life (CSM manufacturer A; Table 62) or a set number of days from product packing (Retailer D; Table 62) or a set number of days before the expiry date of the product (Retailer A; Table 62). As for the initial appropriate refrigeration conditions, the duration of the simulated abuse could be for a percentage of shelf life, a set period, or an interval before the expiry date. All of the protocols subjected the product to a third temperature after the simulated abuse. The third temperature was universally 7^{o}C or 8^{o}C, and meant to represent a simulation of the temperature inside a worst-case domestic fridge after product purchase by a consumer. Two days before the end of shelf life, some CSM manufacturer protocols opened the packs (again a simulation of how the consumer would handle CSM) to allow the MAP to dissipate.

At the time of writing this report, Retailer C was undertaking a review of their shelf life protocols for RTE food generally. Informal discussions with the company microbiologist revealed future plans to supplement *L. monocytogenes* testing with risk assessments and potentially modelling of *L. monocytogenes* growth (with reality checking to take account of imperfect models). For clarity, assessment of risks using models was an aspiration for Retailer C, which had not been implemented at the time of writing. Furthermore, within the general framework outlined, product testing would become less important. Retailer C considered that shelf life determination by challenge testing (i.e. contaminating product with laboratory-cultured *L. monocytogenes*) was a last resort to be used only

when there was no historical testing information derived from naturally contaminated product (or similar products with near identical water activity, pH and osmotic potential). Retailer C felt the best material to use to determine the fate of *L. monocytogenes* over shelf life was naturally contaminated CSM. Retailer C explained their viewpoint that the decisions to move towards theoretical risk assessment was because any competent CSM manufacturer will be improving process hygiene all the time. Designing a shelf life testing programme that relied on naturally contaminated product that was becoming progressively more scarce was not felt to be sustainable or compatible with the hygienic goals set by Retailer C for its suppliers.

12.4.3 INDUSTRY AWARENESS OF CURRENT SHELF LIFE GUIDANCE

In order to assess whether CSM businesses were aware of existing guidance relating to *L. monocytogenes*, eleven businesses were surveyed by telephone. The businesses were five SME CSM manufacturers and three high throughput CSM national manufacturers. In addition, two national and one international contract testing laboratories were surveyed. At the SME CSM businesses, three proprietors and two technical managers were contacted. At the larger throughputs, two plant technical managers and the company microbiologist were contacted. At the commercial testing laboratories, senior microbiologists were contacted. The CSM manufacturer contacts were asked if they had prior knowledge of two documents. The first document was guidance for undertaking *L. monocytogenes* shelf-life studies for ready-to-eat foods generally. The guidance was prepared by the Commission of the European Union to support FBOs with regulation (EC) No 2073/2005 (SANCO-1628, 2008). The second document was guidance for testing laboratories undertaking shelf-life studies for *L. monocytogenes* in ready-to-eat foods issued by the EU community reference laboratory for *L. monocytogenes* (EUCRLLM, 2009). The commercial testing laboratories were asked if they knew about the shelf life determinations guidance only.

None of the contacts in the SME manufacturing plants and at one of larger throughput CSM manufacturer had heard of either of the guidance documents. Both of the remaining CSM manufacturers claimed prior knowledge of both guidance documents. Both of the larger manufacturers however had not specifically incorporated the advice into their shelf life testing protocols. In both manufacturers, the *L. monocytogenes* shelf life testing programmes predated the EU community reference laboratory guidance (EUCRLLM, 2009). In addition, major retailer supplier conditions dictated how both manufacturers undertook shelf life testing. Only one of the three contract testing

laboratories was aware of the shelf-life guidance for *L. monocytogenes* in ready-to-eat foods issued by the EU community reference laboratory (EUCRLLM, 2009).

12.4.4 SUMMARY OF SHELF LIFE DETERMINATIONS AND ISSUES

Larger throughput CSM manufacturers undertake final product testing and have a large database of historical test results. These results were used to support the stated shelf lives of traditional products and to predict the likely safe shelf life for new products that have similar physicochemical properties to existing products. The process is to set a shelf life for a new product, commence microbiological testing to determine if the set shelf life was appropriate, with adjustment as necessary.

The majority of SME CSM manufacturers reported they would benefit from information describing shelf life. In general, there was poor awareness amongst the SME manufacturers of existing guidance prepared to assist RTE manufacturers with shelf life determinations. Larger throughput manufacturers have reported they would like a standardised method for the determination of shelf life that would reduce the amount of temperature abuse testing they undertake using different retailer supply protocols. As reported in section 12.4.1, several SME retailers would also like a systematic guide to shelf life determination. The approaches to shelf life determination for major retailers are all similar, with differences only in abuse temperatures, abuse duration and the stage of shelf life an abuse occurred. A number of larger CSM manufacturers would support an FSA/BRC-endorsed standardised shelf life protocol, which would replace current retailer protocols and reduce the amount of testing required to satisfy the supply criteria for different retailers. In response, a number of retailers said their distribution and retail chains were unique to their organisation and that different products could experience different conditions. In some stores, different products were displayed in different chillers held at different temperatures.

13 CSM RETAILERS

As part of the study, visits were made to a range of butchers and delicatessens that sliced CSM in store. A multi-national sandwich franchise was also visited, but it was determined that all CSM arrived at the store frozen and pre-sliced. The purpose of these visits was to gain an understanding of operations in retail stores, the method used for cleaning and sanitation and the frequency of cleaning. In addition, stores were asked how the shelf lives on their products were determined.

13.1 BUTCHER SHOPS AND DELICATESSENS

The type of retail stores visited are summarised as Table 63 and a summary of the store locations grouped by local authority region is provided as Table 64. As an estimation of the sizes of businesses visited, the number of employees on site was noted and a summary is provided as Table 65. The masses of CSM sliced in the stores visited varied between 5 kg and 50 kg per week.

Table 63 Number of visits undertaken to each store type

Shop type	Number of visits
Butcher	13
Delicatessen	3
National retailer	2
Symbol group retailer	2
Independent SME retailer	2
Total	22

Table 64 Locations of the shops visited

Store location	Number of visits
Aberdeen	3
Aberdeenshire	2
Antrim	6
Breckland	1
Cardiff	1
Dundee	1
North Somerset	2
Norwich	1
Sedgemoor	4
Wolverhampton	1
Total	22

Table 65 Numbers of employees at each premises visited

Number of employees	Count of businesses within the range	Total number of new employees within the range within the last 12 months
1-5	8	1
6-10	7	2
11-15	3	2
>15	4	0

Different members of the project team had different strategies for arranging visits. In England, Southern Scotland and Wales, the favoured approach was to turn up at a store and ask if the person behind the counter could spare 10 minutes for a chat about their operating practices with an assurance of anonymity. Assuming the shop was quiet, the approach was successful most of the time, after the purpose of the interview was explained. In Northern Ireland and Northern Scotland, shops were contacted by telephone and asked if they wanted to participate. In Northern Scotland, it was a challenge to recruit stores willing to participate. In Northern Ireland, most of the stores that were contacted in advance agreed readily to be involved, again after the project purpose was explained.

Once stores had been recruited, the majority of the interviews were undertaken in the same general manner. A questionnaire, which had been checked by a social scientist to ensure neutral phrasing and no leading questions (provided as section 16), was used as the basis of the interview. Each of the questions was asked and the responses were noted. The order the questions were asked was varied to protect against later sections of questionnaire being under represented, were shop staff to feel the interview was taking too long. The following sections in this chapter of the report are a summary of the responses.

13.1.1 CLEANING AND SANITATION

13.1.1.1 MANUFACTURERS' ADVICE FOR THE CLEANING OF SLICING EQUIPMENT

The name and model of each slicer was noted and a summary of manufacturers is provided as Table 66. Shops were asked if the slicer instruction manual was available and if so, the manufacturers cleaning instructions were photographed. For those stores that did not have a manual, an attempt was made to find an instruction manual for the slicer model using a web search.

