CHAPTER 2.9.7.

LISTERIA MONOCYTOGENES

SUMMARY

A wide variety of animal species can be infected with Listeria monocytogenes, but clinical listeriosis is mainly a ruminant disease, with occasional sporadic cases in other species. The main clinical manifestations of animal listeriosis are encephalitis, septicaemia and abortion, and the disease is often associated with stored forages, usually silage. Post-mortem findings and histopathology depend on the clinical presentation.

Listeriosis is one of the most important food-borne diseases of humans. The disease manifestations include septicaemia, meningitis (or meningoencephalitis) and encephalitis, usually preceded by influenza-like symptoms including fever. In pregnant women, intrauterine or cervical infections may result in spontaneous abortion or stillbirths. Listeria monocytogenes has also been associated with gastroenteric manifestations with fever. Although the morbidity of listeriosis is relatively low, the mortality of the systemic/encephalitic disease can be very high, with values in the vicinity of 30%. The elderly, pregnant women, newborns and the immunocompromised are considered to be at high risk of contracting the disease.

A number of molecular and cellular determinants of virulence have been identified for this intracellular pathogen, and although there is evidence of polymorphism among different strains of L. monocytogenes for some of these virulence determinants, this heterogeneity cannot be correlated with the ability or inability of the organism to produce disease. Therefore, all L. monocytogenes strains are considered to be potentially pathogenic.

Identification of the agent: A variety of conventional and rapid methods are available for the detection and identification of L. monocytogenes in food samples and specimens from animal listeriosis. Conventional methods remain the ‘gold standard’ with which other methods are compared. They are usually very sensitive. These methods use selective agents and enrichment procedures to reduce the number of contaminating microorganisms and allow multiplication of L. monocytogenes.

Although not required for regulatory purposes, different levels of subtyping L. monocytogenes strains are available, including serotyping, phage typing, multilocus enzyme electrophoresis, DNA restriction enzyme digestion patterns (conventional and pulse-field gel electrophoresis), nucleic acid sequence-based typing and random amplification of polymorphic DNA.

Serological tests: Serological tests for the detection of antibodies have not been traditionally used for the diagnosis of listeriosis. A number of formats have been tried and they have all been found to be largely unreliable, lacking sensitivity and specificity. Experimental serological assays based on the detection of anti-listeriolysin O have been used in some epidemiological investigations and as support for the diagnosis of culture-negative central nervous system infections. Immunohistochemical detection of L. monocytogenes antigens is a useful tool for the diagnosis of the encephalitic form of the disease.

Requirements for vaccines and diagnostic biologicals: It has proven very difficult to develop effective vaccines against L. monocytogenes which, as an intracellular organism, requires effector T cells for an effective immune response. Experimental vaccines in laboratory animals are being explored to confer protection to L. monocytogenes infection by a number of different approaches, including immunisation with plasmid DNA, CD40 signalling along with heat-killed L. monocytogenes, listeriolysin O-deficient mutants inoculated along with liposome-encapsulated listeriolysin O, and immunisation with listerial antigens and IL-12.
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A. INTRODUCTION

A wide variety of animal species can be infected by Listeria monocytogenes, including mammals, birds, fish and crustaceans, although most of the clinical listeriosis occurs in ruminants; pigs rarely develop disease and birds are generally subclinical carriers of the organism. Most infections in animals are subclinical, but listeriosis can occur either sporadically or in epidemic form. In addition to the economic impact of listeriosis in animals, there is a link between animals and their role as a source of infection for humans primarily from consumption of contaminated animal products. Infection can be as a result of direct contact with infected animals, especially during calving or lambing (68); however, these infections are very rare. The relative importance of the zoonotic transmission of the disease to humans is not clear, and contamination from the food processing environment is apparently more relevant to public health (62).

The clinical manifestations of listeriosis in animals include encephalitis, septicemia and abortion, especially in sheep, goats and cattle. The septicemic form is relatively uncommon and generally, but not invariably, occurs in the neonate. It is marked by depression, inappetence, fever and death. The encephalitic form is sometimes referred to as ‘circling disease’ because of a tendency to circle in one direction, and it is the most common manifestation of the disease in ruminants. The signs include depression, anorexia, head pressing or turning of the head to one side and unilateral facial paralysis. Abortion is usually late term (after 7 months in cattle and 12 weeks in sheep) (34, 67). Only one clinical form of listeriosis usually occurs in a particular group of animals. Bovine and ovine ophthalmitis have also been described (66). Rarely mastitis of ruminants has been associated with L. monocytogenes infection. Gastro-intestinal infections can occasionally occur in sheep (21). When listeriosis occurs in pigs, the primary manifestation is septicemia, with encephalitis reported less frequently and abortions rarely. Although birds are usually subclinical carriers, sporadic cases of listeriosis have been reported, most frequently septicemia and far less commonly meningoencephalitis. Avian listeriosis may be the result of a secondary infection in viral disease conditions and salmonellosis (68).

The post-mortem findings and histopathology, in animal listeriosis, depend on the clinical presentation. In the encephalitic form, the cerebrospinal fluid may be cloudy and the meningeal vessels congested. Gross pathological lesions of the brain are rare. On occasion, the medulla shows areas of softening. However, the histopathology is characteristic of the disease, consisting of foci of inflammatory cells with adjacent perivascular cuffing, predominantly of lymphocytes and histiocytes, plasma cells and occasional neutrophils. The microabscesses in the brain stem often more severely affect one side of the brain. More extensive malacic pathology may occur. The medulla and pons are most commonly involved. In the septicemic form, multiple foci of necrosis in the liver and, less frequently the spleen, may be noted. Aborted fetuses of ruminants show very little gross lesions, but autolysis may be present if the fetus was retained before being expelled (48, 67).

The evidence indicates that animal listeriosis is predominantly associated with stored forage and with the environment as the main source of contamination. Silage is the most frequent source (28, 69). The intestinal mucosa is the main route of entry, after oral ingestion, in the case of septicemic/abortive listeriosis. The incubation period can be as short as 1 day. The incubation period for the encephalitic form is usually 2–3 weeks, and the course of the disease is usually short in sheep and goats; 1–4 days (52), although it can be more protracted in cattle.

Although Listeria monocytogenes has been recognised as an animal pathogen for many years, its significant role as a food-borne human pathogen became evident only in the 1980s, when documented reports of listeriosis outbreaks, traced to contaminated food, started to appear in the literature (57). Today, L. monocytogenes is considered to be one of the most important agents of food-borne disease. Possible explanations for the emergence of human food-borne listeriosis as a major public health concern include major changes in food production, processing and distribution, increased use of refrigeration as a primary preservation means for foods, changes in the eating habits of people, particularly towards convenience and ready-to-eat foods, and an increase in the number of people considered to be at high risk for the disease (elderly, pregnant women, newborns, immunocompromised) (53, 63).

