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Comparison of Cold Enrichment and U.S. Department of Agriculture Methods for Isolating *Listeria monocytogenes* from Naturally Contaminated Foods

PEGGY S. HAYES,* LEWIS M. GRAVES, GLORIA W. AJELLO, B. SWAMINATHAN, ROBERT E. WEAVER, JAY D. WENGER, ANNE SCHUCHAT, CLAIRE V. BROOME, AND THE LISTERIA STUDY GROUP†

Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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We compared the cold enrichment (CE) and U.S. Department of Agriculture (USDA) methods for isolating *Listeria monocytogenes* by examining 402 food samples. The food samples were collected from refrigerators of listeriosis patients as part of a multistate active surveillance project to determine the role of foods in sporadic listeriosis in the United States. *L. monocytogenes* was isolated from 51 food samples (13%). The USDA method was significantly better ($P < 0.001$) than the CE method. The isolation efficiencies of the USDA and CE methods were 96 and 59%, respectively. Quantitation of *L. monocytogenes* in the food samples revealed that many food samples containing <0.3 CFU/g were negative as determined by the CE method but positive as determined by the USDA method.

Listeria monocytogenes is an important pathogen that is found in a variety of foods. Four major outbreaks of listeriosis in the 1980s were linked to the consumption of cole slaw, milk, and cheese (5, 14, 28, 32). Listeriosis may also occur sporadically, although the sources of infection in sporadic listeriosis are largely unknown. We estimate that the incidence of sporadic, culture-confirmed listeriosis is 7 cases per 10^6 people or a projected 1,700 cases with more than 400 deaths annually in the United States (15). The Meningitis and Special Pathogens Branch of the Centers for Disease Control initiated active surveillance in 1988 in five areas of the country to identify cases of human listeriosis and to determine the role of foods in this disease.

L. monocytogenes is difficult to isolate from specimens such as foods, which may contain other organisms. Cold enrichment (CE) has long been the standard isolation procedure used for examining nonsterile specimens (17). Recently, however, other isolation methods have been developed, including the U.S. Department of Agriculture (USDA) method (30), the Food and Drug Administration (FDA) method (29), and a method developed in The Netherlands by van Netten et al. (35); these relatively rapid methods are widely used for quality control in the food industry.

In 1986 we compared the original FDA method, in which modified McBride medium is used, with the CE method and found that the FDA method as originally described was unsatisfactory (1). Other investigators (24, 25) have compared the CE and USDA methods. However, because the sample sizes used by these workers were small and *L. monocytogenes* was isolated only rarely, a meaningful comparative analysis of the data could not be made. During our active surveillance for sporadic listeriosis, we compared the CE and USDA methods for isolating *L. monocytogenes* by examining a large number of naturally contaminated food samples.

MATERIALS AND METHODS

Food samples. The food samples were collected from the refrigerators of patients with listeriosis within 1 week of detection of illness. The food samples were placed in Whirl-Pak bags and shipped by air either on ice or frozen; they arrived in Atlanta, Ga., within 24 h. The samples were analyzed as soon as possible, usually within 5 days. If necessary, they were stored frozen or refrigerated until they were analyzed.

Sample preparation and direct plating. Food samples were homogenized 1:1 in 0.1% peptone at room temperature in a Stomacher instrument (Tekmar Co., Cincinnati, Ohio) or in an Osterizer blender (Oster, Milwaukee, Wis.). A 0.1-ml portion of each homogenate was plated directly onto lithium chloride-phenylethanol-moxalactam (LPM) agar (27). Portions (50 ml) of the homogenate were used for each selective enrichment procedure. The remainder of the homogenate was stored at −70°C after glycerol was added to a final concentration of 10%. The frozen homogenate was used for quantitation of *L. monocytogenes* as described below.

CE method. We used the CE method developed by the Epidemic Investigations Laboratory, Meningitis and Special Pathogens Branch, Division of Bacterial Diseases, Centers for Disease Control (21). Briefly, 50 ml of a food homogenate

* Corresponding author.