Table 66 Slicing machine manufacturer and cleaning instructions provided in operations manual

Slicing machine manufacturer	Manufacturer's cleaning instructions	Year provided
Bizerba	Newer models: Yes: comprehensive instructions including how to disassemble the machine. The equipment manufacturer sells a range of cleaning/sanitising chemicals and promotes purchase of these chemicals in the cleaning instructions	2006
Berkel	Yes: "Clean and sanitize removed parts. Wash these parts in hot water with detergent, rinse in hot water and rinse again in sanitizing solution. Let the parts air dry."	2008
Braher	Unknown – no manual; unable to find	-
Chefmate	Yes: "Clean the disassembled components with a mild detergent and warm water solution. Allow parts to air dry."	1997
Chefmate	Yes: "Place all removable parts in a three compartment sink with warm water and mild detergent solution; soak, clean, and scrub all surfaces thoroughly using a solution of mild detergent and warm water. Rinse removed parts with fresh, clean water. Following the instructions on your quaternary sanitizer label, soak the food chute, knife cover, and slice deflector in a proper solution of quaternary sanitizer for a minimum of two minutes. Allow these parts to air dry.	2011
Hobart	Yes: "… use a mild soap (such as spic and span*) and hot water solution and a clean cloth. Wipe all surfaces of the machine then rinse using another cloth and fresh water"	©1993 (revised 1998)
Medoc	Unknown – no manual; unable to find	-
Parry	Unknown – no manual; unable to find	-
Sirman	Yes (and machine supplied with a blade cleaning kit which included detergent): "[Use] warm water, the equipped detergent or with neutral detergent (pH 7)"	2011
Topkitch	Unknown – no manual; unable to find	-
Vector	Yes: "The machine must be cleaned once a day or more frequently if necessary. Clean all the parts using a cloth sprinkled with water and washing up liquid (do not use strong detergents) and a brush"	2009

*Spic and Span is a popular North American domestic surfactant product for "multi surface and floor cleaning".

Slicers manufactured by Berkel and Bizerba were the most commonly encountered in the stores visited. When store workers were asked the reason why these brands of slicer were so common, the responses were typically that there were two 'types' of slicer. Expensive slicers in the price range £1000-£3000 were considered to be reliable and likely to operate for many years. Cheaper slicers (<£750) were perceived as less reliable with working lives of only a few years. The opinion of one store owner was that an expensive slicer such as those manufactured by Berkel will last "a lifetime" and although the motors and occasionally bearings can wear out, parts are available and they can be replaced. Slicer blades need replacing every few years (depending on usage and sharpening frequency; a blade can be sharpened 50-75 times), but the slicer frame itself will last for decades.

There is a demand for expensive slicers and a healthy second hand market in the UK. Based on the opinions of the shop staff that were asked, an almost unanimous consensus was it was best to buy a second-hand quality slicer rather than cheap new one.

Based on the instructions in the slicing manuals, it was apparent that 10-15 years ago, many slicer manufacturers advised that the cleaning of their slicing machines should be using dish soap and water. In addition, a number of manufacturers specifically advised against the use of steam or pressure washing for the cleaning of the machine frame and a dishwasher for cleaning removable food contact surfaces (FCS) such as blades and guards. The reason for not using a dishwasher is that most blades and guards are made from steel. The carbon in steel can oxidise inside a dishwasher causing rusty spots on the blades. Sometime in the 2000s, the advice to use dish soap was updated by most slicer manufacturers to include the use of a sanitiser. Thus for modern slicers, the cleaning instructions were appropriate and likely to prevent biofilm growth of *L. monocytogenes*. However, given the preference for a high-quality used slicer over a cheap new machine, there are still older slicers operating in the UK with instruction manuals that specifically advise only soap and water.

There were other issues with old equipment, directive 98/37/EC states that "....machinery must be designed and constructed in such a way that it is possible to clean internal parts which have contained dangerous substances or preparations without entering them; any necessary unblocking must also be possible from the outside. If it is absolutely impossible to avoid entering the machinery, the manufacturer must take steps during its construction to allow cleaning to take place with the minimum of danger". However, the observations made during the premises visits was there were slicers in use in commercial premises in the UK that predate the regulation, which are difficult to disassemble and clean effectively.

13.1.1.2 IN STORE CLEANING PRACTICES

A range of responses were recorded relating to cleaning in butchers, SME retail and delicatessen stores. Table 67 gives a summary list of good and bad practices. At the lower end of the scale, there were SME stores in small rural villages that did not use their slicer(s) every day. In such stores, on the day that the slicer was used, it would typically be cleaned and possibly sanitised at the end of the day. Cleaning universally consisted of removing gross detritus, and removing the blade and slider and washing these in dish soap or a specially formulated degreaser. The remainder of the FCS on the slicer were wiped

with a dish cloth soaked in hot soapy water before rinsing the cloth and re-wiping to remove the soap. When no additional sanitation was undertaken, the slicer, blade and slider were typically allowed to drain and air dry overnight before reassembly. In those stores where a sanitiser was applied, there was a variety of practices. At the top end of the scale, accurate dilution of adequate volumes of sanitiser were applied for a defined contact time, at the lower end of the practices much consideration was not given to how long was required to achieve effective sanitation.

In those stores where slicer use was infrequent, if the slicer was not used the next day, it would typically be covered and not re-cleaned the next evening or until it had been re-used. A representative end of day clean down was not observed, but if the dish soap treatment was unable to remove all of the meat residues, there existed a possibility that *L. monocytogenes* growth could occur on the slicer surfaces on the days that it was not used. In contrast to many food contact surfaces, slicers and blades are not refrigerated and are held at ambient temperatures, which can allow rapid bacterial multiplication.

At the other end of the scale, there were (mostly) butchers with specialist cleaning chemicals and SOPs describing the cleaning and decontamination process for their slicers. Typically, these premises had modern slicing equipment that had been designed to be disassembled easily. The food contact surfaces on these slicers were all easily removable without tools for cleaning and sanitation. It was apparent that some local authorities (LA) have 'approved' lists of cleaning and sanitising chemicals. For those LAs, the EHOs agreed with butcher shops which chemical they should use, and how to undertake cleaning. The approved list was drawn up as a response to the FSA guidance of *E. coli* cross contamination rather than specifically for *L. monocytogenes*. The approved list was provided by LAs to ensure cleaner/disinfectant compliance with British Standards BS EN 1276 or BS EN 13 697. The LA-butcher agreements formed the basis of the SOPs and could include fine details such as minimum chemical contact times, which were derived typically from the chemical manufacturer's instructions. A typical high-end practice was to disassemble the machine and soak the blade, guard, slider and landing tray in a purpose-formulated degreaser for a specified amount of time. If required, the parts were scrubbed with a one-time-use pad to remove any fat or protein residues. The parts were water rinsed in either hot or cold water and then soaked in a purpose-formulated sanitiser, again for a stipulated amount of time, before rinsing (if required and dependent on the sanitiser used) and drying. In almost all of the SME stores visited, the drying was in air overnight although there were a small number of stores that dried using one-time-use paper towels. Although the slicer bodies were non-food contact surfaces, these were also wiped with a degreaser in some stores to remove any meat residues. In roughly 50% of stores, the non-food contact slicer surfaces were re-wiped with a sanitiser.

Alternatively, it was noted that in around 20% of the stores visited, alcohol wipes were used to clean and sanitise non-food contact surfaces on slicers as a single action.

There was an even distribution of cleaning and sanitation practices in-between the best and worst practices. A number of premises visited used specialist cleaning chemicals that were required to be diluted before use, but not all stores had equipment to measure volumes accurately. The SOP in one store provided instructions for the use of product scales to weigh volumes of water and sanitiser to ensure accurate dilutions. For those stores that used specialist chemicals, around half used products that were ready to use without dilution and half used chemicals that were required to be diluted. Almost exclusively, the required dilution for cleaning and sanitising chemicals was 1:10.

