LISTERIA

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LISTERIA

A practical approach to the organism and its control in foods

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FOREWORD

The independent investigations some 70 years ago by E.G.D. Murray and colleagues in Cambridge (UK) and J.H.H. Pirie in Johannesburg (South Africa) resulted in the first detailed descriptions of listeriosis (in both instances in small animals), together with the isolation and naming of *Listeria monocytogenes*. These descriptions in 1926 and 1927 show the precision and care of these experimentalists, for not only did they show much skill and attention to detail but also great insight in surmising that the consumption of contaminated food was associated with the transmission of listeriosis. In the words of Pirie in 1927, 'Infection can be produced by subcutaneous inoculation or by feeding and it is thought that it is by feeding that the disease is spread in nature.'

These observations were largely forgotten and listeriosis was regarded as a rather obscure disease of animals and occasionally humans. However, the 1980s saw dramatic changes and the 'elevation' of *Listeria* to a topic of concern not only amongst microbiologists (particularly food microbiologists) but also the general public. The reasons for this change can be linked to: (i) the recognition of severe outbreaks amongst people in 1981 (Nova Scotia, Canada), 1985 (California, USA) and 1983–87 (Vaud, Switzerland) and the realization that these resulted from foodborne transmission; (ii) the rises during the early 1980s in the numbers of reported human listeriosis cases in several countries; and (iii) a general increase in interest in foodborne disease. My own involvement with *Listeria* started in 1981 and coincided with a small (possibly foodborne) outbreak in Cumbria.

Since the early 1980s there has been much new information published on *Listeria* and listeriosis. This has appeared not only in medical and scientific journals, but also in trade journals, scientific conference proceedings, government documents, industry codes of practice, etc. Not only is there now a better understanding of the epidemiology of the

disease, the disease itself and the physiology and distribution of the organism but, more importantly, of effective methods of controlling the organism in the food chain. Bell and Kyriakides have drawn together information from the extremely wide range of available sources (including their own extensive personal experiences) and have provided an up-to-date, practical and instructive text on listeriosis, *Listeria* and food processing. All those with an interest in the food industry should appreciate the potential hazards presented by listeriosis and take heed of the practical advice contained in this book.

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1

BACKGROUND

INTRODUCTION

Listeria monocytogenes is an organism that until comparatively recently was not regarded as a significant foodborne pathogen and consequently had not received much attention from the food industry Levels of listeriosis in the human population have always been hugely overshadowed by other foodborne illnesses, such as salmonellosis or campylobacteriosis, and substantiated foodborne outbreaks of listeriosis were rare Outbreaks of foodborne listeriosis in the early 1980s, however, demonstrated the severe nature of the illness with exceptionally high levels of mortality, particularly in the most vulnerable members of the community such as unborn babies, the elderly and the immunocompromised This prompted widespread public fear about the pathogen (Figure 1 1) and resulted in a massive programme by the public health authorities and the food industry to control the organism and the illness

Figure 1.1 Public concern

C. Bell et al., *Listeria*

This book aims to give the reader an overview of *Listeria*, but it is primarily intended as an aid for those persons who want to understand the nature of the hazard it presents to food products and the means for controlling it.

'*Listeria monocytogenes* is a widely distributed environmental contaminant whose primary means of transmission to humans is through contamination of foodstuffs at any point in the food chain, from source to kitchen. The total elimination of *Listeria monocytogenes* from all food is impractical and may be impossible.' This quote comes from the Conclusion and Recommendations in the Report of a World Health Organization informal working group convened in 1988 to discuss foodborne listeriosis (Anon., 1988). It is clear that eliminating the hazard associated with *L. monocytogenes* from most foods is both an impractical and an impossible task but it is possible to reduce and control this hazard in foods, thereby minimizing the risk presented to public health.

L. monocytogenes has been recognized as an animal pathogen for over 70 years (Murray *et al.*, 1926) but it is principally in the last two decades that concern has been focused on the role of food in the transmission of human listeriosis. Public and regulatory concern relating to the organism has led to the implementation by many countries of mandatory microbiological standards aimed at regulating the levels of *L. monocytogenes* in foods. In addition, concerted action has been taken by the food industry to minimize the incidence and growth of this organism in foods by enhancing standards of hygiene, reformulating foods and reducing product shelf lives.

TAXONOMY OF *LISTERIA* SPP.

The genus *Listeria* is named after the British surgeon Lord Joseph Lister who pioneered the concept of antiseptic surgery in the 1860s to prevent surgical sepsis. For many years the genus consisted of one species, *L. monocytogenes* (Figure 1.2), originally named *Bacterium monocytogenes* (Murray *et al.*, 1926) because of the large mononuclear leucocytosis that it caused in infections of rabbits.

In recent years the taxonomic position of *Listeria* species has been the subject of much work and debate. The ninth and most recent edition of Bergey's *Manual of Systematic Bacteriology* (Seeliger and Jones, 1986) recognizes five clearly distinguishable species (*L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri* and *L. ivanovii*) whilst

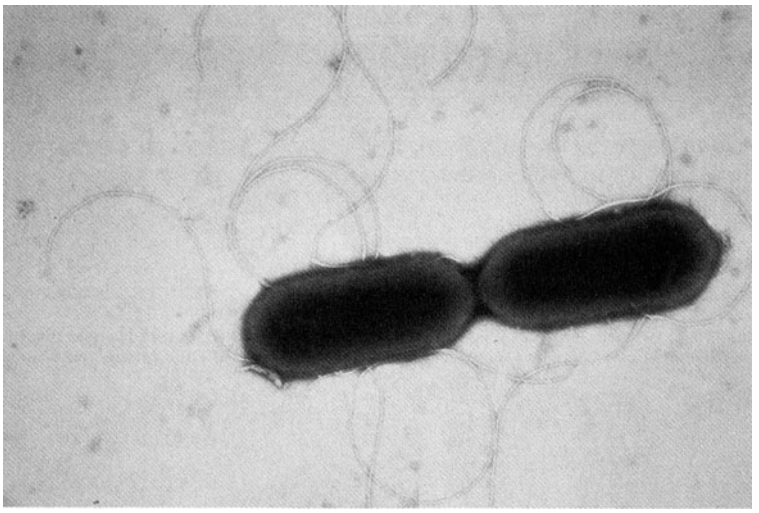

Figure 1.2 *Listeria monocytogenes* By kind permission of Oxoid Ltd, UK

L denitrificans, *L grayi* and *L murrayi* are listed as species *incertae sedis* Recent work investigating the position of *L denitrificans* has identified that it does not belong to the *Listeria* genus and it has therefore been reclassified as *Jonesia denitrificans* *L grayi* and *L. murrayi* have, however, been retained in the genus but are not considered to be sufficiently different to warrant being separate species These have therefore been reclassified as a single species for which the name *L grayi* has been proposed Proposals have also been made for two subspecies of *L ivanovii* *L ivanovii* subspecies *ivanovii* and *L. ivanovii* subspecies *londoniensis* (Boerlin *et al.*, 1992) The most recent information therefore suggests that the genus *Listeria* consists of the species listed in Table 1 1 As further work is reported, however, it is likely that other changes in classification will follow The taxonomic relatedness of the different *Listeria* species is shown in Figure 1 3 The most commonly occurring species in food are *L. innocua* and *L. monocytogenes* (Jay, 1996, Kozak *et al*, 1996)

LISTERIOSIS THE ILLNESS

Listeria monocytogenes is widely recognized as the principal human pathogen of the *Listeria* genus (Jones, 1990) However, *L. seeligeri* and *L. ivanovii* have, on rare occasions, also been implicated in human infections (McLauchlin, 1996) The Public Health Laboratory Service (PHLS) of England and Wales identified the strains of *Listeria* isolated from 2237

Table 1.1 Members of the genus *Listeria*

Listeria species	Previous species names	Origin of species name*
Listeria monocytogenes	*Bacterium monocytogenes*	Increased monocyte production
Listeria innocua		Innocuous/harmless
Listeria welshimeri		After H.J. Welshimer, American bacteriologist
Listeria seeligeri		After H.P.R. Seeliger, German bacteriologist
Listeria grayi	*L. grayi, L. murrayi*	After M.L. Gray, American bacteriologist
Listeria ivanovii subspecies *ivanovii* subspecies *londoniensis*	*L. ivanovii* *L. monocytogenes* serovar 5	After I. Ivanov, Bulgarian bacteriologist

* Seeliger and Jones, 1986.

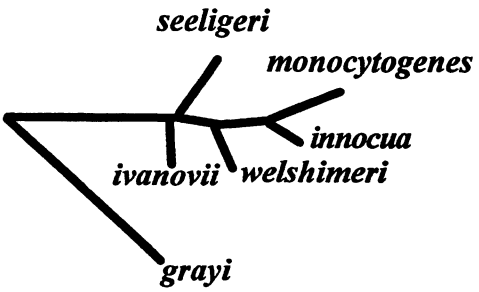

Figure 1.3 Taxonomic relatedness of the *Listeria* species based on 16S ribosomal RNA sequence data (adapted from Collins *et al.*, 1991).

human listeriosis cases in the UK; all but two strains were *L. monocytogenes* (McLauchlin, 1996). The mechanisms of pathogenicity of the organism are not clearly understood but infection involves a variety of factors including host immunity, level of inoculum and virulence of the specific *L. monocytogenes* strain. Haemolysis is understood to be a major virulence factor of *L. monocytogenes* (Rocourt, 1994). In the vast majority of large foodborne outbreaks the epidemic strain has been one of two serogroups: 1/2a or, more frequently, 4b. It has been suggested that factors such as serogroup may be of importance when assessing the risk to an individual consuming a food containing *L. monocytogenes* (Rocourt,

1994). It is possible that future risk assessments carried out by national authorities in accordance with the guidelines being formulated by the Codex Alimentarius Commission (Codex) may have to take account of the apparent differences in the potential of different serogroups to cause extensive foodborne outbreaks.

The PHLS define a case of listeriosis as a 'patient with a compatible illness from whom *Listeria monocytogenes* was isolated from a normally sterile site (usually blood or cerebrospinal fluid, CSF)' (Anon., 1997a). The primary groups of individuals at risk from listeriosis have been reviewed by Rocourt (1996) and in order of descending risk are as follows: organ transplant patients, patients with AIDS, HIV-infected individuals, pregnant women, patients with cancer and the elderly. *L. monocytogenes* can cause a variety of infections (Table 1.2) but listeriosis most commonly takes the form of an infection of the uterus, the bloodstream or the central nervous system (McLauchlin, 1993). In pregnant women this can result in spontaneous abortion, stillbirth or birth of a severely ill baby due to infection of the foetus. Listeriosis may also be acquired by new-born babies due to postnatal infection from the mother or other infected babies. The mother is rarely severely affected by listeriosis as the disease appears to focus on the foetus (Rocourt, 1996). Healthy non-pregnant adults may also suffer listeriosis with the most vulnerable groups including the immunocompromised and the elderly due to the reduced status of their immune systems. In such groups, listeriosis usually presents as meningitis (infection of the tissues surrounding the brain) and septicaemia (infection in the bloodstream). Of 2005 listeriosis cases in England and Wales between 1983 and 1996, a total of 659 (33%) were associated with pregnancy, although there were significant variations from year to year (8% to 48%) (Anon., 1997a). In general, a decrease in pregnancy-related cases has been recorded in recent years following advice in the late 1980s regarding foods to be avoided during pregnancy (Department of Health, 1989). The remaining 1346 cases of listeriosis primarily related to immunocompromised and elderly groups. This probably reflects the increasing proportion in recent years of this vulnerable sector of the community within the total population.

Listeriosis, although often acquired by ingestion of food, has until recently not been recognized as causing symptoms of the more familiar food poisoning. However, recent documented foodborne outbreaks of listeriosis include cases where the presence of extremely high levels of *L. monocytogenes* has resulted in the rapid onset of symptoms of vomiting and diarrhoea with few apparent cases of classical listeriosis (Salamina *et al.*, 1996; Dalton *et al.*, 1997).

Table 1.2 Illness caused by *L. monocytogenes*

Type of listeriosis	Nature of infection	Severity	Time to onset
Zoonotic infection	Local infection of skin lesions	Mild and self-resolving	1–2 days
Neonatal infection	Infection of new-born babies from infected mother during birth or due to cross-infection from one neonate in the hospital to other babies	Can be extremely severe, resulting in meningitis and death	1–2 days (early onset) usually from congenital infection prior to birth 5–12 days (late onset) following cross-infection from another infant
Infection during pregnancy	Acquired following the consumption of contaminated food	Mild flu-like illness or asymptomatic in the mother but serious implications for unborn infant including spontaneous abortion, foetal death, stillbirth and meningitis. Infection is more common in third trimester	Varies from 1 day to several months
Infection of non-pregnant adults	Acquired following the consumption of contaminated food	Asymptomatic or mild illness, which may progress to central nervous system infections such as meningitis. Most common in immunocompromised or elderly	Illness may occur within 1 day or up to several months
Listeria food poisoning	Consumption of food with exceptionally high levels of *L. monocytogenes*, $> 10^7$ per ml	Vomiting and diarrhoea, sometimes progressing to bacteraemia but usually self-resolving	< 24 h after consumption

Listeriosis is a rare illness with an incidence of 2 to 3 cases per million of the total population of England and Wales (McLauchlin, 1993). Levels rose from 115 to 149 cases per year in the period between 1983 and 1986 to a peak of nearly 300 in 1988 followed by a decline to 127 and 112 in 1991-92, respectively. These lower levels have been maintained over the last few years (Anon., 1997a). It is recognized that the sharp fall in the listeriosis figures from the peak in 1988 may have been due to public health advice relating to the consumption of higher risk food commodities and concerted industry action to control the organism (Gilbert, 1996). Indeed, it is believed that the sharp rise in cases in the period between 1987 and 1989 (Figure 1.4) was due to a foodborne outbreak associated with contaminated pâté from a single manufacturer (McLauchlin *et al.*, 1991).

The incidence of listeriosis in other countries is similar to that in England and Wales (Table 1.3) and similar decreases in infections to those experienced in the UK have been reported in the USA following improvements in regulatory/industry control and the issuing of advice to susceptible groups (Tappero *et al.*, 1995).

In spite of the relatively low incidence of disease, listeriosis is a serious illness and this is reflected by the apparent high mortality rate

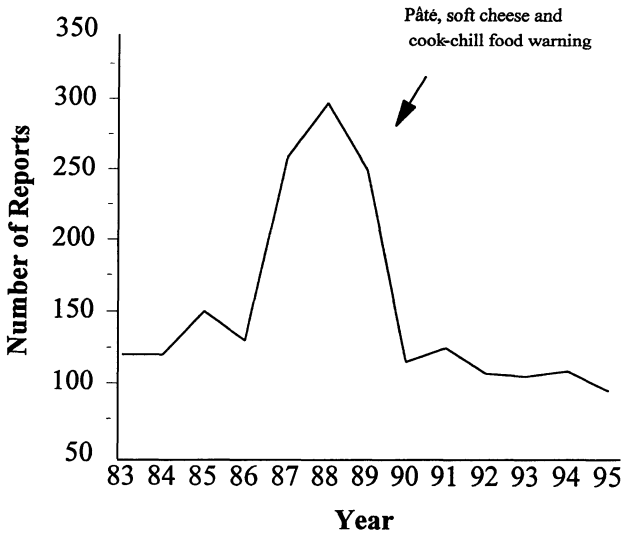

Figure 1.4 Listeriosis in England and Wales 1983-95 (adapted from Anon., 1997a).

Table 1.3 Incidence of listeriosis in different countries

Country	Cases per year (approximate)	Cases per million population	Reference
England and Wales	100	2-3	McLauchlin, 1993
Denmark	20-25	4-5	Qvist, 1996
USA	1000	4.2	Tappero *et al.*, 1995
Canada	40-60	-	Farber and Harwig, 1996
France	-	11	Bille, 1990

in many cases with fatalities averaging approximately 30% (Newton *et al.*, 1992).

SOURCES OF *LISTERIA* SPECIES

Listeriosis is transmitted via three main routes: contact with animals, cross-infection of new-born babies in hospital and foodborne infection.

Wild and domestic animals are known to carry *L. monocytogenes* and many can become infected and suffer listeriosis. Septicaemia and abortion are known to occur in sheep and other animals and, although rare, the hazard of infection to pregnant women, young children and the immunocompromised from such sources must be recognized. Most farm-associated incidents occur in the form of skin infections of farmers and veterinarians acquired by direct contact with infected animals. These are rarely more than mild self-resolving local infections although more serious infections can develop (McLauchlin, 1993).

Listeriosis in new-born babies has occurred on a number of occasions with over 29 incidents being recorded in the UK (McLauchlin, 1996). Cross-infection from single infectious sources has caused a number of large hospital-acquired (nosocomial) outbreaks but in most cases only a limited number of secondary cases were caused. Such cross-contamination has been estimated to be responsible for 25% of hospital-acquired infections (McLauchlin, 1993).

Listeria species are widely distributed in the environment: in soil (MacGowan *et al.*, 1994), vegetables (Beuchat, 1996), meat (Jay, 1996), milk (Kozak *et al.*, 1996) and fish (Ben Embarek, 1994). In addition, 2-6% of healthy people are reported to be asymptomatic faecal carriers of *L. monocytogenes* (Rocourt, 1996). Although the incidence may vary,

it is likely that from time to time most unprocessed (raw) or lightly processed materials will be contaminated with *Listeria* species and *L. monocytogenes*. The presence of the organism should therefore be expected in most food materials of this nature. Because of this, the hazard that *L. monocytogenes* presents to the food production processes and the potential safety of finished products must be assessed.

2

OUTBREAKS: CAUSES AND LESSONS TO BE LEARNT

INTRODUCTION

A large number of listeriosis outbreaks linked to the consumption of food products has occurred in the last two decades. Table 2.1 summarizes information concerning a few of these outbreaks, indicating both the worldwide nature of outbreaks and the wide range of foods involved. These have been summarized by McLauchlin (1996).

A significant feature of many of these outbreaks is the high level of mortality associated with them. Foods implicated in outbreaks of listeriosis have included products from all of the major groups: dairy, meat, vegetables and fish/shellfish. The outbreaks summarized in Table 2.1 represent merely the larger outbreaks that have occurred and there is also a large number of sporadic cases that have occurred throughout the world (McLauchlin, 1996). In addition, there must also be a number of sporadic cases that, due to their nature, cannot be definitively linked with a particular foodstuff. Analysis of foodborne outbreaks indicates that foods that meet the criteria listed in Table 2.2 are far more likely to be implicated in a listeriosis outbreak than others.

The outbreaks very clearly demonstrate that control of *L. monocytogenes* in food processing and supply systems is absolutely essential. The following sections consider some of these outbreaks in more detail, review what went wrong and identify some measures that could be taken to prevent such incidents occurring again. Outbreaks are usually investigated from a clinical perspective and complete information about the nature of the food involved and the production processes used is often not recorded. Tables 2.3 to 2.8 represent a combination of the known information together with the most likely reasons why these incidents occurred and

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Table 2.1 Foodborne outbreaks of listeriosis

Year	Country	Cases (deaths)	Food	Outbreak serotype	Reference
1980–81	Canada	41 (18)	Coleslaw	4b	Schlech *et al.*, 1983
1983	USA	49 (14)	Pasteurized milk	4b	Fleming *et al.*, 1985
1983–87	Switzerland	122 (34)	Vacherin cheese	4b	Bille, 1990
1985	USA	142 (48)	Mexican-style soft cheese	4b	Linnan *et al.*, 1988
1987–89	UK	> 350 (> 90)	Belgian pâté	4b	McLauchlin *et al.*, 1991
1992	New Zealand	4 (2)	Smoked mussels	1/2a	Baker *et al.*, 1993
1992	France	279 (63)	Pork tongue in aspic	4b	Goulet *et al.*, 1993
1994	USA	45 (0)	Chocolate milk	1/2b	Dalton *et al.*, 1997
1995	France	20 (4)	Raw-milk soft cheese	4b	Goulet *et al.*, 1995

Table 2.2 Factors associated with foods linked to outbreaks of listeriosis

Risk factors
Use of raw ingredients not subjected to listericidal process or product susceptible to post-process contamination
Refrigerated storage
Product formulation allowing growth
Extended shelf life (> 10 days)
Ready to eat

ways of controlling them. By analysing outbreaks in this way, practices compromising product safety with respect to *L. monocytogenes* can be better understood. Such understanding can be used to improve the safety of processes and products.

COLESLAW: CANADA

One of the first foodborne outbreaks of listeriosis reported in North America occurred in Nova Scotia between 1981 and 1982 (Schlech *et al.*, 1983). The outbreak was estimated to have resulted in 41 cases of listeriosis with 18 deaths: 2 adults and 16 foetal or new-born. The outbreak, caused by *L. monocytogenes* 4b, was epidemiologically linked to consumption of coleslaw (Table 2.3) with the same serogroup (4b) being isolated from a patient suffering listeriosis, a sample of coleslaw retrieved from the patient's refrigerator and unopened packs from supermarkets. It is important to note that in many outbreaks foods are implicated in the absence of unequivocal microbiological evidence but based on a survey of common foods consumed by infected individuals. Even where the outbreak strain is isolated from a food this may only be from food retrieved from the patient's home, which may have been contaminated within the household. The most conclusive evidence implicating food is usually derived from unopened samples of food or from food obtained from a manufacturing site where the isolated strain can be proven to be identical to the strain isolated from infected individuals, as occurred in this outbreak.

The coleslaw implicated in this outbreak of listeriosis was apparently a dry mixture including carrot and cabbage. Coleslaw may be manufactured in two ways. Traditional coleslaw is manufactured by shredding vegetables, which may be trimmed, washed, and then mixed together prior to packing in a plastic container. This is retailed with a short shelf life (4–7 days) under refrigerated display. Coleslaw can also be made by mixing the shredded vegetables with a pH-controlled dressing, usually

Table 2.3 Outbreak overview: coleslaw

Product type:	Chopped cabbage and carrot
Year:	1981
Country:	Canada
Levels:	Not known

Possible reasons

(i) Contamination of cabbage with *L. monocytogenes* from uncomposted sheep manure
(ii) Extended cold storage of cabbage allowing extensive growth of *L. monocytogenes*
(iii) Use of cabbage in ready-to-eat product without listericidal process

Control options*

(i) Controlled use of manure to prevent contamination of growing crops
(ii) Storage at temperatures to prevent *L. monocytogenes* growth (< 1°C) or storage for short periods of time (< 10 days)
(iii) Washing of cut cabbage with chlorinated water (100–200 ppm free chlorine) to reduce loading
(iv) Control of retail shelf life and cold storage conditions

* Suggested controls are for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

mayonnaise. The acidity in the dressing, its low pH (usually 5.0), together with storage at chill temperatures (5–8°C), offers some protection against the growth of any potential pathogens such as *L. monocytogenes*. However, in this outbreak it is reported that cabbage used for the coleslaw was sourced from fields where both raw and composted manure from a flock of sheep with known recent incidence of listeriosis were being applied. It is also believed that this coleslaw was a type that did not contain a pH-controlled dressing. It is possible that the cabbage may have been extensively contaminated with *L. monocytogenes* although it is important to note that environmental samples, including raw manure, taken from the farm during subsequent investigations did not reveal any *L. monocytogenes*. However, it was normal practice to store the raw cabbage for extended periods (from October to early spring) at low temperatures before use and it is believed that even low levels of *L. monocytogenes* may have been capable of extensive growth during this period (Beuchat, 1996). Additionally, because the coleslaw involved was a dry mix, the pH is likely to have been above pH 5, thus providing conditions suitable for growth of the organism in the finished product.

It is clear that even without detailed knowledge of the entire process and product it is possible to identify stages that, if controlled, could have

significantly reduced the hazard from *L. monocytogenes*. Avoiding contamination in the first instance is usually the best policy and application of uncomposted manure is likely to introduce extensive and high levels of microbial contaminants. For vegetables used without a listericidal process, consideration should be given to avoiding the use of any animal manure as fertilizer or only using material where the composting temperatures are known to 'pasteurize' the manure. However, even given the best farming practices, it should be recognized that any raw agricultural crop is likely to be occasionally contaminated with pathogenic microorganisms. As *L. monocytogenes* can grow in vegetable materials when stored for extended periods under cold conditions, the cabbage should have been stored under temperatures precluding growth, i.e. 0°C to -1°C. Reducing the period of storage to <10 days would also have significantly reduced the hazard from *L. monocytogenes*. A further step in the process, which could have reduced any incoming levels of contamination on the cabbage, is a washing stage. Raw vegetables such as cabbage are usually prepared by taking the core out and removing outer leaves before shredding. Some factories operate a chlorinated water washing system, which can reduce levels of contamination mainly by a rinsing effect but also by the lethal effect of chlorine. Other processors avoid washing some vegetables due to a concern over the distribution of spoilage contaminants such as yeast and residual water on the vegetable material, which may diminish the preservative effect of any mayonnaise dressing that may be applied. If chlorination is employed, it is essential to maintain sufficient free chlorine to prevent the wash water being a source of cross-contamination from batch to batch.

Listeria growth in a dressed coleslaw is inhibited by the pH-controlled mayonnaise dressing, which exerts some antimicrobial effect due to the combination of the preservative effect of acetic acid and the low pH. Maintenance of a pH below 4.5 will prevent the growth of *Listeria* but pH levels in excess of 5 will allow growth, albeit slowly, during the shelf life.

Distribution and retail display of finished products at temperatures between 0 and 5°C, combined with a limited shelf life (<10 days), will also assist in the control of growth and the delivery of safe products for consumption.

VACHERIN MONT D'OR CHEESE: SWITZERLAND

Between 1983 and 1987 a total of 122 cases of listeriosis, resulting in 34 deaths, was recorded in the western part of Switzerland (canton de Vaud). On detailed investigation these cases were epidemiologically linked with

Table 2.4 Outbreak overview: Vacherin Mont d'Or

Product type:	Surface-ripened soft cheese
Year:	1983–87
Country:	Switzerland
Levels:	10^4–10^6 per gram

Possible reasons

(i) Possible use of unpasteurized milk contaminated with *L. monocytogenes*
(ii) Maturation of product in environment that is difficult to clean and open to potential for *L. monocytogenes* to cross-contaminate products, e.g. wooden shelving
(iii) Growth of culture during surface ripening of product increases the pH and may allow growth of *L. monocytogenes*
(iv) Maturation usually occurs at temperature within growth range of *L. monocytogenes*
(v) Final product capable of allowing the growth of *L. monocytogenes*

Control options*

(i) Pasteurize milk or ensure selection of highest quality milk from selected farms known to be operating stringent hygiene control and monitoring hygiene regularly
(ii) Use of hygienic processing equipment capable of being cleaned to high standards of hygiene
(iii) Operate to the minimum time and the minimum temperature necessary to achieve correct product quality
(iv) Control of retail shelf life and cold storage conditions

* Suggested controls are for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

the consumption of Vacherin Mont d'Or cheese (Bille, 1990) (Table 2.4). The outbreak strain of *L. monocytogenes* was isolated from the surface of the cheese, from a piece of cheese obtained from a patient suffering listeriosis and from areas in the cheese production environment, with the wooden shelves and brine brushes used in the ripening rooms being particularly heavily contaminated.

Vacherin Mont d'Or is an example of a surface-bacterial-ripened soft cheese that is frequently made using unpasteurized milk, although the nature of the milk in the variety implicated in this outbreak is not clear from the published reports. Raw milk is a potential source of a wide range of bacterial pathogens. In surveys of raw milk, *L. monocytogenes* is commonly reported to be present (Kozak *et al.*, 1996). The organism is derived either from infections of the udder, contamination of the external

surface of the udder or contamination of milking equipment and storage vessels.