† The members of the Listeria Study Group are as follows: Jo Taylor, Peggy Rados, and Louis Lefkowitz, Department of Preventive Medicine, Vanderbilt School of Medicine, Medical Center of Nashville, Nashville, Tenn.; Chris Harvey and David Stephens, Veterans Administration Medical Center, Decatur, Ga.; Gretchen Anderson, Elizabeth Stone, and Art Reingold, San Francisco Department of Health, San Francisco, Calif.; Pam Archer, Barbara Gildon, Jane Strack, and Greg Istre, Oklahoma State Department of Health, Oklahoma City, Okla.; Laurene Mascola and Maribel Castillon, Los Angeles County Health Department, Los Angeles, Calif.; Stanley M. Martin and Ray Ransom, Statistical Services Activity, Division of Bacterial and Mycotic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Ga.; and Richard Pierce, Katherine Deaver, and Michael Neuenschwander, Meningitis and Special Pathogens Branch, Division of Bacterial and Mycotic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Ga.

was enriched in 450 ml of Oxoid nutrient broth no. 2 (Unipath Co., Columbia, Md.) at 4°C for 8 weeks. After 1, 4, and 8 weeks 1-ml aliquots of the CE broth were subcultured in 9-ml portions of a selective broth (Oxoid nutrient broth no. 2 supplemented with 100 μg of nalidixic acid per ml and 3.75% potassium thiocyanate) for 24 h at 35°C before the preparations were plated onto LPM agar and acriflavine-ceftazidime (AC) agar (4).

USDA method. We used a modified version of the USDA method developed by McClain and Lee (30). This method, as modified by Lee, omitted the KOH treatment of enrichment broths before plating (26). The food homogenate (50 ml) was enriched in 450 ml of USDA selective enrichment broth at 30°C (primary enrichment). After 24 h, 0.1 ml of the selective enrichment culture was plated onto LPM agar and 0.1 ml was transferred to 10 ml of USDA secondary enrichment broth. This secondary enrichment broth was incubated for 24 h at 30°C before the preparation was plated onto LPM agar.

Isolation, identification, and serotyping. The selective agar media were incubated at 35°C for 48 h and were examined by using the Henry method of oblique lighting (20). Suspect colonies were streaked onto Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 5% sheep blood and incubated for 18 h at 35°C. Isolates were confirmed as *L. monocytogenes* and serotyped (21).

Quantitation. Quantitation of *L. monocytogenes* in food samples was accomplished by using the three-tube most probable number (MPN) method essentially as described by Swaminathan et al. (34). Frozen homogenates were thawed quickly by placing them in a 37°C water bath. Each homogenate was diluted 1:10 (1 ml of homogenate and 9 ml of broth), 1:100 (0.1 ml of homogenate and 9.9 ml of broth), and 1:1,000 (0.01 ml of homogenate and 10 ml of broth) in the USDA primary enrichment broth (three tubes per dilution). The broth media were incubated at 30°C for up to 48 h. The cultures in enrichment broth media were streaked onto LPM agar after 24 and 48 h. The plates were incubated at 35°C for 48 h. Presumptive positive cultures were confirmed by using the procedures described above. Plates that yielded one or more confirmed colonies of *L. monocytogenes* were recorded as positive. The MPN values (numbers of colony-forming units per gram) were obtained by referring to previously published MPN tables (31). The homogenates for which the MPN estimates were greater than 220 CFU/g were directly plated (0.1-ml portions of undiluted homogenate and 1/10, 1/100, and 1/1,000 dilutions plated onto LPM agar) to get a better estimate of the numbers of *L. monocytogenes*.

Statistical analysis. The McNemar test for paired samples was used to compare the efficiencies of the CE and USDA methods for isolating *L. monocytogenes* from food samples.

RESULTS

Of the 402 food samples examined, 51 (13%) were positive for *L. monocytogenes* as determined by at least one method; 28 food samples were positive as determined by both enrichment methods. A total of 21 food samples were positive only as determined by the USDA method, and 2 food samples were positive only as determined by the CE method. The isolation efficiency of the CE method was 59%, and the isolation efficiency of the USDA method was 96%.

Eight food samples were positive as determined by both methods at all sampling times. The results of each sampling for each method are shown in Fig. 1. The time of sampling influenced the number of positive samples in the CE procedure. After 1 week of CE 13 food samples were positive, after 4 weeks 11 additional food samples were positive, and after 8 weeks 6 additional food samples were positive. However, five food samples which were positive after 1 or 4 weeks were negative after 8 weeks.

