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Comparisons of Selected Methods with the Fung-Yu Tube Procedure for Determining *Listeria monocytogenes* and Other *Listeria* spp. in Meats

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ABSTRACT

The ability of the motility enrichment Fung-Yu tube procedure with Oxyrase™ enzyme to detect the presence of *Listeria monocytogenes* inoculated into ground beef samples was compared to the USDA-FSIS method. Three strains of *L. monocytogenes* (LM 10IM, LM 103M, and Scott A) were inoculated separately into sterilized ground beef or culture broth. The inoculum levels used were as low as 1 to 1000 *Listeria* cells per g of meat or per ml of broth. The Fung-Yu tube procedure produced results as sensitive as the USDA procedures and provided a shorter detection time of 26-48 h.

A total of 215 retail-level meat and poultry products were analyzed comparatively by the Fung-Yu tube and the GENE-TRAK® DNA hybridization methods for *Listeria* detection. Six *Listeria* spp. (*L. denitrificans, L. grayi, L. innocua, L. ivanovii, L. monocytogenes*, and *L. murrayi*) were identified among the isolates. All 48 presumptively positive samples determined by the Fung-Yu tube method were further confirmed to harbor *Listeria* by biochemical tests. Eleven samples were missed by the GENE-TRAK® procedure, probably because of the enrichment procedure. *L. monocytogenes* was isolated from ground beef, pork sausage, smokie links, and cheese hot dogs. Among cooked samples examined, only cheese hot dogs and macaroni and cheese loaf showed substantial incidence of *Listeria* contamination.

Listeria monocytogenes is distributed widely in foods and has been isolated from a large number of plant and animal sources. Dairy products such as pasteurized whole and 2% milk (*11*) and soft cheese (*1,6*) have been implicated as vehicles of infection for foodborne listeriosis. Fruits and vegetables are less frequently found to be sources of *L. monocytogenes*. However, Ho et al. (*16*) have reported the involvement of contaminated lettuce, cabbage, celery, and tomatoes in human outbreaks of listeriosis. Preliminary results from a recent study in the United States showed that about 49% of ground beef, 52% of pork sausage, and 32% of poultry are contaminated with *L. monocytogenes* (*19*). Contamination also may occur in processed meats such as fermented sausage (*9*) and turkey frankfurters (*2*). Furthermore, the Centers for Disease Control were able to link consumption of uncooked hot dogs and undercooked chicken to sporadic cases of human listeriosis in an epidemiologic study completed during 1986 and 1987 (*23*). The potential contamination of ready-to-eat meat products with *L. monocytogenes* is of special concern for public health because of this organism's ability to survive refrigeration temperature, its high fatality rates (up to 33% in foodborne listeriosis) (*5,11,15,22*) and its pathogenicity for the immunocompromised population (*17*).

McClain and Lee (*20*) have developed a conventional culture-based procedure for isolating *L. monocytogenes* from meats. It involves a two-stage enrichment, which uses the broths of Donnelly and Baigent (*8*) and Fraser and Sperber (*12*) and an isolation agar that is a modification of Oxford agar made more selective with the addition of moxalactam. Presumptive *L. monocytogenes* colonies are confirmed using appropriate biochemical tests. The most recent methods for detecting *Listeria* in foods involve the use of gene probes (*5*). The progress in recombinant DNA techniques now offers opportunities for their application as analytical tools in food and clinical microbiology. Methods are being developed to detect microorganisms by their nucleic acid sequence using hybridization procedures. Labelled DNA fragments (probes) are hybridized with a complementary base sequence present in microorganisms (*21*). Rapid method test kits utilizing DNA hybridization for the detection of *Listeria* in foods are now available from commercial sources (GENE-TRAK® Systems).

A preliminary study (*29*) described the new Oxyrase™ enzyme and motility enrichment Fung-Yu tube procedure for the qualitative detection of *L. monocytogenes* in foods. This U-shaped glass apparatus coupled with the use of the Oxyrase™ enzyme (membrane fractions of *E. coli*) was designed to facilitate the color change in growth media (both Fraser broth and semisolid modified Oxford agar) of the pathogen, in order to permit rapid identification without the need for special equipment or reagents. In the present study, the Fung-Yu tube procedure was compared with the U.S. Department of Agriculture procedure and a gene probe method by using artificially and naturally contaminated

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ground beef and retail meat and poultry products as test materials.

