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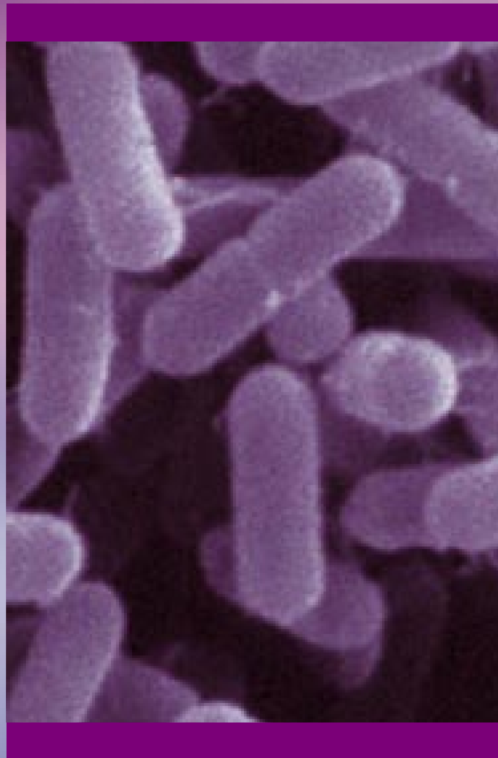

The Control and Management of *Listeria monocytogenes* Contamination of Food

Microbiology

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LIST OF ABBREVIATIONS

a_w	Water Activity
CFU/g (ml)	Colony Forming Units/Per Gram or (Per Millilitre)
DAF	Department of Agriculture and Food
DCMNR	Department of Communications, Marine and Natural Resources
EHO	Environmental Health Officer
EU	European Union
FDA	Food and Drug Administration
FSAI	Food Safety Authority of Ireland
FSPB	Food Safety Promotion Board (*Safe*food)
GHP	Good Hygiene Practice(s)
GMP	Good Manufacturing Practice(s)
GP	General Practitioner
Gy	Gray
HACCP	Hazard Analysis and Critical Control Point
HHP	High Hydrostatic Pressure
HPSC	Health Protection Surveillance Centre*
HSE	Health Service Executive
HTST	High Temperature Short Time
ISO	International Organisation for Standardisation
kGy	KiloGrays
LAB	Lactic Acid Bacteria
MAP	Modified Atmosphere Packaging
MPa	Megapascals
NSAI	National Standards Authority of Ireland
OFML	Official Food Microbiology Laboratory
OJ	Official Journal
ppm	Parts Per Million
Quats	Quaternary Ammonium Compounds
RTE	Ready-to-Eat Food(s)
SCF	Scientific Committee for Food
SCVMRPH	Scientific Committee on Veterinary Measures Relating to Public Health
SI	Statutory Instrument
SOP	Standard Operating Procedure(s)
SPC	Statistical Process Control
SSOP	Sanitation Standard Operating Procedure(s)
USDA	United States Department of Agriculture

* Formerly the National Disease Surveillance Centre.

1. INTRODUCTION

1.1 Background

Listeria monocytogenes is a bacterium that is commonly present in the environment and in food. Most people who are otherwise healthy and not pregnant may tolerate exposure to quite high levels of the bacterium in their food. The organism is important because infection in pregnancy is associated with a risk of severe infection of the foetus or newborn infant that may result in death. Some people are also exceptionally vulnerable to severe *L. monocytogenes* infection as a result of pre-existing illness. Significant progress has been made in recognising foods which are at risk from *L. monocytogenes* contamination and in developing strategies and processes that can minimise these risks. This report was prepared by the Food Safety Authority of Ireland (FSAI) Microbiology Sub-committee and endorsed by the Scientific Committee and the Board of the FSAI.

1.2 Purpose of the Guidelines

The FSAI has published this report to raise awareness and to help minimise the potential risks of foodborne human infection with *L. monocytogenes*.

1.3 Scope

This report is intended to be a source document for food businesses. However, much of the detail will also be useful for members of the public, health care professionals and public policy makers seeking information on foodborne listeriosis. Although control of *L. monocytogenes* contamination is important in all areas of food processing, catering and retailing, *L. monocytogenes* contamination poses particular hazards and challenges in relation to ready-to-eat (RTE) foods and related products.

Due to the specific challenges posed by *L. monocytogenes* for the RTE food sector, this report will place particular emphasis on RTE food production. It is important to recognise that some food products (hot dogs, frankfurters, some composite meals etc.) may frequently be consumed without adequate re-heating or other preparation notwithstanding specific recommendations from the manufacturer with respect to reheating. It is apparent, for example, that failure to reheat thoroughly has contributed significantly to outbreaks of *L. monocytogenes* associated with hot dogs and frankfurters in the United States [1-2].

Ready-to-eat food means food intended by the producer or the manufacturer for direct human consumption, without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganism(s) of concern [3]. Ready-to-eat food intended for infants and young children is food specifically intended for infants and young children as defined in Commission Directives 91/321/EEC and 96/5/EC, when they are used like RTE food [4-5]. RTE food intended for special medical purposes is dietary food for special medical purposes, as defined in Commission Directive 99/21/EC when it is used like RTE food [6].

The effective control of *L. monocytogenes* is product, process and facility* specific. This report is neither an exhaustive nor a definitive statement on prevention of *L. monocytogenes* contamination in every situation. Likewise, not all of the measures recommended apply to every food production facility. Each food business is responsible for ensuring that the measures to control *L. monocytogenes* in operation in their facility are appropriate to local conditions.

1.4 What is *L. monocytogenes?*

Listeria monocytogenes is a ubiquitous bacterium. It is part of the genus *Listeria* which has six identified species: *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri and L. grayi* [7-10]. It may be found in the intestines of animals and humans without causing illness [1]. It is widely distributed in many natural and man-made environments such as soil, water, sewage, vegetation and silage where it can survive for long periods of time [1-2]. *L. monocytogenes* can also be found on walls, floors, drains, ceilings and equipment in food processing environments [7, 11]. It has been isolated from a wide variety of RTE foods including seafood, vegetables, fruit, dairy products, salads and meat products [2, 12-16].

1.5 What Foods are at Risk?

Outbreaks of listeriosis associated with the consumption of coleslaw and soft cheese in the early 1980s led to increased recognition of *L. monocytogenes* as a foodborne pathogen [13, 17-18]. Risk factors typically associated with foodstuffs linked to outbreaks of listeriosis include:

1. The food is ready-to-eat.
2. The food requires chilled storage.
3. The food will have an extended shelf-life.
4. The food receives no listericidal processing.
5. There is a risk of post-process contamination.
6. The product formulation supports the growth of *L. monocytogenes*.
7. The food may be consumed by immunocompromised individuals and pregnant women.

Foods which are typically associated with *L. monocytogenes* contamination include certain meat, poultrymeat and fish products (e.g. frankfurters, pâté, smoked salmon, fermented raw meat sausages etc.), dairy products (e.g. soft cheeses, unpasteurised milk etc.) and prepared salads (e.g. coleslaw, bean sprouts etc.) [1-2].

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* Facility means the buildings and physical structures used for or in connection with processing, storage, packaging, labelling, holding, or transport of RTE foods.

1.6 Health Risks and Clinical Features

The first reported case of listeriosis was reported in 1953 after a stillbirth was linked to a mother's consumption of raw milk from a cow suffering from mastitis [1]. Listeriosis is conventionally considered an uncommon but potentially life threatening invasive infection that primarily affects identifiable "at risk" sections of the population [19]. The median incubation period for the infection is 21 to 28 days but can range between one to 70 days [20].

The minimum number of *L. monocytogenes* that must be ingested to represent a significant risk of disease in humans (i.e. infectious dose) is uncertain. Indications are that the infectious dose is low, possibly less than 1,000 cells [21]. However, investigation of cases of listeriosis indicates that implicated foods are those that support the growth of the pathogen and have elevated levels of the pathogen prior to consumption [21-23]. The minimum infectious dose is also thought to be highly strain and host-dependent [21, 24]. *L. monocytogenes* has 13 identified serotypes [9, 25]. All of these may be associated with human listeriosis however, the majority of human infection is associated with the serotypes 1/2a, 1/2b or 4b [9, 21, 26].

Worldwide serotype 4b has been implicated in up to 50% of all cases of human listeriosis and in particular, 4b is associated with outbreaks [21, 26]. However, the frequency with which these serotypes are isolated from human cases does not parallel the occurrence in food. For example, serotype 4b strains are not the strains most frequently isolated in foods [27] which indicates that this serotype is particularly virulent for humans [9, 25, 28-30]. Variability in virulence can help explain why sporadic cases and outbreaks of listeriosis are uncommon although worldwide the prevalence of *L. monocytogenes* in foods ranges from 1-10% [21, 31].

Healthy, non-pregnant people rarely suffer from life threatening clinical illness as a result of exposure to *L. monocytogenes* in food [21]. When *L. monocytogenes* is acquired through consumption of food, the organism colonises the intestinal tract from which it penetrates the gastrointestinal mucosa, enters the blood-stream and reaches other body tissues. In rare cases, *L. ivanovii* [32-33], *L. seeligeri* [34] and *L. innocua* [35] have been implicated in human listeriosis.

People with impaired immune function due to illness or medication are at risk of invasive disease, as are pregnant women. Features of invasive listeriosis include high fever, shivering, severe headache, neck stiffness and nausea [14]. Listeriosis in pregnancy may be associated with miscarriage, stillbirth or premature birth of an infant with life threatening infection [1-2, 21]. Foodborne invasive listeriosis occurs worldwide as outbreaks or sporadic cases.

In recent years, the distinct clinical entity of *L. monocytogenes* associated febrile gastroenteritis has been recognised. *L. monocytogenes* febrile gastroenteritis affects previously healthy people and is not confined to the groups at risk from invasive listeriosis [36-38]. The clinical illness is characterised by aches, fatigue, fever, chills, nausea, vomiting and diarrhoea and is generally self-limiting.

This clinical entity has been recognised in outbreak settings. As routine examination of specimens of faeces from patients with gastroenteritis for *L. monocytogenes* is rarely, if ever, practiced it is likely that many cases of this condition go unrecognised.

1.7 Surveillance in Ireland

1.7.1 Clinical surveillance

In Ireland, between 2000 and 2003 there was an average of 6.5 cases of listeriosis per annum (i.e. 0.17 per 100,000 of the population per annum) [20, 39-40]. In 2000 there was one reported fatality when an adult patient died from *L. monocytogenes* meningitis [20].

Estimates for the United States place the number of cases annually at 1,850 with 425 listeriosis associated deaths reported annually [41]. The estimated annual incidence of invasive foodborne listeriosis is two to 15 cases per million [42]. A programme of enhanced surveillance for human listeriosis administered through the Health Protection Surveillance Centre (HPSC) has been in operation since 2000. Listeriosis (i.e. *L. monocytogenes* infection) became a notifiable human infectious disease in Ireland on January 1st 2004 [43].

1.7.2 Food surveillance

The microbiological testing of foods is conducted by a number of official agencies under service contract to the FSAI (Table 1).

Table 1. Official Agency Involvement in Microbiological Testing of Foods

Official Agency Laboratories	Samples Taken By:	Sample Type
Health Service Executive (HSE) Official Food Microbiology Laboratory (OFML)	Environmental health officers (EHOs) and in limited cases by other official agency staff	All foods in retail and catering premises Foods of non-animal origin at wholesale and production level
Dept of Agriculture and Food (DAF) [1]	Veterinary inspectors, agricultural officers, dairy produce inspectors/ officers, private veterinary practitioners	Products of animal origin
Dept of Communications, Marine and Natural Resources (DCMNR) [1-2]	Sea fisheries officers	Fishery products and live bivalve molluscs

[1] In addition, both DAF and DCMNR use approved private laboratories
[2] DCMNR does not carry out any in-house analysis

Microbiological results are submitted by the laboratories to the FSAI where they are stored in databases for further analysis. The Official Food Microbiology Laboratory (OFML) database has been in operation since 1999 and is currently the most comprehensive database available. Information stored in this database includes sample type, sample source and parameters tested (Table 1). In 2003, 10,337 qualitative tests (i.e. for presence or absence) and 5,318 quantitative tests for *L. monocytogenes* were carried out in the OFML on RTE foods. Almost 40% of the samples tested quantitatively were from the category of meat and meat products, game and poultry while 28% of samples tested quantitatively were prepared dishes (Table 2).

Table 2. RTE Foods Quantitatively Tested for *L. monocytogenes* [1]

EU Category Code(s)	EU Food Category	No. of Samples	% Total
3	Meat and Meat Products, Game and Poultry	2,119	39.8
17	Prepared Dishes	1,487	28.0
1	Dairy Products	405	7.6
6	Soups, Broths and Sauces	263	4.9
4	Fish, Shellfish and Molluscs	255	4.8
8	Fruit and Vegetables	215	4.0
7	Cereals and Bakery Products	217	3.5
2	Egg and Egg Products	188	3.5
13	Ices and Desserts	136	2.6
5/9/10/11/12/14/15/16/18/19/20/21	Others	33	0.6
Total Number of Samples		**5,318**	

[1] Data source: OFML, 2003

The microbiological safety of these samples (Table 2) was assessed using the national microbiological guidelines for RTE foods [44]. Overall, 5,284 (99.36%) samples were classified as satisfactory, 19 samples (0.36%) as acceptable and 15 (0.28%) as unacceptable/potentially hazardous with respect to *L. monocytogenes* [44].

1.8 What are the Economic Consequences?

A comprehensive economic assessment of the impact of *L. monocytogenes* in the RTE food sector in Ireland is not available. However, the experience of other countries, in particular the United States, suggests that the impact of even a small *L. monocytogenes* outbreak could be significant in Ireland.

In 2002, the second largest poultry company in the United States was forced to recall over 13,000 metric tonnes of chilled and frozen RTE turkey and chicken products suspected to be contaminated with *L. monocytogenes*. The turkey and chicken products were distributed to retail stores, restaurants, schools and institutions across the United States over a five month period. It was the largest meat product recall in American history [45].

Prior to this *L. monocytogenes* outbreak, the facility responsible for the production of the products had been cited for 40 sanitation violations in less than a year, ten of which been issued in the month prior to the outbreak. The violations included meat residues from previous production left on equipment, residues on overhead pipes, leftover meat particles on the conveyor belts and waste water seeping into open containers of frozen meat. At least 50 people became ill from this *L. monocytogenes* outbreak. Most affected individuals were hospitalised, at least seven died and three pregnant women had miscarriages or stillbirths [45]. The human and economic consequences of a *L. monocytogenes* outbreak can be minimised by the implementation of appropriate standards and practices in the food industry.

1.9 What are the Challenges?

1.9.1 Control in the food chain

Consumer demand for minimally processed products with a longer shelf-life has resulted in the mass production and distribution of chilled convenience RTE foods. The challenge of controlling *L. monocytogenes* in RTE foods is considerable as the pathogen has a high resistance to heat, salt and acidic pH and can grow at or below refrigeration temperatures [46-49].

Many of the measures which will control and manage *L. monocytogenes* in the food chain are encompassed within good hygiene practices (GHP), good manufacturing practices (GMP), food safety/hygiene training and the implementation of a Hazard Analysis Critical Control Point (HACCP) system. The implementation of a HACCP system by food businesses and the provision of food safety training for all employees is a legal requirement * [50-53]. However, additional measures may be required to manage and control *L. monocytogenes*.

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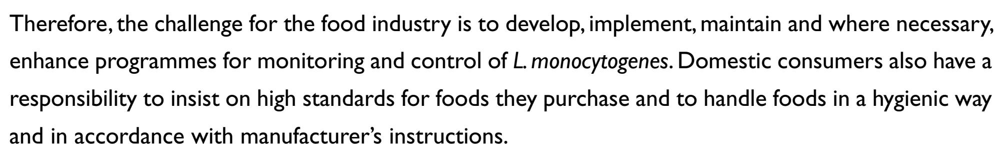
* Regulation (EC) No. 852 of 2004 on the hygiene of foodstuffs will replace Directive 93/43/EEC from 1st January 2006. As a consequence, the current national legislation (S.I. No. 165 of 2000) will have to be revoked by that time. When produced in the European Community and traded between Member States, products of animal origin intended for human consumption must currently fulfil the public health requirements laid down in 17 separate Council Directives (64/433/EEC, 71/118/EEC, 72/461/EEC, 77/96/EEC, 77/99/EEC, 80/215/EEC, 89/362/EEC, 89/437/EEC, 91/492/EEC, 91/493/EEC, 91/494/EEC, 91/495/EEC, 92/45/EEC, 92/46/EEC, 92/48/EEC, 93/43/EEC and 94/65/EC). These Directives will continue to apply until the 1st of January 2006 when they will be replaced with Regulation (EC) No 853/2004. Directive 2004/41/EC (OJ L195, p12, 02/06/2004) will repeal these 17 existing directives and amend Directives 89/662/EEC and 92/118/EEC and Decision 95/408/EC while leaving the implementing decisions in force.



Therefore, the challenge for the food industry is to develop, implement, maintain and where necessary, enhance programmes for monitoring and control of *L. monocytogenes*. Domestic consumers also have a responsibility to insist on high standards for foods they purchase and to handle foods in a hygienic way and in accordance with manufacturer's instructions.

1.9.2 Surveillance, detection and treatment

The challenge for public health care is to ensure that there is sufficient professional and public awareness of listeriosis and its associated risk factors. In addition, resources should be available to ensure that listeriosis is recognised and appropriately treated when it does occur and that the necessary public health action(s) are taken promptly in response to recognised cases.

1.10 Risk Communication

Both the food industry and consumers should be aware of their respective responsibilities and role in the control and management of *L. monocytogenes*. Responsible agencies on the island of Ireland such as the FSAI and *Safe*food-The Food Safety Promotion Board (FSPB) must communicate these risks to the food industry and consumers respectively.

2. FACTORS AFFECTING SURVIVAL AND GROWTH OF *L. MONOCYTOGENES*

2.1 Introduction

Understanding the factors that impact positively and negatively on the ability of *L. monocytogenes* to survive and proliferate in food and in the food processing environment is essential to the development and management of effective *L. monocytogenes* control measures. These factors are reviewed briefly in this section and are discussed in greater detail in Appendix 1.

2.2 Survival and Growth in Food

The growth of *L. monocytogenes* in foods is dependent on the intrinsic characteristics of the product (e.g. pH, water activity), the extrinsic characteristics of the product (e.g. storage temperature, relative humidity) and processing techniques (e.g. cooking, non-thermal processing) used in its production. Some of the growth and survival limits for *L. monocytogenes* are shown in Table 3 [Adapted from 3, 10, 21, 54-60].

Table 3. Growth and Survival Limits for *L. monocytogenes*

Parameter	Minimum	Maximum	Optimal	Can survive (but no growth)[e]
Temperature (°C)	-1.5 to +3	45	30 to 37	-18°C [f]
pH [a]	4.2 to 4.3	9.4 to 9.5	7.0	3.3 to 4.2
Water Activity (a_w)[b]	0.90 to 0.93	> 0.99	0.97	< 0.90
Salt (%)[c]	< 0.5	12 to 16	N/A	≥ 20

[a] Hydrochloric acid as acidulant (inhibition is dependent on type of acid present)
[b] Sodium chloride as the humectant
[c] Percent sodium chloride, water phase
[d] When growth rate is highest
[e] Survival period will vary depending on nature of food and other factors
[f] A temperature of 70°C/2min is required for a 10^6 reduction in numbers of *L. monocytogenes* cells
N/A Not Applicable

The principal factors that influence the survival and growth of *L. monocytogenes* in food are temperature, pH and water activity (a_w). As with other bacteria, the tolerance of *L. monocytogenes* to particular environmental constraints (processing and/or storage conditions) is greatest when all other conditions are optimal for growth. However, it has also been demonstrated that previously stressed cells (e.g. exposure to sub-lethal heating before process heating) can be more resistant to additional stresses [55] (Section 2.3.1).