Most cheese is manufactured by inoculation of pre-warmed milk with a suitable starter culture and coagulation of the milk proteins using rennet. The liquid whey is usually drained from the formed curd by cutting the curd into pieces, filling it into hoops or moulds and allowing whey drainage to take place over 1 to 2 days. Fermentation of the milk by the starter cultures usually reduces the pH to less than 5.0 and growth of contaminants such as *Listeria* spp. in the raw milk at this stage is usually inhibited by acidity development, pH reduction and competitive pressure from the starter culture organisms. As a soft cheese, Vacherin Mont d'Or has a higher moisture content than other cheese such as cheddar. The level of acidity and the low pH of fresh cheese after fermentation usually prevent the growth of *Listeria* during maturation. However, one of the major problems with soft surface-ripened cheese varieties is that during maturation, cultures are inoculated onto the outer surfaces of the cheese and provided with conditions of growth that allow the development of the characteristic visual appearance and flavour. However, the growth of the culture also elevates the pH at the surface of the cheese to near neutral. Under conditions where the pH is no longer inhibitory to growth and where sufficient moisture is available, any *Listeria* present due to introduction in the raw milk or as a post-processing contaminant can proliferate rapidly and easily reach hazardous levels.

In terms of control of *L. monocytogenes* in this product, the starting point has to be the raw milk, from which it is not possible to exclude the potential for *Listeria* to be present. However, by operating to high standards of animal husbandry and hygiene during cleaning, and decontaminating the udder and associated milking equipment, it is possible to minimize the levels of contamination.

As a psychrotrophic microorganism, *Listeria* is selectively favoured by storage at low temperatures for long periods of time and so the use of raw milk as quickly as possible is essential to prevent significant growth of the pathogen in the milk.

In the outbreak of listeriosis associated with Vacherin Mont d'Or cheese, it was reported that drainage and maturation of the cheese was conducted in wooden hoops on wooden shelves where the opportunity for *Listeria* contamination was reported to be very high. Since this coincides with the development of the surface culture growth and elevation of pH at the surface of the cheese, it is highly likely that contamination at this

stage was able to grow to levels sufficient to cause listeriosis in susceptible individuals. In a product capable of supporting growth of *Listeria*, it is absolutely essential that the highest standards of environmental hygiene are maintained in production areas where the product is exposed to the environment; any level of contamination may grow to high numbers rapidly. Environmental hygiene management should encompass areas where *Listeria* may build up, such as floors, walls and drains, but it should particularly focus on product contact surfaces, e.g. shelves and trolleys, and areas where condensation occurs.

In products such as surface-culture-ripened soft cheese made from unpasteurized milk it is not possible to completely eliminate the hazard from *L. monocytogenes* as there is no process step that can assure absence of this organism in the raw milk. However, there are many stages where *L. monocytogenes* can increase significantly. If these stages are controlled properly, the risk of listeriosis outbreaks from these types of cheeses can be significantly reduced.

BELGIAN PÂTÉ: UK

Between 1987 and 1989 the UK experienced a large increase in incidents of listeriosis rising from approximately 100 cases per year to a peak of nearly 300 cases in 1989 (McLauchlin *et al.*, 1991). Following a detailed investigation of the cases involved, it was concluded that the increase was almost entirely due to the consumption of a contaminated pâté imported from a Belgian manufacturer (Table 2.5). Samples of pâté from the implicated manufacturer contained levels of *L. monocytogenes* serotype 4b ranging from $<10^2$ per gram to $>10^6$ per gram (McLauchlin, 1993). Following a general warning issued by the Department of Health in 1989 to pregnant women and those particularly susceptible to infection to avoid eating pâté and soft cheese, together with the removal from sale of pâté from the implicated manufacturer, the level of reported listeriosis dramatically reduced (McLauchlin *et al.*, 1991). The levels of listeriosis have since remained stable at approximately 100 cases per year in England and Wales (Anon., 1997a).

Pâté is manufactured by preparing an emulsion of raw meat, fat, spices and other minor ingredients. For delicatessen counter sale, product is usually filled into large, square-sectioned, hermetically sealed containers and cooked to achieve temperatures in excess of 80°C, which is sufficient to destroy high levels of vegetative microbial contaminants that may be present in the raw material. Following rapid cooling, the pâté is then

Table 2.5 Outbreak overview: Belgian pâté

Product type:	Cooked prepacked pâté
Year:	1987–89
Country:	UK
Levels:	$< 10^2$ to 10^6 per gram

Possible reasons

(i) Post-processing contamination of product during slicing/packing
(ii) Extended shelf life (5 weeks) under refrigerated storage
(iii) Product capable of allowing the growth of *L. monocytogenes* at chill temperatures

Control options*

(i) Control of cooking process
(ii) Hygienic design of equipment, particularly post heat processing
(iii) Strict adherence to effective cleaning schedules of all equipment, especially slicers and conveyor belts
(iv) Regular monitoring of cleaning efficacy
(v) Control of retail shelf life and cold storage conditions

* Suggested controls are for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

removed from the containers, sliced and repackaged for retail sale as prepacks or distributed for bulk display on delicatessen counters. In such circumstances the source of contamination can really only arise as a result of either an inadequate cooking process or cross-contamination after heat processing. The pâté implicated in this outbreak was also given a shelf life in excess of 5 weeks which, given the highly nutritious nature of pâté with neutral pH and few other inhibitory compounds, could readily allow the growth of *Listeria* to very high levels.

In reviewing this outbreak it is evident that there are number of critical control points in a cooked meat process that impact on the control of *Listeria*. Although not implicated as a cause in this outbreak, the most important and obvious stage to control is cooking. It is easy to assume that any cooking process will render the finished product safe irrespective of the contamination level of the raw materials but achievement of the correct process time and temperature to ensure sufficient reduction of the original contamination load of the raw material is critical to the safety of this product.

It is highly unlikely that the problem with this pâté was due to inadequate heat processing and is much more likely to have been due to subsequent

cross-contamination of exposed cooked product. Depending on the post-processing conditions employed in the production of this pâté, potential contamination could arise from the following key areas:

- cooling, if the product is exposed during chilling
- contamination from food contact surfaces or containers used to transport the product within the factory
- slicing machinery or conveyor belts if the product is sold as prepacks
- garnishing, if the product is decorated post processing.

However, an effective way to reduce the hazard associated with *Listeria* is to ensure that high standards of environmental hygiene in the chilling systems are maintained using regular cleaning and sanitizing procedures together with basic standards of good hygienic practice.

In most cooked meat operations the greatest opportunities for contaminating pâté and other cooked meat products arise during slicing and garnishing. The cooked, cooled product is usually manually removed from the containers used for cooking and placed on racks or trays. The product may then be superchilled (-2 to 0°C) to allow for improved slicing performance. Blocks of pâté are usually sliced in a continuous process followed by in-line automatic packing and sealing of product in its final packaging. Bulk product for delicatessen counters may be garnished with herbs and spices prior to packaging. In all cases the opportunities for contamination from the post-heat-process environment and from people are significant.

Contamination can be minimized by focusing hygienic practices on the prime product contact surfaces in the transfer and slicing operation. This includes the product racks used to hold the exposed cooked meat, loading mechanism, slicers and conveyor belts. Minimizing production runs and introducing frequent cleaning and decontamination cycles are essential to prevent levels of any *Listeria* spp. that gain access to the equipment from building up to levels sufficient to allow extensive cross-contamination. Contamination cannot be completely eliminated but by operating to high standards of hygiene it can be reduced to a minimal level. Even with high standards of hygiene practice in place it is likely that sliced meat products may still be found to be contaminated by *Listeria* spp. at incidences occasionally up to 5%, although *L. monocytogenes* would be expected to be present at a very much lower incidence. The goal must be to eliminate *L. monocytogenes* from the environment and product, but as the greatest risk to public health is likely to be associated with high levels of the organism, the primary objective is to ensure

that any contamination with *Listeria* spp. is kept to low levels, i.e. <10 per gram, and that low incidence is also achieved. It is evident from the results of a survey of cooked pâté from the implicated supplier carried out by the UK Public Health Laboratory Service, which showed an incidence of *L. monocytogenes* in excess of 48% (McLauchlin *et al.*, 1991), that this goal had not been achieved. By contrast, the incidence of *L. monocytogenes* in other retail pâté sampled in 1989 was 4%.

Of course, even with low levels of contamination it is possible for *L. monocytogenes* to grow in cooked meat products such as pâté due to the absence of any significant inhibitory factors in the product. Typical pâté has a pH of 6.5 with a water activity of 0.98 and whilst it usually contains sodium nitrite this tends to have a limited effect on the growth of *L. monocytogenes*. Although growth is restricted at chilled temperatures, it is likely that levels of contaminating *L. monocytogenes* could have increased significantly in the 5-week shelf life assigned to this product. Whilst restricting the shelf life would obviously not have an impact on preventing contamination of the product with *L. monocytogenes*, it is clear that it would reduce the time available for the pathogen to increase to an infective dose. Even a basic risk assessment would conclude that a product with a low incidence and low levels of *L. monocytogenes*, together with limited chilled shelf life, would be at low risk of developing high levels of the organism in a large number of the products. This would in turn diminish the risk of one of those products being consumed by a susceptible individual. Therefore, whilst this hazard continues to exist in pâté, by focusing effort on reducing the incidence by applying high standards of hygiene and by introducing a moderate reduction in shelf life, the risk of further outbreaks could be reduced.

PORK TONGUE IN ASPIC: FRANCE

A listeriosis outbreak which resulted in an extemely high number of deaths occurred in France between March and December 1992 (Goulet *et al.*, 1993). Although the suspected vehicle of the outbreak was cooked pork tongue in jelly, it was believed that widespread cross-contamination on delicatessen counters from this point source may have facilitated such a large outbreak (Table 2.6). A total of 279 cases of listeriosis were believed to have been caused, resulting in 63 deaths and 22 abortions.

Table 2.6 Outbreak overview: pork tongue in aspic

Product type:	Cooked meat in jelly
Year:	1992
Country:	France
Levels:	Not known

Possible reasons

(i) Contamination of product during manufacture, probably post processing
(ii) Extensive distribution and sale on delicatessen counters
(iii) Widespread contamination of delicatessen counters and potential for survival and cross-contamination to other products
(iv) Product capable of allowing the growth of *L. monocytogenes* at chill temperatures

Control options*

(i) Hygienic design of all equipment, particularly post listericidal processing
(ii) Regular cleaning of production environment and monitoring of cleaning efficacy
(iii) Procedures to ensure effective cleaning of delicatessen counters
(iv) Retail counter staff training in good hygienic practice
(v) Control of temperature and shelf life of the bulk product, display product (on retail counter) and customer shelf life

* Suggested controls are for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

During the outbreak investigation over 12 000 isolates of *L. monocytogenes* were obtained from product and environmental samples taken from manufacturers, retailers and consumer homes. 203 samples were found to be contaminated with the epidemic strain, mainly in ham, pâté, products in jelly and several cheeses. All manufacturers of the products where the epidemic strains were isolated were investigated to assess the standards of manufacture and cleaning procedures, although epidemiological investigations strongly implicated the pork tongue in jelly. Public health alerts were issued to consumers, as in the UK pâté outbreak, targeted at pregnant women, immunocompromised people and the elderly. In addition, the standards of hygiene at manufacturers and retailers of products were reviewed. It is believed that withdrawal of product and subsequent advice to consumers was primarily responsible for bringing the outbreak to a conclusion (Rocourt, 1996).

In this outbreak, it is apparent that failures must have occurred at a number of stages in the food chain from manufacture to retail and possibly even in

the home. Contamination of the initial product provided the source of the *L. monocytogenes* and measures already described for the UK outbreak associated with Belgian pâté apply equally to avoid and minimize contamination of pork tongue in jelly. Investigations at six manufacturing plants identified *L. monocytogenes* in 33% of environmental samples taken in the finished product area (Salvat *et al.*, 1995). In one plant manufacturing pork tongue in aspic, the epidemic strain of *L. monocytogenes* was isolated from a raw brine sample. *L. monocytogenes* was detected on a variety of product contact surfaces including tables, transport belts, machines, moulds, knives and meat trolleys. It is interesting to note that the manufacturing plant used as a 'control' in the investigation, because it was not implicated in the outbreak, was more heavily contaminated than the plant manufacturing the implicated pork tongue in aspic product. However, in all cases the presence of *L. monocytogenes* was attributed to poor standards of cleaning that in many cases left product contact surfaces with biofilms or with visible product soiling. It was also noted that cross-contamination from raw product to cooked product was a probable reason for high levels of contamination, particularly as trolleys and containers were moved through the raw side to the cooked areas. Finally, the product was allocated a long shelf life of between 30 and 42 days, which would provide ample time for *L. monocytogenes* to multiply to high levels.

Given this account of cross-contamination potential and inadequate cleaning, it is perhaps surprising that the outbreak did not involve other bacterial pathogens in addition to *L. monocytogenes*. However, it is arguable that the outbreak may have been contained or restricted if the hygienic practices in place on the retail delicatessen counters were such that widespread cross-contamination to other products was avoided. The investigation found contamination on products and on utensils from delicatessen counters and it is easy to understand how several products could have become contaminated by the use of common slicing or cutting equipment or utensils in the retail environment. The products from which the epidemic strain was isolated were all capable of supporting the growth of *L. monocytogenes*, including ham, cheese and the pork tongue. The storage conditions of these products and the time they may have been kept in the consumer's home may also have contributed to increases in levels of the epidemic strains.

The primary area to focus upon to prevent such outbreaks is in the manufacturing process, and preventative procedures described for the UK outbreak caused by Belgian pâté would equally apply here. However, there is one key difference in the products and this is the presence of jelly or aspic on the pork tongue product.

A variety of cooked meat products have aspic or jelly applied to them after cooking and this is known to represent a significant risk if not controlled properly. It is not clear in this outbreak whether jelly was applied before or after processing but strict procedures need to be in place to prevent both primary contamination and subsequent growth of *L. monocytogenes* in the jelly system. Usually, the jelly is stored in a warm, molten state and injected into or poured around the product. Storage of the jelly at high temperatures >63°C can be employed to ensure the product remains free of vegetative pathogens. However, this can lead to deterioration of the jelly. Acidified jelly (less than pH 5) could be used at least to inhibit the growth of any contaminants. In many cases it is not the bulk jelly solution that causes the problem but the pipework and equipment used to inject or otherwise apply the jelly to the meat product that is most vulnerable. Particular attention needs to be paid to the hygiene of this equipment using thorough and regular cleaning procedures. To facilitate effective cleaning, it is important to introduce complete breaks in equipment use, at least on a daily basis, so the entire system can be cleaned.

As is the case with many cooked meat products, it is very difficult to ensure complete absence of *L. monocytogenes*; therefore procedures in place at the retail level must be designed with hazards like this in mind. It is probably too much to expect dedicated utensils and equipment for every type of product on the delicatessen counter. However, what is essential is that procedures are in place that minimize cross-contamination and break the cross-contamination chain at regular intervals. In fact this is no different to regular cleaning and disinfection at manufacturing plants. Utensils, counters and trays all need to be cleaned at frequent intervals to ensure that any contaminants that may be present are removed, thereby preventing the potential for them to grow and cross-contaminate other products. Particular attention needs to be paid to areas such as slicing equipment for which regular effective in-process cleaning procedures can be difficult to apply due to problems encountered in dismantling the food contact parts of such machines. Nevertheless, because this is such an important critical control point, regular slicing equipment cleaning procedures must be developed, operated and maintained to minimize the potential contamination hazards posed to products during slicing. In addition, it is frequently found that areas such as knife racks or points of contact between surfaces (joints between chopping boards and the supporting tables, edges and ledges in chill display counters) can be difficult to clean and therefore harbour potential contaminants. Finally, of course, with products that are extensively handled, such as those sold from delicatessen counters, it is imperative to restrict the shelf life of products capable of supporting the growth of contaminants such as *L. monocytogenes*.

SMOKED MUSSELS: NEW ZEALAND

Fish and shellfish have been implicated in few outbreaks of listeriosis (McLauchlin, 1996). However, smoked mussels were responsible for an outbreak in New Zealand in 1992 (Baker *et al.*,1993). The outbreak involved four perinatal cases; although no adult deaths were reported, one case resulted in the abortion of twins and in another case an infant delivered at term developed meningitis after seven days. *L. monocytogenes* serotype 1/2a was isolated from three cases (two of which gave a history of eating mussels of a particular brand) and serotype 4 was isolated from the fourth case, who consumed mussels of a different brand. The outbreak strain 1/2a was isolated from an unopened packet of smoked mussels and proved indistinguishable by 'fingerprinting' methods (phage typing, restriction fragment length polymorphism and pulsed-field gel electrophoresis) from the strains isolated from the two patients with a definite history of mussel consumption of the same brand. The organism was isolated from further samples of this product and also from a number of sites within a factory processing these mussels (Table 2.7). Levels of *L. monocytogenes* found in the products examined from the refrigerator of a patient were approximately 10^3 per gram.

Table 2.7 Outbreak overview: smoked mussels

Product type:	Smoked mussels
Year:	1992
Country:	New Zealand
Levels:	10^3 per gram

Possible reasons

(i) Contamination of product post cooking
(ii) Product capable of allowing the growth of *L. monocytogenes*
(iii) Extended refrigerated shelf life of product

Control options*

(i) Ensure clean growing and harvesting conditions
(ii) Use of fresh brine solutions or limit life of brine
(iii) Hygienic design of equipment, particularly post processing
(iv) Strict adherence to effective cleaning schedules of all equipment
(v) Regular monitoring of cleaning efficacy
(vi) Control of retail shelf life and cold storage conditions

* Suggested controls are for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

Original contamination of the mussels may have occurred in the growing and harvesting grounds and/or during any brining prior to the hot-smoking process. Any *L. monocytogenes* contaminating the product may have survived the smoking process or contamination may have occurred after this process. As the product was capable of supporting the growth of *L. monocytogenes* and the implicated product was reported to have a shelf life of 8 weeks (McLauchlin, 1996), *L. monocytogenes*, even if initially present at low levels, could have significantly increased in number during the extended chilled storage.

Hot-smoked mussels are produced by soaking mussels in a brine solution and then applying a combined smoking/heat-processing step in a smoking chamber. The temperature in the smoking chamber is usually 70–80°C and mussels are smoked for relatively short periods (usually less than 1 h). Product temperatures vary considerably but may range from 55 to 70°C. As with any other process, the time and temperature combination employed is critical. That *Listeria* spp. can be destroyed in a hot-smoking process is not in question but it is clear that minor fluctuations in temperature can have a significant effect on reducing the efficacy of the process.

D values of *L. monocytogenes* in green shell mussels were reported to be 48.09 min and 1.85 min at 56°C and 62°C, respectively, with a z value of 4.25°C (Bremer and Osborne, 1995). Some reports indicate an inhibitory effect of smoke but, depending on the volatile compounds produced during the process, this is likely to be highly variable. Mussels are then packed and chilled for retail sale.

The principal routes of contamination for these products are from environmental cross-contamination before and after heat processing. A most likely contributory cause of this problem is the very long shelf life given to the product; this could have allowed significant growth of any *L. monocytogenes* present in the product. Mussels have a pH close to neutral and a high water activity. Assuming the smoke makes no contribution as an antilisterial agent, it is possible for low levels of contamination, i.e. 10 per gram, to increase significantly within 8 weeks. Therefore, it is clear that unless the product was properly cooked in the pack, the shelf life should have been significantly less than that given and probably no greater than 7 to 10 days under well-controlled refrigeration conditions. Even cooked in the pack, a shelf life validation study is advisable to determine the safe shelf life of the product.

CHOCOLATE MILK: USA

Most incidents of listeriosis tend to be reported as isolated cases and the illness tends to be recognized as a consequence of invasive disease and therefore only those individuals suffering relatively severe symptoms are investigated. Recent outbreaks have indicated that *L. monocytogenes* may actually cause classic symptoms of gastro-enteritis with fever, which may go undiagnosed. This is because stool specimens are not routinely examined for the presence of *L. monocytogenes* when submitted from persons presenting with symptoms of 'food poisoning'.

The most recent evidence for gastro-enteritis caused by *L. monocytogenes* occurred at a picnic at a local cow show in Illinois, USA in 1994 (Dalton *et al.*, 1997). A total of 45 persons suffered symptoms of gastro-enteritis, with most persons suffering diarrhoea (79%) and fever (72%) approximately 20 hours after the consumption of a pasteurized chocolate milk drink (Table 2.8). Four persons were hospitalized and although levels of *L. monocytogenes* detected in the milk ranged from 8.8×10^8 per ml to 1.2×10^9 per ml no deaths are reported to have occurred. Perhaps more astonishingly, one of those affected was a woman who was 40 weeks pregnant, who suffered a six-hour episode of diarrhoea and 5 days later gave birth to a healthy baby. The outbreak strain of *L. monocytogenes* was isolated from stool samples of infected individuals, from a tank drain at the manufacturing plant that produced the chocolate milk and from unopened packs of the implicated chocolate milk product.

The product appears to be similar to many standard pasteurized milk products and the only significant difference was the incorporation of chocolate flavour prior to heat processing. The product was pasteurized at a temperature in excess of 87°C for 18 s and the investigation found no faults associated with pasteurization conditions. The milk was immediately cooled to less than 8°C and, as is common with many pasteurized liquid products, it was passed to a holding tank prior to filling. The holding tank was jacketed and designed to carry refrigerant to maintain the product under chill conditions. However, it appears that it had not been possible to use refrigerant due to the jacket being in a poor state of repair. Product was held in this tank for a period of 2 h prior to being filled into containers over a production period of 7 h.

During the investigation it was found that that the lining of the holding tank jacket was not intact, allowing chocolate milk to leak into the jacket where it stayed as a 'reservoir' until the tank emptied during filling. At this point, product could re-enter the vessel from the lining and contaminate

Table 2.8 Outbreak overview: chocolate milk

Product type:	Pasteurized flavoured milk
Year:	1995
Country:	United States
Levels:	10^7–10^9 per ml

Possible reasons

(i) Contamination of pasteurized milk post heat processing due to damaged product storage tank
(ii) Extensive temperature abuse of product prior to consumption
(iii) Product capable of allowing the growth of *L. monocytogenes* at chill temperatures

Control options*

(i) Hygienic design of all equipment, particularly post listericidal processing
(ii) Visual inspection or checking of vessel integrity
(iii) Regular cleaning and monitoring of cleaning efficacy
(iv) Clear on-pack consumer guidance regarding the necessity to store product at refrigerated temperatures and use by stated date

* Suggested controls are for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

the remaining product being filled. Additionally, the 'spray-balls' used to distribute sanitizer inside the vessel during cleaning were blocked up and this is likely to have prevented the efficient distribution of cleaning chemicals and sanitizer throughout the vessel during the cleaning process.

Packed product was held in a refrigerated room and then despatched in chilled transport to a distributor. 180 cartons were then transported unrefrigerated for over two hours after which they were stored in a domestic refrigerator overnight prior to being placed in an unrefrigerated cooler at 11 a.m. for consumption at the Illinois cow show throughout the morning and afternoon, although most was consumed between 11.30 a.m. and 12.30 p.m.

It is probably not necessary to describe in detail the reasons why such an outbreak occurred and the controls that could have prevented it as these should be obvious. Pasteurization, a key critical control point in the process, appeared to be under good control and the processing conditions were sufficient to destroy even high levels of *Listeria* spp. in the raw material. However, as has been seen with most other outbreaks, the organism appears to have been introduced as a post-processing contaminant. As

a psychrotroph, it is obviously selectively favoured under chill conditions and as the raw milk had been pasteurized at a high temperature, any post-process contaminant would not be subject to the usual competitive pressures as few other microorganisms would have been present. Therefore, even under good conditions of temperature control *L. monocytogenes*, if present, would have represented a significant hazard, particularly as the shelf life of the product was 18 days or more. Clearly, after pasteurization, the additional routes for controlling *L. monocytogenes* should have been to avoid post-process contamination, provide adequate controlled chilled storage conditions for the product and apply a shorter shelf life.

The integrity of enclosed processing equipment and its effective cleaning and sanitization is an important factor in controlling *Listeria* spp. All cleaning schedules should be supported by regular inspection of the vessels to view areas of potential poor coverage by the in-place cleaning system. In addition, tank integrity checks should be scheduled at regular intervals.

The fact that the vessel was not used as a refrigerated vessel due to the poor state of repair of the insulating jacket is only an important factor if the overall holding time in the tank prior to and during filling of the product was sufficient to allow increases in the temperature of the bulk product. With such large volumes of product (>1300 litres) delivered to the tank at less than 8°C, it is debatable whether the product temperature would rise significantly at this stage although this cannot be ruled out.

The main problem is likely to have been the very high levels of contamination that could develop in the jacket and seed the product directly during the filling operation. The subsequent product temperature abuse prior to consumption would allow extensive growth of *Listeria*.

It is difficult to ensure that all consumers hold products at refrigerated temperatures throughout the product shelf life and product labelling should always make such requirements plain. This product was designed to be stored refrigerated and, providing post-process contamination with *L. monocytogenes* was avoided, the shelf life allocated to this product would not have represented a significant hazard.

In this type of pasteurization process, using fully enclosed equipment, extended shelf lives should be readily achievable, but the hazard presented by potential post-processing psychrotrophic pathogenic contaminants such as *L. monocytogenes* should have been identified by a hazard analysis. This would have prompted the implementation of appropriate

process and equipment controls and checks to ensure the integrity of the processing plant, thus avoiding the outbreak by preventative approaches. However, any product being developed by the food industry should build in a degree of safety that accounts for some mild temperature abuse. Under such circumstances, it is likely that this product's shelf life would have been reduced.

3

FACTORS AFFECTING THE GROWTH AND SURVIVAL OF *LISTERIA MONOCYTOGENES*

GENERAL

Listeria spp., including *L. monocytogenes*, are present and will continue to be found in a wide variety of raw food materials. The natural exposure of vegetables, fruits, grains, food animals, fish and poultry to the environmental sources of the organism makes it impossible for such foods to be produced free from *L. monocytogenes* at source. In order to assure the safety of food products in respect of potential foodborne bacterial pathogens, growing, harvesting, handling, storage, processing and associated food supply systems must be managed by food producers and processors in such a way as to reliably control the growth of any pathogens likely to be present. *L. monocytogenes* in particular must be prevented from multiplying to potentially harmful levels. To achieve this, it is necessary to understand the conditions and factors that affect its growth and survival.

Attention to the detail of cleaning and hygiene practices throughout the food supply chain is the prime essential for minimizing the levels of *L. monocytogenes* contaminating plant and animal crops and food production environments. Thereafter, it is the treatment and formulation of the food materials themselves that will determine how any residual contaminating population of *L. monocytogenes* develops.

TEMPERATURE

Within food production processes, a variety of physico-chemical factors, used either singly or in combination, can be effective in controlling the

C. Bell et al., *Listeria*

Table 3.1 Growth rate guide for *L. monocytogenes* (adapted from Mossel *et al.*, 1995)

Lag time					
Temperature (°C)	0–1	2–3	5–6	7–8	9–10
Time (days)	3–33	2–8	1–3	2	< 1.5
Generation time					
Temperature (°C)	0–1	4–5	10–13		
Time (h)	62–131	13–25	5–9		

survival and growth of *L. monocytogenes* both during processing and in the finished food products.

L. monocytogenes can survive for several weeks in many frozen food types although survival may be poorer in products with sub-optimal pH for the organism (International Commission on Microbiological Specifications for Foods, 1996a).

L. monocytogenes is known to grow at the temperatures used for refrigeration, usually 0–8°C. However, the organism only grows slowly at these temperatures (Table 3.1). It is important therefore to operate and apply well-controlled chill holding and storage systems both within the production process for component or part-processed foods and for finished product storage and distribution.

L. monocytogenes is not a very heat-resistant organism (Table 3.2) and properly controlled cooking processes will achieve significant reductions in numbers of the organism. Once cooked, foods must be kept protected from post-process contamination to minimize the potential for any *L. monocytogenes* in the production environment to recontaminate the food.

