

Advances in cultural methods for the detection of *Listeria monocytogenes*

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INTRODUCTION

One of the immediate outcomes of the identification of food as an important epidemiological factor in outbreaks of epidemic listeriosis has been heightened activity to develop improved methods for the detection and enumeration of *Listeria monocytogenes* in foods. Many of the methods available prior to the recent outbreaks were developed for veterinary and medical uses. One of the important differences between medical microbiology and food safety microbiology is the orientation of their methodologies. Typically, clinical microbiologists deal with large numbers of an organism, which is often growing in almost pure culture under essentially ideal conditions. The clinician's primary need is to correctly identify the isolate in the shortest time possible. Alternatively, food microbiologist focus on determining numbers of specific pathogens that may be present in foods in the shortest time possible. This routinely involves detecting very low numbers of the pathogen, often in the presence of millions of other bacteria. The process is often confounded further due to the foods having been subjected to conditions that sublethally stress the target microorganisms. Considering these differences, it is not surprising that the methods used by medical microbiologists were not always directly applicable to the needs of food microbiologists. Some of the spe-

cific problems that are being addressed by various research teams include (1) enhancing selectivity of enrichment and isolation media, (2) improving the ability to detect low numbers of *L. monocytogenes*, (3) shortening assay times, and (4) assuring the detection of sublethally stressed cells.

The classical approach to the identification of bacteria is cultural. This approach involves subjecting samples to a series of tests designed to isolate and identify microorganisms possessing a profile of specific phenotypic characteristics indicative of the species of interest. Such analyses typically include evaluations of structure (e.g. microscopic examination), specific metabolic characteristics or products (e.g. Voges Proskauer reaction), environmental limitations (e.g. temperature ranges), nutritional requirements (e.g. carbohydrate utilization), and resistance to antimicrobials. When applied to the identification of low levels of foodborne pathogenic bacteria such as *L. monocytogenes*, a classical microbiological analysis consists of three overlapping phases; enrichment, isolation, and confirmation.

The first phase, enrichment, is designed to increase the relative numbers of the target species. This is achieved by exposing samples to a set of selective conditions or agents that prevent the growth of other bacteria while still permitting the target species to thrive. The enrichment phase can be most appropriately viewed as an amplification step. The

second phase, isolation, encompasses one or more steps where the organism is presumptively identified. This typically involves the use of one or more plating media that incorporate one or more selective and/or differential agents or conditions. The selective agents are again used to suppress other microorganisms, while differential agents supply a positive identification of the target species based on specific physiological or cultural characteristics. For example, *L. monocytogenes*' resistance to antibiotics such as nalidixic acid is used as a selective factor, whereas its blue-gray color when viewed with reflected light is a differential factor. As the name implies, the final phase involves confirmation of the identity of the presumptive isolates. This entails performing a series of tests that are the basis of a taxonomic designation at the species level.

The objective of the current presentation was to review cultural methods for the enrichment and isolation of *L. monocytogenes*. While a historical perspective is included, new methods developed specifically for the evaluation of food products are

highlighted. The examples cited are not intended to be inclusive, but provide an overview of the approaches that have been employed. A short discussion of confirmation methods is also included.

ENRICHMENT PROCEDURES

The classic method for isolating *L. monocytogenes* from biological samples is cold enrichment which entails incubating samples at 4°C for up to 12 weeks in a suitable non-selective medium [13]. This has become the standard against which other enrichment techniques are evaluated; however, the long incubation times generally preclude it from having much significance in relation to practical food microbiology. Accordingly, emphasis has been placed on identifying effective direct enrichment. Examples of enrichment formulations are presented in Table 1.