L. monocytogenes has an optimum growth temperature of between 30 - 37°C at neutral or slightly alkaline pH (i.e. pH ≥ 7). However, *L. monocytogenes* can also grow at refrigerated temperatures < 5°C (Table 3). The generation time (i.e. growth rate to double population) can vary from 1.1 to 131 hours depending on temperature and other factors [55,58].

The limits for survival and growth outlined in Table 3 are based on research carried out primarily using laboratory media which provided an environment more conducive to survival and growth of *L. monocytogenes* than those which would be found in food. The reasons for this are that interactions in food are more complex than in laboratory media. It has been determined that some non-growth conditions for *L. monocytogenes* in foods would include [3,21]:

1. pH 5 to 5.5 and $a_w < 0.95$
2. pH < 5 at any a_w
3. $a_w \leq 0.92$ at any pH.

2.3 Survival and Growth in the Environment

Extensive research has addressed the survival, growth and control of *L. monocytogenes* in the food processing environment, in particular in relation to the RTE food sector. As in food, the ability of *L. monocytogenes* to survive and grow over a wide temperature and pH range is important in the food processing environment (Table 3). Two additional issues, the general stress response of the bacterium (Section 2.3.1) and biofilm formation (Section 2.3.2) may be particularly relevant to survival in the food processing environment.

2.3.1 General stress response

Under prolonged exposure to adverse environmental conditions such as sub-lethal temperature or acidic conditions, *L. monocytogenes* may develop a stress response (Appendix 1) [55, 61]. This adaptation is an important consideration in the control and management of the *L. monocytogenes*. For example, sanitation, using sub-lethal concentrations of sanitisers, can result in the development of a more resistant *L. monocytogenes* population in the processing environment which may then contaminate food (Appendix 2) [22].

2.3.2 Biofilm formation

L. monocytogenes, like many other bacteria can grow as planktonic cells or as a biofilm. Individual cells, growing dispersed in a liquid or semisolid matrix are the planktonic form and are the classical growth phase studied in microbiology. *L. monocytogenes* can also grow as surface attached communities of cells embedded in an extra-cellular polysaccharide matrix known as a biofilm (Appendix 2.9) [62]. Biofilm growth is important because in this form the bacteria are more resistant to physical and chemical agents intended to kill the bacteria and are able to survive for extended periods with minimal nutrient supply. Surface biofilm particularly in locations which are difficult to identify and clean can act as a persistent source of food contamination through the release of *L. monocytogenes* from the biofilm [62-64].

2.4 Control of *L. monocytogenes*

While there are many different processing methods available to treat food and control *L. monocytogenes*, only two, namely thermal processing and irradiation currently have an established role for the definitive destruction of this and other pathogens in foodstuffs. All other available methods, while having a role as additional safeguards, don't on their own, currently represent acceptable measures for the definitive control and/or elimination of *L. monocytogenes* in foods (Appendix 1.2 - 1.3).

Hurdle technology refers to the concept of achieving control of a risk of contamination of food by combining in series, a number of measures that would not individually be adequate for control. Each individual control measure is considered a hurdle to the survival and growth of pathogens. A hurdle may be based on temperature (e.g. cooking), (e.g. drying, adding salt/sugar), acidity (e.g. pickling), redox potential (e.g. fermentation), preservatives (e.g. adding salt) and other measures [22].

Hurdle technology allows food businesses to address safety issues through application of a series of milder preservation steps and consumer demands for convenient foods that are minimally processed. An example of a food preserved by hurdle technology is a pre-packaged, sliced, cooked ham. This product is preserved using a combination of curing, cooking, chilling and modified atmosphere packaging (MAP) (Appendix 1.6).

Measures to reduce levels and control *L. monocytogenes* in food must go hand in hand with measures to minimise the risk of recontamination of food from the food processing environment. Environmental control is a particular challenge for the food industry. GHP, GMP and a food safety management system based on the principles of HACCP (Section 3) are fundamental for the effective development and implementation of a *L. monocytogenes* monitoring and control programme (Section 4).

3. GENERAL PATHOGEN CONTROL FOR FOOD PROCESSING

3.1 Introduction

It should be assumed that any surface or material which comes into contact with food is a potential source of microbial contamination. *L. monocytogenes* poses a particular challenge in this regard as it is a common environmental pathogen that can become established in a food processing environment and repeatedly contaminate work surfaces.

In the case of RTE foods, the challenges are greatest because production frequently involves extensive processing and packaging after cooking. In addition, there may be an opportunity for *L. monocytogenes* proliferation in the product during storage and distribution and consumers are typically not expected to perform any listericidal step before consumption.

The production of microbiologically safe food is fundamentally based on the implementation and application of general preventative measures, GHP and GMP. These measures are essential controls to implementation of a food safety management system based on the principles of HACCP [53]. Pathogen specific controls such as those targeting the control and management of *L. monocytogenes* (Section 4) can only be meaningful in the context of these controls. Adequate standards must be achieved in respect of the following 16 headings (Sections 3.2 - 3.17). Some controls are particularly pertinent to the control of *L. monocytogenes* and this will be indicated where necessary. More detailed information related to these controls is extensively outlined in Irish standards published by the National Standards Authority of Ireland (NSAI) [65-70].

1. Standard Operating Procedure(s) (SOP)
2. Raw Materials
3. Facility Location, Design and Structure
4. Equipment Design
5. Zoning
6. Environmental and Storage Temperature
7. Water Supply
8. Maintenance
9. Sanitation
10. Personal Hygiene
11. Packaging
12. Storage and Distribution
13. Waste Management
14. Pest Control
15. Recall and Traceability
16. HACCP.

3.2 Standard Operating Procedures

Standard operating procedure(s) (SOPs) are sets of instructions which food businesses follow to complete specific tasks consistently, efficiently and safely. SOPs also allow food businesses to comply with any relevant legislation or standards applicable to their activities [50-52]. SOPs should be written for all food business activities including cleaning, sanitising, raw material handling/delivery, storage and distribution, cooking, packaging, personal hygiene, temperature monitoring, equipment maintenance, recall and traceability, pest control etc. There should also be an SOP for writing an SOP. This should be a template used to write any SOP for any purpose. At a minimum, SOPs should contain the following information:

1. SOP number
2. SOP title
3. Section of food business the SOP is applicable to
4. Approved by
5. Revision number
6. Issue date and review date
7. Number of pages
8. Purpose
9. Scope
10. Responsibility
11. Definitions
12. Related records
13. Procedure to be followed.

3.3 Purchasing and Delivery of Raw Materials

It should be assumed that any raw material entering a facility is a potential source of contamination. Good practice in relation to purchasing and delivery of raw materials is as follows [71]:

1. All food businesses should have a written supplier approval procedure and raw materials should only be sourced from approved suppliers.
2. All raw materials including food-grade packaging materials, gases, lubricants and other chemicals should be purchased to agreed written specifications and comply with any legislative requirements.

3. Written specifications should include information relevant to end-product safety and quality and should be drawn up in consultation with the supplier:
    a. Raw material description and production details
    b. Microbiological, chemical, physical and sensory criteria
    c. Packaging and labelling requirements
    d. Recall and traceability requirements
    e. Legislative requirements
    f. Distribution, delivery and storage conditions
    g. Agreed tolerances if applicable on any of the above
    h. Monitoring procedures to be undertaken by supplier
    i. Notification procedures between supplier and customer.
4. Raw materials should be inspected on delivery to ensure that they are in an appropriate condition (meet the raw material specification) and accompanied by appropriate documentation.
5. Regular supplier audits should be conducted.
6. All raw materials should be stored on site in a manner which prevents chemical, physical or microbiological contamination or deterioration.
7. Raw materials which require controlled temperature storage should be stored at 5°C unless otherwise legislated for, including the following [52]:
    a. Pasteurised milk 6°C
    b. Fresh poultrymeat 4°C
    c. Carcass meat and cuts 7°C
    d. Edible offal 3°C
    e. Prepared cook-chill food 3°C [71]
    f. Chilled minced meat 2°C
    g. Frozen food -18°C or colder.
8. Raw materials should be stored and controlled appropriately after delivery.

3.4 Facility Location, Design and Structure

Important in the control of *L. monocytogenes* (and other pathogens) is the location, design, construction and layout of a processing facility to minimise the growth of *L. monocytogenes* and risks of cross contamination and recontamination. Poor design and layout of premises and equipment will hamper efforts to effectively sanitise facilities (Appendix 2).

The facility should be surrounded by a concrete border/footpath to discourage pest entry. The footpath also allows traps and bait stations to be positioned around the facility. Drainage surrounding the facility should be maintained to reduce or eliminate shelter areas for pests (Section 3.15). Slope the grounds for adequate drainage. Locate outdoor lighting fixtures (including insect control devices) away from buildings and aim them towards the buildings to keep flying insects (that are attracted to light) away

from doors and windows. For similar reasons, internal insect control devices should be located where they will not attract insects from the exterior. In addition, to reduce pest harbourage, weeds under fences or other barriers should be controlled. Grass and shrubs should also be kept short and trimmed regularly.

All areas within a facility and in particular, areas in which RTE foods are exposed to the environment (i.e. high-risk areas) during processing or packaging, should be designed to facilitate sanitation and minimise moisture accumulation. In relation to facility design and layout, general features which will improve food safety include [65-70]:

1. Materials used in construction should be smooth, non-porous, non-absorbent and easily cleaned and sanitised. Walls, floors and ceilings should not have holes or cracks which could act as a reservoir for contamination
2. All windows and doors should have tight fitting insect guards to prevent entry of pests. Window sills and ledges should be sloped
3. Dead spaces should be avoided. Dead spaces are places wherein a product, ingredient, cleaning or sanitising agent, or pest may be trapped, retained or not completely removed during sanitation and processing procedures
4. Where feasible, eliminate overhead fixtures and equipment particularly in high-risk zones
5. Avoid overcrowding of equipment to allow access for effective sanitation procedures.

3.4.1 Floors and drainage

Floors and drains are a confirmed source of *L. monocytogenes* contamination. Therefore, the design and layout of floors and drainage systems is particularly important. General features should include the following [65-70]:

1. Floor drainage commensurate with operation and sanitation procedures should be provided throughout a facility
2. All floors should be designed to drain areas rapidly and prevent pooling
3. All floors should be curved at junctions with walls and sealed to prevent water and residues building up at these junctions
4. Trench drains should be avoided
5. Drains should have adequate capacity and be trapped inside and outside the facility
6. Water and waste material should be directed to grated drains, located away from processing and packaging equipment. The drains should be equipped with rodent screens
7. Equipment which generates waste water should be plumbed directly to a drain
8. Where feasible, manholes within the facility should be avoided. If present, they should be doubly sealed and secured to prevent overflow
9. Drains should not be interlinked, should exit the facility separately and separate systems of drainage should be provided for:
   a. Low and high-risk zones (e.g. effluent water)
   b. Buildings, surrounds, guttering (e.g. storm water)
   c. Amenity and sanitary facilities (e.g. foul sewer)
10. Where feasible, waste from processing should be piped directly to drains.

3.4.2 New facilities and renovations

There is evidence to suggest that construction work in an area where food is exposed can increase the risk of *L. monocytogenes* contamination. The increased risk might be due to dust dispersed during construction [26,72]. However, a greater risk is the introduction of a more virulent strain of *L. monocytogenes* into the processing facility from an outside source (e.g. second-hand equipment) or through disturbance of a potential source of contamination (e.g. replacement of floor drains or walls).

During construction work where normal production continues, it is essential that the construction area be effectively isolated to maintain hygienic operation. Environmental monitoring should be increased during construction, renovation and maintenance (Section 4.6.1). The operating and production capacity of any proposed new facility should be determined taking into account the estimated maximum daily throughput of products expected. Provisions should also be made for the possibility of increased throughput in the future. Any legislative requirements should also be taken into account [50-52]. Reference should be made to the NSAI publications which also outline structural requirements [65-70].

3.5 Equipment Design

Good hygienic design and construction of equipment (i.e. avoidance of poor seals, cracks, crevices, poor welds, hollow tubes, dead spaces etc.) will help reduce the risk of *L. monocytogenes* contamination in the finished product. The following measures are important in equipment design [11-12,26,72]:

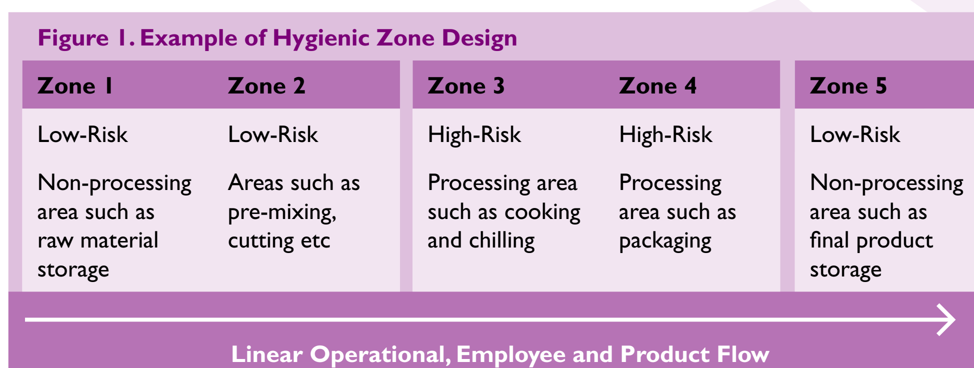
1. Dead spaces in equipment should be avoided. Dead spaces are places wherein a product, ingredient, cleaning or disinfecting agent, or pest may be trapped, retained or not completely removed during cleaning or processing procedures
2. Quality equipment which is easy to clean and made of non-porous, non-absorbent materials such as stainless steel should be used
3. Welds on equipment should be ground and polished
4. Bolts or rivets should not be positioned on or above product contact surfaces
5. Equipment should be moveable, where possible, with surfaces safely accessible for sanitation purposes
6. Conveyor belts should not come into contact with the processing floor
7. All rollers (common with conveyor belts) should be sealed at both ends
8. Simple equipment designs with fewer moving parts should be used were possible
9. Corners or dead space in equipment should be avoided as these are difficult to sanitise. Contour surfaces to facilitate drainage as much as possible
10. Seams, gaps or crevices in equipment should be avoided where possible
11. Taps, hoses, water/steam/condensation lines should not leak

12. Areas where processed product is exposed on equipment should be covered to prevent external contamination from above (e.g. from dripping condensation)
13. Food grade lubricants that contain additives such as sodium benzoate should be used to prevent *L. monocytogenes* growth if the lubricant becomes contaminated with food residue
14. Condensation from air conditioning/cooling/freezing units should be piped directly to drains as condensation is an identified source of *L. monocytogenes* contamination
15. Equipment which generates waste liquids should be piped directly to a drain
16. Equipment which generates aerosols, such as pumps or cleaning/sanitising equipment, should be adequately shielded to prevent spread of aerosols in the processing environment
17. Food contact surfaces of equipment including water hoses should be kept off the floor*
18. Cooling units, refrigerators and insect control devices should not be placed above exposed product
19. Non-hand operated equipment (e.g. knee-operated), particularly at wash-hand basins and footbaths should be used
20. Insulation used in equipment should be covered to prevent it becoming wet.

3.6 Zoning

Facilities should be designed to ensure the physical separation of raw and finished product areas. Separation of facilities into hygienic zones (i.e. hygienic zone design) of low and high-risk, will allow linear product flow (i.e. raw to finished product) (Figure 1) and restrict movement of employees and equipment through the facility [48, 73]. Hygienic zone design should provide physical barriers/partitions between zones (e.g. walls, floors, ceilings, doors and windows) and incorporate positive air pressure systems (Section 4.5.3).

Figure 1. Example of Hygienic Zone Design



Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
Low-Risk	Low-Risk	High-Risk	High-Risk	Low-Risk
Non-processing area such as raw material storage	Areas such as pre-mixing, cutting etc	Processing area such as cooking and chilling	Processing area such as packaging	Non-processing area such as final product storage

Linear Operational, Employee and Product Flow

* Food-contact surfaces are surfaces that contact food for human consumption and include utensils and the food-contact surfaces of equipment.

The compartmentalisation of operations within each hygienic zone will enhance the separation of raw ingredients and finished products. Zone specific facilities e.g. vehicles, equipment, utensils and employees should be considered to prevent cross contamination. Areas (Section 3.13) used for storage of equipment and utensils from raw (i.e. low-risk zones) and finished product (i.e. high-risk zones) should be separated to prevent recontamination of equipment and utensils used for finished products.

3.7 Environmental and Storage Temperature

Environmental temperature is important both for food safety and employee health and safety. Compromises have to be made in relation to employee comfort (i.e. working conditions) and food safety. Most high-risk (e.g. chilled ready meals manufacturer) RTE food processing facilities should be operated at 12°C (ideally 10°C) particularly in areas where product ingredients are prepared, processed, assembled and packaged [(52)]. While these environmental temperatures still permit the growth of *L. monocytogenes*, growth is retarded [(55)]. Temperatures lower than 10°C can make working conditions for staff uncomfortable.

Environmental and storage temperatures must be maintained, controlled and monitored at all times. Storage areas should be designed to maintain the correct product temperature. Raw materials should be stored separately from finished products. Practices which involve the shutting down of power required for environmental and storage temperature maintenance should not be implemented under any circumstances. Contingency plans should be available to ensure the continued safety of products in the event of power failures.

3.8 Water Supply

All water (including ice) used as an ingredient and for preparation of food must be of potable quality [(74-75)*]. Where water entering premises is not of potable quality, or where quality is unreliable, appropriate treatment should be applied to the water before use [(74-75)]. In the production and the placing on the market of live bivalve molluscs, the use of clean sea-water is also permitted [(76)].

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* Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy Official Journal L 327, 22/12/2000 P. 0001 – 0073 will repeal (European Communities Quality of surface water intended for the abstraction of drinking water, Regulations, S.I. No. 294 of 1989) on the 22/12/2007.

3.9 Maintenance

While routine maintenance work is essential in the operation of any food processing facility, it is important to ensure that work of this nature does not lead to contamination of processing areas. Standardised maintenance schedules (Section 3.2) should be implemented to decrease the potential for contamination of equipment due to unscheduled maintenance work. Access restrictions that apply to other personnel in a facility should also apply to maintenance personnel. It is important to ensure that maintenance personnel are provided with protective clothing and instructed in effective facility and personal hygiene practice while work is carried out (Section 3.11).

When non-critical maintenance work is necessary, it should be carried out after normal shift processing has finished. If this is not possible, maintenance work should be scheduled at a normal break in processing. In situations such as equipment breakdown that requires immediate maintenance, production should stop until the problem is rectified. Any product which was in process during the breakdown should be stored appropriately until production begins again. In all cases, complete sanitation of the equipment and the area is essential before processing resumes (Appendix 2). Use of area specific maintenance equipment is recommended to minimise cross contamination risks (Figure 1).