When asked if guidance for effective cleaning and sanitation of slicers would be useful, only ~23% (5/22) of the shop workers thought additional assistance in this area would be of benefit. The apparent basis for the finding was an overall perception by the shop workers that their routine cleaning and sanitation practices for the slicers and FCS were good enough for de-contamination of FCS.

A summary of the captured responses from SME retailers is provided as Table 67.

Table 67 Good and bad practices in butchers, delicatessens and SME in-store slicers for slicing equipment and the storage and display of CSM

Practice/knowledge	Good	Bad
How often was the slicer blade cleaned	All of the shops responded that they would clean the slicer at the end of every day that it was used at the very minimum.	Four stores cleaned the slicer only at the end of day. In these stores, there was a small number of products that were sliced and typically slicers were used only once or twice a week
Blade cleaning procedure during the day	The majority(15/22) of shops stripped down the slicer, removed food residuals, used a separate sanitizer (spray) and cloth for cleaning with optional drying using paper towels. One butcher used a multiple step procedure for cleaning, sanitation and drying (e.g. blade removal, washing in hot water with QBAC, rinsing with potable water and air drying).	Removal of gross detritus only, occurred in four stores. In two stores, blades and FCS on the slicer were washed with hot soapy water as required periodically throughout the day. The criteria for washing was visible detritus on the slicer.
Clean down of general food contact surfaces	All of the stores reported they cleaned FCS such as work surfaces several times per day; the majority (15/22) removed food residuals,	The use of re-useable woven cloths to wipe FCS was observed in a small number of premises. These cloths were typically

	sprayed a sanitizer on FCS and wiped with paper towels. The use of alcohol surface wipes was also observed.	laundered in a domestic washing machine as required.
End of day cleaning (blades and FCS)	All of the butcher stores used a multiple step procedure for cleaning and sanitation: All of the stores reported they stripped down their slicer and removed food residuals 12/13 butchers used hot soapy water and sanitised the FCS/blade using a separate sanitizer, followed by drying in air. A small number of butchers used hot water and QBAC, followed by rinsing with potable water and drying in air overnight.	There were two SME retailers that washed their blades and FCS in hot soapy water to render them visibly clean but did not specifically apply a sanitising chemical. There was evidence of a lack of awareness of the importance of sanitiser contact time in at least three stores.
Diluting the cleaning and sanitising chemicals according to manufacturer's instructions	All of the stores that bought chemicals that required dilution reported they diluted the cleaning and sanitising chemicals according to manufacturers' instructions.	However, the small number of stores that were asked were unable to precisely state how liquids were measured to achieve (for example) a 1:10 dilution.
Is the same slicer used for uncooked (e.g. bacon) and CSM?	All butchers had separate slicers for CSM and uncooked meat. All three delis sold only cooked meats.	There were stores identified with a single slicer that sliced cooked and raw meat.
Knowledge about *Listeria*	11/13 butchers had heard about *Listeria*.	Across the other SME retail sectors, awareness regarding *Listeria* was poor.
Checks performed on CSM at delivery and to ensure that product safety	All of the SME retailers used a combination of visual inspection, taste and smell to determine whether new products were of appropriate quality. Most butchers only sold the product if it was in "used before" date indicated by supplier Some butchers kept half of the meat joint on display in a chilled display and half in a fridge Most of the butchers sold CSM if it was within 2-3 days from opening a loaf and within the manufacturer's expiry date.	Use of temperature probes to check transit refrigeration was not universal for received batches of CSM. At least two butchers would extend a CSM manufacturer's expiry date if the loaf was cut in two and half was VP and then frozen.
Shelf life	Typically, only those butchers that pre-sliced meat into vacuum packs gave a shelf life on their product (typically between five and ten days for ham, 14 days was typically advised by a number of LA EHOs).	For product sliced in front of the customer, it was not common for an expiry date to be included on the bag.
Temperature checks of CSM on display	Most butchers read the temperature indicated by the	A low percentage (32%) of the stores checked the actual

	built-in thermometers on display cases several times /day, usually in the morning and evening	temperature of CSM on display using a thermometer or thermocouple.
Temperature checks of CSM in fridges and freezers	Most SME retailers read the temperature indicated by the built-in thermometers on fridges several times /day, usually in the morning and evening. A small number (at least five) of the SME retailers had a temperature alarm on non-display product storage chillers	For those SME retailers that froze CSM, none checked the actual temperature of CSM held in freezer. For those SME retailers that checked temperatures, few kept records of those temperatures.

13.1.1.3 VERIFICATION OF EFFECTIVE CLEANING AND SANITATION

None of the SME stores visited undertook verifications to determine cleaning effectiveness. In the stores visited, the only testing undertaken was by EHOs, who took swabs periodically (commonly once per year) from cutting boards in shops and other food contact surfaces including knife and slicer blades and slicing machine food contact surfaces. In the SW of England, EHOs had specifically targeted CSM vacuum packing equipment for sample collection.

The laboratory tests that were undertaken were variable between LA region. Commonly, testing was for numbers of indicators such as total aerobic mesophiles (TAMC), *Enterobacteriaceae*, coliforms and *E. coli* (generic). In some regions there was also testing for potential human pathogens such as *Salmonella, E. coli* O157 and *Listeria monocytogenes*, although this was quite rare. In most regions, shops tended to get to see the results only if a result that was a cause for concern was reported by the laboratory. The exceptions were some of the Scottish regions and SW England where the results were provided to FBOs as a matter of course. In one Scottish store, the owner provided a recent lab report, which was discussed. It was apparent that the owner had a basic understanding of the test results but did not know what the results for each of the bacterial types indicated. The store owner was aware however the numbers on the report corresponded to the numbers of 'germs' and that lower numbers were better because that meant less bacteria.

Based on discussions with shop workers, it was apparent there was a sliding scale of shop visits by EHOs. The longest observed time interval between inspections to date was 18 months. It was apparent that the interval between inspections became longer as shops established a history of not undertaking risky practices. The SME store-operator perception is in broad agreement with comments made by a

number of EHOs who said they specifically targeted risky or new businesses for visitation when asked (section 11.1).

In some LAs, the EHOs run periodic training days and workshops for butchers. For that reason butchers in some regions did not think that they needed more training/guidance on *Listeria* spp.

13.1.2 GENERAL INFORMATION RELATING TO SME BUTCHERS AND DELICATESSENS

In all of the delicatessens visited, and some SME retailers, it was not possible for a slicer to slice raw products before CSM because raw products were not stocked. All of the SME butchers and SME stores that sold raw meat such as unsliced bacon said they would never use a slicer to slice cooked meat after slicing uncooked meat without undertaking a thorough cleaning of the slicer. There was a good general awareness in those stores that were asked (i.e. the ones that stocked raw meat) that uncooked meat might be hazardous. Consequently, a variety of strategies were used to ensure that slicing cooked after raw meat did not happen in those stores with a single slicer only. These strategies included pre-slicing raw meat and displaying products such as bacon pre-sliced, and possibly VP. Some stores were able to estimate how much CSM they'd likely sell during a sales day, and pre-sliced that amount. There were some stores that had raw and cooked material in the same display chiller. Hudson and Mott (1993a) reported that raw meat such as bacon and sausages routinely contained *L. monocytogenes* and could act as a source of contamination for RTE cooked meats.

It was noted that in a number of the stores that were visited, there were logs of economy cooked meat on chilled display still inside the casing. In a good proportion of these stores, meats were routinely sliced without removing or peeling back the casing. When asked, the majority of shop workers said it was more hygienic to keep the meat in the casing because it protected the outside of the log from contamination. However, any cross contamination would be to the casing rather than the meat itself. Contamination of meat and slicer blades as a consequence of slicing encased meat with a contaminated casing has been described in the literature (Hudson and Mott, 1993a).