The primary manifestations of listeriosis in humans include septicemia, meningitis (or meningoencephalitis) and encephalitis, usually preceded by influenza-like symptoms including fever. Gastroenteric manifestations with fever also occur. Although the morbidity of listeriosis is relatively low, the mortality can reach values around 30%. In pregnant women, infection may result in abortion, stillbirth or premature birth (53, 61).

Listeria monocytogenes is a Gram-positive rod and is responsible for almost all infections in humans; although rare cases of infection due to L. ivanovii and L. seeligeri have been reported. In animals, L. monocytogenes is responsible for the majority of infections, but L. ivanovii and L. innocua infections have also been recorded. Listeria ivanovii has been associated with abortions and has been reported to very occasionally cause meningoencephalitis in sheep.

Although L. monocytogenes has definite zoonotic potential, it is also an important environmental contaminant of public health significance.
Subtyping of *L. monocytogenes* strains by a variety of methods is available for epidemiological investigations, but the fundamental question of whether all strains of *L. monocytogenes* are capable of causing disease remains unanswered (31, 42, 45, 46).

Several molecular virulence determinants have been identified that play a role in the cellular infection by *L. monocytogenes* and the unravelling of their mechanism of action has made of *L. monocytogenes* one of the most exciting models of host-pathogen interaction at the cellular and molecular levels. These virulence determinants include, among others, the internalins, listeriolysin O (LLO), ActA protein, two phospholipases, a metalloprotease, Vip protein, a bile exclusion system (BilE) and a bile salt hydrolase (17, 22, 25, 29, 60). Although there is polymorphism among different strains of *L. monocytogenes* for some of these virulence determinants, it cannot be correlated with the ability or inability of the organism to produce disease (42).

### B. DIAGNOSTIC TECHNIQUES

#### 1. Identification of the agent

There is a variety of conventional and rapid methods currently available for the detection and identification of *L. monocytogenes* in food samples and specimens from animal listeriosis. Conventional bacteriological methods are important for various reasons: Their use results in a pure culture of the organism, which is useful for regulatory purposes. They remain the ‘gold standards’ against which other methods are compared and validated. These methods are usually very sensitive and they do not require sophisticated and expensive equipment. Some of the disadvantages of this group of methods include the relatively long period of time that the protocols require for completion, several ‘hands-on’ manipulations, the requirement for many different chemicals, reagents and media, the possibility of contaminating microorganisms masking the presence of the target ones, including overgrowth, the potential overlook of atypical variants of the target organism and the relative subjectivity involved when interpreting bacterial growth on selective and differential agar plates (1).

The isolation and identification of *L. monocytogenes* from food, environmental samples and animal specimens require the use of selective agents and enrichment procedures that keep the levels of contaminating microorganisms to reasonable numbers and allow multiplication of *L. monocytogenes* to levels that are enough for detection of the organism. In the early days of listerial clinical bacteriology, cold enrichment (32) was regularly used to this end, exploiting the ability of the organism to multiply at refrigeration temperatures, whereas contaminating bacteria would not multiply under these conditions. However, this procedure requires very long incubation times, often months, making it unacceptable for current investigations of food-borne outbreaks and sporadic cases, as well as for the implementation of effective hazard analysis critical control points (HACCP) programmes in food production and processing establishments. A number of selective compounds that allow growth of *L. monocytogenes* at normal incubation temperatures have been incorporated into culture media, thus shortening the time required for selective growth of the organism. Examples of these selective compounds include cycloheximide, colistin, cefotetan, fosfomycin, lithium chloride, nalidixic acid, acriflavine, phenylethanol, cefazidime, polymixin B and moxalactam (3, 4, 8, 35, 39, 65).

Bacteriological diagnosis of animal listeriosis has traditionally involved direct plating of specimens on blood agar or other enriched media and concomitant use of the ‘cold enrichment’ technique, with weekly subculturing for up to 12 weeks (32, 51, 67). Immunohistochemical detection of *L. monocytogenes* antigens in formalin-fixed tissue has proven to be more sensitive than direct plating and cold enrichment bacterial culture for the diagnosis of the encephalitic form of the disease in ruminants (20, 44). The introduction of alternative enrichment procedures and selective agents for the isolation of *L. monocytogenes* from food and environmental samples has opened up the possibility of using some of these techniques for the bacteriological analysis of samples from animal listeriosis.

In spite of advances made in the selective isolation of *L. monocytogenes* from food, there is still room for improvement in a number of areas. No single procedure can be credited with being sensitive enough to detect *L. monocytogenes* from all types of food (23). In addition, sublethally injured *L. monocytogenes* cells can be found in processed food due to freezing, heating, acidification and other types of chemical or physical treatment. These sublethally injured bacteria require special culture conditions for damage repair, before being able to be detected in culture.

#### a) Isolation methods

Conventional methods for the isolation of *L. monocytogenes* from food that have gained acceptance for international regulatory purposes include the United States Food and Drug Administration (FDA) method (35), the Association of Official Analytical Chemists (AOAC) official method (8), the ISO 11290 Standards (39–41), the United States Department of Agriculture (USDA)-Food Safety and Inspection Service (FSIS) method (65) and the French Standards (3, 4).

Depending on the nature of the sample, a particular method might be more suitable than others. The International Organization for Standardization Technical Committee ISO/TC 34, Agricultural Food Products,
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Subcommittee SC 9, Microbiology, claims that the ISO Standard 11290, parts 1 and 2 (39–41), can be used for the detection of *L. monocytogenes* in a large variety of food and feed products. Although they recognise that this standard might not be appropriate in every detail in certain instances, they recommend that every effort should be made to apply this horizontal method as far as possible.

The FDA and AOAC methods can be used for milk and dairy products. The USDA-FSIS method is recommended for red meat and poultry (raw or cooked ready-to-eat), eggs, egg products and environmental samples.

The traditional procedure for the isolation of *L. monocytogenes* from animal tissues has been direct plating of specimens on sheep blood agar or other rich culture media and concomitant use of the ‘cold enrichment’ technique, with weekly subculturing for up to 12 weeks (32, 51, 67). Isolation of the organism by direct plating is relatively easy when numbers are large in a normally sterile site, like in the case of the septicaemic form of the disease, but isolation is difficult when the organism is present in low numbers, as in the case of the encephalitic form or when samples are heavily contaminated. A comparison of the efficiency of direct plating, cold enrichment and a slight modification of what became the AOAC method, found the latter to be superior over the other two, for the isolation of *L. monocytogenes* from a variety of animal necropsy material, both in terms of the time required for the isolation and identification of the organism, and the isolation rates (26).

For the enumeration of *L. monocytogenes*, the ISO Standard 11290, part 2 (40) applies, as well as optional protocols mentioned in the FDA and USDA-FSIS methods (35, 65).