3.10 Sanitation

Sanitation is cleaning with detergents followed by disinfection (i.e. sanitising) (Appendix 2.1). Sanitising is the treatment of a clean surface with a chemical (e.g. sodium hypochlorite) or process (e.g. ultraviolet light) that is effective in eliminating or reducing numbers of microorganisms without adversely affecting the safety or quality of a product.

To ensure that a facility and equipment are clean, sanitised and suitable for their intended use, company management should draw up and operate a written sanitation programme and SOP (Section 3.2). These are detailed in Appendix 2. The sanitation programme should also include any vehicles which may be used to transport foods. Sanitation programmes designed to control *L. monocytogenes* should take account of the risks posed by the formation of biofilms on food contact surfaces (Appendix 2.9) [63]. Successful control requires consistency and attention to detail. The correct choice of detergent, cleaning equipment, sanitising agent, sanitising procedures and frequency of sanitation are all important (Appendix 2).

3.11 Personal Hygiene of Food Workers

The NSAI and FSAI have produced a range of publications which cover in detail, the main aspects of personnel hygiene [65-70, 77-79]. Training and supervision of all staff in food safety and hygiene, commensurate with their work activity is a legal requirement (Section 4.6.1.6) [50-52]. However, staff working in high-risk areas should receive additional training designed to help minimise the cross contamination of the finished product. Furthermore, staff involved in sanitation procedures should also receive additional training to ensure that general (Section 3) and specific procedures (Sections 4-7) designed to manage and control *L. monocytogenes,* are implemented and operated correctly. In all cases, managers and supervisors should lead staff by example in relation to personal hygiene.

3.12 Packaging

Packaging materials such as plastic wraps should be kept clean and dry to reduce risks of contamination. Packaging storage must be maintained at a high standard of hygiene (e.g. clean, dry and pest-free) as often, packaging material comes into direct contact with finished products. In packaging areas, cooling units should have dehumidifying capability. It is important that floors are kept clean and dry and vehicular and pedestrian traffic through packaging storage areas is restricted. All packaging must conform to the current legislative requirements for materials and articles intended to come into contact with food [80].*

3.13 Storage and Distribution

In storage, foods should be physically separated into raw and RTE products and if possible, each stored in separate storage areas. If pallets are used it is recommended that they are made of plastic, kept in good condition and kept clean and dry. If pallets are kept in good condition there is less risk of *L. monocytogenes* growth, proliferation and transfer from one surface to another. Standards of hygiene should be maintained throughout the distribution process and in particular, maintenance of the cold-chain to minimise survival and growth of *L. monocytogenes* [65-70].

3.14 Waste Management

Adequate facilities for handling waste and inedible and condemned materials should be provided (Section 4.5.7). All waste containers should be sited as far as possible from production and air intake areas. Sufficient lockable, marked and identifiable watertight, insect and rodent proof storage facilities should be provided to hold inedible and condemned materials pending daily removal. The waste storage areas should be physically separated from edible product departments [81]. Mobile waste bins should be cleaned and sanitised before re-entry to production areas.

Adequate demarcation between edible and inedible materials should be maintained during production. The control of inedible materials should be clearly demonstrable by way of properly marked and designated facilities.

* Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food came into force on 3rd December 2004, except for Article 17 on traceability which will enter into force on 27 October 2006.

All external bins should be sited in designated areas, which are suitably paved and drained. They should be covered at all times and emptied as required. The covers should be designed to prevent insect contact with the waste materials. All waste areas, equipment and utensils should form part of the sanitation programme (Section 3.10) [81].

3.15 Pest Control

Pest control is the reduction or eradication of pests. Pests will include rodents (e.g. rats, mice), birds, insects (e.g. flies, cockroaches, weevils) and other animals (e.g. dogs, cats) that can contaminate and infest foods. As far as is practical, pests must be excluded from the facility premises and the immediate area surrounding a facility. Effective pest control will not be possible unless all the other controls (i.e. Sections 3.2 - 3.14) are implemented and monitored.

3.16 Recall and Traceability

The development and implementation of a food recall and traceability system is a legal requirement for all food businesses [50]. Even the best managed and operated food businesses will, on occasion, have a food safety issue which may require recall of product. A reliable recall and traceability system will allow the business to minimise risks to consumer health, financial loss and damage to the businesses reputation. The FSAI and the EU have produced guidance on food recall and traceability for industry [82-83].

3.17 Food Safety Management (HACCP)

Together with the implementation of general controls (Sections 3.2 - 3.16), all food businesses must develop a food safety management programme based on the principles of HACCP. This programme will be a documented plan which describes how the food business monitors and controls their processes to produce safe food. Food businesses carrying out any stage of production, processing or distribution of food, after primary production, must develop, implement and maintain a food safety management system which incorporates the principles of HACCP [51-52].

HACCP is a structured systematic approach to improving food safety, which involves identifying potential hazards and measures for their monitoring and control. HACCP applied in a logical and systematic approach will help reduce or eliminate biological, chemical and physical contamination of products. Recent publications have addressed general and specific aspects of the introduction and implementation of HACCP system under the aforementioned legislation [53,84].

3.18 Recommendations

1. A food safety management system based on the principles HACCP with regular reviews should be developed and implemented.
2. Written SOPs supporting the food safety management system should be developed and implemented for all business practices.
3. Raw materials should only be sourced from approved suppliers.
4. The facility location, design and structure should support separation of processing into hygienic zones.
5. All equipment should be simply and hygienically designed.
6. Operations, product and employee movement within the facility should follow a linear path through hygienic zones.
7. Environmental temperatures in production areas should be 12°C while storage areas should maintain finished products/perishable raw materials at 5°C unless otherwise indicated (Section 3.3).
8. Only potable water (or clean sea-water for live bivalve molluscs) should be used in food processing.
9. Facilities maintenance work should be carefully co-ordinated and controlled to minimise risks of *L. monocytogenes* contamination.
10. A managed sanitation programme should be developed, implemented and maintained.
11. All employees and management should be trained in food safety and hygiene commensurate with their duties and responsibilities and in accordance with FSAI training guides.
12. Packaging storage areas should be maintained to a high standard of hygiene as often packaging material comes into direct contact with finished products.
13. Foods should be physically separated into raw and RTE products and if possible, in separate storage and refrigeration areas.
14. A waste management programme should be developed and implemented with adequate facilities for handling inedible and/or condemned materials.
15. A pest control programme should be implemented.
16. A product recall and traceability system should be developed, implemented and tested regularly.

4. *L. MONOCYTOGENES* CONTROL IN FOOD PROCESSING

4.1 Introduction

As outlined in Section 3, effective pathogen control can only be achieved on a solid foundation of GHP, GMP, recall and traceability and a food safety management system based on the principles of HACCP. Targeted control of *L. monocytogenes*, in RTE food processing and related sectors focuses on three specific objectives:

1. Reduction of the frequency and level of contamination in the processing environment
2. Reduction of the frequency and level of contamination in the finished product
3. Reduction of the frequency and level of recontamination in the processing environment and the finished product.

Implementation of targeted control of *L. monocytogenes* begins with a general review of controls (Section 3) to assess those aspects that are specifically important for the control of this pathogen. After development and implementation of controls and HACCP, *L. monocytogenes* contamination is normally production line, equipment and/or site specific. Often, this will be associated with persistent *L. monocytogenes* present in the processing environment [12, 71, 85-86]. Industrial experience has pointed to recontamination after a listericidal step in the process as the main source of contamination in many commercially prepared RTE foods [62].

4.2 What is *L. monocytogenes* Control?

Control must be facility and process specific. The following elements are required:

1. Inclusion of processing steps such as heat treatment (e.g. pasteurisation or cooking) which will destroy *L. monocytogenes* (i.e. listericidal processing steps)
2. Identification of sources of contamination (i.e. assume everything entering a facility is contaminated)
3. Implementation of measures to eliminate or reduce sources of contamination pre- and post-processing.

4.3 Listericidal Processing Steps

Where feasible, food processors should incorporate a listericidal processing step (e.g. cooking meat products to 70°C for two minutes) into the production of RTE and other at risk foods [59]. Thermal processing steps such as cooking and pasteurisation are very effective in reducing numbers of *L. monocytogenes* as is irradiation (Appendix 1). In some foods, a single intrinsic or extrinsic variable (e.g. pH, a_w, temperature) may be used as a listericidal step. However, it is recommended that a combination of variables is used (i.e. hurdle technology) (Section 2.2). All listericidal steps should be validated to ensure their effectiveness [43]. For foods which receive a listericidal processing step, the risk is largely determined by the potential for recontamination of the product during subsequent processing or packaging [1].

4.4 Identification of Sources of Contamination

Many studies have attempted to identify the sources of *L. monocytogenes* contamination. The results of these studies vary. However, to minimise and control *L. monocytogenes* contamination of RTE foods, it is necessary to understand and quantify the various contributions of different sources of contamination to the contamination of finished products. Three primary sources of contamination identified include [48,89-92]:

1. Raw materials
2. Environment and equipment
3. Personnel.

Research has shown a seasonal effect on prevalence of *L. monocytogenes* in the processing environment, with more positive results detected in the summer months than during the winter months particularly in meat and fish processing [12,86].The exact reasons for increased prevalence in the summer months are unclear, however, changes in throughput and difficulties in controlling temperature in the summer may contribute to increased prevalence. Interestingly, soft cheese made from raw milk was identified as the cause of listeriosis outbreaks in Switzerland between 1983 and 1987. An elevation in the occurrence of listeriosis was observed during the winter months [47]. However, in all cases, the impact of such factors on contamination is likely to be facility and/or line specific.

Studies in different food processing facilities across Europe over the past ten years have indicated that the principal source of *L. monocytogenes* contamination in foods prior to consumer purchase, preparation and consumption is the food processing environment [93]. Research in Northern Ireland indicated that the incidence of *L. monocytogenes* in milk in processing facilities was up to six times higher (33.3%) than in samples of the same milk (5.3%) taken at the farm level [94-95].

Table 4. Sources of Contamination in the Processing Environment

Direct	Indirect
Conveyors	Drains
Spiral/Blast Freezers	Floors/Gangways
Containers	Walls
Hand Tools	Ceilings
Protection Clothing e.g. White Coats	Maintenance Equipment
Racks Used in Transporting Finished Product	Cleaning Equipment
Filling and Packaging Equipment	Transportation Equipment e.g. Forklift
Dicing, Slicing, Blending Equipment	Insulation in Walls or Around Pipes
Injection Brines and Other Solutions	Air, Steam, Condensation
Packaging Materials	

Control of *L. monocytogenes* should emphasise the common or direct routes of product contamination. The most common direct route is contact with a contaminated surface (Table 4). This route is particularly important where a food (e.g. meat) receives its final listericidal processing step (e.g. cooking) and before it is packaged [11, 72]. The environment can also provide an indirect source of contamination (Table 4).

If control of *L. monocytogenes* in the environment can be established, then the risks of product contamination (e.g. food contact surface contamination) are reduced [73]. The significance of a particular route of contamination will depend on the nature of processing (i.e. listericidal step included or not in the process), the type of food processed (e.g. meat, vegetable, egg or dairy product) and the processing environment itself (e.g. plant conditions, air temperature, relative humidity etc) [87].

4.5 Recognising and Reducing Risks of Contamination

4.5.1 General operational issues

Operational issues and problems that increase the risk of contamination may arise on a day-to-day basis. When these issues arise it is important that processors recognise the risks and take extra precautions to prevent *L. monocytogenes* contamination. Many of the issues may be avoided or solved with effective production planning and employee training [92]. Some commonly encountered issues are listed in Table 5.

4.5.2 Control of humidity and moisture

Due to the nature of food production, the facility environment can contain a lot of moisture both on surfaces and in the air. Control of moisture is important because bacteria cannot grow without water and general transfer of contamination from surfaces is minimised if surfaces (e.g. floors, walls and equipment) are clean and dry. Some practices which will help control moisture in the processing environment include:

1. Wet processing areas should be isolated from other areas
2. Areas of standing water should be eliminated or removed as soon as possible
3. Water hoses should be removed from processing area(s) before start of production
4. Practices which produce aerosols should be avoided (e.g. use of high pressure hoses)
5. Cooling units should have a dehumidifying capability
6. Humid air should be exhausted to the outside of the facility to aid drying
7. Air should be heated within zones after sanitation to aid drying
8. Only authorised personnel should alter environmental temperature, air extraction and intake and humidity settings.

Table 5. Issues Which May Lead to Contamination [1]

Category	Issue or Problem
Facility Design and Structure	Construction works (e.g. building new walls)
Facility Services	Rubbish bins not being routinely emptied, maintained or cleaned/sanitised
Employees	Employees who are unfamiliar with business/processing procedures Employees moving between hygienic zones
	Cleaning of equipment parts on the processing floor. Poor personal hygiene practices Inadequate training of employees
Sanitation	Sanitation procedures during production (e.g. mid-shift clean-down). Poor sanitation carried out, resulting in re-sanitation of affected area during production shifts
Maintenance	Repairing equipment, floors, drain blockage etc
	Installation of equipment newly purchased, from storage or from another facility
Equipment	Equipment failure and breakdowns Alteration of production lines (e.g. moving of equipment) Product or product debris left on equipment
	Frequent changing of packaging material and production line speeds due to changes in products being produced Commissioning of new equipment or equipment from another facility or storage Raw product found in cooked area and vice versa
Production	Difficulty in meeting sanitation schedules due to production schedules Movement or modification of a production line Heavy production schedules Using out of date stock (e.g. poor stock rotation) Frequent product changeovers Stagnation of product flow through processing

[1] See sections 3.3 to 3.17 for further details

4.5.3 Control of air quality and ventilation

Air can have a number of uses in food processing including drying, agitation, packaging, conveying, operating equipment, cleaning or refrigeration. Some facilities will operate under positive air pressure on the finished product side relative to the raw side. In this system, high-risk zones (Figure 1) will be maintained at a higher pressure (i.e. above atmospheric pressure) than an adjacent low-risk zone, thereby preventing contaminated air entering the high-risk zone (Figure 1). Calibrated pressure measuring and recording devices should be used to monitor positive air pressure systems.

Compressed air has been identified as a source of *L. monocytogenes* contamination [26]. Compressed air is sometimes used to clean equipment, particularly packing equipment at the end of processing prior to sanitation. However, the use of compressed air as a cleaning aid should be minimised. If compressed air must be used, the air should be dry (i.e. to minimise microbial growth) and filtered at the point of use. The filters should be maintained and replaced as per manufacturer's instructions as these can become contaminated with *L. monocytogenes* also. Air from compressed air lines implicated as a source of *L. monocytogenes* contamination has been traced to niche environments near the point of use (Section 4.5.4) [26].

Research has not identified atmospheric air as a direct source of *L. monocytogenes* contamination in foods [90, 96-97]. However, some research has indicated that *L. monocytogenes* can survive in aerosols for extended periods. This suggests practices which produce aerosols should not be used in the processing environment [98]. In general, air quality and ventilation should be carefully controlled to minimise the formation of visible condensation. Ultraviolet light can be used to treat air at intake points to reduce levels of contamination. It is important to prevent the entrance of non-sterile air during draining or venting. Air vents on equipment, ventilation fans and ducts should also be monitored.

4.5.4 Elimination of niche environments

While isolated contamination with *L. monocytogenes* may occur in the processing environment, repeated contamination is more likely to occur after *L. monocytogenes* has become established in a niche environment. A niche environment is a localised site (e.g. hollow cavity in a roller on a poorly designed conveyor belt) in which food debris and moisture may accumulate. Populations of *L. monocytogenes* may become established and persist in a niche and sometimes form biofilms (Appendix 2.9).

When *L. monocytogenes* becomes established in niche environment normal sanitation procedures may become less effective. Poorly designed equipment is particularly vulnerable to the establishment of niche environments. If *L. monocytogenes* does become established in a niche, the equipment can become a substantial source of contamination. As equipment is operated, *L. monocytogenes* from the niche can become deposited on food contact surfaces. As a product moves over or through this region it can become contaminated and subsequently spread contamination to other areas of the facility as it moves through production.

Table 6 illustrates some identified niche environments associated with food processing facilities. Many of these are associated with equipment design or operational failure [11, 26]. The risk of contamination can be reduced by identifying the niche environment (i.e. source of *L. monocytogenes* contamination), revising the sanitation procedures and improving the sanitary design of equipment. It is important to note that the costs associated with identifying and eliminating a niche are offset by the potential costs associated with a product recall or outbreak of foodborne illness related to contamination of a product.

Table 6. Potential Niche Environments

Area	Example
Ancillary Items	Rubbish Bins, Skips
Ancillary Services	Compressed Air Lines, Hollow Bump Guards on Bottoms of Doors, Plexiglas Shields
Personnel Hygiene	Wash Basins, Aprons, Gloves
Plant Hygiene	Cleaning Equipment
Premises	Cracked Walls, Floors, Ceilings, Wet Insulation, Standing Water, Switches
Equipment	Hollow Rollers on Conveyors, Conveyor Belts, Slicers, Dicers, Mincers, Weighing Scales, Switches, Rubber Seals, Open Bearings, Equipment Motor Housings, Hollow Frames, Ice Makers, Damaged Pipe/Hoses, Hollow Box Cutters, Brine Injection Equipment, Packaging Equipment Hand Tools, Hoppers, Valves

4.5.5 Curing, brine injection and marination

Curing (i.e. direct immersion in a curing solution and/or dry rubbing with salt), brine injection (i.e. using brine solutions and needle injection equipment) and marination (i.e. direct application of marinade pastes or solutions by hand, mechanical tumblers etc.) commonly used with meat, fish and some dairy products (Appendix 1.9 - 1.13) are at high risk from *L. monocytogenes* contamination. When using these techniques the following will minimise risks of contamination [81]:

1. All equipment should be adequately cleaned and sanitised before use. Particular attention should be given to needle injection equipment and mechanical tumblers
2. Brine solutions should be covered and refrigerated (≤ 3°C) until use
3. Where curing or marination is taking place, the room/area or equipment (e.g. temperature controlled mechanical tumbler) should be temperature controlled (≤ 5°C), ideally ≤ 3°C
4. Where brine injection is taking place, the room/area should be temperature controlled (≤ 12°C) using brine solutions at ≤ 3°C
5. Unused and used cure, injection and marination solutions should not be stored for further use
6. Overflow brine solutions produced during injection procedures should be discarded

7. If marinades are to be used for basting during cooking or smoking procedures or as a sauce on a finished product, a portion of unused marinade should be reserved for this purpose
8. All curing, marination and injection equipment should be disassembled and sanitised as per manufacturer's instructions after each use.

4.5.6 Rework (reprocessed products)

Rework is a common consequence of food processing operations. The HACCP system should include provision for the safe processing of rework (Section 3.17). In most processing operations there will be circumstances where finished product may not meet customer specifications and be returned to the processor (e.g. returned under-weight product) or unused product and/or raw materials remain unused after a production shift. In all these cases rework should be viewed as high-risk material. Contingency plans should be available to ensure the safe handling of rework material. Rework material which is deemed unsafe (e.g. microbiological, chemical or physical) or illegally salvaged material (e.g. irradiated, unfit material) should never be re-used or reprocessed [99-100].