A number of good handling practices were observed in some stores. These included having separate staff for the handling of raw and cooked products. In one store, money was also handled by a dedicated member of staff for hygienic reasons. A variety of global currencies obtained from food outlets have been reported as widely contaminated with bacteria (Vrieskoop et al, 2010). Risk factors for banknotes include the value of the note, with higher values harbouring less bacteria (Vrieskoop et

al, 2010). British notes are constructed from a cotton-based substrate, which is particularly unhygienic compared with polymer substrates (Vrieskoop et al, 2010). There is little specific information regarding contamination of money with *L. monocytogenes* in the UK, however one Iranian study reported around one percent of notes were contaminated with *L. monocytogenes* (Moosavy et al 2013).

One of the recommendations of Vrieskoop et al (2010) was that food store handlers should wash their hands after handling money or use one hand for money and the other for food. None of the stores visited used practiced one hand for one item. Hand washing was widespread, as was hand rinsing (i.e. no soap, just water) particularly in butchers where a common aim was to wash between each customer. Most store workers thought it was possible there could be occasional lapses. Also widespread were the use of disposable gloves or polythene sheets (or both) so that products were not handled directly. None of the employees in the stores visited put on a fresh pair of gloves for each customer.

13.1.3 SHELF LIFE

A shelf life was provided to customers only by a small number of butchers. Typically, only pre-cut VP CSM was given a date and it was for a shorter period than the intervals typically suggested by EHOs (Table 67). Butchers that sliced the product at the front counter tended to sell the CSM only if was within the 2-3 days from opening a loaf/case and only if was in the "use before" date indicated by supplier. The notable exception to that rule was those SME slicers that cut a CSM loaf in two prior to opening and froze one-half for later sale.

None of the SME retailers had undertaken any work to determine shelf life with regard to *L. monocytogenes*. There was a commonly encountered opinion in most of the stores that bought in pre-cooked meat for slicing that the original meat manufacturer should be responsible for the determination of shelf life.

13.1.4 PERCEIVED NEEDS OF SME RETAILERS REGARDING *L. MONOCYTOGENES*

Additional store visits were made to an SME butcher, an independent convenience store with a meat slicer and an independently-owned symbol retail store. The purpose of these visits was to discuss the needs and concerns of SMEs relating to CSM and *L. monocytogenes*. All discussions were with the

business proprietors. At all of the stores visited, the FBOs were not particularly concerned about *L. monocytogenes*. At all three stores, the FBOs were of the opinion they were inspected (by EHOs) and if their working practices were not up to the required standard, they would have been told that by the LA. A key issue appeared to be that the FBOs at the stores visited were not very familiar with *L. monocytogenes* as a human pathogen. In addition, the bacterium had not previously caused any issues for their businesses. The strong impression of the project team member that undertook the three visits was FBOs would become interested only if there was an *L. monocytogenes*-related impact on their business e.g. if an FBO was served an improvement notice or similar. When the FBOs were asked what they thought they'd require if there was an *L. monocytogenes* issue with their store, all three answered they'd want advice on how to resolve the issue to the satisfaction of the enforcement authorities. There was universal agreement that advice on how to decontaminate effectively slicing equipment, the shop environment and how to store meat to prevent *L. monocytogenes* growth would all be beneficial.

13.2 LARGER RETAILER IN STORE DELICATESSEN COUNTERS

Most major retailers in the UK have a delicatessen counter in most of their larger stores, where joints or logs of meat are sliced using a slicer, or carved using a knife in front of the customer.

13.2.1 OBSERVED PRACTICES

Most of the larger retail premises visited had multiple slicers in operation (Figure 27). Furthermore, most retailers had adopted similar broad strategies for slicer usage and reducing the potential for cross contamination. The actual number of slicers was roughly correlated with store size. The highest number of slicers observed was four within a single store. Two slicers, one slicer for cooked and another for raw meat was the minimum number observed. There was no apparent pattern as to what slicers were dedicated to what product(s). Slicer usage policy appeared to be set at the store level and was partly related to the number of slicers available. At one store (Retailer D), cooked hams had a dedicated slicer, with the other meat types being sliced on a different dedicated slicer. At another smaller Retailer D store, hams and other meat types were sliced on the same machine, which was dedicated for cooked meat. In another Retailer D store, there were two identical slicers set up to cut different thickness slices of meat. The slicer that was used was dependent on the customer's instructions for slice thickness.

Figure 27 Larger retailers used multiple slicers for different products as a strategy to minimise cross contamination

13.2.2 SHELF LIFE

There were a number of issues regarding in-store sliced products discussed with store staff, that mostly related to keeping track of expiry dates. In brief, in the stores that were visited, up to fifteen types of cooked meats were on display for in store slicing. Discussions centred on how retailers kept track of the expiry dates and how that information was transmitted to customers. Fifteen stores belonging to five national retailers were visited. The results of these visits revealed some complexity with regard to how the CSM reaches larger retail stores and how CSM shelf life is managed.

Generally, providing the customer with the manufacturer-determined expiry date to be printed onto a CSM weight label would involve either keying the correct expiry date in every time a portion of meat was sliced and weighed, or giving each meat type a code that was linked to a database containing the date and keying that in. Both methods are prone to error and the latter could be expensive to implement since scales would require modification to connect to a network. For those reasons, the issue of providing the customer with an expiry date was addressed at every major retail store visited by issuing a short product shelf life of two or three days (dependent on retailer).

By way of example, Retailer A exclusively bought in pre-cooked meat joints/logs for slicing. Retailer A stores kept records of the manufacturer quoted shelf life when a new cooked meat was delivered to the store (commonly 10-14 days). Once a joint of meat had been opened, Retailer A replaced the manufacturer expiry date with four days from the opening day (including the day of opening). In some Retailer A stores, an entire ham could be sliced and sold in less than a day and reaching a four day expiry was considered unusual. In Retailer A customer display chillers, the CSM products were tagged individually with a label. The side of the label facing the customer had the product name and a price per 100g (typically). The reverse side of the label facing the retailer had the original manufacturer expiry date, the date that the meat was opened in store and the new expiry date. Daily checks were made at the start of each retailing day at Retailer A stores to ensure that no meat exceeded either the original manufacturer expiry date or the retailer replacement date.

One retailer, (Retailer B) did not appear to have a consistently applied policy across all of its stores. One Retailer B store in the Midlands sold CSM with two days shelf life stated verbally, but with a label showing the date of printing (with no further text to identify what the date was). Another Retailer B store in the SW of England sold product with the date of slicing printed on the label and staff said in that store said the expiry date was the day of purchase. A third Retailer B store also in the SW England did not know if the same day date applied was an expiry date or a note of the date of slicing. The other retailers all had consistent policies across their stores and summary of the shelf lives is provided in Table 68.

Table 68 Shelf lives provided by national retailers for CSM sliced at delicatessen counters

Retailer	Shelf life
A	Two days, including day of slicing
B	Different information obtained from different stores. Two days was the longest shelf life quoted
C	Three days including day of slicing
D	Three days including day of slicing
E	Two days, including day of slicing

A large store belonging to one retailer (Retailer E) was novel because it cooked pre-cured or pre-brined meat in at least one of their stores for slicing, in essence making the store a CSM manufacturer. The Retailer E store that cooked meat did not slice it in front of the customer, but sliced all of the meat immediately after a post-cook cooling. The CSM was packaged (standard atmosphere) and offered pre-packed to customers with a two days shelf life (including day of sale). However, in other Retailer E stores (one less than 10 miles from the store that cooked its own meat) all CSM was bought in pre-cooked and sliced in front of customers.

13.2.3 CLEANING AND SANITATION OF LARGE RETAILER SLICING MACHINES

Delicatessen staff in major retailer stores were asked about cleaning and sanitation of slicing equipment, knives and ancillary items such as work surfaces and the trays used to display cooked meats. Typically, store staff were suspicious of questions relating to cleaning/sanitation and roughly 50% of those asked were reluctant to speak about their procedures without specific permission from a supervisor or store manager. One store manager further referred the enquiries to the head office of the retailer. For the two retailers where no information was obtained for cleaning and sanitation from store employees, the company microbiologists were contacted. Both of retailer microbiologists that were emailed responded and agreed to be interviewed by telephone. Thus, this section contains information relating to larger retailer cleaning and sanitation from a variety of sources.