In the case of animal listeriosis, the samples should be chosen according to the clinical presentation of the disease: material from lesions in the liver, kidneys and/or spleen, in the case of the septicaemic form; spinal fluid, pons and medulla in the case of the encephalitic form; and placenta (cotyledons), fetal abomasal contents and/or uterine discharges in the case of abortion. Refrigeration temperatures (4°C) must be used for handling, storing and shipping specimens. If the sample is already frozen, it should be kept frozen until analysis.

The protocol recommended for isolation of *L. monocytogenes* from animal necropsy material is described below as originally published (26). It is possible that this protocol could be improved by incorporating the recent updates to the AOAC method and new developments for identification and confirmation of *Listeria* isolates, but there is no published evidence to support this hypothesis yet.

- **Isolation procedure from animal necropsy material**
  
  i) Inoculate 10–25 g or ml of sample (depending on the amount of sample available) into 225 ml *Listeria* enrichment broth. When dealing with samples from animal listeriosis, the size of the sample for inoculation maybe limited and less than the recommended for food samples (25 g or ml). If that is the case, as much sample material as possible (aiming at 10–25 g or ml) should be inoculated (26). (*Listeria* enrichment broth base: Oxoid tryptone soya broth, 30 g; Difco yeast extract, 6 g; water, 1 litre; selective agents: Acriflavine, 2.3 mg; nalidixic acid, 9.2 mg; cycloheximide, 11.5 mg; add selective agents to 225 ml of the broth base).
  
  ii) Incubate broth at 30°C for 48 hours.
  
  iii) Spread 0.1 ml of the enrichment broth culture onto Oxford agar plates.
  
  iv) Incubate plates at 37°C. Examine bacterial growth after 24 and 48 hours.
  
  v) Test five colonies (or all when fewer available) with typical appearance of *L. monocytogenes* for cell shape, Gram reaction, haemolytic activity on blood agar (defibrinated horse blood), tumbling motility at 20°C, fermentation of glucose (+), rhamnose (+) and xylose (–), hydrolysis of esculin and production of catalase.

All culture media prepared should be subjected to quality control and must support growth of the suspect organism from a small inoculum. The reference strain should be cultured in parallel with the suspicious samples to ensure that the tests are working correctly.

Food samples intended for analysis must be representative of the food, including the outer surface and the interior. The conventional culture methods include an enrichment procedure based on the use of liquid culture media containing selective agents. The nature of the media and the selective agents vary with the method. Both the FDA (35) and the ISO methods include a pre-enrichment step that is intended for the recovery of sublethally injured *L. monocytogenes* cells, whereas in the USDA-FSIS (65) and the AOAC methods (8) the samples are processed directly into enrichment broth. In the case of the FDA method, the pre-enrichment is carried out at 30°C for 4 hours in tryptase–soy broth containing yeast extract (TSB YE).
without selective agents. The ISO protocol uses a ‘primary enrichment’ for 24 hours at 30°C in the presence of selective agents, but at half the concentration (‘half Fraser broth’).

Samples are enriched for 24–72 hours at 30°C, 35°C or 37°C, depending on the method. The FDA method uses TSB YE containing acriflavine, nalidixic acid and cycloheximide. The USDA-FSIS method uses two enrichment steps: The ‘primary’ enrichment is done in University of Vermont medium (UVM), containing nalidixic acid and acriflavine; the ‘secondary’ enrichment is carried out in Fraser broth, containing nalidixic acid, lithium chloride and acriflavine. The ISO standard indicates Fraser broth for the ‘secondary’ enrichment, containing the selective agents at full concentration, whereas the ‘primary’ enrichment is carried out in ‘half Fraser broth’, as indicated above. The AOAC method calls for selective enrichment in tryptone soy broth containing acriflavine, nalidixic acid and cycloheximide (‘selective enrichment medium’).

After selective enrichment, cultures are then plated on to selective/differential agar plates for isolation of presumptive colonies of \textit{L. monocytogenes}. All methods, except that of the ISO standard, use Oxford agar or a modification, MOX agar (USDA-FSIS). Oxford agar contains lithium chloride, cycloheximide, colistin, acriflavine, cetotetan and fosfomycin as selective agents, and typical colonies of \textit{Listeria} spp are small, black and surrounded by a black halo. In addition to Oxford agar, the FDA includes lithium chloride/phenylethanol/moxalactam (LPM) or PALCAM agar, which contains lithium chloride, polymixin B, acriflavine and ceftazidime. The MOX agar, used in the USDA-FSIS method, contains lithium chloride, colistin and moxalactam. The two selective plating media used in the ISO standard method are: agar \textit{Listeria} according to Ottaviani and Agosti (ALOA), which contains lithium chloride, nalidixic acid, ceftazidime, polymixin B and amphotericin B (or cycloheximide), and any other selective medium, of each laboratory’s choice, such as Oxford or PALCAM. Typical colonies of \textit{L. monocytogenes} in ALOA agar are green-blue, surrounded by an opaque halo (41).

Bio-Rad Laboratories have developed RAPID’L.Mono, a selective chromogenic medium for direct detection and enumeration of \textit{L. monocytogenes}, based on selective isolation, chromogenic detection of phosphatidylinositol-specific phospholipase C (PI-PLC) and xylose use. \textit{Listeria monocytogenes} develops blue colonies (PI-PLC positive) without yellow halo (xylose negative); \textit{L. ivanovii} produces greenish-blue colonies (PI-PLC positive) with yellow halo (xylose positive). Other \textit{Listeria} spp. colonies are white (PI-PLC negative).

b) Conventional identification methods

Typical \textit{Listeria} spp. colonies, on the above selective/differential agar plates, are then selected for further identification to the species level, using a battery of tests. The tests include the Gram-staining reaction, catalase, motility (both in a wet mount observed under phase-contrast microscopy and by inoculation into motility test media), haemolysis and carbohydrate use. The Christie–Atkins–Munch–Peterson (CAMP) test is a very useful tool to help identify the species of a \textit{Listeria} spp. isolate. It is used in the ISO and AOAC protocols and it is considered to be optional in the FDA and USDA-FSIS methods. The test is simple to perform and easy to read. It consists of streaking a ß-haemolytic \textit{Staphylococcus aureus} (ATCC strain 49444 or 25923, NCTC strain 7428 or 1803) and \textit{Rhodococcus equi} (ATCC strain 6939, NCTC strain 1621) in single straight lines in parallel, on a sheep blood agar plate or a double-layered agar plate with a very thin blood agar overlay. The streaks should have enough separation to allow test and control \textit{Listeria} strains to be streaked perpendicularly, in between the two indicator organisms, without quite touching them (separated by 1–2 mm). After incubation for 24–48 hours at 35–37°C (12–18 hours if using the thin blood agar overlay), a positive reaction consists of an enhanced zone of ß-haemolysis, at the intersection of the test/control and indicator strains. \textit{Listeria monocytogenes} is positive with the \textit{S. aureus} streak and negative with \textit{R. equi}, whereas the test with \textit{L. ivanovii} gives the reverse reactions (51).