MATERIALS AND METHODS

Preparation of L. monocytogenes *inocula for ground beef*

Stock cultures of *L. monocytogenes* strains Scott A (serotype 4, human isolate), LM 101M (serotype 4, meat isolate), and LM 103M (serotype 1, meat isolate) were grown on tryptic soy agar supplemented with 0.6% yeast extract slants and incubated for 18-20 h at 35°C. Cultures were transferred twice before being washed from the slants with sterile Butterfield phosphate buffer (pH 7.2). The culture suspensions were collected and adjusted to McFarland standard #1 (approximately 10^8 cells/ml) with phosphate buffer. For inoculation into sterile (autoclaved) ground beef, the cells of each strain were decimally diluted to yield 10^2 to 10^5 cells/ml with phosphate buffer. A 1-ml aliquot of suspension containing serially diluted cells was aseptically inoculated into 25 g of ground beef to achieve 10^0 to 10^3 cells/g. Colony forming units (CFU) were enumerated by pour-plating 1 ml of inocula with modified Oxford (MOX) agar *(20)* after appropriate dilutions.

For each test strain, the ground beef inoculated with diluted cultures was analyzed in duplicate using the U. S. Department of Agriculture/Food Safety and Inspection Service (USDA-FSIS) and Fung-Yu tube procedures to determine the sensitivity and detection time of each method. All experiments were replicated twice.

Sublethally injured L. monocytogenes *by heating*

Thermal injury of *L. monocytogenes* was done by following the procedures described by Golden et al. *(13)*. Each test strain in brain heart infusion broth (Difco Laboratories, Detroit, MI) was subjected to heating at 56°C for 20 min in a water bath. After heating and cooling, the culture was diluted in phosphate buffer to give approximately 10^0 to 10^3 CFU/ml. Both heat-injured and uninjured cells were plated onto tryptic soy agar. Plates were incubated at 35°C for 48 h before colony counting.

USDA-FSIS's Listeria *isolation procedure*

The isolation of *Listeria* was performed as suggested by McClain and Lee *(20)*. Each inoculated ground beef sample (25-g) was blended with 225 ml of University of Vermont (UVM) broth *(8)* in a Stomacher bag using a Stomacher (Tekmar Co., Cincinnati, OH). After 24 h incubation of the UVM broth at 30°C, three 0.1-ml aliquots of enrichment culture were transferred into tubes of Fraser broth (10 ml) *(12)*. Blackened Fraser broths were streaked onto MOX agar plates. After 24 and 40 h incubation at 35°C, plates were examined for typical *Listeria* colonies (Fig. 1).

For the recovery of heat-stressed or uninjured *L. monocytogenes* in pure culture suspension, 0.1 ml of diluted cultures (10^2 to 10^5 CFU/ml) was introduced into 10 ml of UVM broth, followed by the same procedures described above.

Fung-Yu tube procedure

A 25-g portion of each meat sample (inoculated or noninoculated) was aseptically spooned into 225 ml of Fraser broth with 25 units of Oxyrase™ (Oxyrase Inc., Ashland, OH). The sample mixture was stomached for 2 min and incubated at 30°C. After the meat slurry blackened, three 0.1-ml portions of the 10^{-1} diluted samples (Fraser broth) were transferred into three Fung-Yu tubes (Fig. 2). All tubes were incubated at 35°C and examined for a color change, indicating the presence of *Listeria*.

Sample collection

A total of 215 fresh, processed, ready-to-eat meat and poultry products including ham, bologna, hot dogs, sliced turkey, fermented semidried sausage, and cooked roast beef were purchased locally at five retail supermarkets. All food samples were stored at 4°C and analyzed within 24 h. Definitive isolation of *Listeria* was performed by the GENE-TRAK® *Listeria* assay and Fung-Yu tube procedures, with duplicate samples being taken from the same package for each analysis.

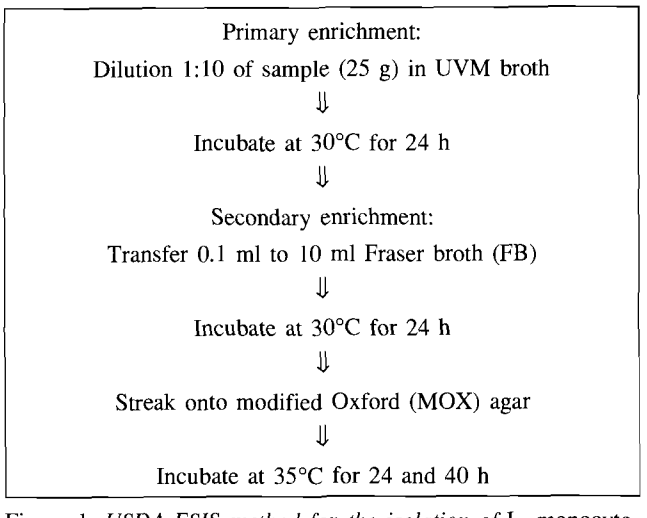

Figure 1. *USDA-FSIS method for the isolation of* L. monocytogenes *from processed meat and poultry products (20).*