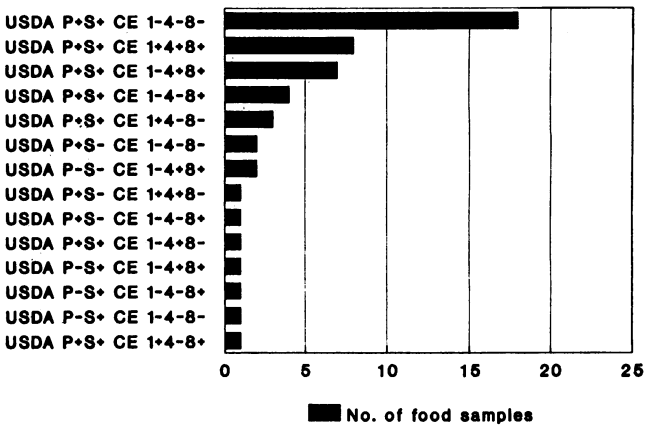

FIG. 1. Comparison of the CE and USDA methods for the isolation of *L. monocytogenes* from food samples obtained from refrigerators of listeriosis patients. USDA P, USDA method primary enrichment; USDA S, USDA method secondary enrichment; CE 1, 4, and 8, CE method after 1, 4, and 8 weeks, respectively; +, positive for *L. monocytogenes*; −, negative for *L. monocytogenes*.

When the USDA isolation method was used, 42 food samples were positive in both the primary and secondary enrichment broth media. Four were positive only in the USDA primary broth, and three were positive only in the USDA secondary broth. The efficiencies of isolation of *L. monocytogenes* from the USDA primary and secondary enrichment broth media were 90 and 88%, respectively.

The CE broth cultures were plated onto both LPM agar and AC agar. *L. monocytogenes* was isolated 49 times on both media, 4 times on LPM agar but not on AC agar, and 1 time on AC agar but not on LPM agar.

Quantitation of *L. monocytogenes* (MPN estimates) was done for 49 of the 51 food samples (Table 1). The homogenates from two food samples were not available for quantitation. Of 21 food samples that were positive only as determined by the USDA method, *L. monocytogenes* was quantitated in 20. The MPN values for *L. monocytogenes* for these 20 food samples ranged from <0.3 to 100 CFU/g. The MPN value for *L. monocytogenes* for one of the two food samples that were positive only as determined by the CE method was <0.3 CFU/g. Eight food samples were positive as determined by both enrichment methods at all sampling times. The MPN estimates for these food samples were <0.3 (two food samples), 0.9, 2.8, 150, >220 (67 CFU/g as determined by direct plate counting), >1,100, and 1.4×10^3 CFU/g. Six food samples were positive as determined by direct plating. Three of these were positive as determined by both enrichment methods at all five sampling times (the MPN values were 150, >1,100, and 1.4×10^3 CFU/g). The other three food samples were positive as determined by both methods but not at all sampling times; the MPN values were 100, 100, and >220 CFU/g (67 CFU/g as determined by direct plate counting).

We observed inconsistencies in the isolation of *L. monocytogenes* from heavily contaminated food samples as determined by direct plating and by the two enrichment methods. Some examples of these inconsistencies are described be-

TABLE 1. Results obtained when the USDA and CE methods were used to isolate *L. monocytogenes* from 51 food samples, serotypes, and quantitation estimates