A variety of selective agents have been used by various investigators for *Listeria* enrichment broths; however, nalidixic acid has been a common

Table 1

Formulations of various enrichment media for detection of *Listeria monocytogenes*

Mavrothalasistitis enrichment broth [21]		Thiocyanate-nalidixic acid enrichment broth [30]	
Todd-Hewitt broth base	30 g	Nutrient broth base	8 g
Potassium dichromate	0.83 g	Potassium thiocyanate	37.5 g
Chromium trioxide	200 mg	Nalidixic acid	100 mg
Thionin (0.25%) in		Water	1000 ml
Glycerol	20 ml		
Nalidixic acid	100 mg		
Amphotericin B	3000 IU		
Water	980 ml		
FDA enrichment broth [18]		Doyle and Schoeni enrichment broth [7]	
Trypticase soy broth	30 g	Tryptose broth base	9 g
Base		Glucose	5 g
Yeast extract	6 g	Dipotassium phosphate	1.5 g
Acriflavin HCl	15 mg	Polymixin B	16 000 IU
Nalidixic acid	40 mg	Acriflavin HCl	12 mg
Cycloheximide	50 mg	Nalidixic acid	40 mg
Water	1000 ml	Blood	50 ml
		Water	950 ml

Table 1. Continued.

Rodriguez enrichment broth [24]

Tryptone	5 g
Lab-Lemco powder	5 g
Yeast extract	5 g
Glucose	5 g
Esculin	1 g
Sodium chloride	20 g
Na ₂ PO ₄ ·H ₂ O	24 g
Potassium phosphate, monobasic	1.35 g
Ferric ammonium citrate	1 g
Trypan blue	40 mg
Nalidixic acid	30 mg
Agar	3 g
Water	1000 ml

Fraser enrichment broth [9]

UVM-LEB base	52 g
Lithium chloride	3 g
Acriflavin HCl	12 mg
Ferric ammonium citrate	0.5 g
Water	1000 ml

UVM *Listeria* enrichment broth [6]

Proteose peptone	5 g
Tryptone	5 g
Lab-lemco powder	5 g
Yeast extract	5 g
Sodium chloride	20 g
Na ₂ PO ₄ ·H ₂ O	12 g
Potassium Phosphate, monobasic	1.35 g
Esculin	1 g
Nalidixic acid	40 mg
Acriflavin HCl	12 mg
Water	1000 ml

Buffered TPB-claforan-acriflavin enrichment broth [4]

Tryptose phosphate broth base	29.5 g
Disodium phosphate	7.1 g
Monosodium phosphate	1.35 g
Claforan	8 mg
Acriflavin HCl	12 mg
Water	1000 ml

ingredient in a large percentage of the formulations [6–9, 14, 16, 18, 21, 24, 30]. Mavrothalassitis [21] reported that nalidixic acid in conjunction with chromium salts, thionin, and amphotericin B was effective for the isolation of *Listeria* from environmental samples. The medium's primary limitation was the growth of enterococci such as *Streptococcus faecalis* which were equally resistant and generally more competitive. Watkins and Sleath [30] used naladixic acid and potassium thiocyanate as the basis for an enrichment broth. They reported that this combination was effective if employed as a secondary enrichment following primary cold enrichment, but was not particularly effective when used alone. Rodriguez et al. [24] evaluated several enrichment formulations, recommending a broth containing nalidixic acid and trypan blue as its selective agents. This formulation also included esculin and ferric ammonium citrate which allowed detection of esculin hydrolyzing *Listeria* by the presence of a black

precipitate. However, such a reaction also would occur with enterococci which would be resistant to the selective agents.

A second selective agent that has been used extensively in combination with nalidixic acid is acriflavin. Doyle and Schoeni [7] employed these antimicrobials and polymyxin B as the selective agents in their enrichment medium, which was used in conjunction with microaerophilic incubation. Lovett et al. [18] utilized acriflavin and nalidixic in combination with cycloheximide to suppress fungal growth as the selective basis of the FDA Enrichment Broth. Donnelly and Baigent [6] developed UVM *Listeria* Enrichment Broth through modification of Rodriguez Enrichment Broth [24] by substituting acriflavin for trypan blue and eliminating the glucose and ferric ammonium citrate. Lee and McClain [15] used UVM *Listeria* Enrichment Broth as the basis of their two step enrichment protocol wherein the first stage employed the original formulation and