<table>
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<th>Species</th>
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<th>Production of acid</th>
<th>CAMP test</th>
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<td></td>
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<td>Rhamnose</td>
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<td>\textit{L. monocytogenes}</td>
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<td>\textit{L. innocua}</td>
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<td>\textit{L. ivanovii}</td>
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<td>\textit{L. seeligeri}</td>
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<td>\textit{L. welshimeri}</td>
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Serology, lysogenic typing and the immunocompromised mouse pathogenicity assay are considered to be optional methods.

c) Rapid identification methods

The following protocols include conventional and nonconventional commercially available tests, e.g. Vitek, API, MICRO-ID, enzyme-linked immunosorbent assay (ELISA) kits and nucleic acid assay kits, to help in the identification of *L. monocytogenes*. Polymerase chain reaction (PCR), targeting the *hly* gene, has been found to be a sensitive and rapid technique for confirmation of the identification of suspect *L. monocytogenes* isolated on selective/differential agar plates (30). In addition to RAPID'L.Mono and ALOA agars, described above, other chromogenic media have been developed to differentiate *L. monocytogenes* colonies from those of other *Listeriae*: Oxoid chromogenic *Listeria* agar (OCLA), and CHROMagar *Listeria*, developed by CHROMagar Microbiology.

i) MICRO-ID Listeria

MICRO-ID Listeria is a commercially available system (Organon Teknika Corp., 100 Akzo Ave., Durham, NC 27712, USA) that has been validated by the AOAC (method 992.18) (5) for the presumptive identification of *Listeria* species isolated from food and environmental samples. It provides an alternative to conventional biochemical testing of *Listeria* spp. isolates by the FDA and USDA-FSIS methods. It is based on the principle that the test inoculum contains preformed enzymes that can be detected after 24 hours at 37°C. Differentiation of *Listeria* species is based on an octal code derived after adding the numerical values for each group of three tests and on the reactions obtained from the CAMP test and haemolysis characteristics, which are assayed separately.

ii) Vitek Automicrobic System

The Vitek Automicrobic System (bioMérieux Vitek, Inc., 595 Anglum Dr., Hazelwood, MO, USA) is an automated microbial identification system that can be used for the presumptive identification of food-borne *Listeria* species and for screening of non-*Listeria* isolates. It has been validated by the AOAC as method 992.19 (6). The system uses an incubator chamber with an optical reader, a filler/sealer unit for test kit inoculation and a computer. Gram-positive (GPI) and Gram-negative (GNI+) identification cards each contain 30 biochemical tests. Changes are analysed by the computer, which then assigns the test organism a genus and/or species. The identification of the *Listeria* species requires the use of the GPI card and two reactions on the GNI+ card. However, for identification of some Listeriae, the analyst must perform the CAMP, haemolysis and/or nitrate reduction tests as described under the FDA method.

Organisms placed in the ‘LM’ category are identified as *L. monocytogenes* or *L. innocua*; in the ‘LI’ category, as *L. ivanovii* or *L. seeligeri*; in the ‘LW’ category, as *L. welshimeri*; and in the ‘LG’ category, as *L. grayi* or *L. murrayi* (a subspecies of *L. grayi*). Organisms in the ‘O’ category are classified as non-*Listeria* species. Further tests should be conducted to identify the species within each category according to the FDA method.

Other commercially available methods for the identification of *Listeria* species include the API LISTERIA (bioMérieux), the MICROBACT 12L (Microgen), the MicroLog System (Biolog), the Sherlock Microbial Identification System (MIS) (Microbial ID; based on fatty acid patterns) and the Walk/Away System (MicroScan).

iii) Rapid immunological detection methods

A number of immunological methods have been developed to identify *L. monocytogenes* in foods and the following commercially available methods have been validated by one or more recognised formal validation systems (24, 58).

- **Colorimetric monoclonal enzyme-linked immunosorbent assay (Listeria-Tek)**

The Listeria-Tek is AOAC official method 994.03 (9) and it is intended for the detection of *Listeria* spp. in dairy products, seafood and meats. Because the monoclonal antibodies (MAbs) used in the test may cross react with other *Listeria* spp., the test is not confirmatory for *L. monocytogenes*.

The kit is commercially available from Organon Teknika Corp., 100 Akzo Ave, Durham, NC 27704, USA.

Enrichment cultures found positive by this method are streaked on selective media and suspect colonies are biochemically identified as *L. monocytogenes* according to the FDA method. A positive result is only valid when the positive and negative controls give acceptable absorbance readings.
• Colorimetric polyclonal enzyme immunoassay screening method (TECRA® Listeria Visual Immunoassay [TLVIA])

The TLVIA is AOAC official method 995.22 (10) and it is intended for the detection of Listeria spp. in dairy foods, seafood, poultry, meats (except raw ground meat), and leafy vegetables. An optimised version, with enrichment protocols for additional foods and with omission of the toxic antifungal agent cycloheximide, is AOAC official method 2002.09 (14), which is a screening procedure for the detection of Listeria spp. in raw meats, fresh produce/vegetables, processed meats, seafood, dairy foods cultured/noncultured, fruit and fruit juices.

The commercial kit is available from TECRA International Pty Ltd, P.O. Box 788, Willoughby, NSW, Australia.

Enrichment cultures that are positive must be inoculated on to selective media and suspect colonies identified according to the criteria specified under the FDA and USDA methods.

• Assurance® polyclonal enzyme immunoassay method

The Assurance® Listeria Enzyme Immunoassay is AOAC official method 996.14 (11) and it can be used for the detection of Listeria spp., including L. monocytogenes, in dairy foods, red meats, pork, poultry products, fruits, nutmeats, seafood, pasta, vegetables, cheese, bone meal, chocolate, environmental surfaces, and eggs.

Readings above the cut-off value are considered presumptive positive and the enriched cultures are then confirmed by culture and identification procedures as described under the FDA method.

The commercial kit is available from BioControl Systems, Inc., 12822 SE 32nd St., Bellevue, WA 98005, USA.

• Visual Immunoprecipitate assay (VIP™)

The VIP™ assay is AOAC official method 997.03 (12). It can be used for the detection of L. monocytogenes and other Listeria spp. in dairy foods, red meats, pork, poultry and poultry products, seafood, fruits, vegetables, nutmeats, pasta, chocolate, eggs, environmental surfaces and bone meal.