Food		Results as determined by:		Sero-type(s)	MPN value (CFU/g)
Sample no.	Type	USDA method	CE method		
123	Smoked ham	+	+	1/2a	0.9
271	Bacon	+	+	4b, 1/2b	2.8
2	Ground beef	−	+	1/2c, 1/2b	ND[a]
266	Steak	+	−	1/2b	<0.3
267	Sausage	+	−	1/2b	4.0
484	Hot dogs	+	−	3b	40.0
536	Ham	+	−	1/2b	5.6
538	Sausage	+	−	1/2a	<0.3
111	Ground beef	+	−	1/2b	ND
11	Pork roast	+	−	3b	1.8
557	Turkey ham	+	−	1/2a	<0.3
131	Bacon	+	+	1/2a	2.8
539	Raw pork skins	+	+	1/2b	4.3×10^4
147	Steak	+	+	1/2b	0.4
525	Roast beef	+	+	1/2b	3.6×10^4
581	Turkey ham	+	+	1/2a	100.0
443	Pork chops	+	+	1/2b	18.0
450	Pork roast	+	+	4b	100.0
431	Bologna	+	+	1/2b	40.0
132	Turkey frankfurters	+	+	1/2a	>1,100
196	Chicken	+	+	4b	<0.3
283	Chicken	−	+	1/2b	<0.3
139	Chicken	+	−	1/2a	0.9
425	Turkey frankfurters	+	−	1/2a	<0.3
433	Chicken	+	−	1/2b	0.8
546	Chicken	+	−	1/2c	100.0
558	Smoked turkey	+	−	1/2a	<0.3
378	Chicken	+	+	1/2a	40.0
377	Chicken	+	+	1/2a	>220 (100)[b]
93	Cabbage	+	+	4b	150.0
302	Green chili	+	+	ND	<0.3
531	Cabbage	+	+	1/2b	1.4×10^3
92	Celery	+	−	4b	0.9
125	Carrot-raisin salad	+	−	1/2a	<0.3
126	Lettuce	+	−	1/2a	<0.3
304	Squash	+	−	ND	<0.3
458	Celery	+	−	4b	<0.3
541	Tomato	+	−	1/2c	1.2
542	Green pepper	+	−	1/2b	8.0
543	Cucumber	+	−	1/2c	<0.3
286	Hash brown potatoes	+	+	4b	<0.3
253	Lettuce	+	+	1/2a	2.0
456	Lettuce	+	+	4b	<0.3
457	Onion	+	+	4b	<0.3
530	Lettuce	+	+	1/2c	<0.3
535	Chayote	+	+	1/2c	40.0
533	Mozzarella cheese	+	+	1/2b	>220 (67)
261	Cheddar cheese	+	+	1/2a	<0.3
448	Cheese	+	+	4b	<0.3
442	Macaroni and cheese	+	+	1/2a	100.0
411	Pasta	+	+	4	4.0

[a] ND, not determined.
[b] The numbers in parentheses are direct plate counts.

low. Five samples had ≥100 CFU of *L. monocytogenes* per g (MPN estimate) (one sample had 3.6×10^4 CFU/g) but were not positive as determined by direct plating. A raw pork skin sample (Table 1, sample 539) that had 4.3×10^4 CFU of *L. monocytogenes* per g was positive in the USDA primary enrichment broth but negative in the USDA secondary enrichment broth. The same sample was positive after 1 and 4 weeks of CE but negative after 8 weeks of CE.

TABLE 2. Efficiencies of the USDA method and the CE method for isolating *L. monocytogenes* from various food groups

Food group	USDA method		CE method	
	No. of samples found positive/ no. of positive samples tested	% Positive	No. of samples found positive/ no. of positive samples tested	% Positive
Meats	18/19	95	11/19	58
Poultry	9/10	90	5/10	50
Vegetables	17/17	100	9/17	53
Dairy products	4/4	100	4/4	100
Pasta	1/1	100	1/1	100

The efficiencies of the two enrichment methods for isolating *L. monocytogenes* from different food groups are shown in Table 2. The USDA method was significantly better ($P < 0.05$) than the CE method for isolating *L. monocytogenes* from meats, poultry, and vegetables. The two methods appeared to be equally efficient in isolating *L. monocytogenes* from dairy products; however, the number of dairy products examined was too small for a meaningful statistical analysis.

We isolated a variety of serotypes, including serotypes 1/2a, 1/2b, 1/2c, 3b, and 4b, by both methods (Table 1). Neither isolation method selected for a specific serotype.

DISCUSSION

The CE method described by Gray et al. (17) has been used successfully by many researchers to isolate *L. monocytogenes* from diverse sources, such as feces, surface waters, silage, and sewage (7, 13, 22, 37). Watkins and Sleath developed a two-step enrichment technique that combined CE with a secondary enrichment in potassium thiocyanate-nalidixic acid broth to isolate *L. monocytogenes* from river and sewage samples (36). Hayes et al. used the Watkins-Sleath two-step enrichment procedure to isolate *L. monocytogenes* from raw milk (19). The CE method is the standard with which other isolation methods are compared. However, the CE method requires incubation of samples for 2 months or longer and is unsatisfactory in situations in which a rapid result is required. Regulatory agencies and the food industry clearly need a rapid isolation method to examine quality control for *L. monocytogenes*.