the second stage employed a higher level of acriflavin (25 mg/l). However, the efficacy of this step-increase in acriflavin levels has yet to be clearly demonstrated. It could be expected that interfering organisms that were selected by the initial level of acriflavin would also be resistant to the higher level of the antimicrobial. This could be a particular problem if the final isolation medium also contained acriflavin as a primary selective agent. Ideally, completely different sets of selective agents should be used for each step of the enrichment and isolation procedure, thereby maximizing the selection process.

Based on our experience, none of the enrichment components and formulations listed above is totally satisfactory in regard to interfering species. Periodically we have encountered highly resistant *S. faecalis* and other enterococci that have been particularly troublesome, and can greatly impact the effectiveness of *L. monocytogenes* detection. We have also encountered resistant strains of *Staphylococcus aureus* and *Kurthia* spp. that have come through the enrichment process.

Recently, Fraser and Sperber [9] used a two stage enrichment approach as a means of screening for *Listeria*. After a 24-h initial enrichment in UVM Listeria Enrichment Broth, samples are transferred to a modified UVM broth which included lithium chloride to help suppress enterococci and ferric ammonium citrate for visualization of esculin hydrolysis. Samples are incubated in Fraser Enrichment Broth for 24 h and then examined for the black precipitate indicative of esculin utilization. Fraser and Sperber [9] reported that this technique yielded no false negatives when employed for the analysis of a large number of dairy and environmental samples, and recommended it for the rapid clearing of negative samples. The method's primary shortcoming was a very high rate of false positives (@ 18%), largely due to enterococci. This method, like the rest of the enrichment techniques would benefit greatly from the identification of a selective agent that suppresses enterococci while permitting *Listeria* to thrive. This screening technique may prove very helpful for performing quantitative enrichment procedures, which require use of MPN tech-

niques. Employing MPN protocols increases the complexity of analyses by at least nine-fold (for a 3-tube MPN), but is the only effective way of obtaining quantitative data when the level of *L. monocytogenes* is in the below approximate 20–50 cfu/g. Buchanan et al. [3] concluded that unless there was an overriding reason for acquisition of quantitative values, the complexity of the MPN analyses greatly overshadows the usefulness of the data generated in comparison to \pm enrichment protocols.

Our laboratory considers the effectiveness of available enrichment formulations to be one of the most pressing methodological problems currently limiting the detection of *L. monocytogenes* in foods. As should be evident from the preceding discussion, the primary problem is controlling the growth of resistant group D streptococci. We are currently working to develop an improved enrichment system for use with our Modified Vogel Johnson Agar [2]. Preliminary results indicate that buffered Tryptose Phosphate Broth with the antimicrobials, acriflavin and claforan, may be an effective enrichment system [4]. However, we are still not totally satisfied with its ability to control resistant enterococci strains and are still looking for an additional agent(s) to achieve that goal.

ISOLATION MEDIA

After the numbers of a target microorganism has been increased by an enrichment step or if the initial level in the food sample was large enough so that enrichment was not needed, the next phase of a microbiological analysis is presumptive isolation. This is generally done through the use of one or more selective and/or differential plating media. In the case of *L. monocytogenes*, a number of plating media have been developed and used over the years with varying degrees of success [1, 2, 5, 8, 10, 15, 18–24, 26, 28, 29]. Examples of some of these formulations are presented in Table 2.