Rework is a potential source of contamination and is best avoided through careful production planning. The risks of contamination can be high if contaminated returns or rework which becomes contaminated during storage, subsequently contaminates a new batch on a different production run. If rework is unavoidable or permitted it must be carefully controlled. Unprotected food (i.e. not packaged) which comes into contact with a contaminated surface (e.g. falls on the floor) should be discarded to waste (Section 4.5.7) and not reprocessed as rework.

4.5.7 Inedible and condemned materials

All inedible and condemned materials must be disposed of as outlined in Section 3.14.

4.6 Measures to Verify Control of *L. monocytogenes*

4.6.1 Environmental monitoring programmes

It is strongly recommended that all food processors use environmental monitoring programmes. These programmes are effective in identifying, preventing the presence of *L. monocytogenes* in foodstuffs and verifying the control of *L. monocytogenes* in the processing environment [3]. An environmental monitoring programme will help verify the ongoing effectiveness of the sanitation programme and also help determine if further control measures are necessary to eliminate or reduce contamination (Appendices 3 - 4).

Environmental monitoring should be carried out during production shifts (e.g. at break time) and after sanitation prior to production to assess efficacy. An important part of an environmental monitoring programme is the development of a documented sampling programme which will describe:

1. Sampling procedures
2. Sampling responsibilities
3. Sampling sites indicated on a site plan
4. Actions to be taken in the event of a positive result.

Data collected from environmental monitoring programmes should be evaluated as it becomes available, to allow rapid response to positive findings. Regular reviews of data collected over a defined period (e.g. one month) can help in the identification of trends or patterns. Trends can help reveal problems with control in the processing environment. It is then possible to take corrective action. If no positive cultures are obtained over prolonged periods the effectiveness of the monitoring programme should be reviewed.

Statistical Process Control (SPC) may be used to track results, identify trends and identify the necessity to take action. SPC is a method of monitoring food production by collection of data (e.g. swab results) during processing to eliminate or reduce reliance on end product inspection and testing [101].

The effectiveness of an environmental monitoring programme relies on the design of the programme and the response to positive results [21]. A well organised programme will on occasion, detect *Listeria* species (*e.g. L. innocua*) other than *L. monocytogenes*. Occasional positive results (i.e. for *Listeria* species) should not be seen in isolation as a failure of control but as verification that the monitoring programme is effective (Section 4.6.1.4). An environmental monitoring programme which is not capable of detecting contamination may be misleading as the business believes that the environment is under control when in fact it may not be so.

4.6.1.1 Monitoring for *L. monocytogenes*

Detection of *Listeria* species (*e.g. L. innocua*) in the food processing environment, particularly on food contact surfaces, should be viewed as an indicator of an increased risk of *L. monocytogenes* contamination. However, monitoring for *Listeria* species only may result in failure to recognise the presence of *L. monocytogenes*. Therefore, environmental monitoring programmes should focus on detection of *L. monocytogenes* [26,102]. Detection of *L. monocytogenes* with additional investigation as appropriate (e.g. molecular sub-typing) contributes to progressive improvements in the understanding of the biology, epidemiology and control of the pathogen in the food processing environment [93, 103].

4.6.1.2 Frequency of environmental sampling

Under certain circumstances, harmonised sampling frequencies at EU level may be set in order to ensure equal levels of sampling are performed across the EU [3]. However, in general, food businesses should determine their own frequency of sampling in the framework of their procedures based on HACCP principles and GHP and GMP [3]. Frequency of sampling should be facility and/or line specific (Appendices 3 - 4) based on knowledge of specific operations and the controls that have been put in place, as well as any available microbiological data.

A generic sampling frequency cannot be specified due to variability of processing facilities and their practices [12]. Under certain

circumstances, the frequency and extent of sampling may increase due to detection of a positive result on a production line (Appendix 3) or to identify a source of contamination [11]. The number of sample units to be taken in routine sampling may be reduced, if a food business can demonstrate by historical documentation, that effective control procedures are in place.

4.6.1.3 Environmental sampling sites

Potential sites of contamination in each hygienic zone (Figure 1) and at each production line, need to be assessed locally by trained staff and priorities for sampling identified, taking account of past experience within a food processing facility. Some suggested sites for environmental sampling in hygienic zones is outlined in Appendix 3. Both food contact surfaces and non-food contact surfaces should be routinely tested although with the emphasis on the latter. Repeated negative results from a particular sampling site may suggest that the sample site is not a good indicator of control [11,13].

During investigations of contamination incidents, the source of contamination is often undetectable unless equipment is operating. This presents a problem to processors in which a significant period of time may pass between detection of *L. monocytogenes* contamination and identification of its source. Further time will then be necessary to implement corrective action and verify that the source of the contamination has been eliminated.

Floor drains should be included in environmental sampling particularly those drains which are present in high-risk zones (Figure 1). Research has indicated that regular monitoring of drains, combined with molecular sub-typing of the isolates, allows for efficient monitoring of persistent contamination in a processing facility [104].

4.6.1.4 Detection of *L. monocytogenes* on food contact surfaces

If *L. monocytogenes* or other *Listeria* species are detected on a food contact surface, follow-up action should include the following:

1. Production batches* which may have come into contact with a contaminated surface should be put on hold, sampled and tested for *L. monocytogenes*
2. The contaminated surface should be cleaned and sanitised
3. Suspect equipment should be dismantled, cleaned and sanitised before being reassembled. If this procedure is not successful in eliminating the contamination, the equipment should be dismantled, all sensitive (e.g. electronic parts) and hazardous components (e.g. lubricating oil) removed, and heat applied (i.e. water/steam > 70°C) to the remaining parts. Heat can be applied in the form of steam or using an oven for smaller parts. Sensitive components should be cleaned and sanitised in accordance with the manufacturers instructions

* Batch means a group or set of identifiable products obtained from a given process under practically identical circumstances and produced in a given place within one defined production period [3].

4. Lubricating oils and grease should be replaced with oils containing a listericidal component
5. Worn or damaged parts in equipment should be replaced
6. Further environmental samples including samples from all previous sample sites should be tested
7. Sanitation procedures and records should be reviewed
8. Any changes or inconsistencies in sanitation procedures, equipment, personnel and records should be identified
9. The causes of the contamination and steps taken to prevent future incidents should be recorded
10. The control and HACCP systems (Section 3) should be reviewed and revised as necessary
11. It should be verified by microbiological testing that environmental and production control has been re-established.

A site layout map of each hygienic zone (Figure 1) which shows the position of equipment in these zones is useful in control and in determining appropriate action. Positive samples detected through the environmental monitoring programme can be marked and dated on the map with dates and used to identify any trends. Organising data in this way allows identification of where contamination first occurs and which sampling sites test positive most frequently [105].

It is expected, that on occasion, positive environmental *L. monocytogenes* samples will be detected in low-risk zones (Figure 1). Results of this nature are useful in alerting the facility to a risk of transfer of *L. monocytogenes* contamination to high-risk zones. Positive results in high-risk zones indicate a serious deficiency in process hygiene and control and a high possibility of product contamination (Figure 1). Detection of positive samples in any zone should initiate the following responses:

1. Review of SOPs
2. Review of access controls/traffic flow to high-risk zones (Figure 1)
3. Review of production procedures
4. Review of plant/equipment design or services as applicable.

The processor may decide to increase environmental sampling and monitoring until the contamination problem in the affected zones is resolved (Figure 1). In so doing, it should be ascertained if controls to prevent contamination of these zones are effective.

4.6.1.5 Detection of *L. monocytogenes* on non-food contact surfaces

The identification of a positive result for *Listeria* species from a non-food contact surface should initiate a review of sanitation SOPs (SSOPs) and GMPs. If positive *L. monocytogenes* results are found on a non-food contact surface such as floors or drains, follow-up action should include the following:

1. The affected area should be sanitised using the appropriate SSOP
2. All adjacent floors and drains should be sampled if the affected area is not physically divided from other production areas
3. Exposed product should be placed on hold until analysis verifies that *L. monocytogenes* is not detected at unacceptable levels in the food
4. The sanitation procedures and records for the affected area should be reviewed
5. Any changes or inconsistencies in previous sanitation procedures and records should be identified
6. The affected area should be re-sampled after sanitation
7. Production/sanitation/personnel practices and procedures should be reviewed and amended as necessary.

4.6.1.6 Investigating the source of *L. monocytogenes* contamination

It is important to note the role employee practices may have in product contamination [92]. Review employee training records and if possible cross reference the presence of specific employees with the site of contamination. When investigating a source of contamination (e.g. a niche environment), individual samples, not composites, should be collected and tested. In addition to the sites for routine monitoring, additional sites on the suspect equipment or production line should be sampled. In addition, samples should be collected more frequently throughout each production shift. Suspect equipment should be sampled while dismantled for cleaning and sanitising. Lubricating oils and grease should also be sampled (Section 4.6.1.4).

4.6.1.7 Final product and raw material testing

Final product and raw material testing should be viewed as a useful part of the overall control of *L. monocytogenes* but it is not acceptable as the sole means of control and it is not an acceptable substitute for an environmental monitoring programme. The safety of foodstuffs is mainly ensured by a preventive approach, such as implementation of GHP, GMP and application of procedures based on HACCP principles [3]. Microbiological criteria can be used in validation and verification of HACCP procedures and other hygiene control measures (Appendix 6) [3,44].

A level of routine final product testing (i.e. processor decides level) is appropriate for most food businesses and it should be performed in a structured manner with the use of a sampling plan. A testing programme for final products or raw materials should specify:

1. Sampling frequency
2. Sampling method
3. Microbiological methodology
4. Response to positive results
5. Record keeping
6. Storage of isolates for a defined period to assist in investigation of problems.

Final product testing has a number of limitations including:

1. Unless very large numbers of samples are tested, contamination may go undetected for extended periods
2. Final product testing can be expensive

3. If a final product is found to be positive for *L. monocytogenes,* this gives no indication to the source/mode of the contamination and controls needed to prevent further contamination
4. Final product testing is seen as more reactive rather than proactive.

If comprehensive and systematic environmental monitoring is implemented, end product testing can be minimised. End-product testing may be performed in response to a positive environmental sample (in particular a food contact surface), a legal requirement or a customer requirement or complaint. When the aim of end-product testing is to specifically assess the acceptability of a certain batch of food or a process, the sampling plans and microbiological criteria currently being proposed by the EU, when adopted, will become the minimum standard (Appendices 5 - 6) [3].

4.7 Assessment of Individual Production Lines

Some food processors will have more than one production line operating in parallel within a facility. A production line will contain a number of pieces of equipment used to produce the final product. In such facilities, parallel production lines in close proximity to a contaminated line are generally unaffected, [26, 87] therefore each production line should be regarded as an independent unit for *L. monocytogenes* monitoring and control. Microbiological sampling plans should routinely survey all production lines at a level sufficient to ensure detection and prevent loss of control. On production lines where there is or was recently a contamination issue, the sampling frequency should be increased to provide enhanced surveillance.

4.8 Shelf-Life

Shelf-life means either the period corresponding to the period preceding the 'use-by' date or the minimum durability date. Food businesses are responsible for determining the characteristics of the products they produce or pack and the subsequent shelf-life [106]. A 'use-by' date will indicate the shelf-life during which a foodstuff will be microbiologically safe for human consumption provided it has been stored correctly (e.g. storage temperature).

Shelf-life of RTE foodstuffs is important for *L. monocytogenes* control because some products may have extended shelf-life's (e.g. > 5 days) due to the intrinsic and extrinsic characteristics of the foodstuff [3, 107] (Appendix 1). In addition, if a food supports the growth of *L. monocytogenes* (Table 3) it is important to determine if the pathogen will grow to unacceptable/potentially hazardous levels during the shelf-life of that product [3]. If it does grow to those levels, the shelf-life of the product should be reassessed.

4.9 Issues Specific to Particular Sectors

The controls outlined above are applicable across many sectors of the food processing industry. Issues that are specific to or require particular emphasis are presented in Section 5.

4.10 Summary

Listeria monocytogenes will continue to be introduced into the food processing environment from a variety of sources. Control must be directed towards preventing *L. monocytogenes* establishment and growth in the processing environment. When effective controls are in place, the principal sources of contamination are often either a niche within the facility or an operational issue resulting in loss of *L. monocytogenes* control. Therefore, attention to detail is the primary component of effective control. Food processors should always co-operate with competent authorities (e.g. FSAI) and inform them when unsatisfactory test results are recorded. This will enable the competent authorities to take swift and effective action, as necessary.

4.11 Recommendations

1. Where possible, a validated and verified listericidal processing step should be included in production.
2. Potential sources of *L. monocytogenes* contamination should be identified.
3. Issues which may lead to contamination of products with *L. monocytogenes* should be identified.
4. Moisture levels within preparation/processing areas should be controlled to minimise the growth and proliferation of *L. monocytogenes.*
5. The use of compressed air as a cleaning aid should be minimised.
6. Niche environments in process equipment and production facilities should be eliminated.
7. Brine and marination solutions should be covered and refrigerated before use and not reused.
8. The use of rework materials should be minimised or eliminated.
9. Rework material which is deemed unsafe or illegal should never be re-used or reprocessed.
10. Environmental *L. monocytogenes* monitoring programmes should be developed and implemented.
11. Data collected from environmental *L. monocytogenes* monitoring programmes should be immediately evaluated to allow rapid response to positive findings.
12. Environmental *L. monocytogenes* monitoring programmes should focus on detection of *L. monocytogenes.*
13. Food businesses should determine their own frequency of environmental *L. monocytogenes* monitoring.
14. Floor drains should always be included in environmental *L. monocytogenes* monitoring programmes.
15. Food contact and non-food contact surfaces should be routinely monitored for *L. monocytogenes.*
16. Routine end product testing for *L. monocytogenes* should be performed.
17. Individual production lines should be regarded as independent units for environmental *L. monocytogenes* monitoring and control.
18. The shelf-life of all products should be determined and verified prior to products being placed on the market.
19. The increased susceptibility of some population groups *to L. monocytogenes* infection should be considered during the development of HACCP plans.

5. SPECIFIC FOOD SECTOR ISSUES

5.1 Introduction

The controls outlined previously (Sections 3 - 4) are applicable across the food processing industry. However, epidemiological investigations have shown that listeriosis is a foodborne illness particularly associated with raw and RTE seafood, meat, poultrymeat, fruit and vegetable produce and dairy products (Section 1.5) [108]. The association of *L. monocytogenes* with these foods has lead to public health incidents, product recalls and large financial losses across the world [45]. This current section briefly emphasises issues specific to particular sectors within the food industry.

5.2 Smoked Fish

Because smoking conditions are not standardised in Ireland the effect of smoking on pathogens such as *L. monocytogenes*, and the inhibitory effect smoking has during storage, may vary for cold smoked and hot smoked fish from different producers (Appendix 1.12). Typically, cold smoked fish products are produced by filleting raw fish, salting or curing, drying and smoking at temperatures between 20°C and 50°C. The fish is then chilled, vacuum packed and distributed through the chill chain. A danger with cold smoked products (e.g. cold smoked salmon) is that *L. monocytogenes* if present, may during the shelf-life (Section 4.8) of the product, reach levels which at the point of consumption may be sufficient to cause illness. The prevention of recontamination of both cold and hot smoked fish is therefore of great importance in terms of food safety. The following practices should help ensure safe production [59, 109-110]:

1. Good quality raw materials from approved suppliers should only be used
2. Washing raw fish in potable water (i.e. typically chlorinated)
3. Frozen fish should be thawed prior to smoking in a refrigerator ≤ 5°C ideally ≤ 3°C
4. Marination or curing should be carried out at refrigerated temperatures ≤ 5°C ideally ≤ 3°C
5. Two thermometers, one to monitor the fish temperature and the other for equipment (e.g. refrigerator, smoker temperature etc.) should be used
6. Where appropriate (hot smoking), ensure an internal temperature of 70°C for two minutes (or 75°C instantaneously) or equivalent to ensure a 10^6 reduction of *L. monocytogenes* cells
7. Finished products should be refrigerated at ≤ 5°C, ideally ≤ 3°C
8. The 'use-by' date (i.e. microbiological shelf-life) of all products should be determined
9. Freezing of finished product to prevent growth
10. Addition of approved preservatives.

Cold smoked salmon even if processed under hygienic conditions, will carry a risk of *L. monocytogenes* contamination. It has been suggested that labels on cold-smoked fish as well as some other RTE foods should indicate that these products may constitute a health hazard for immunocompromised individuals and pregnant women [31]. In addition, consumers who eat certain types of RTE fish products, particularly raw products such as sushi, should be aware of the safety risks (Section 5.8) [73, 86].

5.3 Meat and Poultry Meat Products

Although the prevalence of *L. monocytogenes* in raw meat and poultry differs significantly, some prevalence rates can be as high as 92% [111 - 112]. At slaughterhouse level, *L. monocytogenes* can be endemic, particularly in drains and on floors [89]. In North America, the application of chemicals such as organic acids (e.g. lactic acid) directly onto animal carcasses for decontamination purposes is extensively used [113-115]. In the EU, only potable water is allowed to remove surface contamination from products of animal origin unless use of another substance has been approved* [52].

Poultry can harbour *L. monocytogenes* in their intestinal tract and as such are a potential source of contamination [115]. However, even the best slaughterhouse operations cannot eliminate *L. monocytogenes* from raw meat which goes for further processing. Therefore, food businesses should assume that all incoming raw meat and poultry products are contaminated with *L. monocytogenes* (Sections 3 - 4).

Minced/chopped and emulsified RTE meat products include cooked sausage, frankfurters, salami, cooked mince beef, cooked burgers, pâté, pies, reformed meats and puddings. All these products, by their nature, undergo extensive processing and handling during their production. This leads to greater opportunities for *L. monocytogenes* contamination [12].

For many RTE meat and poultry meat products, the reduction in a_w through cooking (Appendix 1.2) or drying and addition of salt (Appendix 1.9) can reduce the risks of *L. monocytogenes* survival and growth (Table 3). Similarly, the reduction in pH through fermentation of some products and/or addition of acidulants (Appendix 1.11) or surface deposition of inhibitory compounds via smoking (Appendix 1.12) may also be effective listericidal processes [49]. However, food businesses producing these products must determine these characteristics themselves and the associated risks of *L. monocytogenes* survival and growth.

5.4 Unpasteurised Milk

L. monocytogenes has been associated with dairy products and dairy processing facilities [13,47,94,96,108]. The ubiquitous nature of *L. monocytogenes* means that any control programme should first minimise the contamination of raw milk and the possible cross contamination of pasteurised milk with raw milk (Section 5.5). Where raw milk is delivered to a dairy processing facility the delivery and raw milk storage zone and associated personnel must be hygienically zoned or isolated from the processing and packing zones (Figure 1).

5.5 Pasteurised Milk

The processing of raw milk to pasteurised liquid milk and other milk based products such as cheese, involves the use of complex equipment at a processing level [73]. Pasteurisation is a key listericidal step in controlling *L. monocytogenes* in RTE dairy foods.

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* From January 1st 2006.