Table 3.2 Guide to the time and temperature combinations necessary to achieve a 10^6 reduction of *L. monocytogenes* (adapted from Anon., 1997b)

Temperature (°C)	Time
60	45 min
65	10 min
70	2 min
75	30 s
80	5 s
85	1 s

Table 3.3 Growth-limiting parameters for *L. monocytogenes* (Adapted from International Commission on Microbiological Specifications for Foods, 1996a)

Parameter	Minimum	Maximum
Temperature (°C)	−0.4	45
pH	4.39	9.4
Water activity	0.92	-

pH, WATER ACTIVITY AND OTHER FACTORS

Clean practices and temperature control (heat or chill) are key extrinsic factors that can be applied to help control the contamination load and outgrowth of *L. monocytogenes* in foods. There are other physico-chemical factors that are applicable in different ranges of food types that can contribute to the control of the growth of *L. monocytogenes*. Table 3.3 summarizes the main growth-limiting parameters (temperature, pH and water activity) for *L. monocytogenes*.

For products in which the pH becomes non-optimal (usually acidic) either as a result of the manufacturing process, e.g. cheese or fermented meats in which lactic acid is produced by the metabolic activity of the starter cultures used, or by the direct addition of and mixing with an acidic component, e.g. oil and vinegar (acetic acid) dressing, the pH will contribute to the control of bacterial population growth including any *Listeria* spp. that may be present.

When organic acids (acetic, lactic, citric, propionic, etc.) are used as preservatives in foods, it is important to ensure that the correct concentration of undissociated acid (which is responsible for the antimicrobial activity) is available for bacterial growth inhibition. The proportion of undissociated acid present varies with pH (Table 3.4) so this must be taken into account when determining the amount of total acid required at a specific pH to give a particular concentration of undissociated acid. At neutral pH, most organic acids will have a limited effect on the growth of *L. monocytogenes*.

Heat treatments applied to products with a sub-optimal pH for *L. monocytogenes* and/or containing an organic acid as a preservative are expected to be more effective than the same heat treatment applied to a product at the optimum pH for the organism (International Commission on Microbiological Specifications for Foods, 1996a).

Table 3.4 Examples of the percentage of total undissociated organic acid present at different pH values (adapted from International Commission on Microbiological Specifications for Foods, 1980)

Organic acid	pH value			
	4	5	6	7
Acetic acid	84.5	34.9	5.1	0.54
Citric acid	18.9	0.41	0.006	< 0.001
Lactic acid	39.2	6.05	0.64	0.064

The water activity of a food product is lowered by the addition of sodium chloride, sugars and/or other solutes. The higher the concentration of the solute, the lower the water activity of the product. For products in which the water activity approaches or is lower than the minimum for growth of *L. monocytogenes*, growth of the organism will be prevented or minimized provided the water activity does not increase during the life or use of the product, e.g. by mixing the product with other foods of higher water activity or by allowing condensation to affect the product. The bacteriostatic effect of low water activities on *L. monocytogenes* is enhanced by chill storage of food products (International Commission on Microbiological Specifications for Foods, 1996a).

Combining sub-optimal physico-chemical conditions such as pH, temperature and water activity usually has a greater effect than any of the individual factors used at the same level (Mossel *et al.*, 1995; International Commission on Microbiological Specifications for Foods, 1996a).

Table 3.5 indicates some additional physico-chemical factors used in food industry processes and products and their potential applicability to the control of *L. monocytogenes*. A considerable amount of work is still being carried out around the world to determine the effects of current and novel food industry practices and processes, including the effects of individual and combined physical and chemical systems on the survival and growth of *L. monocytogenes* in foods. Results from such work are published frequently.

Whatever information is or becomes available to the food producer or processor, it is essential that for a specific production unit there is available a thorough knowledge and understanding of:

Table 3.5 Comments on the potential applicability of some additional parameters for controlling *L. monocytogenes*

Parameter	Comments	References
Gas atmosphere	Alterations to the atmosphere in which the product is packaged, e.g. vacuum pack for anaerobiosis, increased carbon dioxide, etc., do not completely inhibit the growth of *L. monocytogenes* but may extend the lag and generation times	International Commission on Microbiological Specifications for Foods, 1996a Anon., 1997c
Gamma irradiation	The organism exhibits a similar resistance to gamma irradiation as other Gram-positive bacteria and doses of > 3 kG are likely to be required to eliminate the organism in meat products	International Commission on Microbiological Specifications for Foods, 1996a
Nisin and other bacteriocins	Nisin and other bacteriocins produced by lactic acid bacteria are being examined for their inhibitory effects on *L. monocytogenes*. The results from published work to date indicate a useful role for bacteriocins in controlling the outgrowth of *L. monocytogenes* in foods	Dean and Zottola, 1996
Sodium nitrite	Nitrite exerts some inhibitory effects against *L. monocytogenes* although it does not prevent growth at the concentrations remaining in many cooked meat products	Anon., 1997c

- the processes actually used to produce the product
- the growth control parameters existing in both the process and the finished product.

From such an understanding, a product shelf life can be assigned and used if necessary, by limiting it sufficiently, to prevent any *L. monocytogenes* that may be present in the final product from multiplying to potentially harmful levels prior to consumption.

4

INDUSTRY FOCUS: CONTROL OF *LISTERIA MONOCYTOGENES*

INTRODUCTION

Accumulated evidence to date clearly demonstrates the need for the food industry to employ measures to minimize the potential for *L. monocytogenes* to be present in foods at the point of consumption in numbers considered hazardous to health. Many products can potentially present a risk of causing outbreaks of listeriosis but in most outbreaks a failure in the control systems can be identified that has lead to the outbreak. In such cases, hazard analysis and implementation of controls at the critical points identified could have prevented the outbreaks, provided the control systems were operated consistently correctly. It is strongly recommended that all persons involved in the primary production, processing and sale of food adopt a hazard analysis approach to consider all relevant pathogens, including *L. monocytogenes*.

To help focus attention on the products representing the greatest concern in relation to *L. monocytogenes* and the areas requiring greatest management control, a series of questions can be applied to each food process/product. Processes and products can be reviewed against the key questions to identify the level of concern that *L. monocytogenes* may present (Table 4.1). As a guide, some familiar products in different commodity groups are given as examples in Table 4.2.

After answering each of the questions in Table 4.1, the product can be assessed against the profiles given in Table 4.3 to determine the level of concern that may be associated with the product. Having done this, the key process areas requiring greatest attention for control of the hazard can be determined (Table 4.4).

C. Bell et al., *Listeria*

Table 4.1 How much of a concern does your product represent?

Question	Yes	No
Is *L. monocytogenes* expected to be present in the raw material?		
Will the organism be destroyed or reduced to an acceptable level by any of the processing stages?		
Will the product be exposed to any post-process contamination?		
Will the finished product allow growth of *L. monocytogenes*, if present?		
Does the product have a long shelf life, e.g. > 7 days?		
Will the product be subjected to a process by the customer which will destroy *L. monocytogenes*?		

Every process and product will differ from those presented in the tables and therefore these tables should be used for guidance purposes only; a full understanding of the hazard and controls can only be gained by applying a full hazard analysis. In addition, it is important to note that even processes and products that are rated as being of very low concern in relation to *L. monocytogenes* may be capable of causing listeriosis outbreaks if the controls inherent in the normal manufacture of these products are not applied correctly. In fact, there is probably no more potentially dangerous a product than one manufactured by a complacent management who believe that their product is safe because of historical precedence; food products are generally made safe or unsafe to eat by human intervention.

The highest concern products with regard to *L. monocytogenes* are those where the organism may be present in high incidence or numbers in the raw material, where no process exists to reduce or eliminate it and where it may grow in the finished product, which is consumed without any further processing. Products such as raw-milk mould-ripened soft cheeses fall within this category and, indeed, such products have on a number of occasions been the cause of foodborne listeriosis (see Table 2.1). Other products included in this category are cold-smoked fish, such as smoked salmon and trout, where the raw fish is processed by mild smoking at temperatures between 20 and 30°C. It is interesting to note, however, that to date such products have not been directly implicated in outbreaks of listeriosis, which is particularly surprising given the fact that *Listeria* spp.

Table 4.2 Examples of the key process stages where *L. monocytogenes* may represent a hazard in different foods*

Product	Product examples	Raw material contamination	Reduction process	Destruction process	Post-process contamination	Product allows growth	Shelf life	Consumer cidal process
Dairy products								
Raw-milk ripened soft cheese	Raw-milk Brie, Camembert	Yes	No	No	Yes	Yes	Yes	No
Raw-milk hard cheese	Raw-milk cheddar, parmesan	Yes	Yes	No	Yes	No	Yes	No
Pasteurized soft cheese	Plain cottage cheese, fromage frais	Yes	Yes	Yes	Yes	No	Yes	No
Pasteurized ripened soft/ semi-hard cheese	Brie, Camembert, Stilton	Yes	Yes	Yes	Yes	Yes	Yes	No
Pasteurized hard cheese	Cheddar, Cheshire	Yes	Yes	Yes	Yes	No	Yes	No
Yoghurts	Yoghurt	Yes	Yes	Yes	No	No	Yes	No
Meat products								
Raw meat and poultry	Beef, lamb, chicken	Yes	No	No	Yes	Yes	Yes	Yes

Table 4.2 Continued

Product	Product examples	Raw material contamination	Reduction process	Destruction process	Post-process contamination	Product allows growth	Shelf life	Consumer cidal process
Meat products (continued)								
Fermented meat	Salami	Yes	Yes	No	Yes	No	Yes	No
Cooked meat (hermetically sealed)	Whole hams, chub pâté	Yes	Yes	Yes	No	Yes	Yes	No
Cooked sliced meat	Ham, chicken, frankfurters, pâté	Yes	Yes	Yes	Yes	Yes	Yes	No
Fish and shellfish								
Raw fish/shellfish	Cod, mussels	Yes	No	No	Yes	Yes	No	Yes
Raw fish/shellfish, consumed raw	Oysters, sushi	Yes	No	No	Yes	Yes	No	No
Cold-smoked fish, consumed raw	Smoked salmon, smoked trout	Yes	No	No	Yes	Yes	Yes	No
Cooked fish/ shellfish/fish pâté	Prawns, crab	Yes	Yes	Yes	Yes	Yes	No	No

Table 4.2 Continued

Product	Product examples	Raw material contamination	Reduction process	Destruction process	Post-process contamination	Product allows growth	Shelf life	Consumer cidal process
Salad and vegetables								
Raw vegetables	Potatoes, broccoli, beans, peas, cabbage	Yes	No	No	Yes	Yes	No	Yes
Raw salads	Lettuce, spring onions, celery	Yes	No	No	Yes	Yes	No	No
Washed, prepared salads (ready-to-eat)	Packed salad	Yes	Yes	No	Yes	Yes	No	No

Raw material contamination: Is *L. monocytogenes* expected to be present in the raw material?
Reduction or destruction process: Will the organism be reduced to an acceptable level or destroyed by any of the processing stages?
Post-process contamination: Will the product be exposed to any post-process contamination?
Product allows growth: Will the finished product allow growth of *L. monocytogenes*, if present?
Shelf life: Does the product have a long shelf life, e.g. > 7 days?
Consumer cidal process: Will the product be subjected to a process by the customer which will destroy *L. monocytogenes*?

* Information given is for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

Table 4.3 Categories of concern*

Level of concern	Product examples	Raw material contamination	Reduction process	Destruction process	Post-process contamination	Product allows growth	Shelf life	Consumer cidal process
Category 1: Highest	Raw-milk ripened soft cheese, smoked salmon	Yes	No	No	Yes	Yes	Yes	No
Category 2: High	Cooked sliced meat, pâté, pasteurized ripened soft cheese	Yes	Yes	Yes	Yes	Yes	Yes	No
Category 3: Medium	Salami	Yes	Yes	No	Yes	No	Yes	No
Category 3: Medium	Prepared ready-to-eat salads, dips, sandwiches	Yes/No	Yes	No	Yes	Yes	No	No
Category 4: Medium	Cooked fish, crab, prawns	Yes	Yes	Yes	Yes	Yes	No	No
Category 4: Medium	Pre-cooked ready-to-cook meals	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 4.3 Continued

Level of concern	Product examples	Raw material contamination	Reduction process	Destruction process	Post-process contamination	Product allows growth	Shelf life	Consumer cidal process
Category 5: Low	Pasteurized hard cheese	Yes	Yes	Yes	Yes	No	Yes	No
Category 6: Low	Chub pâté products cooked in pack	Yes	Yes	Yes	No	Yes	Yes	No

High concern: Where *L. monocytogenes* could be present due to raw material contamination or as a post-process contaminant and where the product allows growth and the shelf life would allow high levels to develop.
Medium concern: Where *L. monocytogenes* may be present in the raw material or as a post-process contaminant and where the organism cannot normally grow or will not be present at very high levels due to shelf life restriction, processing or product conditions or a consumer cidal process.
Low concern: Where *L. monocytogenes* may be present in the raw material but the process applied destroys the organism and it cannot recontaminate the product.

* Information given is for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

Table 4.4 Stages where control of *L. monocytogenes* is critical (based on the categories of concern)*

Category	Product examples	Raw material control	Reduction process	Destruction process	Post-process contamination	Product characteristics	Shelf life	Consumer issues
Category 1: Highest	Raw-milk ripened soft cheese, smoked salmon	Yes			Yes		Yes	Yes
Category 2: High	Cooked sliced meat, pâté, pasteurized ripened soft cheese			Yes	Yes	Yes	Yes	Yes
Category 3: Medium	Salami	Yes	Yes		Yes	Yes		
Category 3: Medium	Prepared ready-to-eat salads, dips, sandwiches	Yes	Yes		Yes		Yes	
Category 4: Medium	Cooked fish, crab, prawns			Yes	Yes		Yes	
Category 4: Medium	Pre-cooked ready-to-cook meals			Yes	Yes	Yes	Yes	Yes

Table 4.4 Continued

Category	Product examples	Raw material control	Reduction process	Destruction process	Post-process contamination	Product characteristics	Shelf life	Consumer issues
Category 5: Low	Pasteurized hard cheese	Yes	Yes	Yes	Yes	Yes		
Category 6 Low	Chub pâté, products cooked in pack		Yes	Yes				

* Information given is for guidance only and may not be appropriate for individual circumstances. It is recommended that proper hazard analysis is carried out for every process and product to identify where controls must be implemented to minimize the hazard from *L. monocytogenes*.

may frequently be present in the raw material and growth is possible during the shelf life of the product.

RAW-MILK MOULD-RIPENED SOFT CHEESE

Description of process

Raw-milk mould-ripened soft cheeses are amongst the highest concern products in relation to the hazard associated with *L. monocytogenes*. Many of the cheeses made from raw milk are manufactured in small farmhouse operations and almost all of the processes are based on traditional practices built up over many years. Although modern equipment for large-scale cheese manufacture has become more sophisticated, the underlying process (Figure 4.1) and skill associated with cheese-making has remained the same for centuries and is considered by most cheese manufacturers as a craft.

Raw-milk mould-ripened soft cheese is manufactured using raw milk from a variety of animals depending on the cheese type. Probably the best selling cheeses of this type are Camembert and Brie, which are traditionally manufactured from cows' milk, whereas the most famous raw-milk French cheese, Roquefort, is manufactured from ewes' milk.

Raw milk is collected on farm in bulk milk tanks and held refrigerated until collection, usually at 24–48-h intervals. On arrival at the cheese manufacturing plant, the milk is usually pumped to a silo where it is stored at <5°C until required for production. It is then heated to a temperature appropriate for the starter cultures, which are added as the milk is filled into fermentation tanks or vats. There then follows a standard cheese manufacturing process that involves coagulation of the milk proteins using rennet, cutting of the curd, separation of the whey and filling of the cheese into moulds, which vary in size and depth depending on the type of cheese being manufactured. The fresh cheese is usually left at ambient temperatures for in excess of a day to allow further activity of the starter culture and drainage of the whey. The cheese may then be removed from the mould and salted by placing in a brine solution or by rubbing dry salt onto the surface. The cheese is transferred to a ripening/maturation room or cellar where the temperatures vary but are usually between 8 and 12°C. During several weeks of ripening, the mould spores applied to the surface of cheeses such as Brie or Camembert or introduced into the cheese together with the starter culture or by wire inoculation, as for blue veined varieties, begin to grow and produce the characteristic white mould coat or blue veins in the respective cheeses. As the mould grows it elevates the pH from approximately pH 5 to pH

Process Stage	Consideration
Milk animal	Health Cleanliness
↓	
Milking	Hygiene – udder/equipment
↓	
On-farm storage	Hygiene Temperature Time
↓	
Milk transport and delivery	Hygiene Temperature Time
↓	
Milk storage at cheese production site	Hygiene Temperature Time
↓	
Cheese-making vat	Hygiene – equipment and personnel
↓	
Add starter culture	Starter activity Temperature Time
↓	
Add rennet and mix	Hygiene Rennet control
↓	
Curd cutting	Hygiene – equipment and personnel
↓	
Whey drainage	
↓	
Cheese forming	Hygiene
↓	
Add mould (if not added with starter)	Culture monitoring
↓	
Maturation	Hygiene Temperature
↓	
Slicing/cutting/packing, where applicable	Hygiene
↓	
Retail distribution, storage, slicing, where applicable	Hygiene Temperature Time: Shelf life
↓	
Consumer	Advice

Figure 4.1 Process flow diagram and technical considerations for a typical raw-milk mould-ripened soft cheese.

6–7.0. The pH can vary considerably throughout the cheese, being highest close to the mould growth and lowest at the centre. At the same time, proteolytic activity causes a softening of the cheese and it is at this stage that the product is most susceptible to growth of microbial contaminants, including *L. monocytogenes*. The conditions of temperature (8–12°C) during maturation, elevation of pH (6–7.0) and the higher moisture content in these soft cheeses (45–55%) make these cheeses especially vulnerable to growth of psychrotrophic bacterial pathogens such as *L. monocytogenes*.

Raw material issues and control

L. monocytogenes, as well as being a human pathogen, can also cause severe infections in animals, usually ruminants, i.e. sheep, goats and cows. Infection may result in abortion, stillbirth or neonatal death if occurring in the pregnant uterus or, alternatively, it may cause septicaemic infections or encephalitis (Prentice, 1994). Encephalitis manifests usually as facial paralysis and walking in circles, which has led to the description of the disease as 'circling disease' in sheep (Gitter, 1989). Listeriosis in animals may occur due to exposure to *L. monocytogenes* in the environment although contaminated feed such as silage has also been implicated as a potential source of the organism as the result of insufficient acidity development during its manufacture (Grant *et al.*, 1995; Fenlon, 1996).

L. monocytogenes can frequently be isolated from ruminant faeces and it is believed that contamination of the udder with faeces is likely to be the primary route of the organism into raw milk. *L. monocytogenes* is a common contaminant of raw milk and may also be derived from milking equipment and subsequent storage vessels. Reports have been documented of *L. monocytogenes* contaminating raw milk by direct shedding into the milk from a mastitic infection in the udder, but this is thought to occur only on rare occasions (Prentice, 1994). A variety of authors have studied the incidence of *L. monocytogenes* in raw milk with reported isolations of <1% to in excess of 20% (Prentice, 1994). Milk used for raw-milk cheese is usually collected in milk silos and used within 1 to 2 days of milking. The bacteriological quality of the raw milk is a critical factor in minimizing the risk of food poisoning outbreaks from these types of cheese. Apart from rare cases of direct shedding of *L. monocytogenes* into milk, it is unlikely that levels of *L. monocytogenes* derived from the dairy animal at this stage would be high enough to result in listeriosis; a fact reflected in the lack of reported listeriosis outbreaks due to the consumption of raw milk. It is more likely that poor control of subsequent stages in the manufacturing process, allowing growth of *L. monocytogenes* to high

levels, is the primary contributory factor leading to outbreaks of listeriosis caused by these product types.

High standards of hygiene in the milking parlour are essential to minimize contamination of the raw milk during milking and they are also necessary to prevent build-up of contamination in storage or transportation equipment. Because of the likelihood of even a low incidence and level of contamination of raw milk with *L. monocytogenes*, it will inevitably contaminate processing equipment. Inadequate cleaning of the milking equipment and storage tanks at the farm, transportation vehicles used to transport the milk to the dairy and the silos in use at the dairy itself presents a potential hazard to subsequent batches of product.

Smaller farmhouse manufacturers have the facility to exert direct control over the quality and hygienic practices from milking to finished product because they usually use milk derived from their own farm. This also reduces any hazards introduced by milk transportation and allows the use of fresher milk that has not undergone extensive storage.

For the larger manufacturers of raw-milk soft cheeses, the milk is often supplied by farm co-operatives and is usually received as bulk milk from a number of farms. The manufacturer is often reliant on third parties for the milk quality at the most important stage of the process and it is essential that the farms supplying the manufacturer are aware that the milk is to be used for raw-milk cheese manufacture and know the importance of and need for detailed attention to good hygiene practices. In some cases a milk payment incentive scheme is operated based on tests for indicators of faecal contamination such as *Escherichia coli*, which are used to routinely monitor the quality of the raw milk.

Milk tankers used to transport the milk from the farm to the manufacturer are themselves a potential source of contamination. Attention to the hygiene and cleaning efficacy of bulk farm tankers is absolutely essential to maintaining a supply of raw milk where contamination with *L. monocytogenes* is minimized. Whilst the tankers are often effectively cleaned, particular attention should be paid to areas such as the tanker manway lids and seals, milk inlet/outlet ports, air elimination vessels and flexible hoses used to connect the tanker to milk intake at the manufacturing site. Milk silos should be designed to prevent the possibility of 'dead' spots occurring where milk can be trapped, e.g. in upturned pipes, redundant sample ports or valves that are inaccessible for cleaning. This applies equally to all the other equipment in place for manufacturing purposes, such as process pipes, tanks in the fermentation room or further downstream in

the process, and smaller equipment including curd cutters, ladles, racks and tables. Monitoring of cleaning efficacy by visual inspection, by the use of specific hygiene tests for examining rinse water samples for indicators of contamination such as coliforms or by the use of alternative test systems such as ATP bioluminescence for measuring the total biological residue on a surface after cleaning is important.

Process issues and control

It is particularly important that the bacterial starter cultures actively ferment the raw milk during fermentation as any pathogens present are equally capable of growth during the fermentation process, which is maintained at approximately 30°C. It is the rapid development of acidity by the starter cultures that inhibits the outgrowth of pathogens and so monitoring acidity development in the cheese is an extremely important (critical) control point.

The pH of fresh cheese usually falls to below pH 5.0 in the first few days of the process and this is sufficient to prevent significant growth of *L. monocytogenes*. Although it is recognized that raw milk is a major potential source of *L. monocytogenes*, it is important to remember that these cheeses are also highly susceptible to environmental contamination during processing, particularly during cheese maturation and ripening. Cheeses are extensively handled and are stored for long periods without being covered; they are therefore exposed to a significant possibility of environmental contamination. Hygiene is particularly difficult to maintain in many of the traditional, older cheese maturation environments due to the poor construction of rooms and shelving, which precludes effective cleaning and sanitizing. Some products, such as Roquefort, are still ripened in caves where the potential for contamination can be significant. At this stage of the process the product is most prone to contamination and, because of the raised pH due to the development of the product's characteristic mould growth, conditions are suitable for growth of *L. monocytogenes*. An effective cleaning schedule is absolutely critical in these areas and it essential that cleaning chemicals, procedures and frequency of cleaning are sufficient to remove product debris and decontaminate surfaces at regular intervals. Particular attention should be given to all areas that come into direct contact with product, e.g. shelving, or indirectly such as forced-air heaters/chillers. In addition, any practices that could contribute to cross-contamination, such as salting or turning the cheeses, should be identified and procedures for ensuring the highest standards of hygiene implemented.

Environmental hygiene should be monitored using specific tests to detect *Listeria* spp. and tests for assessing the overall hygienic status of surfaces, such as ATP bioluminescence.

Process design is extremely important and the flow of the production process should be such that raw milk cannot come into contact with finished product (Figure 4.1). Separation of batches of cheese into different maturation rooms allows for easier operation of cleaning schedules and also precludes the possibility of spreading any contamination that may have occurred to other products in the same environment by aerosols created during cleaning. In addition, such separation will allow individual chillers to be emptied for cleaning.

In terms of survival of *L. monocytogenes* during the manufacturing process, the acidity and low pH developed in the fresh cheese may have some inhibitory effect although this is most likely to merely prevent significant growth rather than cause the death of the organism. Indeed, published information indicates that levels of *L. monocytogenes* remain constant or increase only slightly during the fermentation and early maturation stages of cheddar cheese (Ryser and Marth, 1987a) whilst they increase by 5–10-fold in the first 24 hours of Camembert cheese manufacture (Ryser and Marth, 1987b).

The hazard presented to raw-milk mould-ripened soft cheeses by *L. monocytogenes* as a contaminant from the environment during processing and maturation is actually no greater than the same hazard presented to many other soft mould-ripened cheeses made from pasteurized milk. This is because any of these organisms introduced during these stages are provided with suitable conditions for significant growth during the remainder of the process and throughout the shelf life of the final product.

Final product issues and control

Once the pH has increased in the mould-ripened cheeses, growth of any contaminating *L. monocytogenes* can be significant. In Camembert cheese inoculated with *L. monocytogenes*, levels increased by 4–5 log cycles during a maturation period of 65 days (Ryser and Marth, 1987b); this paralleled the increase in product pH during this stage. Growth predictions using the Food MicroModel (Anon., 1997c) for parameters found in a typical Brie or Camembert cheese with a pH of 6.5, aqueous salt concentration of 1.5% and stored at a temperature of 5°C indicate that an initial contamination level of 1 cell per gram *L. monocytogenes* could increase to in excess of 1000 per gram within 10 days.

An additional stage in the production process that provides opportunities for contamination of the finished product is the slicing and packing operation (Figure 4.1). Effective cleaning of the slicing machine is essential to prevent cross-contamination from the machine itself and from one cheese to others in the batch. Frequent cleaning during production and daily full, deep cleaning involving dismantling the equipment, paying particular attention to the slicer blade, product contact surfaces and the slicer housing, are essential. Clearly, preventing contamination at these late stages of the production process is of great importance to the safety of the product.

The heightened attention to controlling the hazard of *L. monocytogenes* in all types of mould-ripened soft cheese has led to a significant reduction in the incidence of the organism in these types of cheese. A recent survey of unpasteurized milk soft cheeses in the UK (Nichols *et al.*, 1996) found 4/72 samples with *Listeria* spp. present in 25 g with one sample containing levels of 10^3–10^4 per gram. Only 1/72 samples contained *L. monocytogenes*, which was found to be present in 25 g but at levels <10 per gram. A report on the incidence of *L. monocytogenes* in soft cheeses (including pasteurized varieties) sold in England and Wales shows a significant reduction since 1987 (Table 4.5) although a study in Sweden carried out between 1989 and 1993 found 42% of raw-milk cheese samples tested (all imported from France) to be contaminated with *L. monocytogenes* (Loncarevic *et al.*, 1995).

It is apparent that the process of manufacturing mould-ripened soft cheeses from raw milk has many inherent hazards associated with it, the greatest probably being the raw milk itself. Control of the bacteriological quality of the raw milk can reduce the incidence of *L. monocytogenes*, but is unlikely to eliminate it completely, so control of all the subsequent processing steps is critical for minimizing this hazard.

Unfortunately, once present there is clearly much opportunity for *L. monocytogenes* to survive the cheese-manufacturing process and even grow in the product, and many manufacturers resort to high levels of in-process and finished product testing to gain further reassurance that *L. monocytogenes* has not become out of control. As it is not possible to assure the safety of any product with microbiological testing, a residual risk will always remain that such products could present a major hazard to consumers. However, with rigorous attention to hygienic practices from the farm and at all subsequent stages, supported by microbiological analysis, the hazard from *L. monocytogenes* can be significantly reduced. To this end, documents that provide guidance for the safe manufacture of

Table 4.5 Incidence of *L. monocytogenes* in soft cheese in England and Wales (adapted from Gilbert, 1996)

Year	Number of samples	*L. monocytogenes* (%)
1987	222	23 (10.4)
1988–89	1135	67 (5.9)
1989–90	131	0 (0)
1991–92	251	10 (4.0)
1995	1437	16 (1.1)

these types of products are extremely important for smaller farmhouse manufacturers who frequently do not have the technical support of larger operations (Anon., 1997d).