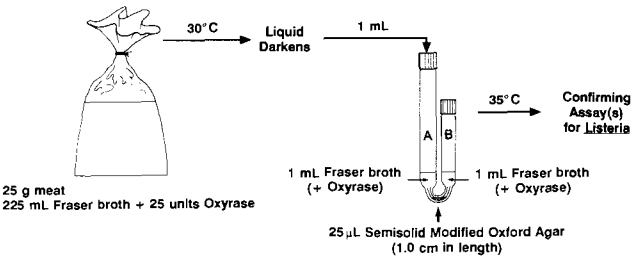

Figure 2. *Fung-Yu tube procedure for* Listeria *isolation.*

GENE-TRAK® procedure

Listeria colorimetric assay kits were supplied by GENE-TRAK® Systems, Framingham, MA. Assays were performed according to the manufacturer's instructions (Fig. 3). Hybridization occurs between fluorescein-labeled detector probes with a poly-deoxyadenosine tail and *Listeria*-specific regions of 16 S rRNA. The probes are captured on poly-deoxythymidine-coated plastic dipsticks. Detection is based on binding of horseradish peroxidase conjugated antifluorescein antibody to the hybridization complexes and enzyme-mediated color development *(18)*. The assay takes approximately 2.5 - 3 h after a 2-d broth enrichment and plate isolation.

Isolation of Listeria *spp. and confirmatory tests*

Pure cultures were isolated from arm B of Fung-Yu tubes and streaked on MOX agar plates. After incubation for 48 h at 35°C, 10 colonies on each MOX agar plate, typical of those formed by *Listeria* (dense yellowish with black halos, slightly raised with sunken center, 0.5 to 1.0 mm in diameter) were selected for confirmation. The tests used to differentiate *Listeria* isolates included: catalase reaction (positive), Gram stain reaction and morphology (gram-positive coccoid rods) determined microscopically, umbrella-shaped motility in semisolid agar stabs at 25°C, β-hemolysis on sheep blood agar plate, CAMP reaction with *Staphylococcus aureus*, carbohydrate fermentation (rhamnose, xylose, mannitol, and arabinose), nitrate reduction, phosphatase test, and reactivity with *Listeria* O antisera (poly, serotype 1; 4) (Difco). Purified *Listeria* cultures were submitted to the

confirmatory tests summarized in Table 1 and specified as described in Bergey's Manual (*24*).

25 g sample + 225 ml University of Vermont (UVM) broth with extra acriflavine

⇓ 35°C, 24 ± 4 h

Swab cultures on LiCl-phenylethanol-moxalactam (LPM) agar plates

⇓ 35°C, 24 ± 2 h

Swab off growth from LPM plates and resuspend in 1 ml of phosphate-buffered-saline (PBS)

⇓ Take 0.3 ml

Pre-treatment

⇓ 37°C, 15 min

Lysis

⇓ 37°C, 15 min

Add *Listeria* probe solution (65°C) for hybridization
Incubate dipsticks with samples

⇓ 37°C, 1 h

Wash twice at room temperature and 65°C, 1 min each

⇓

Incubate dipsticks in enzyme conjugate

⇓ room temp., 20 min

Wash twice at room temp. 1 min each

⇓

Incubate dipsticks in substrate-chromogen

⇓ room temp., 30 min

Stop color reaction and determine A_{450}

Figure 3. *GENE-TRAK® DNA hybridization* Listeria *assay.*

RESULTS

Comparison of USDA-FSIS and Fung-Yu tube procedures for isolation of Listeria *from artificially contaminated ground beef*

The time required for detecting three strains described in Tables 2 and 3 were determined by USDA-FSIS and Fung-Yu tube methods following preenrichment steps. Three *L. monocytogenes* strains were consistently detectable by both methods at 10^0 to 10^3 CFU/g of ground beef (Table 2) or per ml of culture suspension (Table 3). In each case, the time required to attain a *Listeria*-positive result was calculated. A slight nonproportional increase in time for each method was observed at the higher dilutions.