The use, maintenance and sanitation of pasteurisation equipment should be carefully controlled. It is important that the production of pasteurised milk products does not commence until pasteurisation temperatures are reached (i.e. validated and verified) [52]. The use of specialised equipment such as pasteurisers means that sanitation procedures should take special account of issues such as biofouling (Section 2.3.2 and Appendix 2.9) which can reduce pasteurisation efficiency (Appendix 1.2). Maintenance procedures should also ensure that pasteurisation equipment is effective in preventing cross contamination of raw and pasteurised milk (e.g. cracked or broken pasteurisation plates).

5.6 Cheese

Cheeses are made from both unpasteurised and pasteurised milk (Sections 5.4 – 5.5). The survival and growth of *L. monocytogenes* in cheese depends on the intrinsic (e.g. pH, a_w) and extrinsic (e.g. temperature during ripening, competing microflora, pasteurised or not) characteristics of each variety of cheese (Table 3). The key food safety issues with pasteurised cheese are validation and verification of pasteurisation procedures and avoidance of post-pasteurisation contamination. Therefore, validation/verification procedures, GHPs, GMPs and SSOPs are required to prevent post-process contamination (Sections 1.9.1 & 3.10). However, cheeses made from unpasteurised milk have more complex food safety issues.

In 2000, the Institute of Food Science and Technology in the United Kingdom indicated that potential public health hazards posed by pathogens in cheeses made from unpasteurised milk apply particularly to soft and semi-soft cheeses [116]. The temperature of curd used in the manufacture of soft cheeses is often lower than that of hard cheeses and may be insufficient to destroy *L. monocytogenes* which may be present in unpasteurised milk used in the production of the curd. Microbiological surveys of raw milk conducted in Europe have shown the presence of *L. monocytogenes* in 2.5% to 6.0% of samples [116].

Cheese which supports the growth of *L. monocytogenes* has a pH > 4.2 (typically pH > 5.6) coupled with a high moisture content and includes varieties such as Brie, Camembert, Ricotta and soft Hispanic/Mexican style cheeses [117-118].

Other cheese varieties may not support the growth of *L. monocytogenes* but may allow its survival. Typically, these cheeses will have pH values < 5.6 and/or low moisture contents and includes varieties such as cheddar, feta, and cream cheese [116, 119]. Therefore, unpasteurised cheese with pH (≥ 4.2) may present particular challenges with respect to control of growth of *L. monocytogenes* (Section 5.6). In these cases the microbiological quality of the raw milk is particularly important.

In many cheeses, contamination is typically confined to the surface or just under the rind. The crust of soft cheeses such as Camembert or Brie can carry an increased risk of

L. monocytogenes contamination due to an increase in pH (i.e. acidity level decreasing) at the surface during ripening and the high moisture content of the cheese [116]. *L. monocytogenes* can then grow predominantly on the surface crust of the cheese and not in the core of the cheese.

In the United States, the Food and Drug Administration (FDA) has indicated that aged or ripened cheeses made from unpasteurised milk from healthy animals is comparable in safety to cheeses made from pasteurised milk. However, these cheeses must be ripened for at least 60 days at a temperature of not less than 1.7°C to minimise microbiological risks [120].

There are few documented outbreaks of listeriosis linked to consumption of properly ripened hard cheese [116]. Hard cheeses such as traditional cheddar and Parmesan tend not to favour the growth of *L. monocytogenes*. However, recent research has indicated that *L. monocytogenes* can survive for more than 60 days in cheddar cheese prepared from pasteurised milk [73,116].

5.7 Fresh Produce (Vegetables, Mushrooms and Fruit)

It has been reported that 10-20% of prepared vegetable samples may be contaminated with *L. monocytogenes* [121-122]. *L. monocytogenes* has been found on a wide variety of vegetables including bean spouts, potatoes, lettuce, radishes, cabbage, cauliflower, tomatoes, onions, cucumbers and mushrooms [121-122]. Several outbreaks of foodborne listeriosis have been linked to consumption of fresh produce such as coleslaw [123].

Many fresh produce products (e.g. coleslaw, prepared salad mixes) do not receive a listericidal step as part of their processing [124]. Some producers use animal manure as a growth medium or fertiliser, particularly in the case of mushrooms. Although there have been no reported cases of listeriosis attributed to mushrooms, producers should take steps to control the risks of contamination [125]. Use of organic materials such as animal manure should be carefully monitored. An outbreak of listeriosis in Canada in 1981 involving coleslaw was linked to cabbage harvested from fields fertilised with untreated sheep manure taken from a farm with a history of ovine listeriosis [123].

While prevention of contamination is preferred over corrective actions, once contamination has occurred in fresh produce, sanitisation of fresh produce with chlorine to reduce overall microbial loading, is extensively used throughout Western Europe [126]. It is currently one of the most effective ways to reduce microbial loading on fresh produce [127]. However, there are some concerns regarding the use of disinfectants, particularly if pathogens are not fully eliminated and if the natural competitiveness of indigenous microflora is reduced. Therefore, it is difficult to ascertain how effective disinfection is. In addition, the efficacy of different disinfectants may be dependent on the vegetable type and its microbial loading [128]. More research is required to develop effective treatments [122]. In all cases where a food business is using a disinfectant, that food business must not place produce on the market which is unsafe [50-51].

Fresh produce processors should consider these simple control measures [127]:

1. Good quality raw materials from approved suppliers should be used
2. Temperature control of fruit and vegetables should be implemented
3. Environmental monitoring programme should be implemented
4. Sanitation programme for warehouses and equipment should be implemented
5. All fruit and vegetables should be washed and rinsed in potable water.

Washing is a critical component in the preparation of fresh produce, especially if raw processed fresh produce is to be sold as a RTE food. Washing should always use potable water to [127]:

1. Remove pieces of debris and dirt from produce
2. Reduce microbiological and chemical loading of produce
3. Reduce temperatures of produce to help enhance shelf-life.

The FSAI recommends that the washing stage in fresh produce processing should consist of three separate washing stages and three separate tanks, all using potable water (i.e. recycled water should not be used) [127]:

1. A washing tank to eliminate general field debris and dirt
2. A washing tank containing chlorinated water (50 - 100 ppm free chlorine with 1 - 3 minutes contact time at a temperature between 8 - 10°C and a pH of 6.6)
3. A final stage washing tank using non-chlorinate potable water at 1 - 2°C to remove chlorine residues and enhance produce shelf-life.

5.8 Seafood

Estuary and freshwater environments are often exposed to effluent discharges from municipal sanitary services, industry and agriculture which may be contaminated with *L. monocytogenes*. The salinity of estuary waters does not inhibit *L. monocytogenes*. Raw and processed fish are good substrates for the growth of *L. monocytogenes* particularly if there is poor temperature control [129]. Control of post-harvest contamination of freshly caught fish is a vital step in producing safe RTE fish products [73]. All fish should be put on ice, refrigerated or frozen immediately following harvest.

Typically, *L. monocytogenes* will not be found in fish harvested from open waters. However, contamination can occur prior to the fish reaching the processing facility. Sources of contamination on fishing vessels include water, ice, human waste, birds (i.e. seagulls can be intestinal carriers of

* Please refer to Reference 127 for further details.

L. monocytogenes) and improperly sanitised equipment [130]. The processing environment itself has been identified as a significant source of contamination of RTE fish products [2]. Farmed fish may be carriers of *L. monocytogenes*, as rearing waters (i.e. coastal waters, lakes) can contain *L. monocytogenes* [131]. Additionally, feed for farmed fish can become contaminated with *L. monocytogenes* [54].

Listeria monocytogenes is frequently isolated from RTE seafood including crab, shrimp, prawns, oysters, clams and lobster [129]. Many types of seafood may harbour *L. monocytogenes*. In addition, further processing and extensive handling of many of these products post-cooking, can lead to subsequent contamination. For example, cooked, peeled shrimp/prawns are often mechanically peeled and sometimes split with removal of the intestinal tract. Loss of basic control (Section 3) such as movement of operators between raw and cooked areas (Section 3.6) has led to post-cook contamination of these products [73].

5.9 Fermented Milk Products

The intrinsic nature of fermented dairy products such as cultured buttermilk and yogurt make them low-risk products in terms of *L. monocytogenes* growth and survival. The presence of starter cultures, coupled with low pH and the presence of organic acids retards the growth and survival of the pathogen. However, manufacturers of fermented products should follow GMPs and procedures outlined previously to control both *L. monocytogenes* and other pathogens [73].

5.10 Frozen Milk Products

While *L. monocytogenes* doesn't grow at frozen food temperatures (Table 3) it can survive for long periods in frozen products such as dairy ice-cream [132]. Frozen dairy products which have ingredients such as nuts, fruit or confectionary added directly to a frozen ice-cream mix are of greater concern due to an increased risk from contamination. In addition, condensation (Section 4.5.2) in ice-cream hardening rooms has been implicated as a source of *L. monocytogenes* for moulded or extruded ice-cream products [73,117].

5.11 Butter

While butter is typically made from pasteurised milk (Section 5.5) and often salted, it has been implicated in outbreaks of listeriosis illness due to cross contamination (Sections 4.4 - 4.5) of finished product with *L. monocytogenes* from the processing environment (Section 4.6.1) [133-134].

5.12 Egg and Egg Products

Contamination of eggs with *L. monocytogenes* is rare. The presence of the organism in raw, commercially broken egg due to shell contamination from droppings and the processing environment appears inevitable but it has rarely been isolated from raw liquid egg products [42].

6. CATERING/RETAIL/CONSUMERS: SPECIFIC ISSUES

6.1 Introduction

The principles previously outlined for the control of *L. monocytogenes* in the RTE food processing industry should also apply to the catering and retail food sectors and in domestic situations [68-70].

6.2 Issues for Catering and Retail Sectors

Control of *L. monocytogenes* in the RTE food processing industry should be a routine and integrated part of all food business operations. However, in the catering and retail sectors, control of *L. monocytogenes* may be a very different process due to the nature of these food businesses. Problems which may be encountered in the control of *L. monocytogenes* in the catering and retail food sectors include the following:

1. Resources available are often limited.
2. The variety of foods served means increased risk of cross contamination
3. Poor hygienic zoning, separation or segregation practices and procedures
4. Staff normally perform a variety of duties increasing risks of contamination
5. Training of staff in food hygiene is often limited
6. Environmental or product monitoring is often limited or not performed
7. Sanitation facilities are often limited and procedures may not be documented
8. Temperature control is often limited.

However, the risks of *L. monocytogenes* contamination in the catering and retail sectors may be lower because of the following factors:

1. High-risk foods are typically prepared immediately before service and consumption
2. The volume of food produced and the number of customers are typically small
3. Many RTE foods are bought in pre-prepared and often pre-packaged
4. Greater potential to rotate and sell stock early in its shelf-life.

6.3 Issues for Consumers

A press release in 2003 indicated that over six million cases of food poisoning in Europe and America each year are a direct consequence of poor hygienic practices in the domestic kitchen [135]. A 2003 FSPB survey found that in domestic refrigerators in Ireland, pathogens were commonly found. *Listeria* species was found in 6% of domestic refrigerators surveyed, indicating the risks of food contamination in the home [136].

Consumers should be aware that RTE foods are usually not sterile and may on rare occasions, contain pathogens. The measures required in the domestic kitchen to minimise risks of *L. monocytogenes* contamination entails the application of the basic principles of food hygiene. In public awareness campaigns, the emphasis should be consistently placed on these basic principles rather than on the details of individual pathogens. In addition to awareness of basic measures such as hand washing and separation of RTE and raw food, some traditional food preparation practices should be discouraged. For example, the practice of washing dressed poultry carcasses in the kitchen sink is unnecessary and increases the risk of contamination [137].

6.4 Recommendations

6.4.1 Caterers and retailers

1. Facilities, equipment and practices should limit cross contamination.
2. All fruit, vegetables and prepared salads should be washed in potable water before use.
3. The use of tea towels and other re-usable cloths should be avoided.
4. All refrigerators and storage areas should be regularly cleaned.
5. Food should be purchased frequently and manufacturer's instructions for use including 'use-by' and 'best-before' dates observed.
6. Food, once open, should be protected from cross contamination and used as soon as possible.
7. Where possible, consumers should be provided with clear product instructions.

6.4.2 Consumers*

1. Manufacturer's instructions for product use should always be followed.
2. Foods (e.g. especially soft cheese, pâté etc.) which are past their 'use-by' date should never be eaten.
3. All fruit, vegetables and prepared salads should be washed with drinking water before use.
4. Raw foods should not be stored above or beside RTE foods.
5. Tea towels and other re-usable cloths should be replaced at least daily with clean ones.
6. Refrigerators should be cleaned regularly.
7. Consumers who are not satisfied with hygiene standards in food businesses, should inform the management.

* These recommendations are particularly pertinent to high-risk groups and/or farmers/visitors consuming unpasteurised milk and milk products.

7. PUBLIC HEALTH AND REGULATORY AUTHORITIES

7.1 Introduction

The prevention of human *L. monocytogenes* infections should be the primary objective of any *L. monocytogenes* control programme. While the number of cases of listeriosis in Ireland is small in comparison with other bacterial causes of foodborne illness, the high morbidity and mortality associated with this infection make it a significant public health concern [20]. Failures in *L. monocytogenes* control can result in:

1. One or more cases of human infection

or

2. Detection of unacceptable/potentially hazardous levels of *L. monocytogenes* in a product that has been placed on the market.

Both these failures require prompt action by agencies and individuals charged with protecting public health.

7.2 Human Infection

Human *L. monocytogenes* infections are relatively uncommon but a significant public health concern. As of January 2004, there is a statutory obligation on both laboratory directors and other medical practitioners to notify cases of human listeriosis in Ireland*[43]. Prompt notification of human infection provides the basis for rapid action to protect public health. Every case should be carefully investigated.

7.2.1 Major public health issues

Human listeriosis has a number of important distinguishing characteristics which are relevant to the investigation and control of cases:

1. There are two distinct clinical presentations [1,21]
   a. invasive disease, mainly in well defined high-risk groups, with 20 - 30% case fatality and
   b. acute gastroenteritis, self-limiting in otherwise healthy individuals (Section 1.6)
2. Clinical features are non-specific therefore confirmation of diagnosis is dependent on laboratory results
3. Infection occurs as sporadic cases and as outbreaks
4. Outbreaks may be diffused and widespread (multi-county, multi-national potential) and therefore may be more difficult to recognise
5. Unlike many other foodborne infections, secondary person-to-person spread of infection is not a significant concern

* Since January 1st 2004, 8 human cases of listeriosis have been notified to HPSC (Provisional data up to October 15th 2004) [20].

6. Associated food recalls led to large economic losses and generate considerable public anxiety
7. The median incubation period of 21 to 28 days is relatively long (range 1 - 70 days). As food exposure histories tend to be unreliable if time periods of some weeks previously are included, data on food preferences should also be gathered [20]
8. There is no evidence that laboratory screening of samples of faeces from contacts is of any value.

7.2.2 Sporadic cases

Most cases of listeriosis occur in apparent isolation. A single notified case may, however, be part of an outbreak, particularly as *L. monocytogenes* outbreaks are frequently dispersed in space and time. Between 2000 and 2003, data were received on 26 laboratory isolations of *L. monocytogenes* from human specimens in Ireland, an average of 6.5 cases per annum or 0.17 per 100,000 of the population per annum [20]. There was one reported fatality; an adult patient died from bacterial meningitis due to *L. monocytogenes* in 2000 [20]. The essential steps in the investigation of a case are:

1. Prompt clinical and laboratory notification to the relevant public health authorities as required by legislation [43]
2. All human isolates should be characterised in detail (serotyping and/or molecular typing) and stored for at least one year
3. Patient's general practitioner (GP)/clinician should be informed of the diagnosis prior to contacting the patient
4. The epidemiologic questionnaire should include the following points
   a. demographic data including occupational and medical history
   b. clinical features
   c. exposure data including occupational, farming/animal contact and detailed food consumption and preference data
5. All potentially linked human cases (suggestive symptoms, common risk exposures etc.) should be identified and investigated
6. Environmental investigation should focus on inspection of food safety processes and premises that may be implicated with food and environmental sampling as indicated
7. Food and environmental isolates that are detected in the course of investigation of a human case, should be fully characterised to identify any association with the human case
8. Where judged necessary on grounds of protecting public health, prompt food product tracing and recall should be officially sanctioned. This decision should, ideally, be made with the involvement of a multi-disciplinary team. The team should include microbiologists, public health specialists, EHOs and representatives of national agencies. In situations of exceptional urgency it may not be practical to wait for a formal meeting to convene but some inter-disciplinary consultation, for example by telephone, should take place in all but the most exceptional circumstances. Food producers may also wish to initiate product recall voluntarily even if the evidence implicating a specific food is insufficient to indicate a statutory requirement to do so [50].

7.2.3 Outbreaks

Listeriosis outbreaks are often diffused and spread over a wide geographic area with perhaps several countries or states, involving a common food source. From 1991 to 2002 a total of 18 outbreaks of invasive listeriosis, three cases of gastroenteritis listeriosis and one case of invasive and gastroenteritis listeriosis have been reported in nine different countries in Europe, with a total of 526 outbreak related cases. Table 7 details the outbreaks and associated foods [134]:

Table 7. Listeriosis Outbreaks, Europe 1991-2002

Outbreak Type	Number of Outbreaks	Associated Foods
Invasive [1]	6	Processed Meat Products
	5	Cheese [2]
	3	Processed Fish Products
	3	Undetermined
	1	Butter
Gastroenteritis	1	Rice Salad
	1	Corn Salad
	1	Undetermined
Invasive & Gastroenteritis	1	Frozen Cream Cake

[1] The incriminated product of at least six of these outbreaks was known to have been exported

[2] Three of these products were raw milk cheeses

Surveillance systems and prompt thorough investigation of individual cases are required for the early identification of listeriosis outbreaks. When there is evidence of an outbreak (i.e. two or more linked cases) an outbreak control team should be convened at an appropriate level (i.e. local/regional/national). The steps undertaken in the investigation of an outbreak, parallel those taken for sporadic cases (Section 7.2.2). Particular attention must be given to effective management of risk communication. A suspected outbreak of foodborne infection can create widespread anxiety that may impact on individuals and groups that are not at significant risk. Early and effective risk communication with the public is essential to manage the communication of risk and the need for appropriate action.

7.2.4 Control measures

The outbreak control team (Section 7.2.3) should consider prompt public health action guided by epidemiologic evidence while awaiting microbiological confirmation. Options for control of suspect foods range from detention through to product recall. It has not been established if person-to-person spread of *Listeria* is a significant concern. While the source of a proportion of outbreaks remains undetermined, there are no experimental evidence or expert group reports that support a major role for person-to-person transmission. There is, therefore, no reason to call for laboratory screening of faeces from asymptomatic contacts of cases.

A review of outbreaks undertaken by the HPSC revealed no evidence to implicate food handlers in outbreaks of listeriosis [(138)]. However, sensible hygiene precautions are appropriate. Food handlers diagnosed with listeriosis should not return to work until 48 hours after they have fully recovered from their illness. The requirement for a series of negative stool cultures as it arises in relation to some other foodborne pathogens does not arise given that there is little evidence that faecal carriage in food handlers is implicated in outbreaks.