A medium that has served as the basis for a number of other formulations was McBride's *Listeria* Agar [22]. This medium incorporates phenylethanol, lithium chloride, and glycine as selective

Table 2

Formulations of various plating media used for the isolation of *Listeria monocytogenes*

McBride Listeria agar [22]		Modified McBride agar [18]	
Tryptose	10 g	Phenylethanol agar base	35.5 g
Sodium chloride	5 g	Glycine anhydride	10 g
Beef extract	3 g	Lithium chloride	0.5 g
Agar	15 g	Cycloheximide	200 mg
Phenylethanol	2.5 g	Water	1000 ml
Lithium chloride	0.5 g		
Glycine	10 g		
Blood	50 ml		
Water	980 ml		
Mavrothalassitis Listeria agar [21]		Fenlon Listeria agar [8]	
Trypticase soy agar base	40 g	Blood agar base	44 g
Gallocyanin	50 mg	Nalidixic acid	40 mg
Pyronin	5 mg	Acridine	25 mg
Nalidixic acid	50 mg	Blood	25 ml
Water	1000 ml	Water	950 ml
Rodriguez Listeria agar [24]		Modified Despierres agar [10]	
Peptone	3 g	Brain heart infusion broth base	37 g
Neopeptone	5 g	Peptone	10 g
Proteose peptone	3 g	Sodium chloride	5 g
Esculin	1 g	Rhamnose	1 g
Sodium chloride	5 g	Methylene blue	10 mg
Disodium phosphate, monohydrate	12 g	Nalidixic acid	40 mg
Ferric ammonium citrate	1 g	Polymixin B	16 000 IU
Nalidixic acid	40 mg	Acridine HCl	15 mg
Acridine HCl	12 mg	Water	1 000 ml
Agar	15 g		
Blood	50 ml		
Water	950 ml		
Lithium chloride-phenylethanol-moxalactam (LPM) agar [15]		Acridine-ceftazidime agar [1]	
Phenylethanol agar base	35.5 g	Columbia agar base	44 g
Glycine anhydride	10 g	Acridine HCl	10 mg
Lithium chloride	5 g	Ceftazidime Pentahydrate	50 mg
Moxalactam	20 mg	Water	1000 ml
Water	1000 ml		

Table 2. Continued.

Modified McBride agar with lactose and indicator [9]

Phenylethanol agar base	35.5 g
Glycine anhydride	10 g
Dipotassium phosphate	2.5 g
Lithium chloride	0.5 g
Lactose	5 g
Cycloheximide	200 mg
Bromothymol blue	62 mg
Water	1000 ml

Rapamy agar [28]

Columbia agar base	39 g
Nalidixic acid	40 mg
Acridine HCl	10 mg
Cefoxitin	20 mg
Esculin	0.5 g
Ferric ammonium citrate	0.5 g
Phenylethanol	2.5 g
Glucose	1 g
Mannitol	10 g
Phenol red	80 mg
Egg yolk emulsion	25 ml
Water	975 ml

Gray's tellurite agar [12]

Tryptose agar base	41 g
Potassium tellurite	0.5 g
Water	1000 ml

Acridine-nalidixic acid-*R. equi* factor agar [26]

Columbia agar base	44 g
Acridine HCl	10 mg
Nalidixic acid	40 mg
Rhodococcus equi factor	7 500 units
Blood	50 ml
Water	950 ml

Modified vogel Johnson agar [2]

Vogel Johnson agar base	60 g
Nalidixic acid	50 mg
Bacitracin	20 mg
Moxalactam	20 mg
Potassium tellurite	200 mg
Water	1000 ml

agents. Blood is also incorporated to identify hemolytic *Listeria*, a characteristic that is associated with pathogenicity. The medium's primary limiting factor is overgrowth with resistant staphylococci and streptococci. Lovett et al. [18] developed Modified McBride Agar by eliminating blood, adding cycloheximide to suppress eucaryotic microorganisms, and substituting glycine anhydride for glycine. A number of investigators have indicated that glycine anhydride is a more effective selective agent than glycine. Differentiation of *Listeria* colonies on this medium is based on observation of a characteristic blue to blue-gray color when the plates were illuminated with obliquely reflected light (Fig. 1). This method has been used with a number of media for differentiation of *Listeria* colonies. However, a number of investigators have found it not to be a

totally effective means of differentiation, particularly when analyzing foods with high levels of interfering microorganisms.