For all of the retailers that were visited or otherwise contacted, cleaning and sanitation practices for delicatessen counters were set centrally by head office staff. From the viewpoint of the company microbiologists, stores were provided with a standard set of SOPs that detailed how effective cleaning and sanitation should be undertaken. The SOPs were fairly-detailed, stipulated specific cleaning and sanitising chemicals and included minimum scrubbing intervals and chemical contact times for equipment that was immersed such as slicer blades and knives. Two retailers used one time use wipes impregnated with either degreasing, sanitising chemicals or alcohol as appropriate for cleaning non-food contact surfaces, rather than liquid chemicals. A common strategy in stores was to use hot water dishwashers to clean items that could withstand repeated washing cycles (e.g. plastic meat display trays). If required by the SOP, sanitiser would be applied to such items after dishwashing.

Both the company microbiologists took the time to make clear that the SOPs had been validated by microbiological testing to ensure they were effective. For that reason, there was no periodic routine verification of cleaning effectiveness undertaken.

All of the larger retailer staff that were asked said they were provided with appropriate PPE (e.g. anti-slash gloves), to minimise the likihood of injury when disassembling slicer blades and guards and handling knives.

14 SUMMARY AND CONCLUSIONS

The ubiquitous nature of *L. monocytogenes* means that processing plants are subject to a relentless exposure to this pathogen with common sources being raw materials entering the plant and, to a lesser extent, process workers. Potentially any object entering the plant from the outside world can act as a fomite for *L. monocytogenes* which can then become established in the processing environment. Effective cooking of meat is a CCP for *L. monocytogenes* consequently some form of temperature check was undertaken in all of the processing plants visited in this study to ensure adequate processing. For that reason, the principal area of concern is post-cook contamination, supported by reports that established plant-resident strains are most commonly isolated from post-cook final product. Physically damaged or poorly designed processing equipment and floor drains are the common niches for plant resident *L. monocytogenes* strains. The reality is that despite excellent biosecurity practices operating in some (especially the larger) processing plants, sooner or later *L. monocytogenes* will contaminate equipment in the post-cook areas of the plant and hence potentially contaminate final products. The effective response when detected is deep cleaning, to eliminate *L. monocytogenes* in the processing environment. Microbiological testing should be used to confirm effective removal.

At the retail level the premises of large multiples had appropriate facilities and procedures to minimise potential *L. monocytogenes* contamination of RTE meats, and staff were trained to maintain them. In addition, the majority of butchers seemed well-informed and undertook routine cleaning and sanitation of their slicers and FCS during the day, with a deep clean on closing. The overall perception amongst butchers was that routine cleaning and sanitation of slicers and FCS were good enough to eliminate *Listeria*. Consequently, most thought that further guidance of cleaning and sanitation of their slicers was not required. However, in some SME retail premises issues with slicer cleaning and sanitation and in some cases display refrigeration temperatures were noted.

The smallest SME retail premises (e.g. a small rural post office that sold a few basic food items, including sliced cooked meat) would benefit from guidance on how to effectively clean and sanitise older equipment. Some slicer manufacturers historically recommended washing the blades in dish soap (only) in their instruction manuals. Such slicers, complete with the original manuals, were seen to be in use today, mainly in the smallest premises.

Based on the experiences of undertaking this study, the following conclusions were drawn:

- It is recommended that local authorities begin to more assertively target processors that are currently not testing plant environments and products for *L. monocytogenes* to make sure that FBOs are able to demonstrate compliance with Regulation 2073/2005. Smaller businesses are unlikely to have sufficient resources to fund sampling regimes comparable to larger manufacturers and so it would be appropriate to take a risk-based approach to testing environments that reflected the size and nature of businesses.
- EHOs should also focus on identifying and advising businesses using slicing equipment that was never designed to be straightforwardly disassembled to achieve effective cleaning and sanitation, because such equipment could present the most risk to consumers.
- A number of EHOs and smaller processors were not aware of existing guidance on shelf life determination with regard to *L. monocytogenes*. It was stated by a number of processors that an easy-to-follow standardised shelf life protocol for CSM with regard to *L. monocytogenes* would be helpful. Although there is current guidance available, it was felt to be either too general or too complex. In particular, there was felt to be a need across all sizes of processors for universal set of reasonably foreseeable conditions (e.g. specific storage temperatures and storage durations). The current CFA-BRC guidance endorsed by the FSA does not currently contain that level of detail. Pragmatic guidance, in combination with increasing awareness of current guidances would address the technical knowledge gap identified in some SME CSM manufacturers. Larger manufacturers could reduce the amount of testing required to comply with various different retailer protocols. Further, some EHOs also said they would benefit from additional information or training for shelf life determination with regard to *L. monocytogenes*. However, there are barriers to a standardised protocol. Large retailers have distribution chains at slightly different temperatures to each other and customise their supplier protocols to reflect these different temperatures.
- Encouragement should be made towards the use of predictive microbiological modelling as an inexpensive element of shelf life studies. EHOs should be routinely trained in the interpretation of model outputs to help them determine whether predictive modelling carried out by food business operators (or on their behalf by relevant experts) provides appropriate evidence regarding whether or not *L. monocytogenes* may exceed the 100 cfu/g limit during a product's shelf life.
- Since a major route of *L. monocytogenes* entry into CSM plants is via raw materials, consolidating or raising awareness of existing guidance on raw materials acceptance criteria

would help support EHOs, possibly by providing example specifications that they can further distribute to smaller processors.

- Current costs for laboratory testing are perceived by many SME processors to be prohibitive. Whilst EC 2073/2005 stipulates using an ISO testing protocol, it allows the use of alternative methodologies if they are validated as equivalent. Were a less expensive test protocol to be evaluated and validated, it would help address cost concerns from SME processors and would also allow larger manufacturers to undertake a more comprehensive surveillance of processing environments for the same current costs.
- Some EHOs indicated they may require additional training in areas such as control of *L. monocytogenes* and the enforcement of EC 2073/2005 in order to provide them with the technical information they require to support and assist businesses struggling to comply with the statutory obligations.
- To assist EHOs in the above task, guidance on measuring pH and a_w in SMEs and instructing a laboratory to undertake these measurements on behalf of an FBO (subject to the professional standards forbidding EHOs from recommending a testing laboratory to an FBO) would be required.
- For those businesses that have never used an external laboratory, information covering laboratory selection and instruction was perceived to be helpful. Many smaller businesses mentioned they would also like guidance on the interpretation of laboratory test result reports.
- EHO feedback indicated that workshops, and online information, were the preferred mechanisms for the supply of any training.

The development and provision of guidance for processing area cleaning and has been recommended by previous studies, notably including the first Pennington report (1997). FSA have funded previous work to address these types of gaps, which includes advice on cleaning and sanitisation in the Agency's *E. coli* O157 cross-contamination guidance. However, it was apparent that further efforts are required to bridge an important information and training gap for some EHOs and FBOs. In particular, specific advice on cleaning and sanitisation in relation to *L. monocytogenes* is required. Any guidance should contain clear statements regarding the inadequacy of visual cleanliness as a measure of sanitation efficacy. Cleaning, and many of the other issues identified in the current study are similar to those identified previously by other FSA industry practice reviews, such as FS 425012, which dealt with smoked fish, which is also a RTE product. With that in mind, there may be merit in the supply of general information covering RTE food generally rather than, for example, CSM and cold smoked fish individually.

Given the importance of the role of EHOs in the recommendations noted above, there is a need to ensure that environmental health degree courses and CPD activities adequately cover the identified information shortfalls so that all EHOs are cognisant of *L. monocytogenes* control and the related statutory requirements.