The test is performed with an enriched culture of the test samples. Presumptive positive tests must be confirmed by culture and identification procedures as described under the FDA method.

The VIP units are commercially available from BioControl Systems, Inc., 12822 SE 32nd St., Bellevue, WA 98005, USA.

• VIDAS LIS assay screening method

This enzyme-linked immunofluorescent assay (ELFA) is AOAC official method 999.06 (13). It has also been validated by the Association Française de Normalisation (AFNOR) and by the European Microbiological Methods Assessment Scheme (EMMAS) (15). It is used for screening dairy products, vegetables, seafood, raw meats and poultry, as well as processed meats and poultry, for Listeria spp. antigens.

This immunoassay is performed in the automated VIDAS® instrument. The computer compares this value with a standard and a positive or negative report is generated. Positive results must be confirmed by standard culture methods as described under the FDA method. An enrichment modification to this protocol, using demi Fraser and Fraser broths (AOAC official method 2004.06), was evaluated in a multilaboratory study and no significant difference was found in its performance when compared to that of AOAC official method 999.06 (59).

The VIDAS Listeria monocytogenes 2 (LMO 2) method includes specific MAbs to capture L. monocytogenes antigens and it has been validated by AFNOR, including its use with environmental samples.

The VIDAS system is available from bioMérieux, Inc., 595 Anglum Rd., Hazelwood, MO 63042, USA.

Other commercially available immunological methods that have been validated by formal systems include the VIDAS Listeria Species Xpress (bioMérieux), validated by AFNOR; the Transia Plate Listeria ELISA (Transia, Diffchamb Ltd), validated by AFNOR; the EIAFOSS Listeria automated ELISA (Foss Electric), validated by the AOAC Research Institute; the immunochromatographic method REVEAL for Listeria (Neogen Corporation), validated by the AOAC Research Institute; the immunochromatographic method Clearview Listeria Rapid Test (Oxoid), validated by AFNOR, EMMAS and the AOAC Research Institute and the immunomagnetic separation-based Listertest (Vicam), validated by the AOAC Research Institute (15).
Other commercially available immunologically based methods include the Transia Plate *Listeria monocytogenes* ELISA (Transia, Difchamb Ltd), the immunomagnetic separation-based Dynabeads anti-*Listeria* (Dynal Ltd), the *Listeria* UniQue™ ELISA (TECRA), the Microscreen *Listeria* latex agglutination test (Microgen BioProducts Ltd), and the *Listeria* Rapid Test EIA (Oxoid).

d) Nucleic acid recognition methods

A number of methods based on nucleic acid recognition have been developed to identify *L. monocytogenes* in foods. A few of them have been validated by one or more recognised formal validation systems and are commercially available (49). Novel target sequences for diagnostic purposes include the metalloprotease gene (LightCycler foodproof *Listeria monocytogenes* Detection Kit, Roche Applied Science), the *prfA* gene (54) and the *ssrA* gene (50) in a real-time PCR format.

i) GENE-TRAK *Listeria* assay

The GENE-TRAK *Listeria* assay is a colorimetric DNA hybridisation method for the detection of *Listeria* sequences, which has been validated by the AOAC as method 993.09 (7) for use with dairy products, meats and seafood. This assay has also been validated by AFNOR. Due to the possibility of encountering false-positive reactions, positive samples must be confirmed by standard cultural methods.

Test portions found positive by this DNA hybridisation assay must be confirmed by streaking a phosphate buffered saline growth suspension on a *Listeria* selective plate and continuing with biochemical identification of presumptive *Listeria* isolates as described under the FDA method.

The GENE-TRAK *Listeria* assay is commercially available from GENE-TRAK™ Systems, 94 South Street, Hopkinton, MA 01748, USA.

ii) BAX® System

The USDA-FSIS has adopted the PCR-based BAX® System (Qualicon) (64) as their screening method for *L. monocytogenes* in enriched meat and poultry samples. It reduces the report out time for true negative samples by 24 hours and reduces false-positive results, with a detection limit better that 1 cfu/g in a 25 g sample. All samples that are identified as presumptive positive for *L. monocytogenes* are then subject to cultural confirmation by the conventional method.

iii) GENE-TRAK test for *Listeria monocytogenes*

The GENE-TRAK test for *L. monocytogenes* (GENE-TRAK™ Systems) is a hybridisation probe-based method validated by AFNOR (15).

iv) Gen-Probe (AccuProbe®) *Listeria monocytogenes* confirmatory test

The Gen-Probe (AccuProbe®) *Listeria monocytogenes* Confirmatory Test (Gen-Probe) is another hybridisation probe-based method validated by AFNOR (15).

v) AD713 method

The FDA uses a *L. monocytogenes*: a combination of invasion-associated protein and haemolysin (hly) gene probes – AD713 method (33). This method combines the detection of the *L. monocytogenes* haemolysin (also called listeriolysin O) gene by use of the oligonucleotide probe AD13, and the detection of the invasion-associated protein gene by a synthetic probe, AD07. Both probes are used in combination (designated AD713) to avoid false-negative results because of 'silent' mutations in the gene (nucleotide changes that affect DNA probe binding but do not change the gene function). Positive samples must be confirmed with conventional procedures as described under the FDA method.

Other commercially available methods based on nucleic acid recognition include the Foodproof® *Listeria monocytogenes* PCR assay (Biotecon Diagnostics) and the PROBELIA™ (*L. monocytogenes*) PCR assay (Sanofi Diagnostics). The application of the real-time PCR as a quantitative detection method, specific for *L. monocytogenes*, has also been developed (18, 36) and it shows good potential for routine analytical use. The IQ-Check *Listeria monocytogenes* kit (for all food products and environmental samples), developed by Bio-Rad, and the GeneDisc (for all food, except milk products) developed by GeneSystems, France, are based on real-time PCR detection and have been validated by AFNOR (2).
**Table 2.** Some conventional and antibody-based commercial systems for rapid *Listeria* screening and confirmation