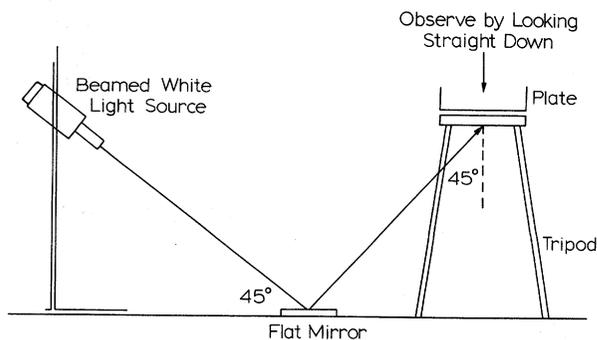


Fig. 1. Apparatus for viewing colonies of *Listeria monocytogenes* with obliquely reflected light. (Adapted from Lovett [17].)

Like many of the *Listeria* enrichment broths, a number of plating media have used nalidixic acid and/or acriflavin as selective agents. Mavrothlassitis [21] developed an isolation agar using nalidixic acid, gallocyanin, and pyronin as selective agents. Rodriguez et al. [24] used a combination of acriflavin and nalidixic acid to suppress other bacteria, and coupled this to esculin hydrolysis (esculin + ferric ammonium citrate) and hemolytic activity (blood) as means of differentiation. Fenlon [8] also used hemolytic activity as a differentiating agent and nalidixic acid and acriflavin as selective agents. Skalka and Smola [26] used this combination of agents and included cell-free supernatant from cultures of *Rhodococcus equi*, one of the two species used for the *Listeria* CAMP test. In the CAMP test, an extracellular product of *R. equi* enhances specifically the hemolytic activity of *L. invanovii*; however, Skalka and Smola [26] reported that its inclusion into the plating medium also enhanced the detection of weakly hemolytic *L. monocytogenes*. Modified Despierres *Listeria* Agar [10] uses acriflavin and nalidixic acid in combination with polymyxin B and methylene blue as selective agents. It also incorporates rhamnose as a primary carbohydrate source to foster the growth of *L. monocytogenes*. Differentiation was again based on observation of colony color under obliquely reflected light.

More recently investigators have been exploring the use of various cephalosporins as selective agents. Lee and McClain [15] further modified Modified McBride *Listeria* Agar by using moxalactam (cefoxitin) as a selective agent. Differentiation of presumptive *Listeria* colonies relies on viewing colonies after 20–24 h with a microscope, using obliquely reflected light. Past experience in our laboratory has indicated that while the medium is highly selective, but can still periodically have problems with enterococci and occasional resistant isolates of *Staphylococcus* and *Kurthia*. We have also found the method of differentiation to be less than ideal in that it is not likely that the characteristic blue color will be evidenced by all the *Listeria* colonies on a plate, which precludes its use for direct quantitative determinations. Further, the use of the microscope tends to become tedious. More recently,

Bannerman and Bille [1] introduced Acriflavin-Ceftazidime Agar. They reported that ceftazidime was more effective than moxalactam, though this medium was still limited by growth of resistant strains of enterococci. Differentiation was again based on use of obliquely reflected light.

While the use of obliquely reflected light classically has been used for differentiation of *Listeria*, this technique is often difficult to use effectively, tends to be subjective, and does not lend itself well for quantitative analyses. Accordingly, a number of investigators have been working towards the development of isolation media that rely on other means of differentiation. Van Netten et al. [28] developed RAPAMY Agar which incorporates nalidixic acid, acriflavin, and phenylethanol as selective agents. The plates were incubated under a microaerophilic atmosphere to retard the growth of aerobes. Van Netten et al. [28] also indicated that moxalactam could also be added to further increase selectivity, though they indicated that the antimicrobial did not significantly improve the medium's performance. Differentiation is based on mannitol utilization and esculin hydrolysis, with mannitol + phenol red and esculin + ferric ammonium citrate being added as indicators, respectively. *L. monocytogenes* colonies are characteristically surrounded by a black halo on a red background, whereas enterococci appear on a yellow background surrounded by a green-blue halo. Van Netten et al. [28] reported that as long as the level of enterococci was not excessively greater than *L. monocytogenes*, the two microorganisms can be differentiated. More recently Van Netten et al. [29] developed modified versions that incorporated lithium chloride or fosfomycin to suppress enterococci.