As part of the increased enforcement activities, there would be merit for EHOs to inform SMEs that historical slicer manufacturer cleaning instructions are no longer adequate. There may also be merit in describing simple and effective decontamination procedures (e.g. heating in a domestic oven to a defined temperature for a defined time) for objects such as slicer blades that have never been routinely sanitised and thus may harbour *L. monocytogenes* biofilms.

From interviews and surveys undertaken in this study training courses or workshops were the favoured approach for information dissemination, and the provision of written online guidance was the next most favoured, with neither of these two options being significantly favoured over the other. The FSA currently have a series of EHO training workshops scheduled around the UK in June and July 2014. Although these are targeted at the smoked fish sector, there are areas of overlap and these workshops could go some way to helping address the identified EHO information requirements.

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16 APPENDIX 1 QUESTIONNAIRE USED FOR SLICING EQUIPMENT IN BUTCHERS AND DELICATESSENS

Question	Response
How many people work in the shop?	
How many people work for the company?	
How many new employees were there in the shop in the last year?	

Question	Response
What is the name of the manufacturer of the slicing machine?	
Does the manufacturer provide cleaning and sanitation advice for the equipment?	Yes No Don't know
Has the slicer been modified in any way?	Yes No Don't know
How often is the slicer used?	Every day Every few days Once a week Less frequently
On average, what mass of CSM is sold each week?	Kg
During the working day: How often is the slicer blade cleaned?	Several times per day Every day After every time used End of day only if it's been used that day
During the working day: If the **slicer blade** is cleaned, how is it undertaken?	Any food residues/pieces removed Stripped down and blade removed

(circle all that apply)	Blade washed in hot soapy water in sink Blade washed in hot detergent in dishwasher A specialist cleaner/degrease chemical is used A separate sanitiser is used A combined cleaner and sanitiser is used Blade is rinsed using potable water Blade is left to air dry Blade is dried using a cloth Other – please describe
During the working day: If there is a clean-down during the working day of the slicer food contact surfaces (FCS) please describe the procedures	
For end of day cleaning: How are **the blade and food contact surfaces** for the machine cleaned?	Any food residues/pieces removed Slicer is stripped down and food contact surfaces (FCS) removed FCS washed in hot soapy water in sink FCS washed in hot detergent in dishwasher A specialist cleaner/degrease chemical is used

	A separate sanitiser is used A combined cleaner and sanitiser is used FCS are rinsed using potable water FCS are left to air dry FCS are dried using a cloth Other – please describe
For end of day cleaning: If sanitising chemicals are used, how long are they left on the surfaces of the slicer?	
For end of day cleaning: How was that contact time determined?	
Are cleaning and sanitising chemicals diluted according to manufacturer's instructions (if applicable)	
Is the same slicer used for uncooked (e.g. bacon) and CSM?	Yes No
If so, is it possible for CSM to be cut on the slicer after uncooked meat without any cleaning and sanitation in-between?	
Has the butcher/deli slicer operator ever heard of *Listeria*?	
If so, has the store ever taken any specific steps to de-contaminate slicing equipment of planktonic	

(unattached) or biofilm (attached to the slicer surfaces and enmeshed in polysaccharides) *Listeria*?
If guidance on how to effectively clean and sanitise slicers was produced would the store make use it?
Are there any barriers that would prevent the store from following the good practice guidance and if so, what are they?
When CSM is delivered, what checks are carried out to make sure the product is acceptable.
Does the store slice and prepack into vacuum packs? Yes No
What shelf life do you give the sliced product(s)?
How was that shelf life determined?
Are the temperatures of the CSM checked when it is on display? Yes No
If so, how are the temperatures measured?
If so, how frequently are temperatures checked?
Are the temperatures of the CSM checked when it is stored prior to display? Yes No
If so, how are the temperatures measured?
If so, how frequently are temperatures checked?

If a thermocouple is used, how is it cleaned and sanitised between temperature checks?

17 APPENDIX 2 QUESTIONNAIRE USED FOR CSM PROCESSORS

Question	Response
How many people work in the plant?	
How many people work for the company?	
How many new employees were there in the plant in the last year?	

What sort of product does the plant manufacture?			
Species	**CSM Type**	**Tick if manufactured**	**Tick if sliced**
Beef	Corned Beef		
Beef	Fermented Sausage		
Beef	Sausage		
Beef	Sliced Beef		
Beef	Tongue		
Chicken	Meatloaf		
Chicken	Sausage		
Chicken	Sliced Chicken		
Chicken	Wafer Thin Chicken		
Mixed	Fermented Sausage		
Mixed	Sausage		
Mixed	Sliced Mixed Species		
Pork	Air-Dried Meat		
Pork	Breaded Ham		
Pork	Brunswick Ham		
Pork	Crumbed Ham		
Pork	Fermented Sausage		
Pork	Garlic Sausage		
Pork	German Ham		
Pork	Honey Roast Ham		
Pork	Luncheon Meat		
Pork	Meatloaf		
Pork	Peppered Ham		
Pork	Sausage		
Pork	Sliced Ham		
Pork	Sliced Pork		
Pork	Smoked Ham		
Pork	Tongue		
Pork	Wafer Thin Ham		
Pork	West Country Ham		
Turkey	Fermented Sausage		
Turkey	Sliced Turkey		
Turkey	Wafer Thin Turkey		
Other (please say what)			

Raw meat received into FBO	
Are raw meats transported using refrigeration?	Yes, chilled transport Yes, packed in ice No
If refrigerated, are transit temperatures monitored	Yes No
If monitored, describe how the monitoring is achieved	
Are any transport crates/boxes/trays designed to permit easy and thorough cleaning and sanitation?	Yes No N/A
Are any the transport crates re-lined every time they are re-used?	Yes No N/A
Are transport crates/boxes/trays cleaned and sanitised?	
Where are they cleaned and sanitised?	
Are any crates ever used for a purpose other than transporting raw meat?	Yes No Don't know
If so, what are the other purposes?	

Are any transport crates/boxes/tray cleaning and sanitation regime validated as effective in controlling *L. monocytogenes* (Plant may not know if it happens offsite)	Yes No Don't know
How are containers that have contact with meat stored when kept on site?	
Is there physical damage (e.g. scrapes and scores) in the surfaces of transport crates that could have contact with raw meat?	
If frozen meat is used, is it thawed at below 7^{o}C before being processed?	
Is meat stored under refrigeration (i.e. in ice or in a chiller) prior to processing?	Yes No
Is the incoming temperature of the raw meat checked?	Yes No
Does the FBO have any criteria for accepting meat?	Yes No
If so, is there any check of the *Listeria* status of the meat coming in	
If so, is the supplier asked to provide test results?	
Has the supplier ever been asked what controls they take with regard to *Listeria*?	Yes No

Does any waste removal (e.g. melted water, meat detritus) from the raw meat receiving and storage areas happen on a continual or near continual basis?	Yes No N/A
Are there knife sterilisers (e.g. >80^{o}C water baths) at any raw meat trimming stations?	
If so, is a two-knife system in use? (one knife in steriliser, the other in use; knives periodically exchanged)	
Plant infrastructure:	
Is the plant infrastructure intact?	Yes No
If not, are there holes in the walls	Yes No
If not, are there broken windows?	Yes No
If not, are there damaged doors?	Yes No
Are plant floors hard surface non-absorbent adequately drained (i.e. no standing water)?	 Yes No Yes No Yes No
Do the drains have removable grates?	Yes, all do Yes, some do No
If so, are the drains fitted with slow-dissolving blocks of sanitiser chemicals?	Yes, all do