<table>
<thead>
<tr>
<th>Test</th>
<th>ID level</th>
<th>Principle</th>
<th>Approx. test time&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Company</th>
<th>Main use</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICRO-ID <em>Listeria</em></td>
<td><em>L. monocytogenes</em>/innocua complex</td>
<td>Enzyme reaction</td>
<td>24 hours</td>
<td>Organon Teknika</td>
<td>Confirmation</td>
</tr>
<tr>
<td>Vitek System</td>
<td><em>L. monocytogenes</em>/innocua complex</td>
<td>Biochemical tests</td>
<td>24 hours</td>
<td>bioMérieux</td>
<td>Confirmation</td>
</tr>
<tr>
<td>API <em>Listeria</em></td>
<td><em>L. monocytogenes</em></td>
<td>Biochemical tests</td>
<td>24 hours</td>
<td>bioMérieux</td>
<td>Confirmation</td>
</tr>
<tr>
<td>MicroLog System</td>
<td><em>L. monocytogenes</em></td>
<td>Carbon source substrates</td>
<td>4 or 24 hours</td>
<td>Biolog</td>
<td>Confirmation</td>
</tr>
<tr>
<td>MICROBACT 12L</td>
<td><em>L. monocytogenes</em></td>
<td>Carbohydrate use and micro haemolysis test</td>
<td>4–6 or 24 hours</td>
<td>Microgen</td>
<td>Confirmation</td>
</tr>
<tr>
<td>Sherlock Microbial Identification System (MIS)</td>
<td><em>L. monocytogenes</em>/innocua complex</td>
<td>Fatty acid patterns</td>
<td>90 minutes</td>
<td>Microbial ID</td>
<td>Confirmation</td>
</tr>
<tr>
<td>Microscreen</td>
<td><em>Listeria</em> spp.</td>
<td>Latex agglutination</td>
<td>1 minute</td>
<td>Microgen BioProducts</td>
<td>Confirmation</td>
</tr>
<tr>
<td>VIP <em>Listeria</em></td>
<td><em>Listeria</em> spp.</td>
<td>Immuno chromatography</td>
<td>Immuno chromatography</td>
<td>BioControl Systems</td>
<td>Screening</td>
</tr>
<tr>
<td>Dynabeads anti-Listeria</td>
<td><em>Listeria</em> spp.</td>
<td>Immunomagnetic separation</td>
<td>48–72 hours</td>
<td>Dynal</td>
<td>Screening</td>
</tr>
<tr>
<td>REVEAL for <em>Listeria</em></td>
<td><em>Listeria</em> spp.</td>
<td>Immuno chromatography</td>
<td>43 hours</td>
<td>Neogen</td>
<td>Screening</td>
</tr>
<tr>
<td>Clearview <em>Listeria</em> (Oxoid Listeria Rapid Test)</td>
<td><em>Listeria</em> spp.</td>
<td>Immuno chromatography</td>
<td>43 hours</td>
<td>Oxoid</td>
<td>Screening</td>
</tr>
<tr>
<td>Listertest</td>
<td><em>Listeria</em> spp.</td>
<td>Immunomagnetic separation</td>
<td>24–48 hours</td>
<td>Vicam</td>
<td>Screening</td>
</tr>
</tbody>
</table>

1: Adapted and expanded from ref. 15
2: When used for confirmation, the test time indicated is after enrichment and agar isolation

**Table 3.** Some ELISA commercial systems for rapid *Listeria* screening

<table>
<thead>
<tr>
<th>Test</th>
<th>ID level</th>
<th>Principle</th>
<th>Approx. test time&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Company</th>
<th>Main use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria</em> Tek</td>
<td><em>Listeria</em> spp.</td>
<td>ELISA</td>
<td>50 hours</td>
<td>Organon Teknika</td>
<td>Screening</td>
</tr>
<tr>
<td>TECRA <em>Listeria</em> Visual Immunoassay (TLVIA)</td>
<td><em>Listeria</em> spp.</td>
<td>ELISA</td>
<td>50 hours</td>
<td>TECRA</td>
<td>Screening</td>
</tr>
<tr>
<td>Assurance <em>Listeria</em> EIA</td>
<td><em>Listeria</em> spp.</td>
<td>ELISA</td>
<td>50 hours</td>
<td>BioControl Systems</td>
<td>Screening</td>
</tr>
<tr>
<td>VIDAS <em>Listeria</em> (LIS)</td>
<td><em>Listeria</em> spp.</td>
<td>ELISA</td>
<td>50 hours</td>
<td>bioMérieux</td>
<td>Screening</td>
</tr>
<tr>
<td>VIDAS <em>Listeria</em> monocytogenes (LMO)</td>
<td><em>L. monocytogenes</em></td>
<td>ELISA</td>
<td>50 hours</td>
<td>bioMérieux</td>
<td>Screening</td>
</tr>
<tr>
<td>Transia Plate <em>Listeria</em></td>
<td><em>Listeria</em> spp.</td>
<td>ELISA</td>
<td>50 hours</td>
<td>Diffchamb</td>
<td>Screening</td>
</tr>
<tr>
<td>Transia Plate <em>Listeria</em> monocytogenes</td>
<td><em>L. monocytogenes</em></td>
<td>ELISA</td>
<td>50 hours</td>
<td>Diffchamb</td>
<td>Screening</td>
</tr>
<tr>
<td>EIAFOSS <em>Listeria</em></td>
<td><em>Listeria</em> spp.</td>
<td>Automated ELISA</td>
<td>48 hours</td>
<td>Foss Electric</td>
<td>Screening</td>
</tr>
</tbody>
</table>

1: Adapted and expanded from ref. 15
Table 4. Some molecular commercial systems for rapid Listeria screening and confirmation

<table>
<thead>
<tr>
<th>Test ID level</th>
<th>Principle</th>
<th>Approx. test time</th>
<th>Company</th>
<th>Main use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen-Probe (AccuProbe)</td>
<td>L. monocytogenes Nucleic Acid Hybridization Probe</td>
<td>30 minutes</td>
<td>Gen Probe</td>
<td>Confirmation</td>
</tr>
<tr>
<td>VIT Listeria Kit</td>
<td>L. monocytogenes/ Listeria spp. Nucleic acid Hybridisation probe</td>
<td>3 hours</td>
<td>Vermicon</td>
<td>Confirmation</td>
</tr>
<tr>
<td>Foodproof Listeria monocytogenes</td>
<td>L. monocytogenes PCR</td>
<td>48 hours</td>
<td>Biotec Diagnostics</td>
<td>Screening</td>
</tr>
<tr>
<td>Gene Trak Listeria Assay</td>
<td>Listeria spp. Nucleic acid Hybridisation probe</td>
<td>50 hours</td>
<td>Gene Trak</td>
<td>Screening</td>
</tr>
<tr>
<td>Gene Trak test for L. monocytogenes</td>
<td>L. monocytogenes Nucleic acid Hybridisation probe</td>
<td>50 hours</td>
<td>Gene Trak</td>
<td>Screening</td>
</tr>
<tr>
<td>BAX for screening L. monocytogenes</td>
<td>L. monocytogenes PCR</td>
<td>48 hours</td>
<td>Qualicon</td>
<td>Screening</td>
</tr>
<tr>
<td>BAX for screening Listeria Genus</td>
<td>Listeria spp. PCR</td>
<td>48 hours</td>
<td>Qualicon</td>
<td>Screening</td>
</tr>
<tr>
<td>PROBELIA</td>
<td>L. monocytogenes PCR</td>
<td>48 hours</td>
<td>Sanofi Pasteur</td>
<td>Screening</td>
</tr>
<tr>
<td>LightCycler foodproof Listeria monocytogenes Detection Kit</td>
<td>L. monocytogenes Real Time PCR</td>
<td>65 minutes³</td>
<td>Roche Applied Science</td>
<td>Screening</td>
</tr>
<tr>
<td>LightCycler foodproof Listeria Detection Kit</td>
<td>Listeria spp. Real Time PCR</td>
<td>75 minutes³</td>
<td>Roche Applied Science</td>
<td>Screening</td>
</tr>
</tbody>
</table>

1: Adapted and expanded from ref. 15
2: When used for confirmation, the test time indicated is after enrichment and agar isolation
3: After enrichment culture

e) Subtyping methods

Regulatory identification of *L. monocytogenes* does not require any specific subtyping of the isolates. However, subtyping schemes can be useful in outbreak investigations, environmental tracking and public health surveillance.