It is the residual risk of the presence and growth of *L. monocytogenes* in raw-milk mould-ripened soft cheeses that has lead to advice being given by the national health departments of several countries concerning the risks to the health of certain vulnerable groups associated with consumption of this type of cheese (Department of Health, 1996; Shank *et al.*, 1996). In the UK, this advice is still applied today and relates to both raw milk and the pasteurized milk varieties of soft ripened cheese because of the potential for contamination of pasteurized varieties by *L. monocytogenes* during the ripening stage that could subsequently grow to levels of concern to public health (Department of Health, 1996).

COLD-SMOKED FISH

Description of process

Cold-smoked fish products, although amongst the highest concern products based on hazard analysis considerations relating to the potential for *L. monocytogenes* to be present, have an excellent food safety record with few, if any, outbreaks involving this organism attributed to these traditional products.

The cold-smoked fish process (Figure 4.2) as employed in the manufacture of smoked salmon and trout begins with the preparation of the raw fish. Fish used for these purposes are usually farmed and killed on site. These fish are then transported on ice to processing plants where they are gutted, filleted and prepared as two sides for salting. Salt is usually applied in the dry form directly onto the surface of the fillets with the skin still attached, although some processors may hold the fillets in

Process Stage	Consideration
Fish farming	Health Cleanliness
↓	
Slaughter/gutting, filleting	Hygiene Temperature
↓	
Storage	Hygiene Temperature Time
↓	
Trimming/halving	Hygiene Temperature Time
↓	
Salting	Distribution of salt Hygiene
↓	
Storage	Hygiene Temperature Time
↓	
Smoking	Hygiene Temperature Time Type of smoke
↓	
Storage	Hygiene Temperature Time
↓	
Slicing	Hygiene Time
↓	
Packing	Hygiene Time
↓	
Distribution	Temperature
↓	
Retail sale	Hygiene Temperature Shelf life
↓	
Consumer	Advice

Figure 4.2 Process flow diagram and technical considerations for a typical cold-smoked ready-to-eat fish.

a brine solution or even inject brine. The fish are then stored at <5°C for several days for the salt to equilibrate in the fish to allow an even distribution of salt to a level in excess of 3.5% in the aqueous phase. The fish are then placed in a smoking chamber for several hours where smoke is generated using wood chips or wood dust and the temperature is maintained at 25–30°C. Following the smoking process, the fish are then chilled to 0–5°C prior to packing as whole sides or being cut into thin slices manually using hand-held knives or slicers, although some automated slicers are now used for further processed products. The sides or slices are usually vacuum packed and retailed with a shelf life of 3–4 weeks or more at chill temperatures (<5°C). In some countries it is common practice to retail these products by mail order and they are often sent by the postal system in insulated packs or with a cold pack for overnight delivery.

Raw material issues and control

Listeria species do not appear to cause infections in fish and do not have a natural reservoir in fish. However, a variety of surveys have shown that they are present in river sediments and water, both fresh and sea water, and therefore may be present in the raw fish as a contaminant from the aquatic environment (Ben Embarek, 1994).

Published surveys of *Listeria* species in salmon farms are few and have not established any incidence of the organism in product or environmental samples (Ben Embarek *et al.*, 1997). Results, however, will only reflect the conditions in the specific site surveyed. Unpublished surveys of raw salmon received at the processing site conducted by smoked salmon processors reveal intermittent contamination that, when it occurs, is at a low incidence (<5%) of *Listeria* species, including *L. monocytogenes*, in the raw material, with levels in all cases of <10 per gram. Therefore, whilst the presence of *L. monocytogenes* may be relatively infrequent in the farmed fish itself, it is unquestionably present on occasion in the raw material and for subsequent processing represents a source for cross-contamination.

Most smoking sites receive the fish already gutted and significant potential exists for cross-contamination of *Listeria* species during this stage of the process. As there is no later stage in the process that will destroy *L. monocytogenes*, poor process or environmental practices may allow *Listeria* to multiply and these will remain with the fish during subsequent stages. Therefore, much attention has to be given to hygienic processing at the gutting stage.

Probably the main contributors to accumulated contamination and cross-contamination are the people working in the area and the utensils and conveyor belts used. Fish are manually gutted and then washed. Regular cleaning and disinfection of knives and gutting boards are critical in the prevention of cross-contamination. In most processing units, extensive use is made of conveyor belts to move fish during processing and as these represent a common product contact surface, it is essential that they are maintained to high standards of cleanliness. They should be frequently rinsed down during shifts and thoroughly cleaned and decontaminated after each shift. Slime and scales from the fish skin can be extremely difficult to remove and it is imperative that appropriate cleaning chemicals are used that are demonstrably capable of achieving an effective clean. If improperly controlled, this stage of the process has the potential to contaminate high numbers of fish. Eklund *et al.* (1995) reported *L. monocytogenes* as a common contaminant of raw, eviscerated salmon supplied to smoked salmon processors with the organism found in 4/19 samples of slime, 30/46 skins, 8/17 heads, 6/9 tails and 1/15 belly cavity and belly flap trimmings.

Fish are transported to the main processing factory packed on ice. Fish are processed to remove the head and then filleted, if this is not already done. They are then placed on racks and salted. At this stage the products are heavily handled on common surfaces with common utensils and regular cleaning is yet again the key to control of the organisms to prevent their build-up.

It is inevitable that *Listeria* species, including *L. monocytogenes*, will contaminate surfaces in fish processing factories and the key effort for controlling the organisms should be focused in two areas: first, preventing them from building up during processing by regular in-process cleaning and disinfection of utensils such as knives and cutting blades; second, breaking any contamination cycle at the end of the day when a full cleandown is implemented. If the full cleandown is not effective in removing *Listeria* species then any subsequent effort to control it during production will be undermined. In processes where there are no stages to destroy *Listeria*, interrupting the contamination cycle and preventing build-up to high levels is a key factor contributing to the safety of these products.

Following the salting stage, the product is cold-smoked in a chamber for several hours at 25–30°C, although some processes may involve smoking at lower temperatures (20°C) for longer times (18–20 h). There is much debate about the effect of this stage on *Listeria* species with some reports

indicating that the smoke may actually have an antilisterial effect. Certainly, this period of smoking is critical to the product as, in theory, any *Listeria* species present could actually grow by several orders of magnitude at such temperatures during the timescale of the process. However, it is apparent that *Listeria* species do survive the cold-smoking process and, although important, this stage is unlikely to contribute to the incidence of *Listeria* contamination but may allow any levels present to increase. Eklund *et al.* (1995) compared the growth and survival of *L. monocytogenes* either inoculated onto the surface or injected into the flesh of brined salmon using two smoking processes: 17.2–21.1°C and 22.2–30.6°C both for 18 h. Levels of the organism on the surface of the fish remained fairly constant when no smoke was applied at both temperatures but decreased slightly when smoke was applied at 22.2–30.6°C and by 10–25-fold at 17.2–21.1°C. When injected into the flesh, *L. monocytogenes* increased 2–6-fold at 17.2–21.1°C and 100-fold at 22.2–30.6°C, regardless of the presence of smoke. It is important for processors of smoked fish to gain an understanding of the effect of their specific smoking process on the growth and survival of *L. monocytogenes* as any changes to the type of smoke or time and temperature conditions applied may actually compromise the safety of the product at this stage. A hazard analysis will undoubtedly identify this stage as a critical control point.

After the smoking process, products are returned to the chill store where they are chilled to 0–5°C prior to slicing and packing.

Process issues and control

Possibly the greatest opportunity for additional bacterial contamination or increasing the contamination load of smoked fish products is at the final stage, after smoking. Smoked, chilled fillets are sliced using knives or, more commonly, manually held slicers with rotating blades. The opportunities for cross-contamination at this stage are extremely high. Common conveyor belts, common tables, common hands, common knives and common blades are all used to cut thin slices of a raw fish. Attention to detail is critical during this stage, and regular and thorough cleaning is essential. Regular problem areas are often the simplest to resolve such as inadequate cleaning of conveyor belts, accumulated debris in poorly accessible areas, poor disinfection of knives and inadequate removal of debris from tables and blades.

L. monocytogenes is difficult to eliminate completely but without removal of obvious product residues it can build up as a major contaminant. In a cold-smoked fish processing environment, such product debris

from small pieces of fish can be difficult to clean and specialist cleaning advice from a chemical company specializing in these areas is recommended.

Personnel operating practices need to be carefully planned to allow the introduction of regular cleandowns and disinfection of surfaces and utensils to help break any chain of cross-contamination and to prevent build-up of high levels of bacterial contaminants. As the smoked fish remain with the skin attached until slicing, always placing the fillet skin side down is advisable when moving the product into contact with surfaces as this provides a layer of protection. Many smoked fish processors use automated equipment for slicing or mincing of fish for further processing. As these machine systems are often complex, more areas may be presented that provide the opportunity for build-up of product debris in slicing blades, on conveyors or in the slicing or mincing chamber itself. Attention must be given to identifying those areas that are difficult to clean effectively in these types of equipment. Thus, 'dead' areas can be prioritized for specific cleaning regimes. Such areas should be regularly (usually daily) stripped down for cleaning and visually inspected after cleaning. Without such an approach, it is inevitable that focal points of contamination will develop, gradually building up until problems occur.

Final product issues and control

Smoked salmon and smoked trout have a pH in excess of 6.0 and are usually salted to achieve an aqueous salt content of 3.5%. Without taking account of the possible antilisterial effect of the smoke, it is clear that significant growth of *L. monocytogenes* could occur if it were present in the final product, given that a 3–4-week shelf life is usually assigned to these products. Growth predictions for *L. monocytogenes* calculated using the Food MicroModel (Anon., 1997c) suggest that levels of the organism could increase by three orders of magnitude in 13 days at 5°C in a product with a pH of 6.2 and a salt content of 3.5% (aqueous salt). However, published data indicate that growth is likely to be slower than this, possibly due to the combined effects of smoke, salt, temperature and anaerobic (vacuum pack) conditions. Hudson and Mott (1993) reported growth of *L. monocytogenes* at 5°C by three orders of magnitude in approximately 20 days, although these same levels were reached within 4 days at 10°C.

Surveys of smoked fish reveal a frequent incidence of *L. monocytogenes* in retail products but rarely indicate the presence of high levels of the

organism, providing further evidence that a combination of factors may be providing inhibitory conditions for the growth of *L. monocytogenes*.

Dillon *et al.* (1994) found 6% (7/116) of samples of cold-smoked fish contaminated with *Listeria* species, including *L. monocytogenes*. McLauchlin and Nichols (1994) reported a 2% (2/86) incidence of *L. monocytogenes* in smoked salmon although a smaller survey (1989–1993) reported in the same paper revealed an incidence of 21% (4/19). Levels found were generally <100 per gram with the highest count between 10^2 and 10^3 per gram. This is broadly in agreement with data on the incidence and levels of *L. monocytogenes* in cold-smoked salmon surveyed in Denmark (Huss, 1997), which indicated an incidence of 31% at the end of shelf life with the highest levels reaching 10^3–10^4 per gram.

With traditional products such as cold-smoked fish it is difficult to make modifications to the process or product in order to enhance food safety. However, as the primary hazard relates to the growth of *L. monocytogenes*, any conditions imposed that can prevent this from occurring are important and minor increases in aqueous salt content or reductions in chilled storage temperatures at the manufacturing site or during distribution or retail sale could enhance product safety. For example, an increase in salt content by 0.5% can reduce the time taken for *L. monocytogenes* to increase by 1000-fold by over 10%. Additionally, reduction in product shelf life can reduce the opportunity for extensive growth of *L. monocytogenes* and thereby reduce the risk of high levels occurring. Minor modifications such as these need to be considered wherever possible to make progress in reducing any risk of listeriosis from these types of product.

COOKED SLICED MEAT AND PÂTÉ

Description of process

Cooked meats have been responsible for a number of listeriosis outbreaks in recent years (McLauchlin, 1996). It is likely that this is due to a variety of factors that, combined, make these products highly susceptible to contamination and growth of *L. monocytogenes*. Cooked meats include products such as pâté, cooked chicken, cooked ham and hot dogs and they represent a very large commodity group. In addition to having lengthy shelf lives (many cooked meats are given shelf lives in excess of 2–3 weeks or even as long as 40–50 days at refrigeration temperatures), they are also consumed commonly in sandwiches or other light snacks by all those groups potentially at risk from listeriosis.

In theory, cooked meat products should be relatively simple to control in relation to the hazard associated with *L. monocytogenes*. In most large-scale processes the raw materials are separated from the finished product with complete segregation by a wall with double-ended ovens built through the wall. Providing the cooking process of the meat achieves temperatures lethal to *L. monocytogenes*, then any contamination at the raw material stage should be effectively eliminated, making the primary hazard post-process contamination.

The process for manufacturing cooked meats (Figure 4.3) involves cooking bulk-packed meat either as joints or as comminuted mixes formed into blocks or sausage shapes of varying sizes. Cooking processes are usually in excess of the equivalent of 70°C for 2 min (Table 3.2) and are often selected to achieve particular product characteristics, e.g. colour and texture. The cooked bulk meat is then blast chilled to prevent growth of surviving spore-forming bacteria and then stored chilled (0–5°C) until required for further processing. Some products may be cooked and despatched in intact packaging to delicatessens or other retailers for slicing on the delicatessen counter and in such cases the post-heat-process contamination hazard at the factory is minimal if the product is cooked in its final packaging and no opportunities exist for cross-contamination until it reaches the retailer. The majority of cooked meat, however, is subject to some form of secondary processing. This may involve simple cutting of the bulk meat into smaller bulk packs or slicing into retail prepacks. Any cooked meat subject to further processing involving extensive handling or slicing is inevitably exposed to significant environmental opportunities for cross-contamination.

Raw material issues and control

The raw materials for cooked meat products vary considerably depending on the nature of the final product. Cooked meats consist predominantly of the raw meat, e.g. pork, beef, chicken or turkey, together with water, salt, emulsifying salts and often herbs and spices. The emulsifying salts, e.g. sodium or potassium phosphate, diphosphate and polyphosphate, are usually injected into the raw meat in a brine solution with sodium chloride and water.

In products such as pâté, a high proportion of the raw material consists of fat and other tissues, e.g. liver, heart, etc., which are likely to be more

Figure 4.3 Process flow diagram and technical considerations for a typical cooked sliced meat or pâté.

Process Stage	Consideration
Animal husbandry	Health Cleanliness
↓	
Animal slaughter and processing	Hygiene Temperature Time
↓	
Meat transport, delivery and storage	Hygiene Temperature Time
↓	
Pâté/comminuted and reformed bulk meats Bowl chopping and addition of other ingredients (spices, herbs, salt, etc.)	Additive distribution Temperature Time
or	
Whole joints of meat Brine injection (where applicable, e.g. hams and cured meats)	Preservative distribution Temperature Time
↓	
Cooking	Temperature Time High/low-risk segregation (post cooking)
↓	
Blast chilling	Hygiene
↓	
Removal from container (where applicable)	Hygiene
↓	
Storage	Hygiene Temperature Time
↓	
Roasting/chilling, where applicable	Temperature Time
↓	
Super chilling	Hygiene
↓	
Slicing, where applicable	Hygiene Time
↓	
Garnishing	Hygiene
↓	
Packing	Hygiene Temperature
↓	
Storage/distribution	Temperature Time
↓	
Retail storage	Hygiene Temperature Time
↓	
Retail slicing, where applicable	Hygiene
↓	
Retail sale	Hygiene Temperature Shelf life
↓	
Consumer	Advice

highly contaminated than muscle meat. Many of these products, especially pâté, are flavoured by the addition of herbs and spices; these can add significantly to the microbial loading of the raw product mix, although they present a greater hazard when added to the cooked product as garnish. Many cooked pork products, including pâté, also contain sodium nitrite, which acts as a preservative, protecting the product from the outgrowth of *Clostridium* spp., and also contributes a characteristic flavour to the product. In general, cooked meats made from beef, poultry or fish do not contain nitrite.

With such a variety of raw ingredients, primarily of animal origin, a high incidence of *Listeria* species is to be expected in the raw mixes destined for cooking. Jay (1996) recently summarized the incidence of *L. monocytogenes* in raw meat and poultry reporting 20% incidence in surveys of fresh and frozen pork from 12 countries, although incidence in individual surveys varied from 0 to 95%. A similar incidence (16%) was found in beef and lamb although this varied from 0 to 77%. In fresh and frozen poultry, the average incidence was found to be 17% (ranging from 5 to 63%). It is clear therefore that whilst every effort must be made to reduce levels of contamination at the raw material stage, the raw material will still represent a significant hazard in these materials and the environments in which they are handled and processed. Therefore, the two critical points that must be effectively controlled are the cooking stage and avoidance of cross-contamination of the final product.

Whilst it is not yet possible to eliminate *L. monocytogenes* from the raw material, it is important that those involved in primary processing at abattoirs, butcheries and in the preparation of the raw meat mix operate appropriate standards of good slaughterhouse and processing practice to avoid any hazard becoming even greater. Differences in slaughter hygiene, storage conditions of the raw meats and cleaning efficacy probably account for the wide variation in the incidence of *L. monocytogenes* found in different surveys of fresh meat, as poor practices will inevitably lead to more widespread and increased levels of contamination. In addition to high standards of hygiene it should be remembered that whilst the ingredients of most cooked meat products with a high water activity are not particularly inhibitory to the growth of *L. monocytogenes* if present in the finished product, some do have ingoing concentrations of salt that can limit its growth rate. In such circumstances it is essential that the correct levels and even distribution of such inhibitory compounds are present and this must be adequately controlled at the raw material stage.

Process issues and control

The key critical control point of any cooked meat operation with respect to vegetative pathogens is the cooking stage (Figure 4.3). Cooked meat processes are designed to significantly reduce levels of vegetative pathogens in the raw materials and usually achieve temperatures in excess of 70°C. However, due to organoleptic considerations and the desire to avoid developing an overcooked flavour, most cooked meat products are cooked to the minimum time/temperature combination to ensure the required visual, textural and flavour changes occur. In the UK, most cooked meat manufacturers follow the guidelines recommended by the UK Department of Health (Anon., 1992), which specify a minimum cook of 70°C for 2 min or an equivalent process to produce a 10^6-fold reduction of *L. monocytogenes*. This is based on work carried out by the Campden and Chorleywood Food Research Association, UK and established now in guidelines produced by the Chilled Food Association, UK (Anon., 1997b). It is important to note that some studies have shown that heat resistance of *L. monocytogenes* is increased by previous exposure to mild heat shock and a slow heating profile may more than double the *D* value (Carlier *et al.*, 1996a,b). These considerations may be particularly important in the cooking of bulk meats where heat penetration to the centre can be very slow. However, the safety margin within a heat process of 70°C for 2 min should greatly exceed any effect of heat-shock-induced heat resistance.

In the cooking process of any meat it is essential that whatever type of cooking equipment is used it must be capable of achieving the desired temperature in all parts of the product and must take into account the different positions the product may occupy in the oven and the temperature of the ingoing raw materials.

A process controlled only by oven temperature and time does not take account of variations in product thickness and density, temperature of ingoing materials and position of the product in the oven. Preliminary studies must be conducted to validate any specific process accounting for 'worst case' product and process deviations attributable to the technical specification of the oven, e.g. plus or minus 2°C across the oven. Each cook should then be monitored with a continuous temperature recorder and, following the cooking process, the centre temperature should be measured in products known to be in the coolest parts of the cooking equipment. Temperature should be taken by inserting a sterilized metal probe of the temperature recorder into the deepest part of the meat.

These principles would appear very simple and basic but it is both surprising and concerning how many manufacturers of cooked meat products have a poor understanding of them. A recent survey of cooked meat manufacturers conducted in the UK by the Ministry of Agriculture, Fisheries and Foods (Anon., 1995a) identified a third of processors who were unable to state the maximum temperature reached at the centre of their cooked meat products during the cooking process. In addition, 36% of processors did not calibrate their temperature monitoring instruments.

The first opportunity for product recontamination arises immediately after heat processing, depending on the cooling method employed. Due to their size, cooked meat products are chilled by forced-air chillers. Preliminary showering using recirculated cold water sprays may also be used to dissipate the initial heat from the product. Contamination can readily occur during these stages from sources within the chilling systems. The use of sealed packs (hermetic or clip-sealed) for processing the product can significantly reduce opportunities for contamination during cooling and such methods are commonly employed in the industry. However, if the pack seal integrity is considered to be the critical barrier to prevent contamination after processing, it is essential to ensure sealing efficacy is adequately monitored, as post-process contaminants readily gain access to the product during cooling due to product shrinkage and the associated vacuum effect.

For product containers that will be subsequently opened to allow jointing, cutting or slicing of product, contamination of the outer surfaces of the pack represents a potential hazard because of the handling that the product receives. This can be reduced by passing the cooked meat in its container through a food-grade disinfectant solution to decontaminate the outer surface prior to further processing of the product, although if handled hygienically at all times after cooking, this should not be necessary. Products that receive further processing, such as sliced meats or pâté, are usually stored in a chiller either in the original packaging or after removal from containers or packaging. Strict attention to hygiene is critical in areas where the product is 'knocked out' of containers and where the packaging is sliced open with knives. Potential hazards are presented in three key areas in this operation: personnel, knives and food contact surfaces, e.g. conveyor belts and tables. Tables, knives and racks used to hold product are all used continuously and come into contact with all cooked products. Regular cleaning of all product contact surfaces during production is essential to prevent build-up of any contaminants. Use of production breaks to implement targeted and 'contained' cleaning can have a significant impact on controlling *Listeria* species.

Regular changing of knives or use of hot water sterilant baths (>80°C) are critical in preventing these utensils from becoming a source of cross-contamination.

Products are usually stored in chillers on racks and the potential for contamination in these areas is again significant. The potential for colonization of the chiller units by *Listeria* species represents a real hazard and forced-air movement by these units creates aerosols, which are readily dispersed to contaminate product. Regular decontamination of chiller units using foam or gel cleaners and disinfectants and/or air fogging systems are useful for preventing build-up of contamination. It is, however, often the simplest of things in chillers that can cause contamination problems such as dripping condensate from the chiller units, ceilings or overhead pipes onto exposed product. Pooling of condensation in areas on the floor together with inadequate removal of product residues from the floor or residues trapped in cracked or poorly maintained floors provide ideal conditions for the survival and growth of *L. monocytogenes*; cleaning schedules for the entire chiller must therefore be frequent and thorough.

After cooking and chilling, a variety of cooked meats are roasted for a period of time to achieve a characteristic flavour and visual appearance on the outer surface. For such purposes, the bulk meat may be placed in an oven at high temperatures (>200°C) for a short period during which time the surface can achieve temperatures in excess of 100°C whereas the centre merely rises to between 30 and 40°C. Having already received a full cook, any post-heat-process *Listeria* contamination of the bulk meat will normally be present on the outer surface; therefore, roasting will effectively repasteurize the surface, destroying any contaminating vegetative organisms. After roasting, the product is rechilled and then processed as for any other bulk or sliced meat.

The greatest hazard presented to cooked meat products arises during the most intensive stage of the process, that of slicing and packing. Bulk meats are usually loaded onto the slicing machine so they present to a rotating blade. Meat slices then drop onto a conveyor belt that transports them to an in-line packing unit where they either fall into a prepack mould or are manually placed into one. The product pack is then vacuum sealed or, more commonly, flushed with a mixture of gases (primarily carbon dioxide and nitrogen) prior to sealing, chilling and despatch.

Depending on the size of the production plant, meat slicing and packing areas may contain several slicing machines side by side. It is preferable to restrict the number of slicing machines in any one area as any cleaning

of one machine can cause contamination of nearby machines. Complete separation of machines into different rooms can significantly reduce problems caused by aerosols created during cleaning of the equipment, whilst maintaining long production runs. This is because machines can be sequentially cleaned without compromising the safety of the product being sliced on an adjacent machine.

Slicing machines are complex pieces of equipment and may have 'dead' areas that can be difficult to clean due to poor access. As the slicing operation may be continuous for many hours in the day, any contaminating *Listeria* have ample opportunity to proliferate and cross-contaminate to other products. Interim cleaning and disinfection of surfaces is important to minimize build-up of contamination and detailed attention must be given to a full daily cleandown involving dismantling of all parts as necessary to allow access to all 'dead' areas and all food contact surfaces including slicer loaders, slicing blades and internal surfaces such as the blade guard. Gross product debris must be removed prior to the cleaning and disinfection procedure using appropriate chemicals.

The slicing equipment should be designed to prevent dead spots from occurring and a full hazard analysis should be conducted on this part of the plant to identify any point where product debris, and consequently *Listeria*, may build up.

If slicing equipment is of most concern in relation to *Listeria* contamination, then the product conveyor belts and associated machinery come a very close second. There is no doubt that a well-maintained one-piece synthetic material conveyor belt is a good option for assisting in the maintenance of hygiene provided it is cleaned and sanitized thoroughly, paying attention to both the upper surface and the underside of the belt and the rollers that drive the belt. Many slicing units are supplied with polycord conveyor belts that, instead of comprising a one-piece belt, consist of 20–30 individual synthetic bands each running between a series of rollers. Meat is transferred more rapidly and effectively on these bands but the problems associated with their cleaning and disinfection are significantly greater than with one-piece belts. This is primarily because each band is wrapped around a groove in the roller and with 20–30 bands in each section of the conveyor, and with most conveyors comprising several sections, the number of grooves where contamination could be trapped runs into hundreds. Clearly very careful consideration needs to be given to designing a cleaning regime to deal effectively with such intricate pieces of equipment.

It is probable that providing the slicers and conveyors are kept free from contamination by *Listeria*, then the product will remain predominantly *Listeria* free. To minimize contamination of slicers and conveyors with *Listeria* it is imperative that the organism is controlled in the rest of the environment. Attention to the hygienic operation and cleaning of slicers and conveyors is largely a wasted effort if *Listeria* is allowed to build up on floors, on walls and in other environmental areas in the slicing room.

Cleaning and disinfection of non-product contact surfaces are important to minimize the potential for *Listeria* to be transferred to product contact surfaces directly or indirectly via personnel or aerosols. It is not possible to identify all of the areas in a post-heat-process production environment that may cause problems without a thorough hazard analysis of specific processing units. However, environmental areas already well known for their potential for contributing to cross-contamination include the following: conveyors, aerosols from poorly cleaned/cracked floors or drains, aerosols from forced-air chillers, tables, product storage containers and racks, condensate from chillers, poorly cleaned ceilings and overheads and engineering activity.

Some products, such as bulk pâtés, are often hand garnished with herbs or spices after cooking. In such situations the garnish must be carefully controlled to prevent the introduction of bacterial contaminants at this final stage prior to repackaging and sale. Wherever possible, garnishes should be treated to eliminate or reduce contamination by dipping in disinfectant such as hypochlorite solution or by using heat-processed garnishes. In addition, many garnishes for this purpose are subject to some form of microbiological testing for the presence of pathogenic microorganisms including *L. monocytogenes*.

Final product issues and control

The presence of *L. monocytogenes* must be anticipated in sliced cooked meat products on some occasions. The aim must be to make such occurrences infrequent and at a low level. One of the greatest problems posed to the supply and sale of cooked meat products is the fact they can support extensive growth of *L. monocytogenes*. Data from the Food MicroModel (Anon., 1997c) show that under conditions found in a typical cooked meat product (pH of 6.4, and aqueous salt content of 3%), an *L. monocytogenes* contamination level of 1 per gram at the beginning of the shelf life may increase to 10^6 per gram within 21 days at a temperature of 5°C. Of course, many cooked meats have other factors that can assist in reducing the growth rate of contaminating *L. monocytogenes*. For

example, the presence of sodium nitrite at 50 ppm can contain the levels after 21 days to 10^4 per gram. It is important to note that these values are predicted values from a mathematical model that is commercially available and actual growth in products may differ from the prediction. Interestingly, studies carried out by Farber and Daley (1994) on naturally contaminated meats and pâtés demonstrated very little growth of contaminating *L. monocytogenes* when the products were stored for 3 weeks at 4°C. Predictive models can be useful for providing guidance on the relative effects of changes in product formulations as the formulation predicted to have the greatest controlling effect on the growth of the organism can quickly be ascertained. However, whichever published study is examined it is clear that one of the most effective ways to significantly reduce the hazard associated with the growth of any contaminating *L. monocytogenes* is to give the organism less time to grow in the product by reducing the product shelf life.