	Yes, some do No
Do the drains have copper linings (or another source of copper ions such as a copper grating)?	Yes, all do Yes, some do No
Are the plant walls? Smooth? Waterproof? Light coloured? Readily cleanable?	 Yes No Yes No Yes No Yes No
Are the ceilings constructed in a manner that prevents accumulation of dust, **condensation** and growth of microorganisms? (look out for pipes, water marks on the ceiling and electrical ducting)	
Are the premises well ventilated (i.e. is there enough airflow to prevent formation of ceiling condensation)? Ask: "Do you ever get condensation dripping off the ceiling?"	
Is there physical separation of: The raw product receiving area from the rest of the plant? The product preparation (trim and cook bag packing) area from the rest of the plant? The cooking equipment area from the rest of the plant? The packing area from the rest of the plant?	 Yes No Yes No Yes No Yes No
Does the plant have a formally-defined high risk area exclusively for cooked product?	Yes No

Is product entry into high risk from an oven using a different exit from the low risk area?	Yes No
Are refuse and processing by-products stored in a physically separate location to final product?	Yes No
Are refuse and processing by-products stored in a physically separate location to raw meat?	Yes No
Does the plant use mains water or a private water supply? Ask: "Do you use mains water?"	
If a private water is used, how is the water made potable? (e.g. chlorine dioxide addition)	
Does the plant have plentiful supplies of hot and cold potable water? Ask: "How is water heated?"	
Does the plant use ice? If so, how is it stored and used?	
What happens to any waste ice?	Melted into drains Other (please state)
Are the food contact surfaces in the high risk area of the plant:	
Hard	Yes No
Impermeable to water	Yes No
Free from cracks and pitting	Yes No
Free from visible corrosion?	Yes No

Are the food contact surfaces capable of withstanding repeated cleaning and sanitation?	Yes No
Are the vats/other containers used for brining or curing:	
Free from corrosion	Yes No
Free from scoring and pitting	Yes No
Constructed in a manner that permits easy cleaning	Yes No
Constructed in a manner that permits complete drainage?	Yes No
Are there machines for:	
Brining (injectors)	Yes No
Tumbling cured raw meats	Yes No
Slicing	Yes No
If so, are these machines specifically designed to be easily cleaned and sanitised?	Yes No
Are bits of meat detritus trapped inside the machines at the end of a day's processing	Yes No
Are there low pressure (i.e. mains pressure) hoses in the processing area?	Yes No
Are there high pressure (i.e. jet washers) hoses in the processing area?	Yes No
Is the temperature monitored in processing areas?	Yes No
If so what is the maximum allowed temperature?	
Is the temperature monitored in the final product storage area?	Yes No
If so what is the maximum allowed temperature?	

Processing staff	
Does the plant have a standard operating procedure (SOP) that describes minimum standards of cleanliness for processing staff?	
Does the plant have a return to work procedure for processing staff (known or suspected of) recovering from gastroenteritis?	
Does the plant have sanitary toilets that allow employees to wash their hands?	
Are there facilities that allow employees to wash and dry their hands in the processing hall?	
Are there facilities that allow employees to dry their hands in the processing hall?	
How often do staff handling raw meat wash their hands?	
How often do staff handling raw meat change their gloves?	
How often do staff handling cooked meat wash their hands?	
How often do staff handling cooked meat change their gloves?	
If used, are gloves changed at appropriate times (i.e. after touching something else)	Yes No
Are staff movements restricted in the direction of clean (i.e. the packing) end of the process towards dirty (i.e. the raw meat receiving) end of the process?	Yes No
Does the plant use casual labour (i.e staff employed for less than one month and paid on a per hour basis)?	Yes No
Do plant employees change their jobs frequently (every few days or more frequently) to prevent boredom?	Yes No
Processing practices	
What is the overall processing environment target air temperature?	

Is salt stored under dry conditions that prevent its contamination?	Yes No
If brine is used, is it made fresh every day (or more frequently)?	Yes No
Does the brine ever accumulate fatty scum or deposit sludges of solid salt mixed with meat residue in the brining container?	Yes No
Is brining undertaken at 3^{o}C or lower?	Yes No
If liquid brine (i.e. not salt crystals) is used, what is the ratio of meat to brine?	Yes No
Is fresh brine used for each batch of meat?	Yes No
Is brine recycled for more than one batch?	Yes No
If dry cure are used, is the brine that forms free draining? (i.e. the meat doesn't sit in the created brine)	Yes No
If dry cure are used, is the meat restacked part way through the salt treatment?	Yes No
Is the dripping/drying area well drained (i.e. no standing water on the floor)?	Yes No N/A
Does waste removal (e.g. melted water, general detritus) from the processing area happen on a continual or near continual basis?	Yes No
What is the height of lowest section of any rack that is used to hold meat? (in cm)	
Are final product packing materials stored under dry conditions that prevent contamination?	
How is the CSM cooked?	Convection oven Steam oven Continuous flow oven

	Other – please state
Is the temperature of the meat monitored?	Yes No
If so, how is the monitoring undertaken?	
How is the meat cooled?	Blast chiller Shower with water Other – please state
Is the temperature of the meat monitored after cooling?	Yes No
If so, how is the monitoring undertaken?	
Does the plant have a target for the rate of cooling?	Yes No
If, so what is the minimum acceptable rate of cooling?	^{o}C per minute
What is the storage temperature of the finished product prior to shipping?	
Are final products Vacuum packed (VP)? Modified atmosphere packed (MAP)?	 Yes No Yes No
If MAP, what gas mix is used?	
If MAP, what ratio of product volume to gas volume is used?	

Are final products and raw meat held in physically separate locations?
Are there safeguards in place (e.g. different colours/types of container) to ensure crates/boxes/trays used for transport of raw meat are not used for finished product?
If so, what are they?
Do you test the final product for *L. monocytogenes*?
Is the testing presence/absence or numbers?
Do you ever get out of specification (OOS) results?
Do you tell your Local Authority (LA) when you get an OOS result? Yes No
If applicable, what corrective actions do you take when you get an OOS result? N/A Undertake a retest Yes No Undertake a deep clean of plant environment and equipment Yes No Use the contaminated product for shelf life testing Yes No Other please state Yes No

Is testing of the processing environment carried out?	Yes No
What methods are used?	
Where are samples taken?	
Are test results stored?	Yes No
If so, how is the information used (e.g. to trend historical data and identify when conditions are moving out of spec)	Trending Effectiveness of cleaning team Stored, not used
Does the FBO determine product water activity?	Yes No
Does the FBO determine product pH?	Yes No
How was shelf life determined?	
Would guidance on shelf life determination be useful?	

What is the preferred format for any guidance	Booklet/Pamphlet Video Workshop Online decision support tool
Are you aware of the BRC/CFA/FSA 2010 Guidance ('Shelf Life of Ready to Eat Food in Relation to *L. monocytogenes* – Guidance for Food Business Operators'	Yes No
Is there product traceability?	
Is the FBO aware of the EC 2073/2005 microbiological criteria regulations for *L. monocytogenes* and ready to eat foods?	Yes No
Does the FBO have a HACCP plan?	
Cleaning and sanitation	
Is there wet cleaning mid- shift? (i.e. is there splashing of *L. monocytogenes* from drains)	
What is the cleaning regime in place?	
Does the plant have equipment washing and cleaning SOPs that are undertaken outside of processing?	

If so, are these SOPs validated as effective for the control of *L. monocytogenes*?	
If applicable, do these SOPs cover:	N/A
Cutting boards	Yes No
Knives	Yes No
Brine injectors (if applicable)	Yes No
Cooked meat slicing machines	Yes No
Chillers	Yes No
Drains	Yes No
Employee PPE (gloves and aprons)	Yes No
Door handles and equipment controls (e.g. electrical switches and oven instrument controls)	Yes No
If applicable, what is the frequency of cleaning/sanitation for:	N/A
Cutting boards	
Knives	
Brine injectors (if applicable)	
Cooked meat slicing machines	
Chillers	
Drains	
Employee PPE (Personal protective equipment e.g. gloves and aprons)	
Door handles and equipment controls (e.g. electrical switches and oven instrument controls)	
Does the plant have an arrangement in place that allows for microbiological testing?	Yes No
If so, is a contract lab used or do they operate their own testing facilities?	Contract lab
	Own lab

Is the effectiveness of cleaning and sanitation periodically checked?
If applicable, how are these checks done? N/A Swabbing and micro lab testing Yes No Agar press slides Yes No ATP machine Yes No Protein residue sticks Yes No
If applicable, what hygiene indicator bacteria do you test for? N/A Total aerobic mesophiles Yes No Total aerobic psychrotrophs Yes No *E. coli* Yes No Coliforms Yes No The *Enterobacteriaceae* Yes No Other (please state) Yes No
If applicable, when are the cleaning efficiency checks done? N/A Immediately after cleaning Yes No Immediately before commencement of the day's production Yes No
Are cleaning and sanitising chemicals changed periodically? Yes No
If so, why does the FBO change their chemicals?