McDonald et al. [19] altered Modified McBride Agar by buffering it to pH 7.7 and adding lactose plus a pH indicator (bromthymol blue). Lactose-negative *L. monocytogenes* appeared as blue-green colonies, whereas the colonies of lactose-fermenting enterococci were yellow to yellow-orange. McDonald et al. [19] cautioned that only filter-sterilized lactose low in glucose be used with the medium. Some clarification of this medium is needed in regard to employing lactose utilization as a differen-

tial characteristic. In the latest edition of Bergey's Manual, Seeliger and Jones [25] indicated that a portion of *L. monocytogenes* strains are capable of utilizing the sugar.

Our laboratory has also worked on the development of an isolation medium with enhanced differentiation capabilities. The medium, Modified Vogel Johnson Agar [2], includes glycine, elevated levels of lithium chloride; nalidixic acid, bacitracin and moxalactam as selective agents. Additionally, the medium includes mannitol as a differential agent and potassium tellurite as both a selective and differential agent. Tellurite was used initially by Gray [12] as a differential/selective agent, but was discon-

tinued when there were reports that it was inhibitory to some strains of *L. monocytogenes* [16]. However, we have not found this to be a problem with Modified Vogel Johnson Agar, possibly due to the tellurite level being 2.5-fold less than that originally employed by Gray [12]. *Listeria* have a characteristic appearance on this medium, being entirely black on a red background. Enterococci and staphylococci resistant to the selective agents are surrounded by a zone of yellow due to mannitol fermentation. Additionally, the outer edge of colonies of enterococci often have a thin band of white. The medium surrounding resistant *Kurthia* turns maroon presumably due to their strong alkaline reaction. The medium effectively differentiates *Listeria* as long as the difference in levels of these occasional resistant bacteria are not so excessive such as to mask a mannitol-negative response. Evaluations have indicated that Modified Vogel Johnson Agar compares favorably with Lithium Chloride-Phenylethanol-Moxalactam Agar [15] in regard to both directly plating (Table 3) and when used with a preliminary enrichment step (Table 4) [2, 3]. The medium tended to give slightly lower quantitative values with direct plating of food samples as compared to LPM Agar; however, it is unclear with the latter medium if the colonies counted were actually all *Listeria*.

Table 3

Isolation of *Listeria monocytogenes* from naturally infected milk samples using direct plating with Lithium Chloride-Phenylethanol-Moxalactam Agar and Modified Vogel Johnson Agar. Adapted from Buchanan et al. [3]

Medium	Log (CFU/ML) ^a
LPM agar	3.67 (±0.07) ^b
MVJ agar	3.26 (±0.11) ^c

^aMean (± standard deviation), *n* = 8. ^bAll colonies counted even though two or more colonial types were evident on most plates. ^cOnly tel⁺ man⁻ colonies counted.

Table 4

Recovery of *Listeria* spp. from retail level foods using LPM and MVJ Agars in conjunction with initial enrichment in UVM *Listeria* Enrichment Broth. Adapted from Buchanan et al. [3]

	MVJ agar		LPM agar		Combined data	
	All <i>Listeria</i>	L.M. ^a Only	All <i>Listeria</i>	L.M. Only	All <i>Listeria</i>	L.M. Only
Fresh meats	11/21	9/21	9/21	8/21	11/21 (52%)	9/21 (43%)
Cured meats	1/12	0/12	0/12	0/12	1/12 (8%)	0/12 (0%)
Poultry products	0/9	0/9	0/9	0/9	0/9 (0%)	0/9 (0%)
Seafood	4/18	1/18	4/18	2/18	5/18 (28%)	2/18 (11%)

^a*Listeria monocytogenes*.