*L. monocytogenes* can be subtyped by a number of different approaches including serotyping, phage typing, multilocus enzyme electrophoresis (MEE), DNA restriction enzyme analysis (either using high-frequency cutting enzymes and conventional gel electrophoresis to separate fragments, or using rare-cutting enzymes and pulse-field gel electrophoresis [PFGE] to separate fragments), nucleic acid sequencing-based typing, microarray analysis, amplified intergenic locus polymorphism (AILP), and random amplification of polymorphic DNA (RAPD).

Because of the requirement for specific reagents, stringent quality assurance procedures and some sophisticated equipment, it is recommended that subtyping of *L. monocytogenes* isolates be referred to the appropriate reference centre. A list of international centres and subtyping methods can be found in ref. 16 (pp. 258–259)

i) Serotyping

Strains of *Listeria* can be assigned to 13 different serotypes, based on their combination of somatic (O) and flagellar (H) antigens. Although all of them are considered to be potentially pathogenic, most (>95%) human clinical isolates belong to three serotypes 1/2a, 1/2b, and 4b. Compared with other subtyping methods, serotyping has poor discriminatory power, but can provide valuable information to facilitate the ruling out of isolates that are not part of an outbreak. Isolates from foods and from environmental sources are frequently nontypable with standard typing antisera.

ii) Phage typing

Bacteriophage typing is a technique with very good discriminatory power that can be used to subtype a large number of isolates. However, the current available phage sets are unable to type a high proportion of strains
repetitive extragenic palindromes (REP), are the best-characterised family of repetitive bacterial sequences. Short repetitive sequence elements are widely distributed among bacteria and palindromic units, known as REPs, can ‘crawl’ through the agarose matrix and are separated according to size differences. This technique is known as restriction fragment length polymorphism (RFLP) analysis. When ribosomal RNA/DNA probes are used, only the particular restriction fragments associated with the chromosomal loci for rRNA are detected. This technique is known as ribotyping and it has been widely used for subtyping L. monocytogenes, mainly through the use of the restriction endonuclease EcoRI. However, the technique was found to be less discriminating than phage typing, REA or MEE. Qualicon has designed an automated ribotyping system, the RiboPrinter, which generates, analyses and stores riboprint patterns of bacteria, including Listeria.

When restriction endonuclease enzymes that cut infrequently are used to digest unsheared chromosomal DNA, such as Apal, Smal, NofI and Ascl, very large fragments are obtained. Because of their size, these large fragments do not separate when run under conventional agarose gel electrophoresis. However, by periodically changing the orientation of the electric field across the gel, through pulses, the large fragments can ‘crawl’ through the agarose matrix and are separated according to size differences. This technique is known as pulsed-field gel electrophoresis (PFGE) and has revolutionised the precise separation of DNA fragments larger than 40 kilobases. PFGE has been applied to the subtyping of L. monocytogenes and has been found to be a highly discriminating and reproducible method. PFGE is particularly useful for subtyping L. monocytogenes serotype 4b isolates, which are not satisfactorily subtyped by most other subtyping methods. The main disadvantages of PFGE are the time required to complete the procedure (2–3 days), the large quantities of expensive restriction enzymes required, and the need for specialised, expensive equipment (31). The Centers for Disease Control and Prevention (CDC) in the United States of America has established PulseNet, a network of public health and food regulatory laboratories that routinely subtype food-borne pathogenic bacteria by PFGE. PulseNet laboratories use highly standardised protocols and can quickly compare PFGE patterns from different locations via the Internet. Listeria monocytogenes was added to PulseNet in 1999 (63).

Restriction endonuclease analysis (REA) of chromosomal DNA is a useful subtyping method for L. monocytogenes. As these enzymes are highly specific in recognising nucleotide sequences, the resulting DNA digestion fragments, of different size and electrophoretic mobility, reflect genomic differences, resulting in specific ‘fingerprints’ among otherwise related strains. Because of the restriction endonuclease specificity, the method is highly reproducible. Of the restriction endonucleases tested on L. monocytogenes in a World Health Organization (WHO) Multicentre study, HaeIII, HhaI and CfoI were the most useful (31). However, because of a potentially large number of enzyme recognition sites in the bacterial genome, sometimes complex fingerprints evolve, with overlapping or poorly resolved bands that are difficult to interpret. The technique is therefore not adequate for comparing a large number of strain patterns or for building dynamic databases (31).

By combining REA with Southern hybridisation, using chromosomal-labelled probes, only the particular restriction fragments associated with the corresponding chromosomal loci are detected, thereby significantly reducing the number of DNA fragments to be analysed. This technique is known as restriction fragment length polymorphism (RFLP) analysis. When ribosomal RNA/DNA probes are used, only the particular restriction fragments associated with the chromosomal loci for rRNA are detected. This technique is known as ribotyping and it has been widely used for subtyping L. monocytogenes, mainly through the use of the restriction endonuclease EcoRI. However, the technique was found to be less discriminating than phage typing, REA or MEE. Qualicon has designed an automated ribotyping system, the RiboPrinter, which generates, analyses and stores riboprint patterns of bacteria, including Listeria.

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Although there have been some reports on the sequence analysis of single genes as a means to type L. monocytogenes strains, determination of allelic variation of multiple genes, has been recently introduced as a very promising subtyping methodology for this microorganism. This approach has been reported for a handful of other microorganisms and it is known as multi locus sequence typing (MLST) (62). Direct amplification and nucleotide sequencing (19, 56), as well as an alternative approach that targets the variable genetic changes directly in a DNA array format (55) have both been used with good discrimination between the strains analysed. Because MLST is based on nucleotide sequence, it is highly discriminatory and provides unambiguous results.