The method currently used by the USDA was developed by McClain and Lee (30) for isolating *Listeria* spp. from meats and poultry. The enrichment broth used in the USDA method was developed by Dominquez Rodriguez et al. (8) and was modified by Donnelly and Baigent (9). Both the enrichment broth and the plating medium are highly selective for *Listeria* spp.

The results of this investigation showed that the USDA method is more sensitive than the CE method for isolation of *L. monocytogenes* from meats, poultry, and vegetables. Two food samples (one meat and one poultry product) were positive only as determined by the CE method. However, the poultry product contained very low numbers of *L. monocytogenes* (MPN, <0.3 CFU/g). The negative result obtained with the USDA method might have been due to

nonhomogeneous distribution of a few cells of *L. monocytogenes* in the homogenate of the poultry product.

The efficiencies of the two methods appeared to be the same for one food group, dairy products, although the number of positive food samples tested was small. Two other groups of investigators tested dairy products by using the USDA method and a two-step CE method. Their findings were similar to our findings. In the study of Lammerding and Doyle all nine naturally contaminated samples of goat milk were positive as determined by the USDA method and eight of the nine samples were positive as determined by the CE method (24). In the study of Crawford et al. the sensitivities for detection of uninjured cells suspended in raw milk were similar when the two methods were used; 0.02 to 3.2 organisms per ml were detected when the two-step CE method was used, and 0.38 organism per ml was detected when the USDA method was used (6).

The time of sampling greatly influenced the results obtained with the CE method. Six samples (roast beef, steak, bacon, turkey ham, cheese, and hash brown potatoes) were negative after 1 and 4 weeks but were positive after 8 weeks. The MPN estimates for *L. monocytogenes* in these samples were as follows: roast beef, 3.6×10^4 CFU/g; steak, 0.4 CFU/g; bacon, 2.8 CFU/g; turkey ham, 100 CFU/g; cheese, <0.3 CFU/g; and hash brown potatoes, <0.3 CFU/g. The low contamination level of four of the six samples and the presence of competing bacteria in the other two samples probably contributed to the negative results obtained after 1 and 4 weeks. Five food samples (raw pork skin, uncooked chicken, pork, pasta, and chayote) were positive as determined by the CE method after 1 and/or 4 weeks but were negative after 8 weeks. The MPN estimates for *L. monocytogenes* in these samples were as follows: raw pork skin, 4.3×10^4 CFU/g; uncooked chicken, >220 CFU/g (100 CFU/g as determined by direct plate counting); pork, 100 CFU/g; pasta, 4 CFU/g; and chayote, 40 CFU/g. The *L. monocytogenes* cells in these samples may have died because of acidification of the medium by bacterial metabolites or because of other adverse conditions.

In two food homogenates (food samples 377 and 533), the MPN estimates for *L. monocytogenes* were >220 CFU/g. However, when the numbers of *L. monocytogenes* cells in these homogenates were determined by direct plating, the numbers were substantially lower (Table 1). This was probably due to the presence of sublethally injured *L. monocytogenes* cells in these homogenates. The sublethally stressed cells were probably sensitive to the selective ingredients of LPM agar, which was used for the direct plating test (23, 33). Sublethal injury in *L. monocytogenes* has been documented by several researchers and is an important consideration in the isolation of *L. monocytogenes* from foods (2, 3, 16, 24).

We failed to isolate *L. monocytogenes* by using the CE method from 21 samples (8 meat samples, 8 vegetable samples, and 5 poultry product samples) that were positive as determined by the USDA method. *L. monocytogenes* was quantitated in 20 of these food samples; 10 food samples had less than 0.3 CFU of *L. monocytogenes* per g, while 10 food samples had 0.8 to 100 CFU/g. Thus, the CE method failed in 10 instances even when 20 or more cells of *L. monocytogenes* were present in the food homogenates.

Doyle and Schoeni (11) tested 90 soft cheese samples by using the CE method, the FDA method, and a method which they developed themselves (10). A total of 21 food samples were positive for *L. monocytogenes* as determined by the CE method. Only 16 samples were positive as determined by the FDA method, and 13 were positive as determined by the Doyle-Schoeni method. Our results cannot be compared with the data of Doyle and Schoeni (11) because these authors used a one-step CE method and did not evaluate the USDA method. Furthermore, the results of our work in progress suggest that the USDA method is better than the FDA method for isolating *L. monocytogenes* from a variety of foods (18). Farber et al. (12) analyzed 445 raw milk samples by using the FDA method and the CE method. Because these authors used various nonstandard modifications of the FDA method and the CE method and isolated *L. monocytogenes* from only five raw milk samples, their data are not useful for comparing the efficiencies of selective enrichment methods.