Many cooked meats have historically been given a long shelf life in most countries with some products even today being retailed with lives exceeding 45 days. However, many manufacturers and retailers who recognize the potential hazard associated with *L. monocytogenes* allocate product shelf lives significantly less than this unless the controlling factors inherent in the product are demonstrated to prevent the growth of *L. monocytogenes* to levels hazardous to health within the designed shelf life of the product.

Surveys of cooked meats have been carried out by a number of workers and frequently reveal varying frequencies of *L. monocytogenes* contamination. There is no question that where effort has been focused on the hazard and attempts made to improve control of *L. monocytogenes* the incidence in the targeted product groups has decreased. A good example of this is given by Gilbert (1996). In routine surveillance carried out between 1989 and 1994 the incidence of *L. monocytogenes* in pâté reduced from 29.7% to 2.6% (Table 4.6). This was undoubtedly attributable to increased awareness of the hazard associated with pâté and cooked meats brought about by the 1989 outbreak of listeriosis associated with pâté imported to the UK from Belgium. A similar reduction in the incidence of *L. monocytogenes* was recorded for cooked chicken (Table 4.7). A recent large survey conducted by the UK MAFF investigated the incidence of *L. monocytogenes* at three stages of cooked meat processing: at the premises of the suppliers of bulk cooked meats, in cooked meat slicing plants and at the retail level. In primary processors *L. monocytogenes* was detected in 3.1% (11/352) of samples of cooked meats with only one sample containing levels above 10 per gram (log 1.3 per gram) (Table 4.8).

Table 4.6 Incidence of *L. monocytogenes* in pâté in England and Wales (adapted from Gilbert, 1996)

Year	Number of samples	*L. monocytogenes* (%)
1989	155	46 (29.7)
1989	1698	162 (9.5)
1989	65	13 (20)
1990	626	25 (4.0)
1990	96	3 (3.1)
1991–92	40	1 (2.5)
1994	3073	80 (2.6)

Table 4.7 Incidence of *L. monocytogenes* in cooked chicken in England and Wales (adapted from Gilbert, 1996)

Year	Number of samples	*L. monocytogenes* (%)
1988–89	527	63 (12.0)
1989	102	27 (26.5)
1991	169	46 (27.2)
1991	983	91 (9.3)
1993	119	6 (5.0)

In secondary processors the samples were taken pre-slicing, sliced and at the final holding stage: 2.9% of samples were found to be contaminated with *L. monocytogenes* (all <100 per gram), although the results indicated that contamination rates increased after slicing (Table 4.9). In the study of cooked meat products purchased from retailers, 5.5% (22/414) were contaminated with *L. monocytogenes* with only two samples contaminated at levels in excess of 100 per gram (log 2.0 and log 4.48) (Table 4.10).

Clearly, cooked meats are a category of food product with an associated risk that appears to be greater than in many other products due to the potential for *L. monocytogenes* to be present and for it to grow during the product shelf life, and because these products are consumed by all vulnerable groups. If listeriosis outbreaks are to be avoided from this product category in the future it will be by attention to the following critical areas:

- effective cooking
- effective segregation of raw material from cooked product
- high standards of personal hygiene by training of operatives
- frequent effective cleaning and disinfection of product contact and

Table 4.8 Survey of cooked meats for *L. monocytogenes* at primary processing sites (Anon., 1995a)

Product	Stage	Number of samples	*Listeria* spp.* (in 25 g)	*L. monocytogenes* (in 25 g)	*L. monocytogenes* (log_{10} per gram)
Ham	Cook	48	0	0	-
Ham	Slice/jelly	48	8	6	-
Ham	Final hold	49	5	3	1.3, < 1.0, < 1.0
Pâté	Cook	16	0	0	-
Pâté	Slice/jelly	13	0	0	-
Pâté	Final hold	16	1	1	< 1.0
Pork pie	Cook	54	0	0	-
Pork pie	Slice/jelly	53	0	0	-
Pork pie	Final hold	55	1	1	< 1.0
Total		352	15 (4.3%)	11 (3.1%)	

* Including *L. monocytogenes.*

Table 4.9 Survey of cooked meats for *L. monocytogenes* at secondary meat processing sites (Adapted from Anon., 1996a)

Product	Stage	Number of samples	*Listeria* spp.* (in 25 g)	*L. monocytogenes* (in 25 g)	*L. monocytogenes* (log_{10} per gram)
Cooked cured pork	Pre-slice	17	2	0	< 2.0
Cooked cured pork	Slice	17	3	2	< 2.0
Cooked cured pork	Final hold	17	3	2	< 2.0
Cooked cured comminuted meat	Pre-slice	13	1	0	< 2.0
Cooked cured comminuted meat	Slice	13	3	0	< 2.0
Cooked cured comminuted meat	Final hold	13	3	0	< 2.0
Poultry	Pre-slice	18	0	0	< 2.0
Poultry	Slice	18	1	0	< 2.0
Poultry	Final hold	18	0	0	< 2.0
Corned beef	Pre-slice	20	0	0	< 2.0
Corned beef	Slice	20	1	1	< 2.0
Corned beef	Final hold	20	2	1	< 2.0
Total		204	19 (9.3%)	6 (2.9%)	

* Including *L. monocytogenes*.

Table 4.10 Survey of cooked meats for *L. monocytogenes* from retail stores (Anon., 1996b)

Product	Number of samples	*Listeria* spp.* (in 25 g)	*L. monocytogenes* (in 25 g)	*L. monocytogenes* (log_{10} per gram)
Cooked cured pork	220	20	19	< 2.0
Cooked cured comminuted meat	47	0	0	-
Cooked poultry	99	4	3	4.48, 2.0 and < 2.0
Corned beef	48	0	0	-
Total	414	24 (5.8%)	22 (5.5%)	

* Including *L. monocytogenes*.

other production areas post cooking (minimizing aerosol formation at all times)
- presence of factors inhibiting growth in the product, including well-controlled chill storage temperature
- reduced shelf life to preclude opportunities for extensive growth.

COOKED READY MEALS

Description of process

Ready meals have frequently been implicated as a reason for the emergence of *L. monocytogenes* as a foodborne pathogen. Ready meals are often referred to as prepared meals, convenience meals, tele dinners, etc. They are all cooked products that are packed and stored under refrigerated conditions prior to consumption without further heating, after a reheat or following a full re-cook by the customer.

Some ready meals may have uncooked processed ingredients such as cheese or fermented meats added after assembly of the cooked components; others may consist of pre-cooked ingredients that are cooled prior to being cold assembled. They vary considerably in type and include pasta dishes, e.g. lasagne and tagliatelle, Chinese dishes, e.g. egg fried rice and spare ribs, and traditional products, e.g. shepherd's pies, cottage pies, roast chicken meals and 'hot pots'.

Ready meals may consist of any individual or combination of ingredients from all sectors of the food industry including dairy, meat, poultry, fish, vegetables, herbs and spices. Although often referred to as a significant

hazard in relation to *L. monocytogenes*, ready meals have rarely been implicated in outbreaks of listeriosis. This is primarily because of the relatively short shelf life often given to these types of products, which usually does not exceed 10–15 days at refrigeration temperatures. This is because of the highly perishable nature of the products brought about by general post-processing contamination during which many of the products are extensively handled (Figure 4.4).

Raw material issues and control

Ready meals may be manufactured from a combination of many different ingredients and it is not necessary for the purposes of this discussion to cover the entire range of this extremely diverse product area.

A typical example of a ready meal may be a chicken curry consisting of cooked chicken pieces in a curry sauce packaged in one compartment of a tray with boiled rice in a separate compartment. Ready meal manufacturers often reduce their exposure to the potential hazards inherent in raw food materials by sourcing pre-cooked or pasteurized ingredients such as cooked chicken, prawns or pasteurized liquid egg. In this way the incidence of pathogens, including *L. monocytogenes*, and the concomitant risk to their own process and products are reduced.

Listeria spp., including *L. monocytogenes*, will be present in some of the raw materials, particularly fresh vegetables where it is known to be occasionally present, albeit at low levels. As with other processes incorporating a cooking stage, the key controlling factors usually involve the cooking process and avoidance of post-cooking cross-contamination.

The most important aspect of raw material control with respect to ready meals is the segregation of components likely to be contaminated with *L. monocytogenes* from those that are destined to be added to the finished product without a listericidal process, for example ready meals that may be topped with grated cheese. Obviously, the cheese should be stored separately from raw food ingredients and when transferred into the high care area of the factory, packs should be dipped through a disinfectant solution so that the outer packaging is decontaminated before the cheese pack is opened in the high care area. The physical characteristics (size, viscosity, etc.) and temperature of raw materials can be critical to the safety of a product as the cooking stage later in the process is often set to a standard time and temperature based on studies with previous supplies of ingredients. Therefore, any variations in the physical nature of the raw material or requirement to cook from a colder temperature must lead to a revalidation

Process Stage	Consideration
Materials intake	Supplier quality assurance (raw/cooked separation at supplier raw/cooked separation at intake)
↓	
Materials storage	Separation Hygiene Temperature Time
↓	
Recipe formulation	Hygiene Time
↓	
Cooking (where applicable)	Temperature Time
↓	
Transfer to high-risk area	Hygiene Segregation
↓	
Product assembly	Hygiene Temperature Time
↓	
Transfer into packs	Hygiene Temperature
↓	
Storage	Temperature Time
↓	
Distribution	Temperature Time
↓	
Retail sale	Temperature Shelf life
↓	
Consumer	Advice

Figure 4.4 Process flow diagram and technical considerations for a typical ready meal.

of the cooking process to ensure the required time/temperature combination is achieved. Because of the potential for post-cooking process contamination, ready meal factories usually have segregated low risk and high risk areas. Raw ingredients destined to be cooked are stored in the low risk area and the cooking stage is used as the division between low and high

risk areas. Personnel hygiene disciplines must be in place to maintain the integrity of high risk areas and the factory structure and process flows should be in accordance with good manufacturing practice (Anon., 1991). Details of the systems and procedures expected of manufacturers of such chilled products are given in guidelines recently published by the UK Chilled Food Association (Anon.,1997b).

Process issues and control

As with any process with a cooking stage, this is the critical control point for destroying vegetative bacterial contamination in the raw materials. There must be effective separation of the raw materials from the cooked product to prevent post-process recontamination. In general, ready meal components are cooked to achieve a centre temperature of at least 70°C for 2 min or an equivalent process, similar to cooked meats. Providing this is carried out properly so that all raw components are fully cooked, then *L. monocytogenes* will be destroyed. Some ready meals are pasteurized in a vacuum pack and then refrigerated for retail sale. These are termed sous vide products and do not represent a hazard with regard to *L. monocytogenes* if the process temperatures achieved are sufficient to destroy initial levels of contamination and the seal integrity of the pack remains intact. However, the majority of ready meal ingredients are subject to a cooking process in bulk followed by chilling. Bulk products are chilled to <5°C within several hours and then stored, usually for less than 24 h, until required. The products are then cold assembled in a high care or high risk area of the factory by manual or automated systems. In some cases, components of ready meals may be cooked and hot-filled into products, as in the case of sauces and some other materials that are difficult to fill in the cold state. This presents an additional hazard to the process as a hot material may be deposited onto a chilled component such as pre-cooked chicken. Sauces are usually filled at temperatures above 60°C but upon deposition onto the chilled product the temperature equilibrates and there is the potential for subsequent growth of any contaminants present on/in the cold-filled component unless the whole product is rapidly blast chilled. In most cases this is in fact what occurs and the blast-chilled product is then sealed, packaged and stored for despatch.

There are many areas of potential bacterial contamination in ready meal operations because of the complex and manual nature of the cold-fill assembly of pre-cooked products (Figure 4.4). Opportunities for contamination arise from the point at which the product passes from the low risk side of the factory through the cooking area into the high care or high risk area. Transfer may be via through-ovens for baked products, via

continuous grills where the product passes under the heat source on a chain belt, via pans where the products are cooked in large frying or boiling pans prior to being tipped into receiving vessels in the high care area or from large enclosed heating vessels that may be piped directly into high care vessels through enclosed pipework, as is often the case with sauces. All of these operations present risks to products from potential contamination with *L. monocytogenes*.

Product contact surfaces always offer the greatest potential for cross-contamination of any contaminating *Listeria* species to cooked product. Particular attention needs to be given to cleaning and disinfection of any vessel used to transfer or receive cooked product from the low risk into the high risk area. Valves, seals and lids are often poorly cleaned on tanks and in pipework systems and unless these are regularly stripped down for cleaning and inspection it is easy to anticipate build-up of product residues, offering environments suitable for colonization by and growth of *L. monocytogenes*.

In the manufacturing environment, aerosols are a significant potential hazard to cooked products. It is extremely important to control aerosols in areas of open cooking where extractor hoods should be used to withdraw steam rising from the product. However, these often create condensation, which can drip back into the cooked product on transfer to the high risk area. Adequate condensation drainage is essential, together with regular daily cleaning and disinfection of areas where condensation occurs. Similar concerns arise in chillers where condensation often builds up on the ceiling, again leading to drips into chilled material if it is left exposed. In many cases, product components are left exposed due to the desire to chill them rapidly thus preventing growth of any surviving spore formers; therefore, mechanisms for preventing condensation from building up and dripping into products are very important. Forced-air chillers are also known to be capable of transferring *L. monocytogenes* to product. This occurs as a result of colonization of the chiller unit with the organism, which may then be blown throughout the chiller during fan operation. Product chillers are probably one of the most important areas for the control of *Listeria* and regular cleaning, disinfection and fogging of the chiller is an important step to minimizing contamination.

As with cooked meats, the area offering the greatest potential for cross-contamination is the assembly area where the product is usually cold-filled into product containers by hand. The two main opportunities for contamination are from personnel handling products and from machinery used to fill or convey the product. Aerosols and condensation created in the environment also present significant hazards to exposed product,

and cleaning regimes need to be carefully controlled to avoid creation of aerosols that may contaminate product.

Considerable attention should be given to maintaining the cleanliness of all product contact surfaces in high risk areas. Cleaning and disinfection of conveyor belts is made easier by the use of continuous one-piece belts that can be removed for cleaning and sanitizing or can be sanitized *in situ*. Areas often neglected are those on the underside of the belt where product debris can accumulate and, due to a commercial desire to operate production runs for long periods, only the areas where product debris is visible, i.e. the surface of the belt, are effectively cleaned. However, *L. monocytogenes* can readily colonize the underside of the belt and from there, via belt movement and moisture channels, eventually contaminate the surface of the belt and become a continuous stream of contamination for personnel and products. Whilst high standards of personal hygiene are usually recognized as important and controlled effectively, it is often such intermediates that can offer a significant hazard by contaminating hands that subsequently come into contact with the product. Other potentially contaminating intermediates include brush handles, handles of spray guns used for ongoing decontamination and knife handles used for opening packaging, which, although they never come into contact with the product directly, act as a source of contamination that then gets passed via hands to products. Attention must be given to cleaning and sanitization of these areas.

Some ready meals receive a garnish or are coated with ingredients not subject to a full cook; for example, some may be topped with grated cheese or fermented meat. In such circumstances the incoming raw ingredient represents a potential hazard to both the environment and the products. Control of these materials needs to be established through supplier quality assurance schemes and the provision of separate and appropriate storage and handling systems within the ready meal site.

Final product issues and control

Ready meals may be chilled or frozen prior to despatch to retailers or caterers for subsequent sale. Frozen ready meals represent little risk with regard to *L. monocytogenes* as no growth will occur after freezing. However, it remains important to control the hazard during manufacture to avoid contamination with high levels that may subsequently present a hazard on defrosting by the consumer.

The greatest potential hazard is presented by chilled ready meals as any *L. monocytogenes* present may grow in the final product. This hazard

is probably less than in most other pre-cooked products such as sliced cooked meats as the shelf lives of ready meals are usually significantly shorter, ranging from 5 to 15 days. Shelf lives are usually limited by the growth of general post-process contaminants such as *Pseudomonas* species or other Gram-negative bacteria and this therefore restricts the time that *L. monocytogenes* has to increase in number. The extent to which *L. monocytogenes* can grow in the product is extremely product variable and is dependent on the factors presenting in the product. However, many ready meals have pH values ranging from 6 to 7 with water activity in the range 0.97–0.99, without any other controlling factors. Under such circumstances the growth of *L. monocytogenes* can occur from initial contamination levels of 1 per gram to in excess of 10^3 per gram within 10 days (water activity 0.99, pH 6.6, 5°C; data from Food MicroModel (Anon., 1997c)). Therefore, avoidance of contamination and restriction of shelf life are important factors in controlling this hazard.

The safety of ready meals, however, is increased in many cases by the inclusion of a further cooking step to be applied by the consumer. For this step, cooking instructions given on the product pack for consumer guidance are often designed to ensure the product is repasteurized by achieving temperatures in excess of 70°C. It is believed that many consumers do not follow cooking guidelines precisely as the pre-cooked appearance of the product may lead them to consider that the guideline is designed only to rewarm the product. Additional advice, including statements to cook the product 'thoroughly' or until it is 'piping hot', is often placed on the pack to support the need to achieve a high temperature re-cook. In some countries susceptible groups such as pregnant women are still advised to fully cook ready meals if they want to completely avoid any hazard associated with *L. monocytogenes* (Department of Health, 1996).

Surveys of ready meals frequently detect *L. monocytogenes*. MacGowan *et al.* (1994) reported a 17% incidence (13/75) of *Listeria* species in cook-chill meals with 9 of the 12 *Listeria* species being reported as *L. monocytogenes* (12% incidence). It is believed that in recent years the incidence of *Listeria* species in these products has significantly reduced to <5%, with *L. monocytogenes* present in <1% of products. The incidence of *Listeria* species is not related to specific product types, but species are found in a variety of products and more frequently found in products that are extensively handled or have greater exposure to environmental cross-contamination. This reinforces the fact that the primary areas allowing *L. monocytogenes* to gain access to these products is via people and the environment in the product assembly areas.

Ready meals with raw components

Some ready meals may contain components that are not fully cooked at the manufacturing site. Such products may contain portions of fish that are not pre-cooked because texture would be lost when a consumer cook was applied or they may contain only blanched vegetables that are refreshed in cold chlorinated water after blanching before being assembled into pack; again this is to maintain product texture for the consumer. These products often have a cooked visual appearance, especially if the fish is covered by a pre-cooked sauce. The incidence of *L. monocytogenes* is likely to be higher in such products due to the uncooked component. The raw components and the assembly process for these products should be completely segregated from any other product process involving only fully cooked components. Advice to consumers given on the pack should also make clear the need for thorough cooking.

RAW DRIED AND FERMENTED MEATS

A variety of products exist in the retail market that are manufactured by drying and/or fermentation of raw meat without any associated listericidal heat process. Traditional fermented meats include salamis, such as Danish salami, peppered salami and German salami, etc., whilst those products that receive only a curing and drying process are termed raw dried or cured meat and include Parma ham, Proscuitto and Bresaola. These products are usually consumed in small quantities because of their strong flavours (and high cost) and are enjoyed by all sectors of society including most risk groups of individuals. These products have a good safety record in respect of *L. monocytogenes* with no outbreaks of listeriosis being reported that implicate such products.

Description of process

The first stage in a salami manufacturing process involves bowl chopping precut raw meat components, usually pork but sometimes also beef (Figure 4.5). These days it is also possible to find salamis manufactured from other meats such as turkey or chicken. The meat is chopped into small pieces together with herbs, spices, salt, sodium nitrite (and sometimes sodium nitrate) and lactose or other fermentable sugars. Some products are inoculated at this stage with starter cultures consisting of lactic acid bacteria and staphylococci, although some of the traditional salamis do not have any starter cultures added but rely on the growth of natural lactic acid bacteria present on the raw meat. The raw meat mix is forced

Process Stage	Consideration
Animal husbandry	Health Cleanliness
↓	
Animal slaughter and processing	Hygiene Temperature Time
↓	
Meat transport, delivery and storage	Hygiene Temperature Time
↓	
Meat trimming	Hygiene
↓	
Bowl chop with herbs, spices, salt, nitrite (and starter culture)	Distribution of additives Starter culture activity Hygiene
↓	
Stuff into casing	Hygiene Casing quality assurance
↓	
Tying	Hygiene
↓	
Fermentation	Hygiene Temperature Time Acidity development/pH profile
↓	
Drying	Hygiene Temperature Time Moisture loss
↓	
Slicing, where applicable	Hygiene
↓	
Packing	Hygiene
↓	
Storage and distribution	
↓	
Retail slicing, where applicable, and sale	Hygiene Shelf life conditions

Figure 4.5 Process flow diagram and technical considerations for a typical raw fermented meat.

into casings, which may be made from animal intestine or from synthetic materials, and then tied with string before being hung on racks. Salamis are placed in rooms of controlled temperature and humidity and allowed to ferment. Process temperatures range from 20 to 37°C, depending on the product, and the meat mix is fermented for several days. European processes usually ferment at 20–30°C whereas US processes operate at higher temperatures of 30–37°C. During this stage the lactic acid bacteria dominate and actively reduce the pH by producing organic acids. The fermentation usually achieves a pH of approximately 5.0 and moisture content is reduced by 10–15% during the process due to drip loss and drying. Following fermentation, products are transferred to drying rooms at temperatures of approximately 10–12°C and controlled humidity where they are left for periods varying between 20 and 50 days or more depending on the product. For most salamis, this stage is designed to achieve further drying of the product whilst at the same time allowing development of textural and flavour changes desirable for the finished product. For some products, a mould coating is allowed to develop on the outer surface of the salami; this imparts a distinctive mould-ripened flavour whilst elevating the pH of the product from 4.5–5.0 to in excess of pH 6, although this varies from the centre to the outer surface of the product. At the end of the process, the finished salami usually has a water activity below 0.94, an aqueous salt content of 5–10% and a pH between 4.5 and 7. Some salamis may also be smoked at < 15°C in large smoking rooms prior to being dried.

Dried meats are manufactured from whole pieces of muscle or whole joints such as Parma ham, which is made from whole legs of pork, without any comminution (chopping) process (Figure 4.6). The primary stage of the process involves regular application of dry salt to the meat pieces, which are then stored under refrigeration at <5°C. The meat is turned at regular intervals and further salt added, which may be rubbed or massaged over the product. Meat may be salted for periods of 1 to 2 weeks and during this time conditions develop that select for the growth of a lactic acid microflora. After salting, the products are then dried at 10–12°C, sometimes with controlled humidity, for several months, during which time a mild lactic fermentation occurs and the moisture content decreases significantly. A smoking stage may also be incorporated prior to drying for some of these products. The final product has a pH between 5 and 6 and water activity which may be as low as 0.85.

Salamis and raw dried meats may be wrapped and sold whole for slicing by the retailer or they may be sliced on site and prepacked for sale.

Process Stage	Consideration
Animal husbandry	Health Cleanliness
↓	
Animal slaughter and processing	Hygiene Temperature Time
↓	
Meat transport, delivery and storage	Hygiene Temperature Time
↓	
Meat trimming	Hygiene
↓	
Salting and storage	Distribution of salt Hygiene Temperature
↓	
Drying	Hygiene Temperature Time Moisture loss
↓	
Slicing, where applicable	Hygiene
↓	
Packing	Hygiene
↓	
Distribution	
↓	
Retail slicing, where applicable, and sale	Hygiene Shelf life conditions

Figure 4.6 Process flow diagram and technical considerations for a typical raw dried meat.

Salamis and dried meats are stored and sold at ambient temperatures or under chilled conditions and have extensive shelf lives that, because of their inherent stability, may be between 1 and 3 months or more.

Raw material issues and control

As with other processes that do not have a true listericidal stage, the quality of the raw material is extremely important in controlling the level of contamination the product may be exposed to during the subsequent

fermentation and/or drying process. The incidence of *L. monocytogenes* in raw meats has been reviewed already (see section on cooked sliced meat and pâté, p. 60) and it is clear that the raw material does present a significant source of the organism and hence a potential hazard to the product. High standards of hygiene and process control to mini-mize additional contamination or growth of existing contamination at the bowl-chopping stage and during casing and tying are essential to prevent any further build-up of *L. monocytogenes*.

Fresh meat is usually processed very quickly from receipt at the processing unit; this is advantageous as it prevents the growth of any contaminating *Listeria* species prior to the processing stage. The requirement for high quality meat needs to be impressed upon the supplier of the raw meat and a supplier quality assurance programme is essential to establish the key requirements for hygienic conditions of slaughter and supply. This may include monitoring for indicators of hygienic processing. Meat is often further processed by the salami or dried meat manufacturer to trim fat to appropriate levels and this is an area where significant cross-contamination can occur from conveyor belts, tables, personnel and knives. Standards of process hygiene must be carefully controlled and cleaning and disinfection must be carried out regularly. Other raw materials, although important, may not be as greatly contaminated by *L. monocytogenes* as the raw meat but it is important to have an understanding of the microbiological status of ingredients such as herbs, spices and casings, particularly natural ones. Hygienic handling procedures minimize any build-up of food debris and dust in ingredient storage areas and primary processes will also control *L. monocytogenes* in the environment.

Process issues and control

The process for manufacturing salami and dried meats is an age-old traditional process. The presence of high levels of starter bacteria, salt and sodium nitrite are important factors in controlling pathogenic bacteria including *L. monocytogenes* in salami. The growth of the starter bacteria to high levels with the concomitant production of organic acids and reduction in pH is believed to have a growth-controlling effect on *L. monocytogenes* that, coupled with reductions in moisture content during subsequent stages, may result in reduction in levels of *L. monocytogenes*. Many of these factors are not present in raw dried meats, which usually have little significant fermentation and may have no addition of sodium nitrite. However, the differences in the relative hazard associated with these products will be recognized by understanding the significance of the

primary stages and the process for each product type. With salami, the meat is comminuted and so any contamination present is transferred throughout the product. With raw dried meats, most are manufactured from whole muscle joints and therefore any contamination with *L. monocytogenes* is usually restricted to the surface. Therefore, when comparing the two processes, pathogenic bacterial contaminants of a salami have to be destroyed throughout the product whereas with raw dried meats the main decontamination must occur at the surface, which, of course, is where the harshest conditions prevail, with continuous application of salt, exposure to competing lactic acid microflora and removal of moisture during drying.

The high incidence of *L. monocytogenes* recorded in various published surveys of salami demonstrates that the process is probably not capable of destroying *L. monocytogenes* but it appears that levels of contamination are reduced or contained. Whiting and Masana (1994) reported a 4 log reduction of *L. monocytogenes* in experiments with a salami-type product, although this related to the higher fermentation temperatures used in US-type salami products.

Although the precise factors controlling *L. monocytogenes* in a salami or dried meat product are not clearly understood, it is important to recognize that the chemical and microbiological changes occurring in these processes must be having an inhibitory effect. Therefore, understanding and close monitoring of the changes that do occur in any particular process are important as any deviation from the normal process profile may make the product unsafe.

Well-controlled process fermentation profiles and drying profiles appear to be the most significant factors in the maintenance of product safety. However, as it is possible that *L. monocytogenes* may survive the process, cross-contamination of finished products needs to be controlled, particularly in slicing operations where heavy contamination of one product could lead to widespread contamination of others. Factors already described for the control of environmental contamination for cooked sliced meats apply equally to its control in the slicing operation of salamis and raw dried meat. However, as the final product is itself inhibitory to the growth of *L. monocytogenes*, care should be taken to avoid excessive wet cleaning and all product residues must be removed effectively prior to disinfection procedures. Failure to remove wet product debris could be more hazardous than failure to clean at all as the inhibitory nature of the debris will be diminished by the added moisture, thus providing

conditions for proliferation of any contamination that may be present in the product at low levels.