7.3 Detection of *L. monocytogenes* in Food

Routine testing of high-risk foods for *L. monocytogenes* is generally carried out directly by the relevant food business and/or by the statutory authorities having responsibility for the food business. Whenever *L. monocytogenes* is detected at any level in food samples, the food business and the statutory authorities should share this information, even if the level of contamination is very low. The food business should review their *L. monocytogenes* control programme at this time (Sections 3 - 4). Where foods are found to be positive for *L. monocytogenes* at retail, wholesale, or catering establishments the storage and handling of the foods in these premises may also need to be examined. Consideration should be given to more frequent visits to and sampling of product from food businesses which have had recent or repeated samples positive for *L. monocytogenes*.

Wherever possible, if positive samples are identified in products early in their shelf-life, repeated samples from closer to the end of shelf-life (i.e. 'use-by' date) should be tested. Risk assessment should be carried out to establish the likelihood of numbers of *L. monocytogenes* reading ≥ 100cfu/g by the end of the product shelf-life. Products that are likely to do so should be withdrawn from sale (Appendix 6).

All isolates of *L. monocytogenes* from food should be stored for a least one year by the primary laboratory or by a reference laboratory. Typing of isolates should be performed where a specific premises or product is repeatedly positive.

Where *L. monocytogenes* is detected in a RTE food on the market at an unacceptable/potentially hazardous level (i.e. ≥ 100/g), the relevant batch of product should be traced and recalled from sale (Section 3.16) [3, 44]. A process of multi-disciplinary/multi-agency consultation, should take place at this time. Consideration should be given to setting up a multi-disciplinary investigation team to follow through on the occurrence. The investigation team, after consultation with the food business, should determine, if in the interests of public health, it is necessary to recall other batches of product and/or cease production [50,82 - 83]. It is not possible in this document to be prescriptive for all situations but the following factors should be considered in arriving at a decision:

1. Has the food actually been implicated in a case or cases of human infection? Where a product is actually implicated in a case of human infection a more cautionary approach is required
2. Is there evidence on inspection of the premises of good manufacturing practices and of the operation of an appropriate food safety programme based on HACCP?
3. Is there an effective *L. monocytogenes* control programme in operation and are results satisfactory on review?
4. Where multiple product lines are in operation are there clear records as to which line each batch of product was produced on? *L. monocytogenes* contamination can be line specific if multiple production lines are operated by a food business
5. Is there evidence of contamination of other batches of product with *L. monocytogenes*? Additional end product sampling should be performed. Where possible, preference should be given to samples close to the end of the product shelf-life
6. What is the profile of intended or expected consumers of the product?

7.4 Recommendations

Relevant agencies should:

1. Ensure that the prevention of human *L. monocytogenes* infections is the primary objective of any *L. monocytogenes* control programme
2. Ensure that food businesses adhere to best practice(s) and comply with relevant legislation regarding food safety
3. Promote and develop links with food businesses to establish monitoring and control strategies for *L. monocytogenes* and share data on *L. monocytogenes*
4. Ensure that all human cases are adequately investigated and the associated isolates are adequately characterised (serotyping and/or molecular typing)
5. Ensure the prompt notification of human infection with effective risk communication to protect public health
6. Ensure continued public awareness campaigns which focus on general measures for safe food handling and preparation
7. Provide targeted information on *L. monocytogenes* to high-risk groups on the hazards of specific foods.

8. KEY RECOMMENDATIONS FOR CONTROL OF *L. MONOCYTOGENES*

The control of *L. monocytogenes* in food requires commitment at different levels within the food chain. The challenges for controlling *L. monocytogenes* are considerable given its ubiquitous nature, high resistance to heat, salt and acidic pH and arguably most importantly, its ability to grow and survive at or below normal refrigeration temperatures. The following recommendations are typically general in nature. However, recommendations specific to *L. monocytogenes* control are highlighted in **bold**:

8.1 All Sectors

1. **Good food hygiene habits should be practised.**
2. **Separate storage areas for RTE foods should be provided.**
3. **Cross contamination of RTE foods should be avoided.**
4. **The susceptibility of specific population groups to *L. monocytogenes* infection should be considered.**
5. **All fruit, vegetables and prepared salads should be washed in potable water before use.**
6. **All refrigerators and storage areas should be regularly cleaned.**
7. Only potable water should be used in the production, preparation, handling and consumption of foods.
8. Manufacturers instructions should always be followed.
9. Food preparation areas should be cleaned and sanitised before and after use.
10. Raw materials and ingredients should only be sourced from approved suppliers.
11. Poor standards of food hygiene in any food business should not be accepted and should be brought to the attention of the business.

8.2 Processors, Caterers and Retailers

1. **The recommendations outlined in Section 8.1 should be complied with.**
2. **Food businesses should be aware that they are responsible for ensuring the safety of the food they produce or pack.**
3. **A food safety management system based on the principles HACCP, with regular reviews, should be developed and implemented.**
4. **A managed sanitation programme should be developed and implemented.**
5. **Facilities, equipment and practices should limit cross contamination.**
6. **Product and environmental temperatures should be controlled.**
7. **The shelf-life of all products should be determined and verified.**
8. Written SOPs supporting the food safety management system should be developed and implemented for all business practices.
9. Raw materials/ingredients should be purchased frequently with good stock rotation.
10. All employees and management should be trained in food safety and hygiene commensurate with their duties and responsibilities and in accordance with FSAI training guides.

11. A product recall and traceability system should be developed and implemented.
12. A waste management system should be developed and implemented.
13. A pest control programme should be implemented.

8.3 Processors

1. **The recommendations outlined in Sections 8.1 - 8.2 should be complied with.**
2. **Where possible, a validated and verified listericidal processing step should be included in production.**
3. **Moisture levels within preparation/processing areas should be controlled.**
4. **The use of compressed air as a cleaning aid should be minimised.**
5. **Niche environments should be eliminated.**
6. **Brine and marination solutions should be covered and refrigerated before use and not reused.**
7. **The use of rework materials should be minimised or eliminated.**
8. **Rework material which is deemed unsafe or illegal should never be re-used or reprocessed.**
9. **Environmental monitoring programmes should be developed and implemented.**
10. **Data collected from environmental monitoring programmes should be immediately evaluated to allow rapid response to positive findings.**
11. **Environmental monitoring programmes should focus on detection of *L. monocytogenes*.**
12. **Routine final product testing should be performed and viewed as a useful part of the overall control of *L. monocytogenes* but it is not acceptable as the sole means of control.**
13. **Individual production lines should be regarded as independent units for environmental monitoring and control.**

8.4 Caterers and Retailers

1. **The recommendations outlined in Sections 8.1 - 8.2 should be complied with.**
2. **Where possible, a validated and verified listericidal processing step should be included in preparation.**
3. **Separate storage areas for raw foods and RTE foods should be provided.**
4. **The use of tea towels and other re-usable cloths should be avoided.**
5. Food, once open, should be protected from cross contamination and used as soon as possible.
6. Consumers should be provided with clear product instructions.

8.5 Consumers

1. **The recommendations outlined in Section 8.1 should be complied with.**
2. **Foods** (especially soft cheese, pâté etc.) **which are past their 'use-by' date should never be consumed.**
3. **At least daily, tea towels and other re-usable cloths should be replaced with clean ones.**

8.6 Public Health and Regulatory Authorities

Public health and regulatory authorities should:

1. **Ensure that food businesses adhere to best practice(s) and comply with relevant legislation regarding food safety and hygiene**
2. **Ensure that the prevention of human *L. monocytogenes* infections is the primary objective of any *L. monocytogenes* control programme**
3. **Promote and develop links with food businesses to establish monitoring and control strategies for *L. monocytogenes* and share data on *L. monocytogenes***
4. **Ensure that all human cases are adequately investigated and the associated isolates are adequately characterised (serotyping and/or molecular typing)**
5. **Ensure the prompt notification of human infection with effective risk communication to protect public health**
6. **Ensure continued public awareness campaigns which focus on general measures for safe food handling and preparation**
7. **Provide targeted information on *L. monocytogenes* to high-risk groups on the hazards of specific foods.**

8.7 Future Research and Surveillance Priorities

Relevant organisations should:

1. **Establish a national *L. monocytogenes* reference laboratory**
2. **Improve data on levels of *L. monocytogenes* in foods, domestic animals and the environment and what the link is between these sources and clinical illness in humans**
3. **Investigate sources of *L. monocytogenes* contamination in processing environments to develop practices specifically targeted at eliminating or minimising contamination**
4. **Develop targeted sanitation procedures for elimination or minimisation of *L. monocytogenes* in the food processing environment at all levels**
5. **Increase and improve research on the pathogenesis of *L. monocytogenes*.**

APPENDIX 1. GROWTH, SURVIVAL AND CONTROL OF *L. MONOCYTOGENES* IN FOOD

1.1 Introduction

Control of *L. monocytogenes* in food can be achieved by using a combination of primary techniques:

1. Prevention of contamination of food with *L. monocytogenes*
2. Destruction of *L. monocytogenes* if present in food
3. Retarding or inhibiting *L. monocytogenes* growth when in food.

Some RTE foods are of low-risk to consumers because they do not support the growth of *L. monocytogenes*. Typically, these foods will have a low a_w and/or pH (Table 3). Other foods which are of low-risk to consumers are frozen products requiring re-heating before consumption (e.g. frozen ready meals), products which are commercially sterile or cooked in their retail container (e.g. canned food) or hot filled into sterile containers (e.g. baby foods), to prevent growth and survival of *L. monocytogenes* [26].

1.2 Thermal Processing

Cooking is the principal thermal process used in the production of many RTE foods. *L. monocytogenes* is considered to be the most heat-resistant vegetative foodborne pathogen and as a consequence there has been extensive research into its survival and heat-resistance in different foods [55-56, 59, 139].

Many RTE foods receive a mild heat treatment called pasteurisation. Pasteurisation is designed to destroy pathogens such as *L. monocytogenes* (or reduce to a safe level) and significantly decrease numbers of spoilage organisms. A common example of a pasteurised RTE food is milk. Typically, in Ireland, milk will receive a High Temperature Short Time pasteurisation process of 71.7°C for 15 seconds or an equivalent time/temperature combination [140]*.

The cooking of food, such as meat, to an internal temperature of 70°C for two minutes (or 75°C instantaneously) is also a pasteurisation process which should ensure a 10^6 (i.e. 1 million) reduction of viable *L. monocytogenes* cells [71]. It should be noted that each RTE food processor should validate their thermal treatment processes at least annually as part of an overall HACCP approach to food safety. Validation will ensure that a thermal process is adequate (e.g. the milk industry use the phosphatase test to verify that pasteurisation of liquid milk is adequate) to destroy and/or reduce to safe levels, numbers of *L. monocytogenes* cells.

1.3 Irradiation

Food irradiation (sometimes referred to as cold pasteurisation) is a process which exposes food to ionising radiation using a radioactive source [141]. Food irradiation will decontaminate foodstuffs (even when packaged) of most spoilage and pathogenic microorganisms including *L. monocytogenes* [142].

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* As a precautionary measure to reduce the likelihood of consumers being exposed to *Mycobacterium paratuberculosis* when consuming pasteurised cows' milk, the FSAI currently recommends that Irish milk manufacturers pasteurise milk for 25 seconds at ≥ 72°C. This is the time temperature combination recommended by the EU scientific Committee on Animal Health and Animal Welfare [178].

Currently, the use of irradiation in Ireland and the EU is not authorised in food processing except for dried aromatic herbs, spices and vegetable seasonings [99]. However, five Member States (i.e. Belgium, France, Italy, the Netherlands and the United Kingdom) do authorise the use of irradiation on other products such as poultry (max 5kGy) in France, shrimps (max 3kGy) in the Netherlands, fish and shellfish (max 3kGy) in the United Kingdom and others*. In the United States, irradiation is used in products such as minced beef to control both *Salmonella* and *Escherichia coli* O157:H7.

Irradiation of food is considered a safe and effective food safety technology by many international authorities [137] although some people have concerns regarding the impact of ionising radiation on the chemical and physical properties of food and on microorganisms in the food [99].

1.4 Freezing

The effects of freezing and frozen storage on the survival and growth of *L. monocytogenes* has been extensively studied. The majority of these studies have confirmed that foods are more protective of *L. monocytogenes* during freezing and frozen storage than laboratory media [132]. Some types of processing have been shown to decrease the protective nature of foods for *L. monocytogenes*. For example, homogenisation of milk destroys macrophages, thus reducing the protection against pasteurisation temperatures afforded by cellular internalisation [55]. While *L. monocytogenes* does not grow below -1.5°C, it can survive at lower temperatures (Table 3). Although freezing and frozen storage can reduce numbers and viability of *L. monocytogenes*, the processes are more likely to injure and/or sensitise than kill *L. monocytogenes* cells [143].

1.5 Microwave Treatment

L. monocytogenes demonstrates no unusual resistance to microwave heating. Regeneration (i.e. re-heating) of all parts of a RTE food to an appropriate temperature (e.g. ≥70°C) will reduce or eliminate the risk of *L. monocytogenes* infection. However, a problem with microwave ovens is uneven heating which may result in parts of the food not reaching the appropriate temperature during regeneration [24].

Different microwave oven models often heat foods differently. Following the manufacturer's instructions and regular stirring during regeneration are important whether a microwave oven has a turntable or not. When the microwave has finished a heating cycle, problems of uneven heating may be offset if manufacturers recommended standing times after regeneration are followed [56]. If no instructions are given, the foodstuff should stand for at least one minute before serving as the food continues to heat for a time after the regeneration cycle has been completed.

* The Gray (Gy) is the international S.I. unit for absorbed radiation dose. One KiloGray (kGy) is 1000Gy.

1.6 Modified Atmosphere Packaging

Modified Atmosphere Packaging (MAP) is a technique commonly applied to high value added products such as RTE foods (e.g. cooked sliced meats). MAP involves the removal of atmospheric air from a package and its replacement with a mixture of inert gases (e.g. nitrogen and carbon dioxide). The gas mixture in conjunction with refrigeration and the proper packaging materials will help inhibit proliferation of some microorganisms without alteration of the foods characteristics.

When properly applied, MAP can extend the shelf-life of products. However, *L. monocytogenes* can grow under refrigerated aerobic and anaerobic conditions. This means that RTE foods (e.g. sous-vide, pre-packed sandwiches) which are MAP packed with standard gas mixtures or are vacuum packed may support growth of *L. monocytogenes* [(144-146)]. High concentrations of carbon dioxide in a MAP food (≥ 70%) completely inhibit *L. monocytogenes* proliferation [(147)]. However, MAP should not be used as a solitary microbiological hurdle. Adding additional hurdles such as controlled refrigeration or incorporation of preservatives (e.g. sodium nitrite in cured meats) will improve the safety of these foods.

1.7 Active Packaging

Recently, packaging materials such as edible films have been developed which can prevent the growth of *L. monocytogenes* on RTE chicken and other pre-cooked foods. These types of packaging have been defined as active food packaging. Active food packaging can provide several functions not available in standard packaging systems and MAP. Active functions can include oxygen and moisture scavenging, flavour release and incorporation of antimicrobial agents [(148)]. One type of active packaging being researched is an edible film incorporating two proteins called zein and nisin [(148-149)].

1.8 High Hydrostatic Pressure

The concept of using High Hydrostatic Pressure (HHP) as a food preservation technique has attracted recognition in the last 15 years. HHP works by submerging food (normally packaged) in liquid such as water, in a vessel which generates high pressure. Pressures normally generated are between 100-1000 megapascals (MPa) [(150)]. For most foods, a ten minute exposure to HHP between 250–300 MPa will result in a 4-6 $\log_{10}$ Colony Forming Units/millilitre (CFU/ml) reduction in vegetative microorganisms without changing the organoleptic characteristic of the product [(150)]. *L. monocytogenes* is more resistant to HHP in milk and milk products than in poultry or laboratory media [(55)]. HHP is a processing technology with potential for the RTE food processing industry and is currently in use in Europe (e.g. apple juice and cooked, sliced meat snacks) and the United States (e.g. oysters and guacamole).

1.9 Salt (Sodium Chloride)

The a_w of foodstuffs (Table 3) is depressed by the addition of salt and sugars. The higher the concentration of solutes in a foodstuff the lower the a_w will be. Growth of *L. monocytogenes* in low a_w conditions also depends on the type of solute (e.g. salt, sugar etc.) present. Typically, salt is inhibitory to foodborne pathogens. However, *L. monocytogenes* is very resistant to salt.

Strains of *L. monocytogenes* can survive salt concentrations in excess of 20% and can grow in concentrations of up to 12% (Table 3). However, salt does decrease growth rates of *L. monocytogenes*. Survival of *L. monocytogenes* in foods which contain salt is largely dependent on storage temperature. Refrigerated storage temperatures will enhance the bacteriostatic effect of a low a_w due to addition of salt [58, 145]. However, the solitary use of brines in foodstuffs (e.g. cheese, meat, fish) for the control of *L. monocytogenes* is not recommended as most foods will be unpalatable if salt concentrations are > 3% [55].

1.10 Sodium and Potassium Nitrate and Nitrite

Sodium and potassium nitrates and nitrites are food additives and as such the type of foods they can be used in and the level of use are regulated by legislation [151]. Sodium nitrate and nitrite is predominately used in cooked cured meat production for development of colour and flavour. Potassium nitrate is used in some cheese and pickled fish products, while potassium nitrite is used in non-heat treated cured and dried meat products [151]. Sodium nitrite has a bacteriostatic effect against specific microorganisms including *L. monocytogenes* and its effect is synergistic with intrinsic and extrinsic variables such as product pH, salt level and storage temperature [145].

1.11 Organic Acids

pH is a key intrinsic property of foodstuffs. While *L. monocytogenes* can grow at pH 4.3 and survive pH 3.3 (Table 3), its inactivation at low pH will depend on the type of organic acid present in the food and also on the constituents of the contaminated food (e.g. glutamate can protect against the inhibitory effect of low pH) [152-153]. When used on an equal weight basis, acetic acid (i.e. vinegar) is more listericidal than either citric or lactic acids [148]. However, the majority of permitted organic acids in foods are not used for control of *L. monocytogenes*.

1.12 Smoking

The smoking of meat, fish, shellfish and some cheeses is a well practised preservation technique which can inhibit the growth of *L. monocytogenes* [154]. Smoke (liquid and natural) is inhibitory to *L. monocytogenes* due to the presence of phenolic (e.g. isoeugenol) and other compounds [55].

Smoking impregnates foods with volatile smoke compounds. Traditionally, two forms of natural smoking are used in food processing. Cold smoking typically takes place at temperatures

of 20°C to 50°C while hot smoking takes place at temperatures > 60°C [155]. The hot smoking process is normally sufficient to destroy *L. monocytogenes* through a combination of both heat and volatile smoke compounds [156]. However *L. monocytogenes* inoculated into fish can grow during the cold smoking process at temperatures between 20 and 30°C [48]. Furthermore, as smoking conditions are not standardised in Ireland, the effect of smoking on *L. monocytogenes* and its inhibitory effect during storage will vary among processors of both cold and hot smoked produce. Therefore, prevention of post-process contamination is vitally important [54]. The cold and hot smoking of fish such as salmon and trout is very common in Ireland (Section 5.2). Many meats are also cold or hot smoked including varieties of ham and beef (e.g. pastrami, beef jerky).

Many RTE food processors use a smoking procedure which combines drying with the addition of liquid smoke flavourings. Liquid smoke flavourings do have some listericidal effect. However, the concentrations of liquid smoke necessary to control *L. monocytogenes* on their own would be organoleptically unacceptable to consumers.