Although we found no significant difference between LPM agar and AC agar, we preferred LPM agar because it was more inhibitory to competing organisms.

In conclusion, we convincingly demonstrated the superiority of the USDA procedure over the CE procedure for isolating *L. monocytogenes* from a variety of foods. In addition to the fact that the CE method is very time-consuming and inefficient, the results obtained by this method are also influenced by the length of incubation at low temperature. While the USDA method is an improvement over the CE method, it failed to recover *L. monocytogenes* from two samples that were positive as determined by the CE method. In four samples, only the USDA primary enrichment yielded positive results, while the secondary enrichment did not. Also, the recovery of sublethally stressed *L. monocytogenes* cells by the USDA procedure may be problematic because of the presence of acriflavine and other selective chemicals in the enrichment medium. We are presently comparing the USDA method, the FDA method, and a selective enrichment and plating method developed in The Netherlands (35) for the isolation of *L. monocytogenes* from naturally contaminated food samples obtained from the refrigerators of listeriosis patients. Our preliminary results suggest that there are no significant differences in isolation efficiency among the three methods; however, our results also indicate that two methods in combination are significantly better than a single method.

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REFERENCES

1. **Ajello, G., P. Hayes, and J. Feeley.** 1986. Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, P-5, p. 276.
2. **Al-Zoreky, N., and W. E. Sandine.** 1990. Highly selective medium for isolation of *Listeria monocytogenes* from food. Appl. Environ. Microbiol. **56**:3154–3157.
3. **Bailey, J. S., and D. L. Fletcher.** 1990. Efficacy of enrichment media for recovery of heat-injured *Listeria monocytogenes*. J. Food Prot. **53**:473–477.
4. **Bannerman, E. S., and J. Bille.** 1988. A new selective medium for isolating *Listeria* spp. from heavily contaminated material. Appl. Environ. Microbiol. **54**:165–167.
5. **Bula, C., J. Bille, F. Mean, and M. P. Glauser.** 1988. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1106.
6. **Crawford, R. G., C. M. Beliveau, J. T. Peeler, C. W. Donnelly, and V. K. Bunning.** 1989. Comparative recovery of uninjured and heat-injured *Listeria monocytogenes* cells from bovine milk. Appl. Environ. Microbiol. **55**:1490–1494.
7. **Dijkstra, R. G.** 1982. The occurrence of *Listeria monocytogenes* in surface water of canals and lakes, in ditches of one big polder and in the effluents and canals of a sewage treatment plant.

Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B **176**:202–205.
8. **Dominquez Rodriguez, L., G. S. Fernandez, J. F. F. Garayzabal, and E. R. Ferri.** 1984. New methodology for the isolation of *Listeria* microorganisms from heavily contaminated environments. Appl. Environ. Microbiol. **47**:1188–1190.
9. **Donnelly, C. W., and G. J. Baigent.** 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl. Environ. Microbiol. **52**:689–695.
10. **Doyle, M. P., and J. L. Schoeni.** 1986. Selective-enrichment procedure for isolation of *Listeria monocytogenes* from fecal and biological specimens. Appl. Environ. Microbiol. **51**:1127–1129.
11. **Doyle, M. P., and J. L. Schoeni.** 1987. Comparison of procedures for isolating *Listeria monocytogenes* in soft, surface-ripened cheese. J. Food Prot. **50**:4–6.
12. **Farber, J. M., G. W. Sanders, and S. A. Malcolm.** 1988. The presence of *Listeria* spp. in raw milk in Ontario. Can. J. Microbiol. **34**:95–100.
13. **Fenlon, D. R.** 1985. Wild birds and silage as reservoirs of *Listeria* in the agricultural environment. J. Appl. Bacteriol. **59**:537–543.
14. **Fleming, D. W., S. L. Cochi, K. L. McDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold.** 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312**:404–407.
15. **Gellin, B. G., C. V. Broome, W. F. Bibb, R. Weaver, S. Gaventa, L. Mascola, and the Listeriosis Study Group.** 1991. Epidemiology of listeriosis in the United States—1986. Am. J. Epidemiol. **133**:392–401.
16. **Golden, D. A., L. R. Beuchat, and R. E. Brackett.** 1988. Evaluation of selective direct plating media for their suitability to recover uninjured, heat-injured, and freeze-injured *Listeria monocytogenes* from foods. Appl. Environ. Microbiol. **54**:1451–1456.
17. **Gray, M. L., H. J. Stafseth, F. Thorp, Jr., L. B. Sholl, and W. F. Riley, Jr.** 1948. A new technique for isolating listerellae from the bovine brain. J. Bacteriol. **55**:471–476.
18. **Hayes, P. S., et al.** Unpublished data.
19. **Hayes, P. S., J. C. Feeley, L. M. Graves, G. W. Ajello, and D. W. Fleming.** 1986. Isolation of *Listeria monocytogenes* from raw milk. Appl. Environ. Microbiol. **51**:438–440.
20. **Henry, B. S.** 1933. Dissociation in the genus *Brucella*. J. Infect. Dis. **52**:374–402.
21. **Jones, G. L., B. Gellin, P. S. Hayes, L. Pine, B. Swaminathan, and R. E. Weaver.** 1989. Isolation and identification of *Listeria monocytogenes*. Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta.
22. **Kampelmacher, E. H., and L. M. van Noorle Jansen.** 1969. Isolation of *Listeria monocytogenes* from faeces of clinically healthy humans and animals. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **211**:353–359.
23. **Lachica, R. V.** 1990. Selective plating medium for quantitative recovery of food-borne *Listeria monocytogenes*. Appl. Environ. Microbiol. **56**:167–169.
24. **Lammerding, A. M., and M. P. Doyle.** 1989. Evaluation of enrichment procedures for recovery of *Listeria monocytogenes* from dairy products. Int. J. Food Microbiol. **9**:249–268.
25. **Leasor, S. B., and P. M. Foegeding.** 1989. *Listeria* species in commercially broken raw liquid whole egg. J. Food Prot. **52**:777–780.
26. **Lee, W. H.** Personal communication.
27. **Lee, W. H., and D. McClain.** 1986. Improved *Listeria monocytogenes* selective agar. Appl. Environ. Microbiol. **52**:1215–1217.
28. **Linnan, M. J., L. Mascola, D. L. Xioa, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekura, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome.** 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. **319**:823–828.
29. **Lovett, J., D. W. Francis, and J. M. Hunt.** 1987. *Listeria monocytogenes* in raw milk: detection, incidence, and pathogenicity. J. Food Prot. **50**:188–192.
30. **McClain, D., and W. H. Lee.** 1988. Development of USDA-FSIS method for the isolation of *Listeria monocytogenes* from raw meat. J. Assoc. Off. Anal. Chem. **71**:876–879;892.
31. **Oblinger, J. L., and J. A. Koburger.** 1984. Compendium of methods for the microbiological examination of foods, p. 99–111. American Public Health Association, Washington, D.C.
32. **Schlech, W. F., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome.** 1983. Epidemic listeriosis—evidence for transmission by food. N. Engl. J. Med. **308**:202–206.
33. **Smith, J. L., and D. L. Archer.** 1988. Heat-induced injury in *Listeria monocytogenes*. J. Ind. Microbiol. **3**:105–110.
34. **Swaminathan, B., P. S. Hayes, and V. Przybyszewski.** 1988. Evaluation of enrichment and plating media for isolating *L. monocytogenes*. J. Assoc. Off. Anal. Chem. **71**:664–668.
35. **van Netten, P., I. Perales, A. van de Moossdijk, G. D. W. Curtis, and D. A. A. Mossel.** 1989. Liquid and solid selective differential media for the detection and enumeration of *L. monocytogenes* and other *Listeria* spp. Int. J. Food Microbiol. **8**:299–316.
36. **Watkins, J., and K. P. Sleath.** 1981. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge, and river water. J. Appl. Bacteriol. **50**:1–9.
37. **Welshimer, H. J.** 1968. Isolation of *Listeria monocytogenes* from vegetation. J. Bacteriol. **95**:300–303.