Salamis are fermented and dried usually by attaching them to racks with string used to wrap the meat mix in the casing. This ensures a free flow of air and allows effective moisture loss. As they are completely exposed during the entire process, significant opportunity exists for contamination from the environment during this process. It would be very easy to operate a relaxed standard of hygiene for traditional products such as these by believing that the hazard present in the raw material is greater than that in the environment, thus making control of the environment less important. However, this is unsound thinking and attention must be given to regular cleaning and disinfection of all process environments to prevent build-up of contamination.

Some salamis have herbs or spices added to them after the fermentation and drying process. This is done by dipping the salami in gelatine and using this to bind the spice mixture onto the external surface. Naturally, the potential for contamination of the raw material needs to be considered although at this stage any bacterial contamination is unlikely to grow on the product. Nevertheless, suitable control needs to be assured.

Final product issues and control

L. monocytogenes cannot grow in most salami and raw dried meats as the water activity is frequently <0.92, below the minimum for the growth of the organism. The facts that the process may reduce contamination levels and the finished product does not support growth probably account for the fact that no reported outbreaks of listeriosis have yet been attributed to these types of products. As the low water activity in these products is such a critical factor for controlling the growth of *L. monocytogenes* and other bacterial pathogens, it is essential that storage conditions throughout their life are such as to prevent moisture contamination, e.g. via condensation, which could raise the local water activity to levels that may allow the growth of these organisms.

Surveys of salami and raw dried meats frequently reveal contamination of the finished product. Jay (1996) reported a variable incidence of *L. monocytogenes* in these products ranging from 0 to >50%. A recent survey conducted in the UK by the MAFF (Anon., 1997e) indicated a high incidence of *L. monocytogenes* in salami and raw dried meats (Table 4.11). Samples

Table 4.11 Survey of ready-to-eat dried and fermented meat for *L. monocytogenes* from retail stores (Adapted from Anon., 1997e)

Product origin	Number of samples	*Listeria* spp.* (in 25 g)	*L. monocytogenes* (in 25 g)	*L. monocytogenes* (log_{10} per gram)
Germany	131	11	8	< 2.0
Denmark	104	1	1	< 2.0
Italy	78	8	4	< 2.0
Others†	142	4	2	< 2.0

* Including *L. monocytogenes*.
† Others include Spain, France, Holland, Belgium, Hungary, Poland, Czech Republic, UK and unknown sources.

included all varieties of imported products, including German salami, Milano salami, Parma ham, etc. Although *Listeria* species were detected in 24 out of 455 samples, of which 15 were *L. monocytogenes*, all samples had counts of <100 per gram, indicating the growth-controlling effect of the inherent physico-chemical characteristics of these products.

PROCESSED FRESH DAIRY DESSERTS

Description of process

Processed dairy desserts include a very wide variety of product types supplied as either fresh or frozen, e.g. cream-filled desserts, cream cakes, trifles, cream-garnished mousses, ice-cream desserts and many similar products. Of greatest concern in relation to *L. monocytogenes* are those products that are subject to post-processing contamination and have extended shelf lives at chill temperatures. Dairy desserts are consumed by all sectors of society but are particularly cherished by young children.

With the exception of soft cheese and pasteurized milk, other processed dairy products have an excellent history of safety with regard to listeriosis. The only reported incident associated with other dairy products was reported in Belgium where listeriosis was said to be associated with the consumption of ice cream made with fresh cream in a restaurant (McLauchlin, 1996). Like ready meals, this low association with listeriosis probably relates to the fact that fresh dairy desserts usually have an extremely short shelf life of <5 days because of spoilage of the product by other contaminating microorganisms.

Manufacturing processes for dairy desserts vary considerably from the automated production of ultra-high temperature (UHT) processed

Process Stage	Consideration
Intake of processed raw materials, including cream	Supplier quality assurance (raw/cooked separation at supplier raw/cooked separation at intake)
↓	
Materials storage	Separation Hygiene Temperature Time
↓	
Mixing	Temperature Hygiene Time
↓	
Filling/product assembly	Temperature Hygiene Time
↓	
Cream garnishing	Temperature Hygiene
↓	
Storage	Temperature Time
↓	
Distribution	Temperature Time
↓	
Retail sale	Temperature Shelf life

Figure 4.7 Process flow diagram and technical considerations for a typical processed fresh dairy dessert.

mousses, which are deposited into their final containers under aseptic conditions, to the hand decoration of trifles with cream (Figure 4.7). In most cases the primary ingredient of concern is fresh cream and most dessert manufacturers purchase cream from a processor who will usually supply the cream in bulk pasteurized form. The cream is usually given a chilled shelf life of 10–12 days and unless a further heat process or controlling factor is applied, e.g. fermentation, then the shelf life of the fresh finished product should not exceed that of the cream itself. This naturally restricts the dessert product's shelf life, usually to 5–7 days.

Fresh dairy desserts may contain a variety of other ingredients, e.g. nuts, fruit, biscuit or other bakery components, but cream is the main component of concern in respect of the potential hazard from *L. monocytogenes*, which may contaminate and grow in the product. Any controls applicable to cream-containing dairy desserts are equally applicable to

any dairy dessert where the final products may support the growth of *L. monocytogenes*.

Cream is stored in chillers until required for production. Depending on the product, the bulk cream container may be attached to a pump and cream pumped directly to depositors for automatic application to the dessert product. Alternatively, it may be decanted into the bowls of machines that whip the cream, after which it is removed for depositing either manually via piping bags or semi-automatically by machine depositors onto the dessert or cake. For many products the cream is pumped into a vessel and mixed with other ingredients such as flavourings and thickeners to make mousse or fruit dairy desserts and, in its simplest form, it may be manually whipped and manually deposited onto the top of a trifle or similar dessert.

Products may be manufactured under chilled conditions, with production rooms often maintained at around 12°C, and after manufacture are wrapped, sealed and blast chilled for subsequent sale.

Raw material issues and control

Although the wide variety of dairy desserts may contain pastry, flavourings, skimmed milk, whey powder, water, sugars, fruit, nuts, etc., the primary ingredient of concern is cream.

Cream is produced from whole milk by a process of separation. It is subject to a pasteurization process of approximately 74°C for 15 s that is effective in destroying *L. monocytogenes* and if correctly applied will result in a product free from contamination with *Listeria*. The cream manufacturing process is highly automated with pasteurization and filling being conducted in enclosed pipe systems with little opportunity for contamination. However, it is important to remember that milk pasteurization is equally simple with apparently very low risk of contamination but there have been two listeriosis outbreaks recorded where improper pasteurization or post-process contamination have occurred. Therefore, the integrity of the pasteurization equipment and all vessels and pipes post pasteurization must come under close scrutiny as part of a supplier quality assurance programme for any manufacturer of a dairy dessert. This should extend to procedures in place to monitor the integrity of pasteurization, including divert valve operation and maintenance. In addition, checks must be made to ensure improperly pasteurized milk or cream will be reliably diverted from properly pasteurized product. Also, monitoring the integrity of the pasteurizer by implementing routine plate checks is essential. Although

not a control, it is common practice for the cream supplier to test pasteurized cream for the presence of *L. monocytogenes* and provide appropriate certification to the customer, although like most microbiological tests this information will be retrospective.

Published surveys for *L. monocytogenes* in cream indicate a very low incidence. Kozak *et al.* (1996) reported that no *Listeria* species were detected in 33 samples of whipping cream or in 19 samples of ordinary cream with only 2/350 pasteurized whole milk samples containing *L. monocytogenes*. Kozak concludes that contamination of finished dairy products occurs due to post-pasteurization contamination from environmental sources, which is undoubtedly the case. It is therefore likely that cream or other pasteurized dairy products received by dessert manufacturers will be infrequently contaminated with *L. monocytogenes* and therefore every effort should be made to process the material in a way that ensures it remains so.

Many manufacturers purchase bulk cream and dairy ingredients and use them over a period of a few days. In such circumstances, it is essential that the processing conditions by which the cream is removed from the bulk container are maintained aseptically as contamination of the outlet valve of the cream vessel or the lid and seal will allow the contaminants to colonize and proliferate. This is indeed true of any product held in large vessels where only a proportion is removed for production. Cleaning and disinfection of the outlet valve is critical to avoid microbial colonization and is an area often overlooked by many processors.

Process issues and control

As the cream ingredient as received is rarely the cause of contamination, the primary route for contamination of dairy desserts is through cross-contamination from people, production equipment or the environment. With UHT processes where product is aseptically filled into containers there is little opportunity for contamination; therefore, provided the heating process and aseptic plant are maintained to appropriate standards, *L. monocytogenes* represents little hazard to the product. With fresh products where cream or milk is mixed with other ingredients the greatest hazard probably arises from improperly cleaned and sanitized equipment. Blades on mixers together with valve seals and lid rims are usually the areas that receive inadequate attention during cleaning. In fact, if such components are under effective hygienic control, products that are subject to automatic mixing and depositing directly into pots or other containers would only be at minor risk from contamination.

Many of these types of operations are often described as 'clean fill', i.e. little opportunity for contamination exists because of the degree of automation and most product is only exposed to the inside of pipes and vessels with the only opportunity for contamination from the manufacturing environment arising between the filling head and the pack. Providing effective in-place cleaning systems are operated for such equipment, these product types are also less prone to contamination with general spoilage organisms, thus allowing a longer shelf life than many other dairy products that may readily pick up post-process Gram-negative contaminants, which can spoil products within several days. As a consequence, if *L. monocytogenes* were to gain access to the extended shelf life desserts and if the final product characteristics supported growth, with little other competition in the product, it could grow unhindered. Many products, such as mousses, fall into this category and cleaning and disinfection of production equipment is critical to the safety of these products.

Effective cleaning procedures must be applied to vessels, pipes and valves. Regular inspection of tank and seal integrity is essential to avoid incidents similar to those that occurred in the chocolate milk outbreak described in Chapter 2.

The products exposed to greatest risk of contamination are those cream-filled desserts that are extensively handled. Products such as cream cakes and cream-topped trifles are often produced semi-automatically using cream machines to deposit the cream into or onto the product. The type of equipment employed to fill these types of product is relatively complex and requires manual cleaning; anything of this nature always represents a major potential microbial hazard.

Attention to detail is required in the removal of food debris and effective cleaning and decontamination of the product contact surfaces in the cream machine together with areas around the shaft seal where cream seepage may occur. In addition, many of the semi-automated or automated machines for cream whipping and depositing usually draw significant quantities of air from the environment and incorporate it into the product. Some of these designs draw air from close to the floor without any form of microbial filter; this poses an obvious contamination risk to product. Safe positioning of air intake, air filtration and cleaning procedures for these systems are critical to avoiding a continuous influx of microbial contaminants, which, of course, may include *L. monocytogenes*. Again, the manual nature of many of these production operations can cause problems due to poor personal hygiene or poor equipment

cleaning procedures. Every utensil or manual operation likely to come into direct contact with the product must be identified and frequent cleaning and disinfection procedures implemented for both staff and equipment.

Scoops and other utensils are often used and may be left lying in the production environment instead of being rinsed immediately and placed in sanitizer tanks until next required. Sanitizer tanks must be of sufficient size to contain all the utensils for which they are required so that overloading is avoided and all utensils are submerged and in direct contact with the sterilant. Sanitizer tanks must be emptied and cleaned regularly and solutions replaced regularly to prevent possible build-up of soil. Utensils must be rinsed and washed prior to being placed in the sanitizing solution. This also prevents build-up of soil.

Final product issues and control

As already mentioned, the extent of the risk associated with these products involves a number of factors. High amongst these factors are the opportunities for cross-contamination, potential for growth in the product and shelf life, during which growth of *L. monocytogenes* may occur. With fresh dairy desserts, the industry has been fortunate in the sense that product shelf life is largely dictated by hygiene in the manufacture of the product. This is because poor cleaning leading to post-processing contamination is likely to introduce Gram-negative pyschrotrophs such as *Pseudomonas* species, which can actually spoil these products far more quickly than *L. monocytogenes* is likely to grow to hazardous levels. Processes operated with inherently higher standards of hygiene, which ensure post-process contaminants are minimal, have led to products being given an extended shelf life. These higher standards of hygiene of course also normally prevent contamination by pathogenic *Listeria* species. There is no question that once introduced into many fresh dairy desserts, which have neutral pH and very high water activities (0.98–0.99), the available nutrients could allow significant growth of *L. monocytogenes*.

Surveys of the incidence of *L. monocytogenes* in dairy desserts have not been widely reported. However, McLauchlin and Gilbert (1990) reported the presence of *Listeria* spp. in 6/116 (5%) samples of cream, none of which were *L. monocytogenes*. 23/274 (8%) samples of ice cream contained *Listeria* spp., of which 17 (6%) were *L. monocytogenes*. Of 135 samples of other foods that contained dairy products, 23 (17%) were also found to be contaminated with *Listeria* spp. with 3 (2%) samples

containing *L. monocytogenes*. A further 7% (12/172) of samples of desserts contained *Listeria* spp. with 5 (3%) containing *L. monocytogenes*. The incidences reported in this survey are comparatively high compared to the known reduction in incidence that has occurred in cream-containing dairy desserts in recent years. Nevertheless, it is clear that such products will be contaminated with *L. monocytogenes* from time to time and it is important that there is a system in place to prevent contamination. Together with the normally limited shelf life of these products, the risk presented to consumers from the hazard of *L. monocytogenes* in fresh dairy dessert products can be kept very low.

GENERIC CONTROL OF *LISTERIA*

Raw material identified as a potential hazard

If the raw material is likely to be contaminated with *L. monocytogenes* and no subsequent process exists for reducing it to an acceptable level, i.e. cooking, then control of the raw material is absolutely essential. In most cases the raw material is not under the direct control of the product manufacturer. This is often case with raw milk for raw-milk cheeses, raw meat for salami, salmon for smoked salmon and so on. In such circumstances, it is absolutely essential to operate a raw material supplier assurance programme. Such a programme requires as the minimum the following.

1. *The user of the raw material must have a detailed understanding of the production process of the raw material and knowledge of the critical control points or those stages in the process influencing the control of the hazard*

Understanding the process involved in the production of the raw material is critical to the ultimate safety of the final product as the safety of the raw material may in fact be the most important stage in the entire production process. Without reliable knowledge of this it is hard to see how a manufacturer using the raw material can make a judgement concerning which raw material suppliers offer the highest standard of product or indeed how their own handling of the raw material during manufacture can influence its subsequent safety. For example, without knowing how *L. monocytogenes* may gain access to the raw-milk supply, any attempt to review the level of control a raw material supplier may be exerting on preventing its access during milking or storage in bulk farm tanks is precluded. As an absolute minimum, implementation of a detailed hazard analysis should be expected of the raw material supplier.

2. *Audit of the raw material supplier to review process control*

Clearly, with critical raw materials it is essential that a supplier audit programme is established to review the control of the hazard in question, e.g. *L. monocytogenes*. Such an audit needs to be conducted at regular intervals and must focus on aspects impacting on the control of the specific hazard. In many cases such audits may be conducted by third parties but they must be given a defined scope and objective and both parties must be fully committed to implementing action points arising from such reviews. Manufacturers of raw fermented meats, for example, must conduct regular reviews of the raw material suppliers, which are usually abattoirs and boning premises, to focus on hygienic processing. A large difference can be made to the incoming incidence of *L. monocytogenes* by selecting raw material suppliers operating high standards of hygiene, even though it may be impossible to eliminate the hazard completely. Successful attempts to control *L. monocytogenes* at this stage can have a major impact on both the degree of exposure to the community generally and the levels of exposure to individuals themselves.

3. *Raw material verification checks*

In addition to supplier audits the product manufacturer should monitor the quality of the incoming raw material. With critical raw materials it is important to review the incidence and levels of *L. monocytogenes* in the raw material, although it may be useful to use broader indicators of hygienic processing such as *Listeria* species or even *Escherichia coli* or coliform contamination.

For raw-milk suppliers to a dairy, it may be important to review the quality of the raw material from each farm supplier and so monitoring of the microbial loading is usually conducted on samples taken ex-farm and delivered to the manufacturer. In some cases payment incentive schemes have been operated based on the level of bacterial loading in the raw milk. This type of scheme was operated for many years by the Milk Marketing Board in the UK. Farms supplying poor quality milk were penalized and received less payment per volume of milk whilst farms with low bacterial counts were paid a premium for the higher quality. This type of scheme is also in operation for suppliers of raw milk for the manufacture of raw-milk cheese in continental Europe and it is recognized that such schemes can positively influence the standards of hygienic operation in the primary process.

4. *Agreed specification with the raw material supplier*

One of the most important aspects of efforts to control the safety of critical raw materials is an understanding by both parties of the need for the highest standards of quality in the production of the raw material. Such understanding can only come from open discussions between the two parties, with it being particularly emphasized to the supplier that the raw material is to be used in the manufacture of high risk products. Details of the expectations of both parties are usually best documented in a product specification, signed and agreed by the vendor and purchaser.

5. *Conditions of storage and use of the raw material*

In all cases, perishable raw materials must be kept as cold as possible to prevent any increase in levels of *L. monocytogenes* that may be present and the raw material should be used within as short a time as possible. This would naturally be the case with raw milk, fish, meat and poultry as they would soon suffer microbial spoilage if subjected to temperature abuse or extended chilled storage.

The tendency to use modified packaging, e.g. vacuum packing, to extend the refrigerated shelf life of raw products such as fish, meat and vegetables poses the dilemma that whilst the conditions may prevent growth of typical spoilage bacteria such as *Pseudomonas* species, they actually may allow growth of *L. monocytogenes* if present. Potential hazards presented by any modification to the storage conditions of these critical raw materials must be considered in any hazard analysis of the product. An example of problems arising in the absence of this control is the outbreak in coleslaw already detailed (Chapter 2).

In most cases, storage of the raw material for extended periods at temperatures slightly below freezing (0 to -2°C) will be sufficient to prevent any significant increase in the levels of *L. monocytogenes* present, and such temperatures are often employed for the storage and transportation of fish, meat and vegetables.

Production incorporates processes to reduce the level of contamination or eliminate the hazard

Many products involve the manufacture of food where *L. monocytogenes* may be present in the raw material but a process is applied to reduce the hazard to an acceptable level or eliminate it completely. In such products

the key control must be exerted at the stage where the antilisterial process is applied. Examples of these stages include:

- heat processing for cooked ready meals, cooked meats, hot-smoked fish, etc.
- pasteurization/UHT processing of milk for cheese, yoghurt and dairy desserts
- chlorination of vegetables for ready-to-eat prepared vegetables and salads
- fermentation and drying for salamis and raw dry meats
- fermentation and maturing for hard cheeses.

As part of any hazard analysis of the production process, key critical control points will be identified that, if kept under proper control and monitored, will maintain the safety of the product. Unless sufficient control is exerted to ensure the process at which a critical control point exists is carried out effectively, the subsequent safety of the product can be compromised. One of the key things that must be understood for these stages is the minimum process requirements to ensure reduction of the hazard to an acceptable level.

In the example of a product with a cooking stage it is essential that the cooking times and temperatures that must be achieved to ensure destruction of the hazard are defined. With cooked meats this may be 70°C for 2 min or an equivalent time/temperature combination; for pasteurized milk it may be 71.7°C for 15 s. These are usually considered as the temperatures below which the product may be unsafe. Systems must be designed and operated to ensure it is not possible for products to fail to reach the required temperature process at all points within the product. Thus, in most cases processes are designed with a built-in safety margin set at slightly higher temperatures or for slightly longer periods than the minimum to be achieved. If process control systems are designed using target levels and critical limits, the latter as defined in the Codex documents (Anon., 1996c), then under conditions where the process begins to drop below the target level it is possible to take action to rectify the process and bring it back to, or above, the target before the critical limit is reached. This avoids the expenditure in time and production costs associated with process failure.

It is important to ensure that any system designed to reduce contamination is based on a sound knowledge of the likely impact of changes to the raw material conditions on the subsequent effect of the process. For example, in the cooking of prawns in an immersion system, the process

is usually operated by immersing the raw prawns in the heated water for a certain period of time followed by cooling. The process control is carried out by ensuring the temperature of the water prior to immersion is above a certain figure and that the residence time of the prawns in the water is above a certain time. However, the efficacy of this process will be dependent on variables such as the temperature of the incoming raw material prawns and the size of the prawns. If some of the prawns are larger than those originally used during derivation of the process times and temperatures, then the existing process may now be insufficient to achieve the correct temperature throughout the prawn. Likewise, if the process was established with prawns entering the water at a temperature of 4–5°C but a batch is cooked with an ingoing temperature of 0–1°C, then the process will probably not achieve the correct cook.

Such problems should not occur with the implementation of correct HACCP-based procedures as relevant raw material characteristics would be identified for monitoring and documenting. It is often in situations of food poisoning outbreaks that relatively simple issues such as these have been overlooked because of the pressure to manufacture product to meet tight delivery schedules or because of a fundamental ignorance of the importance of raw material and process control for the safety of the final product.

With the pasteurization of milk, both the process and product temperatures are monitored and so inadequate pasteurization is usually unlikely. In addition, pasteurizers are usually set to automatically divert milk away from the finished product if the target or critical temperature is not achieved. The use of manual override switches on pasteurizers needs to be carefully controlled as inappropriate use can lead to improperly pasteurized milk being sent forward during periods of manual override.

With processes where the listericidal effect is less pronounced, but where control is nevertheless important for its contribution to final product safety, it is still essential to employ similar process controls. For example, the chlorination of salads and vegetables for use in ready-to-eat prepared products is not recognized as giving a large reduction in the levels of *L. monocytogenes*. However, it does reduce levels of contamination by one or more orders of magnitude (Zhang and Farber, 1996) and as such should be effectively controlled. The use of common bulk tanks of chlorinated water to carry out washing could itself represent a major opportunity for widespread cross-contamination of other salads and vegetables if chlorine levels are not adequately maintained. In such

circumstances, the control of chlorine levels is essential and continuous dosing systems are preferable, with frequent monitoring of the levels of free chlorine in solution. For the best effect on reduction of microbial load, the pH of the chlorine treatment water should be maintained between pH 6 and pH 7. In addition, contact time with the product and the nature of any system used to facilitate contact, e.g. submersion and agitation, are important areas warranting control and monitoring.

Where control is exerted by fermentation and drying it is equally important that the fermentation profiles and drying profiles that are established to ensure control of the hazard are actually monitored and adhered to for subsequent batches. It is common with most fermented and dried products, such as salami or cheeses, to control the process on the basis of process times, temperatures and humidity where relevant. Again, such process parameters do not necessarily reflect the factors controlling *L. monocytogenes* in the product and specific product characteristics need to be considered. Therefore, whilst milk may normally be expected to produce an acidified curd after incubation with a starter culture for a defined period of time at an appropriate temperature, it is essential to monitor the activity of the starter culture in the product by way of pH decrease or acidity increase. In this way, any factors interfering with the activity of the starter culture and hence product safety will be manifest by changes in the fermentation profile. This is also true of pH decreases or moisture loss in salami manufacture.

It is most important in the safe manufacture of food to clearly identify what is controlling the hazard, i.e. *L. monocytogenes*, and also what the process limits are that will allow effective control. The position of the salami or raw dried meat in the drying chamber may influence its rate of fermentation or drying. The position of a cooked meat in an oven may influence the achievement of the desired heat process. These factors must be taken into account in the design of a safe process before any production ever begins.

Product could be recontaminated with *L. monocytogenes* as a post-processing contaminant

Clearly, *Listeria* species are widely distributed and if a processed product is exposed to equipment, people or the environment prior to being placed into packaging, opportunity exists for contamination to occur. The degree to which this represents a hazard really depends on whether the product allows growth of the organism or not. Table 4.12 summarizes some potential sources of contamination from *Listeria* spp. and

Table 4.12 Some approaches to the control of *L. monocytogenes* in manufacturing environments

Area	Problems	Controls	Additional information
Floors	Damaged areas that can act as traps/reservoirs for debris and water (ponding), allowing *Listeria* spp. to multiply	Repair damage Minimize use of water Remove excess surface water Resurface floor area if required	All floor surfaces must be kept in a good state of repair, including grouted areas and expansion joints. Floors should be kept dry wherever possible; in any case excess water must not be allowed to stand. All floors must be included in a regular cleaning schedule
Drains	Damaged areas that can act as traps/reservoirs for *Listeria* spp.	Repair damage	Drain flow must be designed in such a way as to avoid any possible contaminating connection between high risk and low risk areas, e.g. ensuring drainage flow away from high care areas. All drains must be kept in a good state of repair and constructed to be easily accessible for cleaning and maintenance. All floors must be included in a regular and effective cleaning schedule
	Product debris trapped under drain cover	Always remove drain cover during cleaning and clean manually	
	Overflow/blocked drain leading to excess contaminated water flooding the production environment	Stop production in the area, clear blockage (not with a high pressure hose) and clean and sanitize the production area prior to restarting	

Table 4.12 Continued

Area	Problems	Controls	Additional information
Ceilings, walls and overheads, e.g. pipes, ducts and gantries	Damaged areas that can act as traps for debris and bacteria and sources of contamination to the production environment	Repair damage. Regular and appropriate cleaning schedules must be operated for all overheads and the surfaces of ceilings and walls	All ceiling and wall surfaces must be kept in a good state of repair, including all joints. Where possible, exposed overheads should be minimized by routing pipes above the ceiling or running them in properly constructed boxing that allows access for maintenance and appropriate cleaning
Cleaning equipment, e.g. brushes, vacuum cleaners, mops, squeegees, buckets	Contamination build-up leading to sources of high numbers of *Listeria* spp. Aerosol spread of *Listeria* spp. by use of hoses	All cleaning equipment must itself be cleaned after use and stored dry or left immersed in a sanitizer bath Hoses must be kept clean and stored off the floor. Hoses should not be used when production is in progress	Equipment used for cleaning floors and drains must be kept separate from equipment used for cleaning product-contact surfaces. Proper use of disposable cloths and cleaning pads can be helpful in minimizing the spread of bacterial contamination in production environments
Floor contact items, e.g. wheels on trollies, racks and bins	These items assist the spread of water and bacteria through the production environment	Where possible, dedicate such equipment to specific areas. Low risk equipment must not be used in high risk areas	All wheeled equipment must be properly cleaned (including wheels) as part of a regular cleaning schedule

Table 4.12 Continued

Area	Problems	Controls	Additional information
Non-routine equipment, e.g. ladders, engineers' equipment/tools	Import contamination from external areas	Where possible, dedicate such equipment to specific areas. Low risk equipment must not be used in high risk areas	Appropriate cleaning systems, e.g. alcohol wipes for water-sensitive items, must be applied to minimize contamination from these items
Food contact surfaces	Cross-contamination potential from inadequately cleaned surfaces with product soiling on table, ledges, under conveyors, in seals, etc.	Identify all direct product contact surfaces and ensure they are all specified in cleaning schedules with appropriate frequencies defined	Full cleaning regimes, usually carried out daily, should consist of debris removal, appropriate equipment 'strip down', a detergent wash followed by rinsing and sanitizing procedures. Intermediate cleaning procedures should be appropriate to the type of production processes operated
Refrigeration units	Accumulation and dissemination of *Listeria* spp. via the moist air blown through the units and condensation dripping from pipework	Operate regular cleaning schedules for the refrigeration unit, drip trays and pipework. Ensure drip trays are maintained appropriately and not routed directly above exposed product Repair leaks	Refrigeration systems should be designed to allow easy access for cleaning and to avoid the collection of water in the system or drainage of water (condensate) directly onto the floor. Efficient insulation of pipes will help minimize the formation of condensation

Table 4.12 Continued

Area	Problems	Controls	Additional information
Air-handling systems, e.g.ventilation, extraction systems and air conditioning	Accumulation and dissemination of *Listeria* spp.	Operate regular cleaning schedules for all hoods/canopies, ducting, pipework, drainage channels and vents or air socks, where applicable. Air filtration to a minimum standard of 95% removal of 5 μm particles will minimize dust levels	Air-handling systems should be designed to minimize the potential to trap dirt or accumulate moisture and to facilitate cleaning procedures. Air intakes must be sited to avoid cross-contamination from low risk to high risk areas
Equipment and tray washing	Inadequate cleaning leading to cross-contamination	Washing equipment must be maintained in a good state of repair. Automatic and manual cleaning systems must be operated with the correct chemicals and a specified cleaning sequence appropriate to the items being cleaned	Washing equipment and procedures must be suitable and sufficient for the purpose intended. To help maintain operational standards and minimize build-up of debris and other deposits that could facilitate cross-contamination of items passing through, the equipment itself must be subjected to a cleaning schedule (internally and externally)
	Cross-contamination of cleaned equipment from dirty equipment	A unidirectional flow of dirty to clean equipment must be maintained and a dedicated area designated for storage of cleaned equipment	
	Aerosols created during washing	Washing areas should be sited away from production areas in a dedicated room with air extraction hoods, where appropriate	

Table 4.12 Continued

Area	Problems	Controls	Additional information
Personnel	Product contamination by inadequate hygiene and production practices	Staff must be adequately and appropriately trained and equipped to maintain practices that will minimize contamination of finished products by *Listeria* spp.	Protective clothing, including coats, hats and sometimes footwear, must be provided for all staff and specifically designated clothing provided for staff working in high risk areas. Facilities must be provided and clear procedures laid down for the adequate washing of hands, changing of dirty clothes and cleaning of footwear (the welts of wellington boots and other footwear collect moist debris and act as harbourages of *Listeria* spp. that are then transferred readily between production areas)
Cleaning procedures	Inadequate for destroying *Listeria* spp.	Use food-safe chemicals and cleaning regimes that have been demonstrated to be effective in destroying *L. monocytogenes*	The responsible suppliers of cleaning chemicals to the food industry are able to supply the necessary information concerning the efficacy of their products in respect of the destruction of *L. monocytogenes*. As a matter of due diligence, food manufacturers should ensure they obtain such information and that it does meet with their requirements prior to purchase

L. monocytogenes in high risk food production areas and the controls applicable for minimizing contamination from these sources.