1.13 Spices and Herbs

The majority of spices and herbs and other plant extracts such as essential oils used in RTE food processing, are used for flavouring and seasoning purposes rather than for their antimicrobial properties. Many plant extracts (e.g. cinnamon, pimento leaf, hops, rosemary, cloves and horseradish) contain compounds that can inhibit or destroy pathogens such as *L. monocytogenes*. However, the levels of these herbs and spices usually required to inhibit *L. monocytogenes,* will make the food organoleptically unacceptable [157].

1.14 Bacteriocins (Biopreservation)

Biopreservation is the use of microorganisms and/or their metabolic by-products to inhibit or destroy other microorganisms [158]. Biopreservation using lactic acid bacteria (LAB) is the most common biopreservation technique. The biopreservation effect of LAB in foods is through competitive inhibition and/or production of antimicrobial compounds such as hydrogen peroxide, acids or bacteriocins. Many RTE food processors are now adding patented bacterial cultures (i.e. probiotic bacteria) such as Lactobacillus species to food products particularly in the dairy sector where milk based products are being marketed with live cultures of probiotic bacteria. *L. monocytogenes* is a poor competitor and growth of non-pathogenic microorganisms such as LAB may retard its growth (i.e. Jameson Effect) [159].

L. monocytogenes may be sensitive to a number of commercially available bacteriocins, including nisin (i.e. E234), and its use has been approved for use in foods such as processed cheese spread [151]. Nisin may have only a slight antimicrobial effect on *L. monocytogenes*, but other bacteriocins have been identified which have a much higher specific activity against *L. monocytogenes*, including the so called anti-*Listeria* Class IIb group of bacteriocins [158]. While such bacteriocins

may play an important role in controlling *L. monocytogenes*, it is advisable that bacteriocin based control strategies should be combined with another listericidal treatment [55]. Overall, the inhibition and destruction of *L. monocytogenes* in RTE foods using biopreservation technology can be aided by [158]:

1. Direct addition of purified bacteriocins to food products
2. Direct incorporation of ingredients containing bacteriocins into the food
3. Direct addition of bacteriocin producing bacteria to foods
4. Using bacteriocin producing LAB starter cultures in fermentation processes
5. Incorporation of bacteriocins into packaging materials.

1.15 Lyophilised Cultures

Currently licensed in Denmark (but not in other EU States) is the use of lyophilised cultures of *Leuconostoc carnosum* on cooked sliced meats as a means of biological control of *L. monocytogenes* [160]. These cultures are either sprayed directly onto cooked meat or the meat is dipped in the culture before it is packed normally under MAP conditions. The cultures inhibit the growth of *L. monocytogenes* through production of bacteriocins and competitive inhibition.

1.16 Naturally Occurring Antimicrobial Compounds

Some RTE foods will naturally contain antimicrobial compounds which may help inhibit *L. monocytogenes*. Lysozyme is a heat stable (i.e. 4 - 95°C) enzyme found in foods such as hen's eggs and milk which has inhibitory effects against *L. monocytogenes*. The enzyme is destroyed by pasteurisation and is not at present a significant component of *L. monocytogenes* control programmes in the RTE food industry [161].

1.17 Food Structure and Composition

The structure and composition of a food, for example, the fat content, can affect the ability of *L. monocytogenes* to survive [10]. Heat-resistance studies for inactivation of *L. monocytogenes* have shown that heat-resistance increases with increasing fat content [56].

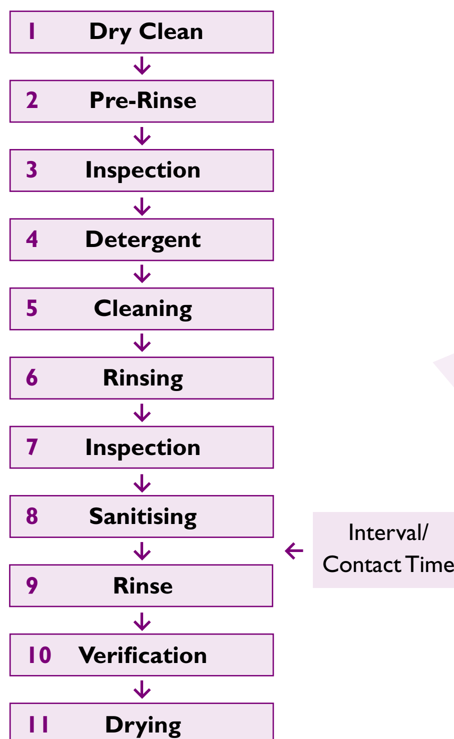
APPENDIX 2. SANITATION

2.1 Introduction

Sanitation is a multi-stage procedure which consists of cleaning followed by sanitising (Figures 2 - 3) [84]. To ensure that a facility and equipment are clean, sanitised and suitable for their intended use, management of the food business must draw up and operate a written sanitation programme and SOPs (Section 3.2) for the sanitation of all equipment surfaces and structures within the facility.

The sanitation programme should address sanitation before, after and during production if a production line change is required. The pre-operational programme should address food contact surfaces of facilities, equipment and utensils. The programme should indicate the frequency with which each task should be carried out and the individual responsible. Records of all activities and corrective actions taken should be maintained on a daily basis. A written sanitation programme should be signed and dated by the person with overall authority for the plant, before it is implemented [81].

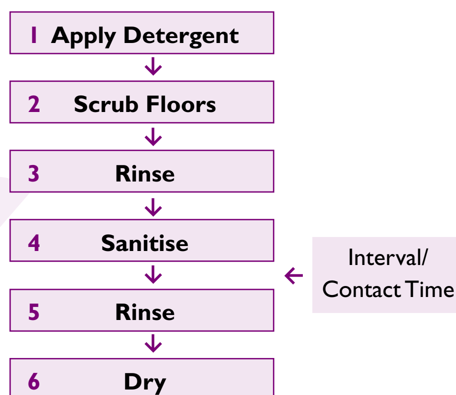
Figure 2 Sanitation Procedure for Processing Equipment*



* The procedure will vary depending on the individual facility. Some steps in the above procedure can be repeated as necessary.

1. Used as applicable.
2. Used to remove gross organic residue using potable water
3. Visual inspection of adequacy of cleaning
4. Detergent is applied to remove gross organic residue (always follow manufacturer's instructions for detergent used)
5. Using brushes/detergent etc. to remove organic residue (some equipment can require disassembling prior to sanitation, and may need to be re-sanitised after re-assembling)
6. To remove detergent residue using potable water
7. Visual inspection of adequacy of cleaning
8. Application of sanitising agent (always follow manufacturer's instructions for detergent used as contact times/concentrations vary with sanitiser)
9. To remove sanitiser residue using potable water (will depend on type of sanitiser used)
10. Verification of adequacy of sanitation (e.g. visual, microbiological, chemical)
11. Removal of any water pooling particularly on floors.

Figure 3 Sanitation Procedure for Processing Floors*



1. Apply a powdered caustic cleaner with water to the floor
2. Manually scrub the floor using brushes dedicated for floor use only
3. Rinse with water using a low volume, low pressure hose
4. Sanitise with a high concentration sanitiser (e.g. 1,000 ppm Quats)
5. Rinse with water using a low volume, low pressure hose
6. Dry.

* The procedure will vary depending on the individual facility. Some steps in the above procedure can be repeated as necessary. An important part of cleaning floors is cleaning drains. Drains should never be cleaned with high pressure water hoses as this can result in the formation of contaminated aerosols in the processing environment and/or contamination of food contact surfaces with spray from the drains.

2.2 Sanitation

Employees involved in sanitation should receive specific training in sanitation procedures to control *L. monocytogenes*. Sanitation should be performed as specified in SOPs and production schedules should not impinge on time spent on sanitation. The SOPs for sanitation should include specific information (in terms of cleaning and sanitising) for different zones (Figure 1) within a facility, utensils or equipment. The SOP should also cover items not directly related to processing such as electrical plugs, cabling, control panels and switches. All areas of the plant and equipment should be inspected before production to ensure that sanitation is satisfactory. In addition, environmental microbiological sampling (Section 4.6.1 and Appendices 4 - 5) is recommended to determine the effectiveness of sanitation procedures. The results of these checks and analyses should be documented [81].

2.3 Cleaning

Cleaning is removal of visible dirt and other extraneous material from surfaces using detergents. Cleaning within a sanitation programme is typically divided into a series of stages for equipment and the facility environment, each with a defined purpose prior to sanitising [81]. Because detergents used in food processing facilities are typically not formulated to destroy *L. monocytogenes*, (i.e. sanitise) they can lead to the spreading of the pathogen from equipment, to areas of the facility which are not sanitised. *L. monocytogenes* may thus survive in the non-sanitised areas and subsequently be transferred to sanitised surfaces and contaminate food [162].

Priority should be given to equipment and zones (Figure 1) used for holding final product either packaged or unpackaged. Inadequately cleaned equipment in raw food processing areas has not been associated with *L. monocytogenes* contamination in RTE finished product. Consideration should be given to assigning experienced and highly motivated employees to zones where RTE products are handled and packaged [11].

Infrequent or inadequate cleaning of refrigerated storage units used for holding finished unpackaged RTE food (e.g. cooked meat) can be a potential source of *L. monocytogenes* contamination, particularly during peak production periods. Refrigerated storage units should be emptied and cleaned regularly. Maintaining dry walls, ceilings and floors in cooling units is also important.

Spiral freezers should be cleaned at least annually but ideally twice a year. Infrequent defrosting, cleaning, and maintenance of spiral freezers used for freezing unpackaged product can contribute to the persistence of *L. monocytogenes* contamination. Coolers and other rooms should not be cleaned while exposed RTE food is present. Remove all unpackaged product from the room before beginning sanitation [11]. Reliance on covering the product with plastic or paper should be avoided.

Racks used to transport cooked food, particularly if uncovered, (e.g. cooked hams removed from the cooker being transported to the chiller) should be carefully designed and maintained. The racks should be constructed using a good quality stainless steel and designed to be

easily dismantled for cleaning. Splash guards should be provided over the wheels to prevent splash from the floor via the wheels onto the exposed product as the rack is moved. Because racks can be used in cooking, food residues will adhere to the rack surface. If residues become baked onto racks they are difficult to remove. Therefore, thorough cleaning is necessary after each use.

2.4 Cleaning Utensils

Cleaning utensils can be a source of *L. monocytogenes* contamination. The use of coloured disposable paper towel (i.e. coloured to distinguish them from food) is preferable to the use of cleaning cloths as cloths may spread contamination. Cleaning utensils should only be used for cleaning purposes and should be zone specific (Figure 1) and not moved from low-risk zones to high-risk zones or vice versa. Floor scrubbers can be helpful, particularly for cleaning large open spaces such as hallways. Cleaning utensils which are used to clean floors, drains, walls etc., should not be used to clean processing equipment. After use, cleaning utensils should be sanitised with quaternary ammonium compounds (Quats) at a concentration of 600-1,000 parts per million (ppm) (Appendix 2.6). The utensils should be stored dry if possible or in a strong Quats solution maintained at 1,000 ppm [11]. Quats solutions used to store cleaning utensils should be changed regularly.

2.5 Sanitising

Sanitising (i.e. disinfection) is the inactivation (generally using chemicals) of microorganisms. It should be carried out after cleaning [73]. Sanitisers will only be effective if the surface being sanitised has been thoroughly cleaned. Research has indicated that *L. monocytogenes* will have different degrees of sensitivity to different sanitisers [73, 163]. Acquired resistance to sanitisers by *L. monocytogenes* has been documented [164-166]. Therefore, it is recommended that a system of sanitiser rotation be adopted by RTE food processors [163-167].

2.6 Sanitising Agents

2.6.1 Heat/hot water/steam

Racks are typically sanitised by heating cleaned empty racks in a cooking oven to temperatures ≥ 82°C. Hot water (≥ 82°C) can be used in conjunction with a chemical sanitiser if manufacturers instructions permit preparation of the sanitiser in hot water [54, 81]. Hot water with a chemical sanitiser is particularly useful in the removal of biofilms (Appendix 2.9). The use of hot water alone should be avoided due to the difficulties in maintaining a consistently high enough temperature to destroy *L. monocytogenes*. Steam can be used as an alternative to chemical sanitisation. However, the use of steam should be limited to equipment which is difficult to clean and to closed systems such as Clean-In-Place, due to the hazards of aerosol formation and moisture condensation on equipment surfaces (Section 4.5.2).

2.6.2 Chlorine

Chlorine based sanitisers are among the most widely used sanitising agents in the RTE food

processing industry and *L. monocytogenes* exhibits no unusual resistance to these chemicals [168]. However, chlorine sanitiser efficacy can be influenced by the following [127, 135]:

1. Free chlorine concentration (follow manufacturers instructions for use)
2. Contact time (antimicrobial activity increases with longer exposure times)
3. pH
4. Temperature (reactivity doubles for every 10°C increase up to 52°C)
5. Presence of organic matter (such as food will decrease sanitisers effectiveness)
6. Surface attachment of bacteria
7. Formation of biofilms
8. Type of surface.

Chlorination of water must be carried out using a metering device which ensures that the correct concentration of chlorine is added and which incorporates an alarm if the device malfunctions. The residual chlorine level in chlorinated water should be measured regularly and records documented. In general, a free chlorine level of 0.2 to 0.5 ppm should be adequate and levels should not exceed 1.0 ppm [127]. Free chlorine is defined as the concentration of residual chlorine present in water as dissolved chlorine gas, hypochlorous acid, and/or hypochlorite ion. The three forms of free chlorine exist together in equilibrium. Their relative proportions are determined by the pH and temperature of a solution. As the pH falls below 2, the predominant form is chlorine gas. Between pH 2 - 7 the predominant form is hypochlorous acid.

At pH 7.4, hypochlorous acid and hypochlorite ions are about equal, while above this increasing proportions of hypochlorite ion are present. In use, chlorine based sanitisers are most effective at pH 4 - 5 and in storage they are most stable at pH 10 - 11. Aqueous chlorine solutions are not stable; therefore where these solutions are used they should be freshly prepared, diluted and monitored accordingly. Therefore, chlorine based sanitisers are not suitable for use in footbaths as they are quickly dissipated. Regular measurement and monitoring of chlorine and other sanitiser concentrations will help maximise their antimicrobial effectiveness [169].

Chlorine dioxide based solutions are effective surface sanitisers. These solutions may be used for items that are not subject to corrosion. Solutions can have rapid action times.

2.6.3 Iodophors

Iodophors are iodine based compounds. Iodophors are recommended for use in dairy processing as they do not inactivate starter cultures, and at recommended concentrations, are effective against *L. monocytogenes*. Iodophors are not recommended for use on white vinyl surfaces as they will stain from repeated exposure to iodine. Unlike chlorine, iodophors have reduced efficacy against *L. monocytogenes* at low temperatures of ≤ 4°C [168].

Iodophors are unstable at high temperatures and lose antimicrobial activity in the presence of hard water and alcohol. Distilled or softened water is recommended to dilute iodophors before use. Typically, iodophors will require

between 10 and 20 minutes exposure time to ensure maximum antimicrobial effect on a surface. Concentrations of iodophors at 200 ppm are effective on equipment and other surfaces [104].

2.6.4 Quaternary ammonium compounds

Quaternary ammonium compounds are complex synthetic derivatives of ammonium chloride. They are not recommended for direct use on food contact surfaces or in facilities which produce RTE foods using starter cultures such as cheese, as LAB starter cultures are inactivated by small residues of Quats (i.e. Quats leave a residual germicidal effect on surfaces) [73]. However, Quats are very effective against *L. monocytogenes* on areas such as floors, walls, drains, coolers, spiral freezers, footbaths and condensation drip pans [163]. If footbaths are used in a facility, they should be well maintained and contain a strong concentration (i.e. 1,000ppm) of sanitiser such as Quats.

2.6.5 Other chemical sanitisers

Peracetic acid and peroctanoic acid have been shown to be effective against *L. monocytogenes* including organisms in biofilms [11].

2.6.6 Alternative sanitisers

If alternative sanitisers are used, such as ultraviolet light, membrane filtration, etc., then the facilities and the system testing regime may differ considerably from those required for chemical sanitisation [81]. It is recommended that in all cases, manufacturer's instructions are followed.

2.7 Use of Sanitisers

The concentration of specific sanitisers used in food processing facilities will vary to compensate for factors such as water hardness. However, manufacturer's recommended concentrations should not be exceeded. Using high concentrations of sanitisers can increase risks to employee health, contamination of foods and in some cases, damage of equipment through corrosion. Chlorine based sanitising foams are highly corrosive and therefore their use on food contact surfaces should be minimised. Furthermore, using highly concentrated sanitisers will not compensate for poor cleaning.

The use of foam based sanitisers is widespread particularly on floors but on specific surfaces they may not be suitable. On walls, for example, foams may flow downward decreasing the contact time of the sanitiser on the surface. New brands of sanitisers incorporate gels which allow the sanitiser to cling to the surface and improve contact time.

Using combination detergent/sanitisers should be avoided as sanitisers typically require a defined contact period with the surface they are sanitising. If a cleaning procedure does not allow this contact time, then the effect of the sanitiser is diminished. Furthermore, sanitation procedures which use a combination detergent/sanitiser will have one less step in that procedure than a sanitation procedure which uses a separate detergent and sanitiser. This means there is less opportunity to compensate for a deficiency in either the cleaning or sanitising procedure using a combination detergent/sanitiser.

Application of powdered/crystalline citric acid to certain areas of the floor can be effective in controlling *L. monocytogenes*, provided the floor has been properly cleaned and dried before applying the citric acid. For maximum effectiveness, the surface of the floor should be maintained at ≤ pH 4.5. Litmus paper can be used to check the pH. While this may help control *L. monocytogenes*, the condition of the floor should be monitored, as the use of acids will cause deterioration that eventually will necessitate replacing the floor (Figure 3) [26, 54].

2.8 Frequency of Sanitation

The frequency of a sanitation programme is critical in the control of *L. monocytogenes* contamination. However, a food business should decide on the frequency of sanitation required. A sample schedule is shown in Table 8. In some circumstances, sanitation of production lines during daily production schedules will be required (in addition to wash-downs before staff breaks or after use of equipment). In well maintained, fish processing facilities, mid-shift sanitation procedures may be of benefit in lowering the risk of *L. monocytogenes* contamination [54].

Table 8. Example Sanitation Schedule [1]

Surface	Frequency	Suggested Sanitiser [2]
Equipment	Daily	Chlorine, Quats, Iodophors, Acid Sanitisers
Floors and Drains	Daily	Quats, Acid Sanitisers [3]
Walls, Coolers, Drip Pans	Weekly	Quats, Acid Sanitisers [3]
Final Packing Lines	Monthly	Quats, Acid Sanitisers [3]
Spiral Freezer	Bi-Annually	Quats, Acid Sanitisers [3]

[1] Assign a responsible person to ensure that the sanitation schedule is correctly followed

[2] The use of a particular sanitiser should be rotated

[3] Sanitisers containing peracetic or peroctanoic acid

However, evidence from the meat and dairy industries suggest that mid-shift sanitation can increase the level of contamination in facilities [26]. The procedures can be counter-productive, increase the risk of *L. monocytogenes* contamination and can lead to contamination of product with sanitation chemicals [11]. In relation to staff breaks, it is important not to allow product to be processed during breaks. Batches of product should be finished before breaks, or ideally, schedule staff breaks to facilitate processing schedules.