What water source is used for cleaning?
If applicable, are dilutions of cleaning and sanitising equipment undertaken according to manufacturer's instructions Yes No
Product packaging
Where does the FBO store packaging?
How does the FBO ensure that packaging does not become contaminated?
Is packaging periodically tested to ensure it has not become contaminated during storage? Yes No

18 APPENDIX 3 QUESTIONNAIRE USED FOR ENVIRONMENTAL HEALTH OFFICERS

Dear [*EHO Name*],

The FSA aims to achieve a reduction in the number of human cases of, and deaths from, listeriosis in the UK by 2015, to be accomplished through consumer- and industry-focused activities. Recently, the Food Standards Agency asked our research team to gather evidence and determine the key risks and control points in the production of cooked sliced meats (CSM). The ultimate output is to inform the development of tailored support tools to assist food businesses operators (FBOs) and environmental health officers (EHOs) in their work. Outputs from this work should improve the collective understanding of *Listeria* control in businesses producing these foods, particularly small and medium-sized enterprises (SMEs), aiding risk assessment and the implementation of interventions to reduce *Listeria* in the food chain.

We are inviting your input to help us identify what aspects of CSM processing (defined as the manufacture of CSM from raw meat and excluding those plants that buy pre-cooked meats just for slicing) FBOs and/or EHO enforcers would like help with to ensure the hygienic production of CSM. In particular we need to determine:

1. What areas of CSM inspection you perceive to be problematic or otherwise difficult to enforce, causing you concern.

2. The best way that additional technical guidance could be supplied to you and FBOs

Please can you answer the questions below. Answering a question with a score of 1 means that it is something you consider to be a low priority, either because there are no issues with the topic or because you think the topic is trivial. A score of 5 is for something that you think should have the highest priority because there are knowledge gaps in the field or because the topic is important.

There are two columns for your answers, the one on the left is for your opinion in the context of FBOs. The left hand column is for you to inform us of any areas that you think FBOs struggle and would benefit from assistance. The right hand column is for your opinion in the context of EHOs and allows you to inform us of areas that you think you and your colleagues would find additional information helpful. After you are finished with your answers, please click the button at the bottom of the screen to save your responses. All of the responses are completely confidential.

Basic Food Safety Principles	Rating (1=low, 5=high)	
Would there be benefit in providing assistance with:	Answer for FBOs	Answer for EHOs
1. Basic HACCP and the concept that HACCP principles can help with the control of generic microbiological hazards (i.e. general microbiology that is not specific to *Listeria* spp.)?	Not answered	Not answered
2. Characteristics and good practices **targetted for the control of key hazard organisms such as *Listeria***	Not answered	Not answered

monocytogenes

3. Understanding, controlling and monitoring salt, pH, temperature and the use of vacuum/modified atmospheres as multiple interventions that can be used in combination to help prevent the growth of *L. monocytogenes*	Not answered	Not answered
4. Assessment of the impacts of changing product formulation using predictive modelling tools	Not answered	Not answered
5. Is there anything you consider important for basic food safety that is currently poorly understood? (500 character limit)		

Food handlers	Rating (1=low, 5=high)	
Would there be benefit in	Answer for FBOs	Answer for EHOs
6. Enhanced training specific to *L. monocytogenes* that should be completed by CSM food handlers	Not answered	Not answered
7. Information regarding return to work procedures for food handlers known (or suspected) to be recovering from an infection by an agent capable of causing foodborne disease	Not answered	Not answered
8. Is there anything you consider important for food handlers that is currently poorly understood? (500 character limit)		

Raw materials	Rating (1=low, 5=high)	
Would there be benefit in information describing good practices for:	Answer for FBOs	Answer for EHOs
9. Raw materials sourcing criteria (e.g. controls in place slaughterhouses, environmental temperatures, packaging and other conditions during transit to the CSM plant)	Not answered	Not answered
10. Common raw material acceptance criteria on delivery (e.g. *Listeria* status of the meat, physical condition, odour, temperature abuse evidence such as excessive drip, yellow fats)	Not answered	Not answered
11. It is understood that cooking CSM is a critical control point (CCP). However *Listeria* can become long term resident in the environment of a CSM plant and raw meat received can be the source of the plant resident strains. In that context, is there anything you consider important that is not well understood for raw materials received? (500 character limit)		

Plant infrastructure and hygiene	Rating (1=low, 5=high)	
Would there be benefit in information describing good practices for:	Answer for FBOs	Answer for EHOs

12. Requirements for physical separation of the raw product receiving area and product preparation, processing, final product storage and packing areas.	Not answered	Not answered
13. High care area requirements for cold-smoked or dried sliced meats	Not answered	Not answered
14. High risk area requirements for CSM	Not answered	Not answered
15. Water quality for brines and cures in contact with meats	Not answered	Not answered
16. Management of cleaning and sanitation of the factory and the frequency and type of monitoring effectiveness	Not answered	Not answered
17. Is there anything you consider to be important for plant infrastructure hygiene (500 character limit)		

Processing and shelf life	Rating (1=low, 5=high)	
Would there be any merit in good practices relating to	Answer for FBOs	Answer for EHOs
18. The effective monitoring and control of cooking processes	Not answered	Not answered
19. Post process handling of products including strategies for keeping cooked products free from *Listeria* until they are packed.	Not answered	Not answered
20. Determination of shelf life (including frequency of day of production and end of life sampling and testing for *L. monocytogenes*)	Not answered	Not answered
21. Is there anything you consider to be important, but not well understood or that you have particular concerns about relating to processing and product shelf life (500 character limit)		

Statutory testing for *L. monocytogenes*	Rating (1=poor understanding, 5= excellent understanding)	
	Answer for FBOs	Answer for EHOs
22. Do you think that people have a good working understanding of EC 2073/2005 and *L. monocytogenes* testing for CSM, which is a ready-to-eat food?	Not answered	Not answered
23. Do you think that people in your region know that if they have not undertaken work to show that *L. monocytogenes* numbers stay below 100 CFU/g CSM **for the entire shelf life of the product**, that the testing they should do is to confirm the absence of *L. monocytogenes* in 25g of CSM at the end of manufacture?	Not answered	Not answered
24. Do you think that people in your region know that the statutory test method for *L. monocytogenes* numbers is ISO 11290-2; and for presence/absence testing is ISO 11290-1 and if a lab uses a different test method, the method has to be validated against the reference test	Not answered	Not answered

method?

25. Do you think that staff in your region know that FBOs manufacturing RTE, which may pose a *L. monocytogenes* risk for public health, are required to sample the processing areas and equipment for *L. monocytogenes* according to ISO 18593:2004?

Not answered | Not answered

26. What areas do you consider to be problematic, difficult to enforce or high risk for CSM manufacture?

27. What environmental and food sampling, if any, do you carry out?

28. On what basis do you decide how and when to carry out inspections and samplings?

29. How do you decide when enforcement action should be taken?

In what format should any guidance be provided?	Rating (1=low, 5=high)
30. Web-based written guidance	Not answered
31. Web-based interactive guidance	Not answered
32. Training courses and workshops	Not answered
33. Booklets or pamphlets	Not answered
Another format you think would be best (500 character limit)	
34. Roughly, how many plants manufacturing CSM from raw meat do you estimate to be in your region?	Not answered