Post-process contamination but no subsequent growth

In products where growth of any contaminating *L. monocytogenes* is unlikely to occur due to the presence of inhibitory compounds, the primary concern is to prevent high levels of cross-contamination occurring that could present a hazard to the consumer by mere survival. Products in these categories include hard cheese, such as cheddar and edam, dried foods and yoghurts. Here, the hazard occurs from production environments where, due to possible complacency about the finished product not supporting the growth of the organism, *Listeria* may be allowed to colon-ize and proliferate. In the majority of cases, basic standards of good manufacturing and good hygienic practice will reduce any risks associated with this type of product to a minimum. It remains important even in these factories to understand where *Listeria* species may colonize and grow to high levels and to ensure adherence to effective cleaning schedules for production equipment and the environment, especially in the areas identified as a particular hazard.

Post-process contamination and subsequent growth

The greatest need for control in the process environment is required for products that are exposed to post-processing contamination and where product conditions prevail that allow extensive growth of the contaminating organism. In such circumstances it is absolutely essential to control the presence and build-up of *Listeria* in the environment; hygiene and cleaning schedules must be focused on the requirement to remove *Listeria* from key areas of the environment.

To ensure control of *Listeria* it is important to conduct a review of the processing plant incorporating all areas where the product could become contaminated. With cooked products this would begin after the oven; with pasteurized products it would begin post pasteurization, although the pasteurizer itself must be regularly checked to ensure no cross-contamination potential exists in the cooling section of the heat exchanger.

All areas of the production environment should be subject to detailed examination and areas identified where clear potential contamination hazards exist. The results of this examination should then be used to identify the cleaning and personnel operating practices necessary to ensure the hazard is controlled. This study should be carried out with the

assistance of engineering, production and microbiological personnel. Their joint expertise will be valuable in identifying the most likely potential hazards. Particular areas for consideration include:

(i) *Conveyor belts* The surface area offered by exposure of product to conveyor belts is often extensive in some of the highest risk products such as cooked sliced meats or ready-to-eat foods. The design and construction of the belts can have a significant impact on the potential for *Listeria* to both colonize and build up in the factory.

Conveyor belts need to be cleaned and sanitized on the surface and the underside, where debris may build up. One-piece synthetic conveyor belts are often more suitable as they are more readily cleaned and offer less opportunity for product debris to build up. Stainless steel chain belts are very difficult to clean and allow extensive retention of product debris but are more suitable for high temperature sterilization. Conveyor belts made from individual bands, as used in many sliced meat production facilities, offer extensive opportunity for contamination due to build-up of product debris in the grooves of the interspacing cogs around which the belts are wound. Regular cleaning and disinfection of these belts is ideally carried out by complete removal of the belt from the carrier and is important in preventing build-up of contamination on such equipment.

(ii) *Racks and trolleys* Any open products stored in contact with racks or trolleys, usually for transportation purposes, may become contaminated directly from the equipment surfaces. Such equipment should be cleaned and sanitized after use using a purpose-designed trolley or rack washer and the system used must be demonstrably capable of eliminating contamination. Washing processes often create large amounts of aerosol and should be sited in a segregated area away from exposed product.

(iii) *Pumps, vessels and pipework* Although usually completely enclosed, even the most modern of equipment can lead to extensive product contamination. In fact, the very nature of enclosed lines means that inspection of many of the internal surfaces is not possible and so in-line cleaning systems must be designed to ensure that efficient flow dynamics result in the effective removal of debris on all internal surfaces. Pipes and tanks should be inspected to ensure no 'dead' spots are present. These are often caused by redundant pipe bends when new flow patterns are created or by the introduction of in-line sampling devices. Pipe joints need to be sealed with hygienic welding seams to prevent local 'dead' spots building up. Vessels should be frequently inspected to monitor for

any 'dead' spots that are not effectively cleaned by the in-line spray-ball-type devices. In addition, the integrity of vessel chambers must be checked to ensure prevention of contamination from external sources or indeed from coolant used on jacketed vessels. Seals in valves or seals need to be regularly stripped down and cleaned as debris often accumulates underneath the seals. Care must also be taken when operating automatic cleaning sequences to ensure no 'dead' spaces are created by valves that trap product debris during the cleaning cycle and subsequently push it back in line when the valve is closed at the end of the cycle.

(iv) *Sample points, pressure gauges, temperature gauges and other in-line monitors* The introduction of any device welded in-line creates an intrusion that affects the flow of product and cleaning efficacy. In addition, poor welding may result in the creation of microenvironments capable of shielding microbial contaminants from subsequent cleaning. The inappropriate positioning of gauges may actually create crevices or chambers in which product can be trapped for extensive periods of time, creating areas where microbial contamination may build up. Any inspection of plant must first and foremost identify any areas like these to ensure that they are installed properly or removed completely.

(v) *Slicing/chopping/mixing machines* The complex nature of many slicing machines makes them an obvious focus for attention when prioritizing areas for *Listeria* control. Slicing machines may run for extensive periods without full cleaning and the intricate nature of the machinery offers a number of areas where product debris can build up and, if contaminated, provide conditions for cross-contamination. Manufacturers should understand the construction of the equipment they use and must ensure that they understand where contamination could be building up, how to dismantle the equipment appropriately for cleaning and how cleaning schedules need to be concentrated to remove debris and contamination. The hygienic status of loading devices used to introduce the meat to the slicer, the slicer itself, the slicing blade and any moving machinery parts must all be closely monitored. Similar hygiene management is required for bowl choppers, dicers and other general mixing equipment in which the product is subjected to extensive contact with blades or paddles where contamination may build up.

(vi) *Tables, chopping boards, cutting boards and other work surfaces or cutting utensils* Any surface where the product is placed prior to cutting, chopping or further processing is a potential contamination point and needs to be cleaned rigorously. Chopping boards, work surfaces and tables all have the potential to become contaminated with *Listeria* and if

inadequately cleaned and sanitized will allow cross-contamination to subsequent products.

Particular attention should be paid to the design of these items to avoid surfaces with lips where product may accumulate or areas where the chopping board may be joined to its support, as debris will accumulate in the joint between the two surfaces. Ideally, the work surface should be removable to allow cleaning and sanitation of the top and underside of the surfaces. Tables with lips curling under the table are often sources of contamination due to product debris accumulating underneath the table. This contaminated debris can be transferred by people's hands onto products. The design of the table and efficient cleaning are the best ways to deal effectively with these problems.

It is important that knives, automated cutting or slicing machines and hand slicers such as those used in smoked salmon plants are cleaned effectively as such utensils come into contact with a high proportion of products passing through the production plant.

Machinery in direct contact with products, such as carrier belts with needle points used in the automated slicing of smoked fish and piercing machines used for creating aeration of stilton and other blue veined cheese, all provide opportunities for contamination of many products if not maintained hygienically.

(vii) *Personnel handling product* Any product that is extensively handled is undoubtedly exposed to a high risk of contamination. It is likely that most contamination does not originate from the personnel themselves but probably arises from contaminated surfaces and utensils and is then transferred to the product by the personnel. High standards of personnel hygiene and practices are essential to minimize contamination from these sources, but the most important aspect is staff education.

It is essential that any person involved in the manufacture or handling of food is fully aware of the hazards that they may contribute to the product by their actions. An understanding of the basic principles involved in transferring microorganisms from floors, walls, knives or other equipment as a result of inappropriate practices will be far more beneficial than any strict work instruction. An educated individual can make an informed decision about practices that are not detailed in work instructions. Uneducated adherence to instructions will allow improper practices to develop and continue until they are identified by trained individuals or after a problem occurs.

The primary area of cross-contamination from personnel arises from the hands and clothing. Irrespective of the policy for use of gloves or direct handling of food, the most important aspect is operation of hygienic practices to avoid the hands coming into contact with contaminated equipment or surfaces and then into contact with the product. Frequent changing of gloves and hand washing together with hand cleaning or glove changing after handling any item or surface where contamination may arise is essential. Touching the sides of equipment or the handles of brushes when sweeping up debris during production and then returning to product-handling duties are simple things that happen frequently and can be avoided. In addition, sleeves and aprons are often neglected areas of concern where continuous build-up of contamination can be readily transmitted by contact with food.

(viii) *Non-product contact surfaces that come into contact with personnel handling the product* Many items of equipment are not considered to be of high concern in relation to *Listeria* control because they are not considered to come into direct contact with the product. As a consequence they are often neglected in cleaning and sanitization regimes and when used can offer significant potential for cross-contamination. Good examples of these areas are the handles of brushes, knife racks used for storage of knives, other utensil racks, handles on storage chillers or oven doors, which are often touched and then the hands used for handling product and so on. Many examples exist in this area and it is essential to identify the surfaces and utensils coming into contact with items and hands that may also come into contact with the product and ensure that all such items are cleaned and sanitized properly.

(ix) *Non-product contact surfaces and the creation of aerosols that come into contact with the product* Some of the greatest hazards to the product arise because of contamination from the structures and environment of the factory itself. Contamination on floors, walls, drains, forced-air chillers, air socks, ceilings, pipework overheads and many other such areas clearly cannot contaminate products directly. However, the creation of aerosols or subsequent condensation can facilitate cross-contamination from some of these heavily contaminated areas to product. It is not possible to completely prevent *Listeria* from being present in some of these areas but the objective of attention to the cleaning and sanitization of these areas is to prevent the build-up of high levels of potential contamination that, by distribution in aerosols, can contaminate the product or product contact surfaces. The greatest hazard is usually presented in chillers from condensation and from

forced-air movement produced by the fan, which has the potential to blow contamination and condensate throughout the chiller room and onto exposed product. A large amount of exposed product is held in chillers as the obvious aim with blast chillers is to cool down products quickly. Therefore, whilst attention must be paid to providing protection to products by enclosing them in lidded containers or covering them with clean plastic sheeting, the emphasis must be on the prevention of build-up of *Listeria* in the chiller. This is achieved by regular cleaning and sanitization of the chiller, which includes the condensers and the fan area, removing condensate and in many cases using a sanitizing fogging system throughout the chiller after cleaning.

Other environmental areas for attention, such as floors and walls, must be regularly cleaned and sanitized but in a way that avoids filling the entire production facility with aerosols. Manual cleaning using soft brushes and without the use of pressure hoses is usually the best system. Grills should be removed from drains to clean the underside of the grill and the drain itself. Cleaning equipment for floors and drains should be separate to that used for product contact surfaces as contamination may be spread by these utensils.

Whilst attention must be paid to the cleaning and sanitization operation itself, it is often hindered by the presence of inadequately maintained buildings with cracks in the floors and walls where removal of contamination is extremely difficult. Cleaning cannot be a substitute to the provision of adequate building structure facilities, but is solely there to maintain the hygienic status of properly maintained manufacturing environments.

Product where *L. monocytogenes* may be present and customer cooking is designed to eliminate the hazard

Products that are either raw or processed and where *L. monocytogenes* may be present but where the product is designed to be fully cooked do not present a hazard unless the consumer does not cook the product appropriately. Under such circumstances the product cooking guidelines are critical in conveying the message to the consumer about the need to ensure the product is fully cooked. Cooking guidelines can never cover all eventualities but it is essential that when they are provided they are based on trials that show they can achieve the specified cooking time and temperature. Cooking instructions must be validated by the manufacturer or retailer generating them. They must take into account the different types of cooking appliance used in domestic kitchens and

should evaluate the effect of distance from the heat source, turning frequency, thawing conditions, etc. on the achievement of the final cooking temperature. In most cases a cooking instruction for a raw product should be accompanied by a statement that reminds the customer to ensure the product is cooked until it is 'boiling' or 'piping hot' as additional advice to supplement the instruction itself.

Advice for susceptible groups

Some product types have a significant history of causing outbreaks of listeriosis and hold a greater risk of infection to susceptible groups. If it is not possible to reduce the risk to those groups to a sufficiently low level then it is important to inform those groups about the hazards inherent in the products such that they can avoid them. Such action is usually taken during outbreak situations, as was seen during both the French listeriosis outbreak with pork tongue in aspic and the UK outbreak attributed to Belgian pâté. Indeed the advice given by the UK Department of Health, together with the withdrawal of the product, resulted in a dramatic reduction in the number of cases of listeriosis in the following years. In the UK the advice for pregnant women to avoid the consumption of soft ripened cheeses, such as Brie and Camembert, and pâté, and to cook poultry and other ready meals until they are hot throughout remains to this day. This is supported by manufacturers and retailers who actively label products as being unsuitable for consumption by susceptible groups.

5

INDUSTRY ACTION AND REACTION

INTRODUCTION

Listeria monocytogenes as a species is subdivided into serotypes and genetic techniques are used to further differentiate strains of the organism. There has been a great deal of debate concerning the specific pathogenicity of individual strains of *L. monocytogenes* and their relevance to public health. In 1995, in a case concerning fitness for human consumption of some batches of Lanark Blue cheese allegedly found to contain high levels of *L. monocytogenes* serotype 3a, the food condemnation orders requested by the local authority under the UK Food Safety Act, 1990 (Anon.,1990a) were refused. This was in part due to evidence presented indicating that this serotype had never been associated with listeriosis caused by the consumption of contaminated food. In 1996, the UK Health and Agriculture Departments asked the Advisory Committee on the Microbiological Safety of Food (ACMSF) for advice on whether all strains of *L. monocytogenes* were pathogenic or should be regarded as pathogenic. Following consultation and discussion with other expert groups, the Committee advised that, for public health purposes, it would be prudent to treat all strains of *L. monocytogenes* as pathogenic. In a detailed review of the pathogenicity of *L. monocytogenes*, McLauchlin (1997) concluded 'in the interests of public safety and for considerations for public health purposes, all *L. monocytogenes*, including those recovered from food, should be regarded as potentially pathogenic.' It is also relevant to note that the Codex Committee on Food Hygiene treat the species *L. monocytogenes* as a whole and not in terms of individual strains when discussing sampling plans and industry management controls. Where the organism is included in legislation, *L. monocytogenes* is not qualified as to serotype or other subdivision of the species. Since *L. monocytogenes* was first recognized as a foodborne pathogen, as a matter of due diligence and concern for food safety, the food industry has always acted on positive findings of the species and not specific

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serotypes of the species. Indeed, many manufacturers also use the presence of *Listeria* spp. as a general indicator for the presence of *L. monocytogenes*, and many industry guidelines and specifications reflect these approaches.

A microbiological criterion consists of statements concerning the microorganism or microbial toxin of concern, the specific food and sample type, the sampling plan to be used, the test method to be used (the method must have been validated for the microorganism or toxin of concern in the food being examined) and the microbiological limit(s) to be applied (also indicating the interpretation to be placed on the result and a reaction procedure for those results that are in excess of the upper limit set). There are a variety of texts available that address these areas in detail (NRC, 1985; International Commission on Microbiological Specifications for Foods, 1986; Anon.,1996d).

For the food industry, microbiological criteria fall into three categories:

1. Standards: these are microbiological criteria contained in a law. Compliance is mandatory. Examples include most criteria in European Union (EU) Directives and Statutory Instruments of England and Wales. Standards are monitored by enforcement agencies.
2. Guidelines: these are criteria applied at any stage of the food production and distribution system to indicate the microbiological condition of a sample. They are for management information and to assist in the identification of potential problem areas.
3. Specifications: these are microbiological criteria applied to individual raw materials, ingredients or the end product. They are used in purchase agreements.

LEGISLATION AND STANDARDS

The general approach taken to legislation in Europe and North America in the context of food safety is to indicate the clear responsibility of food business proprietors to produce and supply safe and wholesome foods. For instance, in the UK, the Food Safety (General Food Hygiene) Regulations, 1995 (which implement parts of the European Union Directive 93/43/EEC of 14th June 1993 on the hygiene of foodstuffs), Section 4(1) states:

> 'A proprietor of a food business shall ensure that any of the following operations, namely the preparation, processing, manufacturing,

packaging, storing, transportation, distribution, handling and offering for sale or supply of food, are carried out in a hygienic way.'

Further, in Section 4(3):

'A proprietor of a food business shall identify any step in the activities of the food business which is critical to ensuring food safety and ensure that adequate safety procedures are identified, implemented, maintained and reviewed on the basis of the following principles:

(a) analysis of the potential food hazards in a food business operation;
(b) identification of the points in those operations where food hazards may occur;
(c) deciding which of the points identified are critical to ensuring food safety ("critical points");
(d) identification and implementation of effective control and monitoring procedures at those critical points; and
(e) review of the analysis of food hazards, the critical points and the control and monitoring procedures periodically, and whenever the food business's operations change.'

Because it is a ubiquitous organism in the general environment and consequently a frequent contaminant of food raw materials, *L. monocytogenes* is one of the potential microbiological hazards that needs to be considered in the hazard analysis of many food business operations.

In addition to this general but important and necessary responsibility imposed by legislation on food business proprietors, other legislation (sometimes referred to as vertical legislation because it deals with a specific food in contrast to horizontal legislation, which applies to generic food production controls, e.g. food hygiene) may also apply depending on the food type and business. It is, of course, the responsibility of the food business proprietor to know and understand which legislation applies to the business and ensure compliance. Some legislation contains microbiological standards and compliance is compulsory. For example, *L. monocytogenes* is specified in the Dairy Products (Hygiene) Regulations, 1995. In Schedule 6 (Anon., 1995b) 'Requirements for Milk-Based Products. Part 1. Microbiological Criteria' the following standards are laid down for products upon removal from the processing establishment:

(i) cheese, other than hard cheese: *L. monocytogenes* absence in 25 g where $n = 5$, $c = 0$ (Note: n = number of samples; c = number of samples allowed to exceed the limit)
(ii) milk-based products, other than cheese covered by (i) above: *L. monocytogenes* absence in 1 g.

Where these standards are exceeded, the relevant products are to be excluded from human consumption and withdrawn from the market.

L. monocytogenes must, of course, also be included for those generic standards which state that pathogenic organisms must be absent in 25 g, where $n = 5$ and $c = 0$, in any random sampling checks carried out in the treatment establishment such as that for heat-treated drinking milk (Schedule 4, Part III of the Dairy Products (Hygiene) Regulations, 1995 (Anon., 1995b)) and any required action must be taken.

Subsequent to the outbreak of listeriosis in the US traced to Mexican-style soft cheese (Linnan *et al.*, 1988), surveys were carried out that revealed widespread contamination of food industry environments and products with *L. monocytogenes*. This led to the US Food and Drug Administration and Food Safety Inspection Service implementing a policy of 'zero tolerance' (negative in 25 g) for the organism in cooked and ready-to-eat foods (Shank *et al.*, 1996). In pursuing this policy, many product recalls have been made for ready-to-eat foods found to be contaminated with *L. monocytogenes*, involving cheeses, ice cream, milk, fish, prepared salads, sandwiches, crab meat, smoked fish and bakery products, and the industry has carried out a significant amount of work to establish the sources of the organism and mechanisms for its control. The continuing US policy considers the detectable presence of *L. monocytogenes* in ready-to-eat foods to be a hazard to health. Ready-to-eat foods found positive for the organism are considered by the US Food and Drug Administration, Department of Agriculture and Food Safety Inspection Service to be adulterated and subject to action, e.g. seizure (Shank *et al.*, 1996). This policy is believed to have contributed to a 40% decline in listeriosis in the US (Tappero *et al.*, 1995).

In a document prepared for the Codex Committee on Food Hygiene by the International Commission on Microbiological Specifications for Foods (International Commission on Microbiological Specifications for Foods, 1996b), the Commission considered (on the basis of epidemiological information from several countries) that for foods in international trade, a concentration of *L. monocytogenes* not exceeding 100 per gram of food at the point of consumption is of low risk to consumers. However, in food

intended for consumption by high risk groups, e.g. the elderly and babies, absence in 25 g in a certain number of sample units was still considered appropriate. It is likely that any standards ultimately endorsed by Codex will be used as a basis for amending national legislation.

GUIDELINES

Microbiological guidelines used in industry are rarely published as they are generally developed in association with particular processes and products; consequently a degree of confidentiality applies and industry guidelines remain in-house and self-imposed. Results from tests for *Listeria* spp. and *L. monocytogenes* exceeding the anticipated normal levels for the sample in question should lead to investigation to identify the source and rectify the cause.

General guidelines on the levels and types of microorganism relevant in specified foods produced under good manufacturing practice may be provided by industrial associations for their members, e.g. in the *Airline Catering Code of Good Catering Practice* (Anon., 1990b). This includes microbiological guidelines for *L. monocytogenes* for non-manipulated items and manipulated cold meal items with limits of not detected in 25 g and less than 100 per gram, respectively. In the UK, the Public Health Laboratory Service has published microbiological guidelines (Anon., 1996e) to assist food examiners and enforcement officers in assessing the microbiological quality of foods and to indicate levels of certain types of bacterial contamination considered to be a potential health risk in ready-to-eat foods at the point of sale. Table 5.1 shows how various levels of *L. monocytogenes* are classified for all food categories considered in these guidelines. Although the PHLS (Anon., 1996e) guidelines have no statutory position, certain actions to be taken in respect

Table 5.1 *L. monocytogenes* - guidelines for the microbiological quality of various ready-to-eat foods (Anon., 1996e)

Category	Level of contamination
Satisfactory	Not detected in 25 g
Borderline - limit of acceptability	Present in 25 g to less than 200 per gram
Unsatisfactory	200 per gram to less than 1000 per gram
Unacceptable/potentially hazardous	Greater than or equal to 1000 per gram

of results being found in the 'unsatisfactory' and 'unacceptable/potentially hazardous' categories are discussed. For example, if a result is obtained from a test for *L. monocytogenes* classifying a food 'unacceptable/potentially hazardous', urgent action is recommended to locate the source and identify the risk this presents to consumers. Results in this category could also form the basis for a prosecution by enforcement officers.

SPECIFICATIONS

Product specifications drawn up between a food manufacturer and a customer (often a retailer) usually include information concerning the physical appearance of the product, physico-chemical characteristics of importance to the safety and/or quality of the product and microbiological parameters relevant to the safety and quality of the product.

There has been a tendency in the recent past (still persisting in some areas of the industry) to establish and maintain a fixed list of microorganisms (potential pathogens as well as non-pathogens and general microbiological tests such as total colony counts and coliforms) that is then included in all product specifications regardless of relevance to the product or the processes by which it was made. *L. monocytogenes* has frequently been included in this list because of media attention and tests are often indiscriminately applied to many products and processes where the use of this microbiological test has little significance. If, after consideration of all the issues relevant to determining where and how processes and products need to be monitored, microbiological testing and associated criteria are regarded as useful, then it is important to ensure that only those organisms relevant to the raw materials, processes and finished product are selected (Anon., 1997f). Because *L. monocytogenes* is ubiquitous in the environment, effective and reliable hygiene systems are required to minimize its presence throughout the food production environment. In addition, because it is able to grow at refrigeration temperatures, its absence or presence at only very low levels in ready-to-eat foods is necessary. Therefore, it is common for microbiological specifications to include a target of 'absence' of *L. monocytogenes* in 25 g of product with unacceptable levels commonly specified as 'presence in 25 g' or greater than 10 or 100 per gram. Products not complying with specifications result in investigative action to identify and rectify the cause. In turn, this may lead to removal of products from the distribution and retail system.

MONITORING FOR *LISTERIA* SPP. AND *L. MONOCYTOGENES*

The success or otherwise of any systems put in place to control *L. monocytogenes* is usually monitored by testing samples taken from selected points. Food samples are commonly taken from incoming raw materials, food materials in process, e.g. after washing procedures, cooking procedures or slicing operations, and finished products.

The buying specification for some raw material supplies may include criteria to be met for *L. monocytogenes*, e.g. prepared cooked meats for sandwich production. In addition to obtaining a microbiology report from the supplier, incoming batches may be sampled for testing by the manufacturer. In this case, small portions (10–20 g) may be aseptically removed from several packs, combined and mixed prior to a test portion of 25 g being used in the laboratory examination.

Where in-process samples are required to be taken to monitor the efficacy of production processes, then random single samples (>25 g per occasion) may be taken during the production run and tested individually. The results from these tests are assessed against in-house criteria.

Finished product samples are usually taken as complete finished packs from the end of the production line. Single packs may be taken at intervals during the production run. The numbers and frequency of sampling are usually in accordance with a customer's requirements. For monitoring purposes it is often useful to take product samples from the start of the production run as any residual contamination on the equipment may be detected in the first batches of product from the line. In the laboratory, it is important that a representative sample is removed from the pack for testing. For multicomponent products this may involve selecting small quantities from each of the components in similar proportion to their percentage in the product so as to make up a final test portion of 25 g. Alternatively, the test may comprise 25 g of each individual component or 25 g made up from those components deemed to be the highest risk in respect of *L. monocytogenes* contamination and potential growth. Again, the approach taken is usually in accordance with a customer's requirements but for most buying specification purposes the first approach is used. The other options may be applied in problem-solving situations, i.e. if *Listeria* is found in the composite sample tested and a component analysis is warranted.

Environmental samples are commonly taken from food contact surfaces, e.g. mixer bowls, tables, conveyor belts, floors, drains, wall/floor junctions and floor contact items, e.g. wheels, footwear and air. If meaningful results are to be obtained, then it is important that samples taken are relevant and representative of the area or material targeted. Table 5.2 indicates some of the points that should be considered when selecting the means for sampling specific environmental and associated areas.

Because the processing environment (particularly moist or wet areas) is such an important potential source of *L. monocytogenes* it is essential to employ the best means for detecting the organism if it is present. The more reliable the information can be from the test carried out, then the more effectively the control measures can be applied. The objective of any sampling programme must be to find the organism so as to focus improved control in areas where the hazard is identified.

Medical swabs are usually inadequate and inappropriate for application to environmental sampling. In order to ensure the best opportunity for detecting listerias if they are present, samples taken should be large in volume or weight or from large areas so as to be representative of the environmental situation observed.

It is common practice to examine environmental samples for the presence of *Listeria* spp. on the grounds that if any of the species are present, then *L. monocytogenes* is also likely to be present and appropriate control measures are instigated following the detection of *Listeria* spp. Examination of environmental samples specifically for the presence of *L. monocytogenes* are usually instigated in problem solving, e.g. when it is necessary for tracing a specific strain that has been found in a product. In this case, fingerprinting of the strains found may be usefully employed.