The frequency of a sanitation routine should be determined upon individual experience within a facility and the collection of microbiological data. Routine environmental microbiological testing allows the development of baseline microbiological data which can be used to identify trends and inadequacies in the sanitation programme [3].

In many food business sanitation is undertaken by designated cleaning crews during the night or early morning. While the timing of this sanitation suits many production schedules it can lead to problems if the staff involved are untrained, unmotivated and working against a tight schedule. Therefore, it is prudent to have a trained member of staff present during these procedures to monitor the process and to ensure that sanitation is carried out correctly and that the facility is ready to begin operation at the appropriate time.

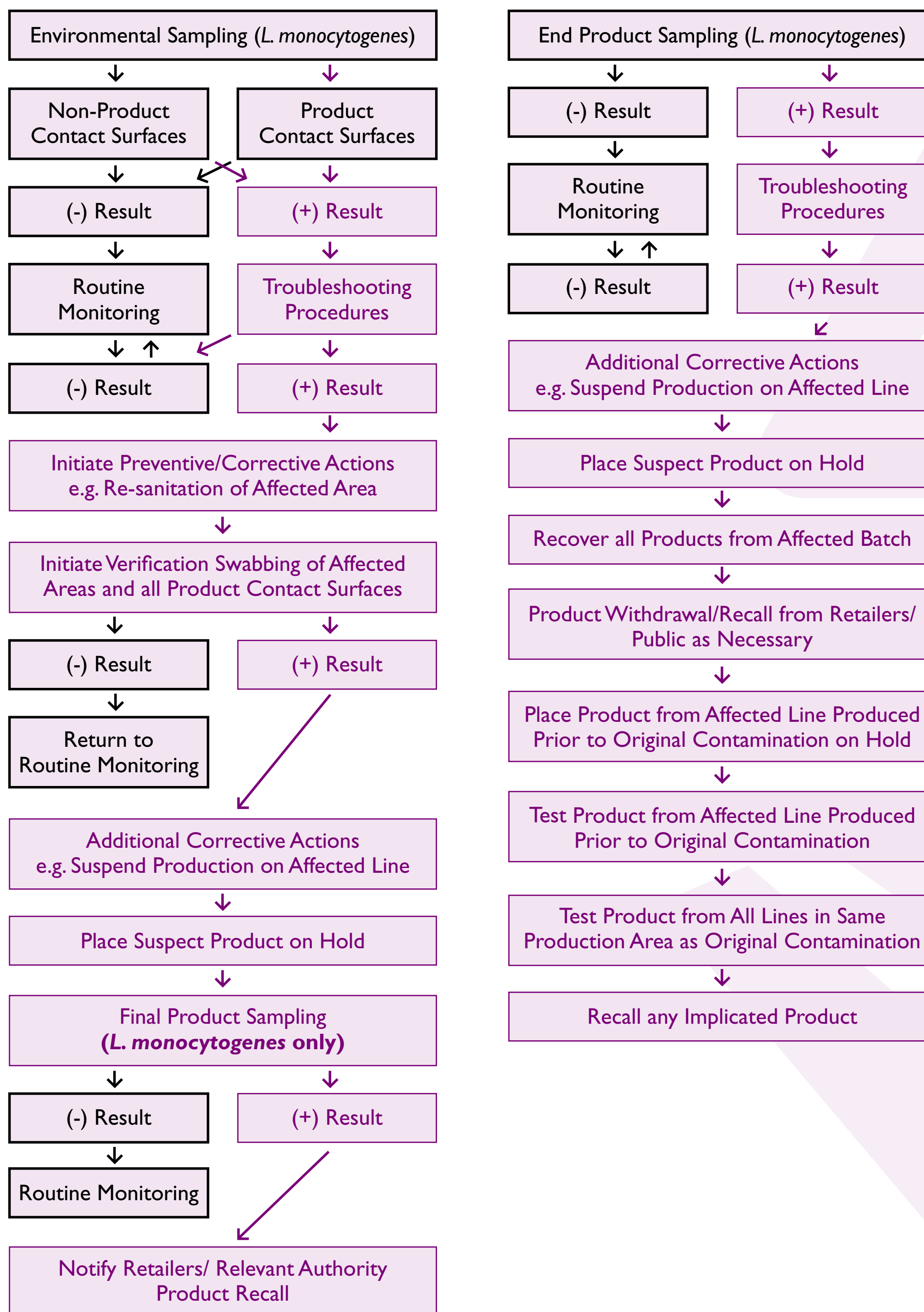
2.9 Biofilms

Listeria monocytogenes can form biofilms (also known as biofouling), on equipment, utensils and other surfaces. Microorganisms in biofilms are difficult to remove by cleaning and can show increased resistance to sanitisers [62-64]. Biofilms serve as reservoirs from which *L. monocytogenes* can contaminate products during processing. Stringent measures to control *L. monocytogenes* biofilms in RTE food processing facilities are necessary. The control of biofilms begins with a sanitation programme [64]. Furthermore, spoilage microorganisms (e.g. *Pseudomonas* species) can also form biofilms and while not normally associated with foodborne disease they can contaminate food during production and accelerate spoilage. *L. monocytogenes* can also become entrapped inside biofilms formed by spoilage bacteria and contaminate the product.

L. monocytogenes adheres to stainless steel, Buna-n-rubber, polypropylene plastics and several other food contact surfaces commonly found in RTE food processing equipment [170]. The exposure time to chlorine necessary to kill attached *L. monocytogenes,* varies depending on the type of surface. The activity of chlorine against *L. monocytogenes* biofilms decreases on porous surfaces such as polypropylene plastic in contrast to non-porous surfaces such as glass. In comparisons between common materials used in food processing, *L. monocytogenes* biofilms have been shown to be more resistant to chlorine based sanitisers on Buna-n-rubber (e.g. used in gaskets), and Teflon than on stainless steel [73, 168, 171].

Research has indicated that sodium hypochlorite used at a concentration of 200 ppm on a non-porous surface requires two minutes to effect an adequate inactivation of *L. monocytogenes* in contrast to two minutes for a 100 ppm solution used on a porous surface [172]. Recent research has indicated that on stainless steel surfaces destruction of *L. monocytogenes* is optimal when the pH of chlorine solutions is adjusted to 6.5 [169]. While research differs on the effectiveness of different sanitisers on different surfaces, best practice is to use nonporous material such as stainless steel in food processing as these materials are easier to maintain and sanitise.

APPENDIX 3. EXAMPLE PROCEDURES FOR SAMPLING



APPENDIX 4. ENVIRONMENTAL MONITORING SITES

4.1 Introduction

Environmental monitoring of the processing environment is important in identifying and controlling pathogens such as *L. monocytogenes*. The success of environmental monitoring in identifying and controlling *L. monocytogenes* is related to the correct selection of monitoring sites. Selection of the correct monitoring sites is necessary to allow food businesses to assess the level of control in the processing environments.The sampling technique used will also influence the success of environmental monitoring (Appendix 3).

Hygienic zone design (Figure 1) will assist in environmental monitoring by allowing logical and consistent selection of monitoring sites. Environmental monitoring will help determine what control measures are needed to prevent contamination of finished products and to verify the effectiveness of sanitation procedures. Outlined below are some suggested environmental monitoring sites based on hygienic zone design as previously described in Figure 1. However, sample sites should always be facility specific. In addition, food processing facilities will not always have five hygienic zones.

4.2 Low-Risk Area (Zone 1)

This will typically include low-risk areas which are not directly involved in processing such as raw materials or packaging storage. Environmental samples taken from this zone can be useful in assessing the risk to processing from immediate surroundings of the facility, thereby alerting management to potential problem sites within this zone (Table 9).

Table 9. Suggested Environmental Sampling Sites in Low-Risk Areas

Low-Risk Area (Zone 1)	Low-Risk Area (Zone 2)
Suggested Sampling Sites	
Rubbish collection sites	Slicers, cutters, mixers, hoppers, peelers
Service sites	Injection equipment
Raw materials/packaging storage	Chill water or brines
Waste disposal sites	Table, benches and countertops
Pallets	Conveyor systems/belts/chains/hollow rollers
Recycling sites	Cold storage units/exterior/interior of chill tanks
Traffic areas	Floors/walls/ceilings/drains
Vehicles	Window ledges/door handles/frames
Access-ways	Shelves/racks
Gutters	Waste collection points
Roofs	Knifes and other utensils
Difficult to clean areas	Personnel safety/hygiene items
Floors/walls/ceilings/drains	Any area which is wet or soiled by food residues
	Vehicles such as forklifts/trolleys
	Difficult to clean areas

4.3 Low-Risk Area (Zone 2)

This will typically include areas where raw materials are decanted, prepared or stored prior to further processing such as cooking. Processing in this zone can include brine injection, mixing and cutting procedures (Table 9).

4.4 High-Risk Area (Zone 3)

This will typically include areas where products receive final processing such as cooking and where processed product is handled prior to final packaging. It has been confirmed that *L. monocytogenes* is commonly introduced to a RTE food after final processing such as cooking. Therefore, post-process product contact surfaces should form an integral part of environmental sampling sites (Table 10).

Table 10. Suggested Environmental Sampling Sites

High-Risk Area (Zone 3)	High-Risk Area (Zone 4)	Low-Risk Area (Zone 5)
	Suggested Sampling Sites	
Product contact equipment	Packaging equipment	Floors/walls/ceilings/drains
Tables/benches/countertops	Packaging materials	Door handles/frames
Conveyors/belts/chains	Weighing scales	Personnel safety/hygiene items
Hollow rollers	Gas lines/connections	Cleaning equipment
Filling heads/mandrels	Sealing equipment	Waste disposal areas
Seals/gaskets/welds/joints	Conveyors/belts/chains	Pallets
Switches/levers/control units	Hollow rollers	Flow wrap machines
Storage vessels	Switches/levers/control units	Forklifts/trolleys
Floors/walls/ceilings/drains/ledges	Floors/walls/ceilings/drains	Cold/frozen storage units
Door handles/frames	Door handles/frames	Switches/levers/control units
Shelves/racks	Personnel safety/hygiene items	Difficult to clean areas
Weighing scales	Wet or soiled areas	Wet or soiled areas
Knives/utensils	Cleaning equipment	Specialised equipment
Personnel safety/hygiene items	Difficult to clean areas	Air vents/filters/ducts
Blast freezers, cold storage units	Waste disposal areas	
Exterior/interior of chill tanks	Specialised equipment	
Wet or soiled areas	Seals/gaskets/welds/joints	
Specialised equipment	Air vents/filters/ducts	
Air vents/filters/ducts		
Cleaning equipment		
Waste disposal areas		
Difficult to clean areas		

4.5 High-Risk Area (Zone 4)

This will typically include areas where products are packaged after final processing. These are high-risk areas where processed product and packaging may be handled prior to completion of packaging (Table 10).

4.6 Low-Risk Area (Zone 5)

This will typically include low-risk areas where processed products may receive final chilling or freezing, are loaded onto trucks for delivery or stored prior to dispatch (Table 9).

APPENDIX 5. MICROBIOLOGICAL ANALYSIS

Results from microbiological analysis are dependent on the analytical method used, and therefore, reports of microbiological analysis of samples for *L. monocytogenes* should specify the method used. Currently in the EU, the reference methods are the International Organisation for Standardisation (EN/ISO) 11290-1 and EN/ISO 11290-2 [173-174]. However, as microbiological techniques for analysis of samples for *L. monocytogenes* advance, food businesses should be able to use analytical methods other than reference methods, in particular, rapid methods [3].

The use of an alternative analytical method for detection and/or enumeration *L. monocytogenes* is acceptable, when the method is validated against the current EU reference method and certified by a third party in accordance with the protocol set in EN/ISO standard 16140 or other internationally accepted similar protocols [3,175]. If a food processor wishes to use analytical methods other than those validated and certified as described above, the methods shall at least be well documented, scientifically validated and provide equivalent results.

In the case of final product or ingredient testing, intact product packages should be sent for microbiological analysis, by quality control staff, as this provides better control of samples. Otherwise, samples should be aseptically collected in sterile bags or other sterile container for analysis by quality control or laboratory staff.

The analysis of all samples for *L. monocytogenes* must be carried out by approved external or on-site laboratories. It is important to stress that *L. monocytogenes* and other foodborne pathogens should not be cultured in on-site laboratories unless the laboratory is completely separated from the main processing facility. Laboratories performing these analyses should participate in a proficiency scheme, have suitably trained staff and a certified quality assurance programme.

Environmental samples should be collected by a suitable method. Swabs should examine as large an area as possible and the same size area should be tested at each sample site. Swabs or sponges must be free of substances that may inhibit bacterial growth. Duplicate samples should always be taken with one sample retained for further verification if necessary.

Many food businesses may practice compositing* of samples particularly if low levels of positive results are routinely recorded. However, it is recommended that where composite sampling is used all samples used, in the composite should have a duplicate in storage until results of the composite test are known so they can be tested individually, if necessary. Compositing of samples can help minimise costs particularly for smaller food businesses. However, cost should not impinge on proper sampling and monitoring. Environmental monitoring programmes (Section 4.6.1) should be viewed as routine investigative studies designed to target specific sites and detect loss of control [26].

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* Compositing or composite samples are materials for testing composed of more than 1 sample.

APPENDIX 6. MICROBIOLOGICAL CRITERIA

Microbiological criteria can provide guidance on the acceptability of foods and the processing environment (e.g. processing area) in terms of microbiological safety. Currently, there is no European or international agreement on microbiological criteria for *L. monocytogenes* in foods or the environment. However, within the EU, *L. monocytogenes* must be absent in 25g of cheese other than hard cheese and absent in 1g of other milk products [51 - 52, 140].

The European Commissions Scientific Committee on Veterinary Measures Relating to Public Health (SCVMRPH) has indicated that not > 100 CFU/g of *L. monocytogenes* at the point of consumption is a low-risk to consumers [21]. However, it has also been indicated by SCVMRPH that because of the uncertainty related to the estimation of this risk and because of the potential for proliferation of *L. monocytogenes* in food during storage, levels of < 100 CFU/g may need to be applied to foods in which growth can occur. The Scientific Committee for Food (SCF) agreed with these recommendations in its opinion of 22 June 2000 [176].

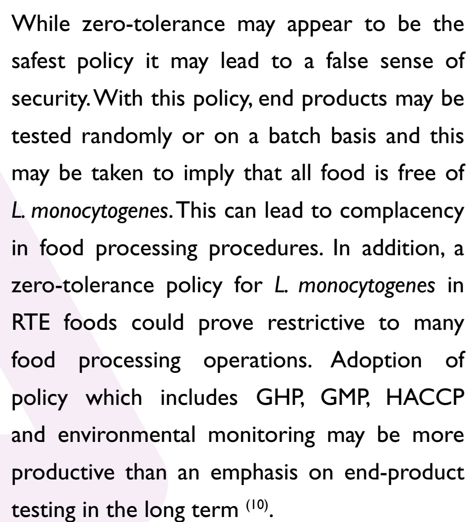
The EU is currently revising all legislation related to microbiological criteria of food. A regulation on harmonised microbiological criteria for foods (which will include criteria for *L. monocytogenes*) will come into force in 2006 [3].

It has been estimated that < 100 CFU/g of *L. monocytogenes* at the point when food is eaten (or sold) provides a similar level of consumer protection to a standard which requires the absence of *L. monocytogenes* in 25 or 50g [3, 124].

In Ireland, DAF and DCMNR indicate that the isolation of *L. monocytogenes* in RTE foods should result in the initiation of a food businesses withdrawal programme (i.e. essentially a zero-tolerance policy). The FSAI indicates that the presence of *L. monocytogenes* in RTE foods > 100 CFU/g is unacceptable [44]. A similar situation is seen in the United Kingdom, France, Germany and Holland. However, Germany also recommends that products which contain between 100-1,000 CFU/g *L. monocytogenes* should be reprocessed. There appears as yet to be no policies for levels of *L. monocytogenes* in foods among Asian countries.

Countries including the United States, Australia, New Zealand, Italy and Austria apply a zero-tolerance policy (absent in 25g/ml of food) towards *L. monocytogenes* in foods [1]. Denmark and Canada adopt a mixed policy of zero-tolerance for some foods and acceptable levels of < 100 CFU/g for others [177]. With this mixed policy particular reference is given to foods which support growth of *L. monocytogenes* or have a long shelf-life. Some types of food are considered to be of low risk to consumers as they do not support the growth of *L. monocytogenes*. Typically, these foods will have a low pH and/or a_w such as dried meats and pickled vegetables. Other foods have no apparent associated risks as they are frozen and then heated before serving, hot filled at temperatures sufficient to destroy *L. monocytogenes* or cooked in the container in which they are sold [55].

Zero-tolerance requirements for *L. monocytogenes* in processed RTE foods are controversial.



While zero-tolerance may appear to be the safest policy it may lead to a false sense of security. With this policy, end products may be tested randomly or on a batch basis and this may be taken to imply that all food is free of *L. monocytogenes*. This can lead to complacency in food processing procedures. In addition, a zero-tolerance policy for *L. monocytogenes* in RTE foods could prove restrictive to many food processing operations. Adoption of policy which includes GHP, GMP, HACCP and environmental monitoring may be more productive than an emphasis on end-product testing in the long term [10].

In the United States, current policy not only is for a zero-tolerance of *L. monocytogenes* in end-products but also for zero-tolerance of *L. monocytogenes* on food contact surfaces. Where *L. monocytogenes* is detected on a food contact surface, all food which came into contact with the contaminated production line before the production line is cleaned and sanitised is regarded as contaminated [26].

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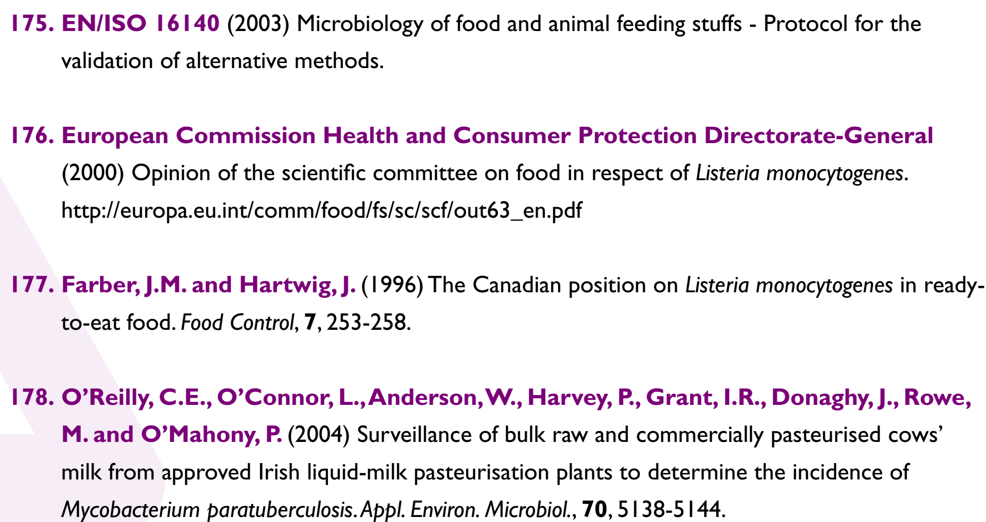

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