While other chapters will deal with the subject more extensively, it is important to note that one of the areas that must be considered when employing cultural methods is the impact that injury may have on the efficacy of detection methods. It has been demonstrated that *L. monocytogenes* can be sublethally stressed by environmental challenges such as heating, exposure to acids, or, to a lesser extent, freezing [11, 27]. In this state, the microorganism is less tolerant of other environmental or chemical stresses, and requires a period of repair before it regains its normal resistance. In terms of detection methodologies, the microorganism's resistance to selective agents can be decreased substantially, resulting in the detection of only a small fraction of the viable cells that may actually be present in a food sample. Smith and Archer [27] evaluated a variety of enrichment and isolation media, as well as individual selective agents, and found in general that the greater the selectivity of a media, the less likely it was to detect sublethally stressed cells effectively. To date, we have not found any highly selective medium to be capable of quantitative recovery of injured cells. Currently, a non-selective pre-enrichment is recommended, particularly if dealing with very low levels of *Listeria*.

CONFIRMATION

The final phase of an analysis for *L. monocytogenes* is the confirmation of the genus identification and subsequent speciation of positive *Listeria* isolates. Our laboratory currently uses nine determinants to confirm to the genus level, including Gram-positive, coccoid to short rod, tumbling motility, catalase-positive, oxidase-negative, VP-positive, esculin-positive, tellurite-positive, and acid production from glucose. Experience with meat and poultry products has indicated that this latter assay is particularly important since it is the only one that differentiates the genus *Kurthia* (Table 5). This microorganism appears to be relatively common to these types of products and can be readily confused for

Table 5

Characteristics of the genus *Listeria* and *Kurthia*

	<i>Listeria</i>	<i>Kurthia</i>
Gram stain	+	+
Cocoid to short rod	+	+
Growth at 35°C	+	+
Catalase	+	+
Oxidase	-	-
Motility	+	+
H ₂ production	+	+
Tellurite reduction	+	+
Acid from glucose	+	-
O ₂ requirement	Facultative	Aerobic

Listeria on a number of commonly used isolation media. It should also be noted that a number of these other confirmation assays have specific requirements or are not as straight-forward as one would expect. For example, the motility testing must be done at temperature below 30°C. Likewise, it is often difficult to distinguish true cocci from short rods. It is recommended strongly that a known reference culture be carried through each group of confirmations to ensure adequacy of these assays and the tests for speciation.

There are a number of potential tests that can be performed to achieve speciation. However, unless there is some underlying reason for proceeding further, we rely on four or occasionally five tests. The four tests include utilization of xylose, mannitol, and rhamnose, and the CAMP test. The fifth test, nitrate reduction, is used for distinguishing *L. denitrificans*. However, it does not appear to occur commonly in foods, and is no longer classified as a member of the genus *Listeria*.

CONCLUSIONS

During the past three years there has been a significant amount of activity in the development of rapid and accurate cultural methods for the detection, enumeration, and confirmation of foodborne *L. monocytogenes*. The result of this activity has been the development of a battery of media and tech-

niques that can be used effectively either by themselves or in conjunction with other 'rapid methods' to accurately detect *L. monocytogenes* in food systems. These improved techniques have largely eliminated the need for extended periods of cold enrichment. However, additional work is still needed and warranted, particularly in the area of improved enrichment media. This is currently the limiting step for both classical and non-classical methods for detecting *L. monocytogenes*. The most critical need is identification of selective agents or conditions that can suppress effectively enterococci while still permitting *L. monocytogenes* to thrive. Availability of such an agent would greatly enhance cultural methods and would similarly improve the effectiveness of the various rapid methods that are currently in use.

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