All sampling procedures, methods, criteria and reaction procedures to be followed in the event of positive results must be clearly indicated in standard operating procedures and the responsibilities of key personnel at all monitoring, reporting and control levels clearly defined. Table 5.3 indicates some approaches to action which may be taken in response to results from tests carried out to detect or count *L. monocytogenes* in environmental or food samples. These would normally form part of an internal quality system.

Table 5.2 Guide to testing for the presence of *Listeria* spp. and *L. monocytogenes* in processing environments

Point of sampling	Method and consideration
Floors	Collect volumes of liquid from puddles by pipette or using a large sponge swab Cracked or broken flooring should be targeted. Floor/wall junctions can accumulate debris and extensive junction sections (minimum 1 metre lengths) should be swabbed
Drains	Collect a minimum 25 ml from the liquid in the drain using a pipette Damaged areas that have caused puddling should also be sampled by collecting a volume of liquid by pipette Large sponge swabs can be used to collect biofilm from drain surfaces and the grooves/joints in the drainage channel within the floor
Wheels	Use a large moistened sponge swab to thoroughly swab both sides of the wheel
Wellington boots	Use a large moistened sponge swab to thoroughly swab the entire surface of the sole of the boot making sure that the welts are well swabbed. If large quantities of debris have accumulated in the welts, then a knife or spatula should be used to remove as much as possible for testing as a solid sample
Joint sealant	Use a large moistened sponge swab to thoroughly swab at least 1 m length of the sealant. Where sealant is cracked, broken or loose, then the areas under the damage should also be swabbed or a quantity of sealant removed for testing. For large pieces of debris, a plastic bag, e.g. stomacher bag, can be inverted over a hand to form a glove and the debris picked up; re-forming the bag when pulling it off the hand automatically contains the sample within the bag (inverted bag technique)
Food contact surfaces, e.g. conveyor belts, tables, mixers, blenders, choppers, trays, bins, hoppers and utensils	Use a large moistened sponge swab to thoroughly swab a large defined area on surfaces such as tables and conveyor belts, e.g. 1 m^2. Use a moistened sponge or cotton swab to thoroughly swab a defined area of smaller items. For bowls and trays, the whole food contact surface can be swabbed; however, a minimum of 100 cm^2 should be used. For knives and spoons, the whole blade or spoon bowl is swabbed

Table 5.2 Continued

	Point of sampling Method and consideration
Air	Prepared plates of *Listeria*-selective agar media can be directly exposed for defined periods of time in the working environments of the production areas. Alternatively, commercially available air-sampling devices, which draw environmental air over the surface of agar plates or strips for preselected time periods, can be used. The air contamination level in all high risk production areas, including refrigerators and blast chillers, should be monitored regularly
Condensate from chillers	Collect volumes of condensate by pipette or using a large sponge swab
Cleaning equipment and engineering tools	Swabs and physical samples should be taken by any means appropriate to the object. For cloths or sponges used for cleaning purposes, the entire item can be removed using the inverted bag technique
Points to remember when sampling	All equipment used for sampling, e.g. pipettes, swabs, bags, etc., must be sterile and aseptic procedures must be used at all times All samples must be fully labelled (date, time, description, sampler) at the time of collection A standard operating procedure should exist, which includes a basic rota for environmental monitoring and methods to be used. This should be reviewed regularly to ensure any new areas, equipment or process introduced are included in the rota. Look out for other areas of potential contamination when sampling and always take extra swabs for this purpose Additional sampling may be necessary when investigating known problems, in support of new product development or for due diligence demonstration When sampling post sanitization, a neutralizer may need to be added to the medium to inactivate any terminal disinfectant that may be present Remember to inform building maintenance or cleaning personnel where any damaged areas in need of repair or areas inadequately cleaned are found

Table 5.3 Considerations to be taken when finding *L. monocytogenes* in product or environmental samples*

Consideration	Not detected in any samples	Present in environmental samples	Present in 25 g but < 10 per gram (food)	Present at 10 per gram to < 100 per gram (food)	Present at > 100 per gram (food)
No action	Yes				
Environmental monitoring: increase sampling points to identify source		Yes	Yes	Yes	Yes
Cleaning efficacy: check procedures/cleaning records and monitor pre and post cleaning		Yes	Yes	Yes	Yes
Raw material testing: increase testing to identify any contamination			Yes	Yes	Yes
Intermediate product testing: increase testing of in-process material to identify stages of contamination			Yes	Yes	Yes
Finished product testing: increase to identify point of production where contamination is occurring			Yes	Yes	Yes
Review efficacy of process controls: check all process records to ensure current controls have been carried out properly			Yes	Yes	Yes
Stop production: cease production until the problem is identified and resolved					Yes
Withdrawal of product: consider the need to withdraw products from sale			†	†	Yes†

* Information given is for guidance only and may not be appropriate for individual circumstances.
† Consider the percentage incidence and the potential for initial levels of contamination to increase during the remaining shelf life of the products when assessing the action to be taken.

In any investigation by public health or enforcement officers of a case of foodborne listeriosis, the ability to demonstrate well-structured and reliably operated procedures targeted to control *L. monocytogenes* will prove invaluable.

6

TEST METHODS

CONVENTIONAL METHODS

Methods for the detection and identification of *L. monocytogenes* were first developed for use in clinical laboratories (McBride and Girard, 1960; Wood, 1969). Since the mid-1980s, when *L. monocytogenes* became recognized as a significant foodborne human pathogen, a considerable amount of method development has occurred around the world to determine the most effective means for isolating the organism from clinical samples and foods and its subsequent identification (Lovett *et al.*, 1987; McClain and Lee, 1988; Fraser and Sperber, 1988; Curtis *et al.*, 1989; van Netten *et al.*,1989; Bille *et al.*, 1992; Curtis and Lee, 1995).

Food industry laboratories generally use conventional methods for detecting and identifying *Listeria* spp. and *L. monocytogenes*. Tests for presence/absence (detection) per 25 g are most commonly used for foods but counts per gram are carried out particularly when an investigation involving a specific public health concern is necessary. Tests for presence/absence (detection) per swab or other specified material are usually applied in respect of environmental samples.

Detection methods involve selective enrichment in a broth, e.g. FDA Listeria Enrichment Broth (unbuffered, LEB, or buffered, BLEB), University of Vermont Medium primary and secondary enrichment broths (UVM1 and UVM2), Fraser Broth and Half Fraser Broth followed by the isolation of colonies on selective agar, e.g. LPM Agar, Oxford Agar, PALCAM Agar. These selective media contain various combinations of antibiotics and other selective/diagnostic chemicals, e.g. acriflavine hydrochloride, cycloheximide, nalidixic acid, cefotetan, polymixin B, fosfomycin, colistin sulphate, lithium chloride and aesculin (Curtis and Lee, 1995). Colonies growing on the selective agar and exhibiting a morphology typical of *Listeria* spp. on that medium are designated presumptive (suspect) *Listeria* spp. and are

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Table 6.1 Characteristics used to identify *Listeria* spp.

Test	*Listeria* spp. reaction
Gram stain	Positive
Cell morphology	Non-spore-forming rod
Motility	Positive tumbling motility at 20–22°C
Catalase	Positive
Oxidase	Negative
Aesculin hydrolysis	Positive
Indole	Negative
Urease	Negative
Growth conditions	Aerobic and facultatively anaerobic

further identified to species level using biochemical and haemolytic characteristics (Tables 6.1 and 6.2). Where enumeration of the organism is required (colony count/colony-forming units per gram), volumes (0.1–0.5 ml) of appropriately serially diluted sample are inoculated directly by spread technique to the surface of the selective agar plate.

As more than one species of *Listeria* may be isolated from any one sample, particularly from environmental samples, a minimum of three colonies exhibiting characteristic morphology for *Listeria* spp. on the selective agar are usually selected for identification to genus and species level. These 'presumptive' colonies are first purified by streaking onto non-selective agar such as nutrient agar or tryptone soya agar with yeast extract (TSAYE) prior to identification. Current published International Standard methods (Anon., 1993; Anon., 1995c) use a similar approach. Figure 6.1 illustrates the general procedure followed for the isolation of *Listeria* spp.

Where processing may have caused injury to listerias, e.g. frozen or dry foods, there may be a need to resuscitate cells prior to exposure to the selective agents used in selective enrichment media. For this purpose, pre-incubation of samples in a non-selective medium or a basal medium prior to the addition of selective agents can be useful.

Most workers have found the use of buffered LEB incubated at 30°C for 48 h or UVM1 incubated at 30°C for 24 h followed by subculturing into Fraser Broth to be the most reliable conventional enrichment procedures. In most cases, enrichment broths are subcultured on Oxford Agar, modified Oxford Agar or Palcam Agar.

When only low levels of *Listeria* may be present in foods, more reliable detection may be achieved by examining samples taken at the end of the

Table 6.2 Conventional differentiation of *Listeria* spp.

Species	β-haemolysis	Production of acid from		Christie, Atkins, Munch-Petersen (CAMP) reaction on sheep blood with	
		L-rhamnose	D-xylose	*Staphylococcus aureus*	*Rhodococcus equi*
L. monocytogenes	+	+	−	+	−
L. innocua	−	V	−	−	−
L. ivanovii	++	−	+	−	+
L. welshimeri	−	V	+	−	−
L. seeligeri	(+)	−	+	(+)	−
L. grayi	−	−	−	−	−

++: strong positive reaction
+: positive reaction
(+): weak positive reaction
−: negative reaction
V: variable reaction

Day 1: 25 g/ml test portion + 225 ml selective enrichment medium

30°C ↓ 48 hours

Day 3: Subculture by streaking from incubated broth to selective agar

30°C ↓ 48 hours

Day 5: Subculture selected suspect colonies to Tryptone Soya Agar with Yeast Extract for purification

30°C ↓ 24 hours

Day 6: Commence tests for identification on purified colonies

Figure 6.1 General conventional procedure for the detection and identification of *Listeria* spp.

shelf life of the food. Therefore, testing products both at the beginning and end of shelf life is important when monitoring the incidence of *Listeria* in products that support its growth.

ALTERNATIVE METHODS

Conventional method development has exploited the characteristics of *Listeria* spp. using the traditional microbiological approaches that are based on a knowledge of the organism's growth requirements and biochemical characteristics built up since microbiologists first recognized the organism (Seeliger and Jones, 1986). These methods are, however, both labour-intensive and lengthy, with results for samples not containing *Listeria* (negative results) being available only after three to four days. If colonies are present on the selective agar that exhibit the correct morphological characteristics for *Listeria* spp., then a further two to seven days are necessary to confirm these suspect colonies as *Listeria* spp. and identify which species is present. There is, therefore, a need to supplement conventional methods with simpler, labour-saving methods, preferably capable of delivering results more quickly than is possible using conventional methods.

When selecting any method for use all of the following attributes need consideration:

- sensitivity
- specificity

- simplicity
- robustness
- reliability
- the need for additional tests to confirm presumptive results, 'hands-on' time and time taken to obtain the final result
- requirement for trained staff and/or special equipment
- cost per test.

A variety of alternative methodologies currently exist for the detection and/or identification of *Listeria* spp. (Table 6.3) and the list is growing rapidly as new technologies are exploited for application to the needs of microbiologists. Regular updates on commercial alternative microbiological test methods are available together with useful key references and the validation status of the method (Betts, 1996).

Many alternative tests depend on the presence of a minimum number of target cells for reliable detection and the procedures specified for use with a manufacturer's kit or test are designed to ensure a reliable result. To avoid false negative or false positive results, it is always important to read and understand the kit/test manufacturer's technical information supplied and carefully follow the instructions for carrying out the test.

The following sections describe some of the alternative test methods more commonly used in routine testing laboratories. Table 6.4 summarizes some of the practical information concerning the use of some of the technologies applied for the specific isolation and/or identification of *L. monocytogenes*. When conducting tests for confirming the identity of *L. monocytogenes* it is good practice to use purified cultures. Where applicable, the time necessary for the production of pure cultures has been included in the total approximate times to produce a result from the examination of presumptive positive broths or colonies given in the table.

Biochemical identification kits

These are some of the simplest forms of labour-saving test systems for identifying *Listeria* spp. They consist of a range of biochemical reactions that occur in preformed chambers supplied in a disposable unit. Following inoculation and incubation of the test chambers, reactions are assessed usually by a specified colour change in the medium. Following assessment, a profile of the organism is obtained, which is used to determine the identity of the *Listeria* species. For the specific identification of *L. monocytogenes*, it may be necessary to supplement the test results with separate haemolysis and CAMP tests.

Table 6.3 Examples of alternative methods available for the detection and/or identification of *Listeria* spp. and *L. monocytogenes*

Test type	Name of test	UK supplier
Miniaturized biochemical test kits	API Listeria Microbact 12L *Listeria* MicroID	bioMérieux UK Ltd Microgen Bioproducts Ltd Organon Teknika Ltd
Enzyme screening test	Key ID *Listeria*	LabM
Enzyme-linked immunosorbent assay (ELISA)	*Listeria*-Tek™ *Listeria* Visual Immunoassay *Listeria* Detection Kit Unique *Listeria*	Organon Teknika Ltd TECRA diagnostics Transia Ltd TECRA diagnostics
Enzyme-linked fluorescent immunoassay	VIDAS *Listeria* VIDAS *Listeria monocytogenes*	bioMérieux UK Ltd bioMérieux UK Ltd
Latex-linked immunoassay	Oxoid Listeria Rapid Test (Clearview)	Oxoid Ltd
Immunomagnetic separation	Listertest Lift	Vicam L.P., USA
Latex agglutination	Microscreen *Listeria*	Microgen Bioproducts Ltd
Nucleic acid hybridization probe	Gene Trak test for *Listeria* Gene Trak test for *Listeria monocytogenes* Gen-Probe (Accuprobe) test for *Listeria monocytogenes*	Gene Trak Systems Gene Trak Systems Eurogenetics UK
Molecular typing using DNA fragmentation, gel electrophoresis and chemiluminescence	RiboPrinter™	FQMS, LLC, Dupont, USA
Polymerase chain reaction	TaqMan System Bax™ system	Perkin Elmer Applied Biosystems Qualicon, Dupont, USA

Table 6.4 Some aspects associated with the use of conventional and alternative methods for the detection and identification of *Listeria* spp. and *L. monocytogenes*

Test	Presumptive/ confirmed result	Further confirmation/ identification required	Approximate time to carry out the specific test	Approximate total time to negative result from initial sampling*	Approximate total time to confirmed result from initial sampling*
Conventional: broth enrichment and plating	Presumptive	Yes	4 days	4 days	6 days
Biochemical tests	Confirmation and identification of a purified isolate	No	24 h	6 days	6 days
Enzyme screening test	Presumptive	Yes	15 min	4 days	6 days
Immunoassays/ELISA	*Listeria* Tek: presumptive	Yes	2 h	50 h	6 days
	Tecra Visual: presumptive	Yes	2 h	50 h	6 days
	Transia *Listeria*: presumptive	Yes	1.5 h	50 h	6 days
	Tecra Unique: presumptive	Yes	8 h	32 h	5 days
	Vidas *Listeria*: presumptive	Yes	45 min	48 h	6 days
	Oxoid Clearview: presumptive	Yes	45 min	43 h	6 days
Immunomagnetic beads	Dynal: presumptive	Yes	1 h	3–4 days	5–6 days
	Vicam Listertest Lift: presumptive	Yes	24 h	24–30 h	2–3 days
Latex immuno-agglutination	Presumptive	Yes	< 5 min	48 h from broth 4 days from plate	6 days 6 days

Table 6.4 Continued

Test	Presumptive/ confirmed result	Further confirmation/ identification required	Approximate time to carry out the specific test	Approximate total time to negative result from initial sampling*	Approximate total time to confirmed result from initial sampling*
Electrical	Presumptive	Yes	up to 30 h	54 h	6 days
Nucleic acid hybridization probes	Gen-probe: confirmed	No	45 min	48 h from broth 4 days from plate	48 h from broth 4 days from plate
	Gene Trak: confirmed	No	3 h	50 h	50 h
Polymerase chain reaction	Bax: confirmed	No	5 h	24 h	24 h

* Includes time which may be required for any associated culture work, e.g. prior to conducting the test, purification of suspect positive cultures prior to conducting relevant confirmatory tests and the confirmatory tests.

Specific enzyme reaction kits, e.g. Key ID *Listeria*, are also available and these can be used to rapidly differentiate colonies of *Listeria* spp. from colonies of other organisms that are aesculin positive on Oxford Agar.

Enzyme-linked immunosorbent assays (ELISA)

ELISA tests make use of the high specificity of an antibody to its target antigen; in this case, *Listeria* spp. The anti-*Listeria* antibodies are bound to a solid substrate, e.g. the internal surface of the wells in a microtitre plate, and these are used to capture *Listeria* antigens present in the treated enrichment broth placed in the well. Following a sequence of manipulations involving washings, addition of further reagents and incubations, a coloured end product is obtained in those wells containing *Listeria*. Sample enrichment based on the use of conventional enrichment broths is necessary to ensure sufficient target cells are available (10^5–10^6 per ml) for detection in a positive sample.

Positive results obtained using ELISAs are usually confirmed by streaking the original enrichment broth onto selective agar and then following the conventional approach to identify the *Listeria* species present.

ELISAs can offer rapid screening of samples with a negative result available in 48 h and a potential early indication of a positive result.

Immunomagnetic separation

This technique is used to improve the sensitivity and specificity of conventional selective methods in detecting the presence of *Listeria* spp. in samples. It employs small (<100 μm) magnetic beads coated in antibodies to *Listeria*. The beads are used to specifically capture cells of *Listeria* from enrichment broths. The magnetic property of the beads is used to separate the cells from the broth and these are plated out onto selective agar and the conventional approach then followed to identify any *Listeria* spp. isolated. In another application of the technique not yet widely used, the beads are used to capture and concentrate *Listeria* spp. directly from a sample suspension. The beads are then removed and transferred directly to the surface of a selective agar plate. Plates are then incubated to produce microcolonies, which are removed by membrane imprint for examination using a chromogenic immunoblot technique to count positive *Listeria* spp. colonies (Listertest Lift).

Immunochromatography and latex agglutination tests

Antibodies to *Listeria* flagella antigen are immobilized in a line on a membrane. Cultured enrichment broth is heat treated before being added to *Listeria*-specific antibody bound to coloured latex particles dispersed at the base of the membrane. If *Listeria* antigens are present, the latex particles bind to them via the specific antibody and they are drawn up the membrane. The immobilized antibody captures the *Listeria*-antibody-latex complex and as the latex particles are concentrated together a coloured line forms, indicating a positive result.

Antibody-coated latex beads can also be used in agglutination tests to detect *Listeria* spp. directly from enrichment broth culture, although they are more commonly used to screen presumptive *Listeria* colonies on selective agar as high levels are required before clumping of latex particles becomes visible.

Such methods offer specificity and time saving; however, due to potential false positive reactions, the *Listeria* spp. present are usually confirmed by streaking the original enrichment broth onto selective agar and then following the conventional approach for identification.

Gene probes

The most specific techniques currently available for routine use in the detection and identification of *Listeria* species are based on the use of gene probes. These are synthetically produced sequences of DNA that are complementary to sequences of DNA or RNA that are unique to the target organisms: either *Listeria* species or *L. monocytogenes*.

Gene probes are usually applied to colony growth on selective or nutrient agar media (Klinger *et al.*, 1988; Bobbitt and Betts, 1992). They can be applied directly to broths after an incubation period of 48 h but this approach may give false negative reactions if the *Listeria* cells do not achieve a sufficiently high concentration (10^6 per ml) to give a positive reaction.

The high specificity of these tests means that once a result is obtained no further confirmatory tests are necessary unless specific 'fingerprint' information is required for the isolate. 'Fingerprint' tests can be complex and are best carried out by a national centre for typing *L. monocytogenes* (see Appendix).

Electrical techniques

Electrical techniques for the detection of microorganisms are based on the ability of instruments to monitor and detect small changes in the electrical properties of a medium in which a microorganism is growing.

Specific broth media have been developed that favour the growth of *Listeria* spp. As the organism grows, particular substrates in the broth are utilized, yielding products of metabolism giving detectable changes in conductance. The changes occurring are recorded as a conductance curve. The curve generated is monitored by computer; if it meets certain preset criteria, the sample is registered as positive and the broth is considered to be presumptive positive for *Listeria* species. Confirmation of any presumptive positive broths is carried out by streaking from the broth onto selective agar and then following the conventional approach to identify the *Listeria* spp. present.

'Fingerprinting' methods

Usually, once *L. monocytogenes* has been isolated and identified to species level using conventional methods, this level of identity together with the associated sample information is sufficient for the food microbiologist to interpret results against the original requirements for the test, e.g. a product-buying specification. However, it may sometimes be necessary to further discriminate the identity of the strain isolated, particularly if the origin of the strain needs to be traced in connection with cases of suspected illness.

The ability to subtype or 'fingerprint' *L. monocytogenes* is important in the surveillance of listeriosis as well as in the investigation of outbreaks of the illness and traceability in food processing and environmental/ecological situations. Techniques are available that can 'fingerprint' strains of *L. monocytogenes*, allowing confident traceability of strains in the factory environment. Techniques include serotyping, phage typing, production of listeriocins and their ability to inhibit the growth of a panel of indicator strains, ribotyping, pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD). Should this level of identity be required, then the services of a laboratory expert in these techniques and the interpretation of the results should be obtained (see Appendix).

It is always important to ensure that the method used will reliably detect *Listeria* spp. and *L. monocytogenes* (if it is present) in the food being examined. It may be necessary to initially confirm by experiment that a

particular method will be suitable for the purpose intended. Subsequently, in routine use, quality assurance systems (internal and external) should be employed to verify ongoing test efficacy.

A variety of sources exist that may be used for guidance in appropriate method selection. National and international standard methods published, for example, by the British Standards Institution, the International Standards Organization and the International Dairy Federation are available. In addition, methods have been reviewed, practised and validated by reputable bodies such as the Public Health Laboratory Service (UK), the Association of Official Analytical Chemists (USA) and the Campden and Chorleywood Food Research Association (UK).

7

THE FUTURE

Over the past decade in particular, commercial considerations have driven food product development to become increasingly innovative. The ready availability of food raw materials from anywhere in the world leading to the use of more 'exotic' ingredients and the closure of the 'season gap', which has made normally seasonal foods available all year round, has given free rein to innovative food technology.

Developments in such raw materials availability and novel applications, combined with developments in processing technologies, packaging technology and storage and distribution systems, are all providing continuing challenges to food microbiologists and technologists to devise and maintain controls of food safety.

The market for chilled food products has grown significantly over the last decade, including savoury ready meals (recipe dishes), dairy and dessert products, sandwiches and other snack meals. Increasingly novel and complex combinations of raw materials are being manufactured in large-scale commercial systems using the wide variety of meats, poultry, game, fish and shellfish, milk and milk products, eggs, vegetables (root, leafy and salad), grains, nuts, herbs and spices available.

Alternative food processing technologies are in development and new applications of existing technologies are being explored for use in food production processes. Ohmic heating processes, irradiation, ultra-filtration, high pressure and high intensity light are examples of such technologies. The effects of these on the survival of microorganisms in respect of food safety and food spoilage have, in many instances, yet to be determined. Clearly, the list of already known bacterial pathogens, including *L. monocytogenes*, must be included in any consideration of the implications of new combinations of approaches to food product development and the application of alternative technologies in food processing.

C. Bell et al., *Listeria*

It is essential that HACCP-based considerations are applied at an early stage in all new food product and process developments to minimize the potential public health problems that could arise from the presence and outgrowth of *L. monocytogenes*.

GLOSSARY OF TERMS

Bacteriocins Protein antibiotics produced by a variety of strains of Gram-positive and Gram-negative bacteria. They are inhibitory or lethal to other bacteria (often related strains).

Biotyping The conventional method for distinguishing between bacterial types using their metabolic and/or physiological properties.

CAMP test (Christie, Atkins, Munch-Petersen test) A synergistic haemolytic reaction in which a characteristic enhancement of the haemolytic reaction is obtained on sheep blood agar when a specific weakly beta haemolytic strain of *Staphylococcus aureus* is grown in close proximity to *Listeria monocytogenes* but not when grown in close proximity to *Rhodococcus equi* (see Table 6.2).

D-value The time required (usually expressed in minutes) at a given temperature to reduce the number of viable cells or spores of a given microorganism to 10% of the initial population.

Genotyping Methods used to differentiate bacteria based on the composition of their nucleic acids.

Incertae sedis Of uncertain taxonomic position.

Indicator organisms Those organisms whose presence suggests inadequate processing for safety.

Multilocus enzyme electrophoresis (MEE) A method used to distinguish between isolates of the same species on the basis of water-soluble enzyme migration patterns obtained from electrophoresis of cell extracts.

Pasteurization A form of heat treatment that kills vegetative pathogens and spoilage microorganisms in milk and other foods; for example, for milk a common pasteurization process is 71.7°C for 15 s.

Phage typing A method used to distinguish between bacteria within

the same species on the basis of their susceptibility to a range of bacterial viruses (bacteriophage).

Phenotype The observable characteristics of an organism, which include biotype, serotype, phage type and bacteriocin type.

Polymerase chain reaction (PCR) A technique used to amplify the number of copies of a preselected region of DNA to a sufficient level for testing.

Polymorphism The ability to occur in two or more morphologically distinct types (morphotypes) depending on the conditions prevailing.

Psychrotrophic Organisms that can grow at temperatures as low as -5°C but which have an optimum growth temperature in the mesophilic range (20–30°C).

Pulsed-field gel electrophoresis (PFGE) A technique that allows chromosomal restriction fragment patterns to be produced.

Random amplified polymorphic DNA (RAPD) A modification of PCR that allows random amplification of unknown segments of DNA using a single oligonucleotide primer. This produces a number of fragments, the size and distribution of which is used to compare isolates.

Restriction enzymes (restriction endonucleases) Enzymes that attack DNA. Each enzyme recognizes a particular and different nucleotide sequence and cuts the DNA at a specific site.

Restriction enzyme analysis (REA) A method for discriminating between isolates of the same species on the basis of patterns obtained from the separation of DNA fragments in agarose gel after they have been digested with one or more restriction enzymes, e.g. *Eco*R1, *Hae*III, *Hha*I or *Xho*I. Differences in the banding profiles of two isolates is referred to as a restriction fragment length polymorphism.

Ribotyping A method for characterizing bacterial isolates according to their ribosomal RNA pattern (ribotype) and identifying the isolate by comparing the pattern obtained to a database of patterns.

Serotyping A method of distinguishing bacteria on the basis of their antigenic properties.

Sous vide Usually composite foods pasteurized in a vacuum pack intended for catering outlets. Such products are often given an extended shelf life at refrigeration (≤2°C) temperatures.

Southern hybridization (Southern blot) A method used to transfer single-stranded DNA from agarose gel to a cellulose nitrate filter where it

can be fixed and labelled probes used to identify complementary DNA molecules among other DNA fragments.

Strain An isolate or group of isolates that can be distinguished from other isolates of the same genus and species by phenotypic and/or genotypic characteristics.

Ultra-heat treatment A high temperature heat treatment (138–142°C for 2–5 s) applied to liquid foods usually followed by aseptic packaging for the production of long-life ambient stable products.

Water activity (a_w) A measure of the availability of water for the growth and metabolism of microorganisms. It is expressed as a ratio of the water vapour pressure of a food or solution to that of pure water at the same temperature.

z-value The increase in temperature (°C) required to decrease the D-value by 10-fold.

APPENDIX: NATIONAL CENTRES FOR TYPING *LISTERIA* CULTURES

Public Health Laboratory Service
Food Hygiene Laboratory
Listeria Section
61 Colindale Avenue
London NW9 5DF
UK

Centre National de Référence pour la Lysotypie et le Typage
Moléculaire de *Listeria*
WHO Collaborating Center for Foodborne Listeriosis
Département de Bactériologie
Institut Pasteur
28 rue du Dr Roux
75724 Paris Cedex 15
France

Foodborne and Diarrheal Disease Branch
Division of Bacterial and Mycotic Diseases
Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta
Georgia 30333
USA

Swiss National Centre for Listeriosis
WHO Collaborating Centre for Foodborne Listeriosis
CHUV Lausanne
Switzerland

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