Short repetitive sequence elements are widely distributed among bacteria and palindromic units, known as repetitive extragenic palindromes (REP), are the best-characterised family of repetitive bacterial sequences. REP elements are present in L. monocytogenes and a PCR based on the sequence of these elements (rep-
PCR) has been used successfully to subtype strains of the organism. The four major strain clusters identified by this method matched the origin of their isolation (43).

vi) Random amplification of polymorphic DNA

When arbitrarily selected primers are used in the PCR under low stringency conditions, with chromosomal L. monocytogenes DNA as a template, amplicon patterns are generated that are useful for subtyping strains. RAPD is a viable alternative to phage typing and is highly discriminating. However, despite its relative simplicity and discriminating ability, its main drawback is the inconsistent reproducibility of patterns. The low stringency conditions for primer annealing results in polymerisation with various efficiencies, and therefore the quantities of DNA produced may be widely variable among the different amplicons from a given isolate, which makes it difficult to compare and interpret the RAPD patterns. The technique requires a great deal of standardisation and consistency to obtain reliable results (31).

Based on the results of the WHO multicentre L. monocytogenes subtyping study (27), which compared several different subtyping methods using a well defined set of isolates, serotyping, phage typing, REA, PFGE and RAPD were selected for standardisation in Phase II. This effort should eventually result in a selected set of standardised L. monocytogenes subtyping methods (31).

2. Serological tests

Serological tests for the detection of antibodies have not been traditionally used for the diagnosis of listeriosis. They have been largely unreliable, lacking sensitivity and specificity. A number of formats, including ELISA, complement fixation and microagglutination have been largely unsuccessful in the diagnosis of culture-proven human listeriosis, even in the absence of immunosuppression. Considerable cross-reactivity with antigenic determinants of other Gram-positive organisms has been observed. On the other hand, L. monocytogenes is a ubiquitous organism, and regular exposure of animals and humans to this microorganism is very common. Many healthy individuals are intestinal carriers (2–6%) and anti-L. monocytogenes serum antibody prevalence as high as 53% have been reported in humans. Carriage rate for animals is similar to that of humans, with some differences depending on the species and a little higher rate during indoor season, as compared to animals on pasture (37, 38).

The discovery that the L. monocytogenes haemolysin, listeriolysin O (LLO), is a major virulence factor and that it can stimulate an antibody response, has recently renewed interest in the possibility of using serological tests for the diagnosis of listeriosis, particularly in central nervous system patients, with sterile blood and cerebrospinal fluid, and in perinatal listeriosis. An indirect ELISA based on the detection of anti-LLO was used for the diagnosis of experimental listeriosis in sheep (47). However, LLO is antigenically related to a number of cytolysins, including streptolysin O (SLO) from Streptococcus pyogenes, pneumolysin from S. pneumoniae and perfringolysin from Clostridium perfringens. Problems of cross-reactivity of anti-LLO antibodies with these cytolysins, particularly SLO and pneumolysin, have hampered the development of specific reliable serological tests based on the detection of anti-LLO antibodies. In addition, anti-LLO antibodies have been found in a proportion of healthy individuals and patients with other bacterial, fungal or viral infections (27%, all combined), although at lower titres than in patients with listeriosis. Absorption of diagnostic antisera with SLO is only partially effective in eliminating all cross-reactivity. These experimental assays have been used in some epidemiological investigations and as support for the diagnosis of culture-negative central nervous system infections. Recombinant forms of LLO have been explored as alternatives to wild LLO as a diagnostic antigen in Western blot assays. This is currently an evolving field and we will have to wait for the development of reliable, validated serological tests for the diagnosis of listeriosis.

Immunohistochemical detection of L. monocytogenes antigens in formalin-fixed tissue has proven to be more sensitive than direct plating and cold enrichment bacterial culture for the diagnosis of the encephalitic form of the disease in ruminants (20, 44).

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

It has proven very difficult to develop effective vaccines against L. monocytogenes which, as an intracellular organism, requires effector T cells for an effective immune response. Experimental vaccines in laboratory animals are being explored to confer protection to L. monocytogenes infection by a number of different approaches, but these are still far from becoming available for human and farm animal use. These experimental approaches include immunisation with plasmid DNA, CD40 signalling along with heat-killed L. monocytogenes, LLO-deficient mutants inoculated along with liposome-encapsulated LLO, and immunisation with listerial antigens and IL-12.

Genetically modified L. monocytogenes is also being considered as an effective vaccine vector for the expression, secretion and intracellular delivery of foreign antigens for the induction of potent immune responses against viral antigens and tumour cells.
However, the most feasible and practical means to reduce the risk of listeriosis in humans is through dietary and food preparation measures that not only decrease the risk of acquiring listeriosis, but also contribute to the prevention of other common food-borne infections such as those caused by *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter*. These preventive measures include thorough cooking of raw food of animal origin, keeping uncooked meats separate from vegetables, cooked foods, and ready-to-eat foods, thoroughly washing raw vegetables before eating, washing hands, knives, and cutting boards after handling uncooked foods, and avoiding unpasteurised milk or products made from it. Immunocompromised persons, pregnant women and other groups at increased risk of listeriosis should avoid foods that have been epidemiologically linked to this disease, e.g. soft cheeses and pâté. These individuals should also avoid other ready-to-eat foods, unless they are heated until steaming hot before being consumed.

The food industry and public health agencies play a pivotal role in the prevention of food-borne listeriosis by developing and implementing effective HACCP programmes to reduce the presence of *L. monocytogenes* at all critical points in the food production and distribution chain (from the farm to the market).

Likewise, the lack of well designed and tested vaccines for animal use, means that control of listeriosis in animals is most feasible by preventing the environmental conditions that favour its presentation. There is a well-established linkage between silage feeding and listeriosis and, as *L. monocytogenes* is widely distributed in nature, with animals and birds acting as carriers, contamination of silage is not uncommon. Emphasis should therefore be placed on reducing the likelihood of the multiplication of the organism, which occurs more frequently at pH values greater than 5, particularly where ineffective fermentation has occurred and where there is concomitant growth of moulds. Every effort should be made to produce silage of good quality, with early cutting of grass, minimal contamination with soil or faeces and ensuring optimal anaerobic fermentation, which will insure that the pH falls below 5.0; at that level, growth of *Listeria* spp. is inhibited. The best silage for feeding should be selected, especially in the case of sheep, discarding material that has obvious signs of contamination with mould. Material a few centimetres from the top, front and sides of an opened bale or bag, should also be discarded. Leftover silage should be removed (48).

**REFERENCES**


---

1 AFAQ: Association française pour le management et l’amélioration de la qualité
2 AFNOR: Association Française de Normalisation
3 AOAC: Association of Official Analytical Chemists
Chapter 2.9.7. - Listeria monocytogenes

Methods of Analysis of AOAC INTERNATIONAL, Volume I, Agricultural Chemicals; Contaminants; Drugs, Horwitz W., ed. AOAC INTERNATIONAL, Gaithersburg, MD, USA, 138–141.


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