The effectiveness of the Fung-Yu tube procedure was assessed initially by comparison against the USDA-FSIS method for detecting *L. monocytogenes* in ground beef or culture broth. The USDA-FSIS and Fung-Yu tube methods use different enrichment protocols. The UVM broth enrichment is used in the USDA-FSIS method, followed by Fraser broth (FB) for secondary enrichment, whereas FB is the only one specified for the Fung-Yu tube method. Oxyrase™ enzyme is added to the latter to stimulate the growth of *Listeria* and the formation of black precipitate in FB from the hydrolytic product of esculin, which is reactive with ferric ions in the biochemical assay. The Fung-Yu tube method shortens the detection time from that required for the USDA-FSIS method by utilizing the motility of this organism and the growth-enhancing effect of Oxyrase™. When half of the semisolid agar column in the Fung-Yu tube turned black because of the motility and esculin hydrolysis of the organisms (ca. 3 h after FB in tube A turned black), the test samples could be considered as positive for *Listeria*. The new procedure takes 23-33 h for the detection of *Listeria* in meat samples (see the column of "Total[b]" in Table 2). The Fung-Yu tube procedure produced

TABLE 1. *Confirmatory tests to distinguish* Listeria *spp.*[a]

Listeria spp.	Mann.	Xylo.	Rham.	Arab.	Phos.	Nitr. redu.	β-hemo.	CAMP-test with *S. aureus*
L. monocytogenes	-	-	+	-	+	-	+	+
L. innocua	-	-	+/-	-	+	-	-	-
L. ivanovii	-	+	-	-	+	-	++	-
L. seeligeri	-	+	-				+	+
L. welshimeri	-	+	+/-				-	-
L. grayi	+	-	-	-	+	-	-	-
L. murrayi	+	-	+/-	-	+	+	-	-
L. denitrifcans	-	+	-	+	-	+	-	-

[a] All organisms that were catalase-positive, gram-positive rods and demonstrated umbrella-like growth in motility medium were submitted to above scheme (*24*).
Mann.: Mannitol, Xylo.: Xylose, Rham.: Rhamnose, Arab.: Arabinose, Phos.: Phosphatase, Nitr. Redu.: Nitrate Reduction, β-hemo.: β-hemolysis.

TABLE 2. *Comparative detection time of Fung-Yu tube and USDA methods to recover inoculated* Listeria monocytogenes *from ground beef.*

Strain	Cells/g in ground beef	Time (h)								
		USDA method				Fung-Yu tube method				
		UVM	FB[1]	MOX	Total	FB[2]	tube A	tube B	Total[a]	Total[b]
LM 101M	$2.7x10^3$	24	9	24-40	57-73	19	3	12-13	34-35	25
	$2.7x10^2$	24	10	24-40	58-74	20	3	12-13	35-36	26
	$2.7x10^1$	24	10	24-40	58-74	22	4	13-14	39-40	29
	$2.7x10^0$	24	11	24-40	59-75	26	4	14-15	44-45	33
LM 103M	$3.0x10^3$	24	7	24-40	55-71	18	2	9-10	29-30	23
	$3.0x10^2$	24	8	24-40	56-72	20	2	10-11	32-33	25
	$3.0x10^1$	24	8	24-40	56-72	22	3	10-11	35-36	28
	$3.0x10^0$	24	9	24-40	57-73	25	3	12-13	40-41	31
Scott A	$2.7x10^3$	24	9	24-40	57-73	23	2	12-13	37-38	28
	$2.7x10^2$	24	11	24-40	59-75	24	2	15-16	41-42	29
	$2.7x10^1$	24	11	24-40	59-75	25	3	15-16	43-44	31
	$2.7x10^0$	24	11	24-40	59-75	26	3	15-16	44-45	32

Total[a]: Total time required to complete Fung-Yu tube procedure.
Total[b]: Total time required to obtain blackening of FB in tube A and blackening of half MOX agar column.
FB[1]: Time required to obtain blackening of FB in test tubes.
FB[2]: Time required to obtain blackening of FB in Stomacher bags.

results as sensitive as the USDA-FSIS procedure and provided 26-48 h shorter detection time (comparisons between USDA method's column of "Total" and Fung-Yu tube method's column of "Total[b]", Table 2).

Listeria *spp. isolated from naturally contaminated meat and poultry products by Fung-Yu tube and GENE-TRAK® methods*

A retail sample was considered positive if *Listeria* spp. could be detected by either method and if isolates could be confirmed. A distinct difference was observed between 48 and 37 positives isolated from 215 meat samples examined by the Fung-Yu tube and GENE-TRAK® methods, respectively (Table 4). All GENE-TRAK® positive samples were also tested positive by the Fung-Yu tube. However, 11 samples were positive by the Fung-Yu tube but negative by GENE-TRAK®, giving a false-negative rate of 22.9% for GENE-TRAK®. The Oxyrase™ enzyme used in the Fung-Yu tube system might have stimulated the growth of *Listeria* far better than in the enrichment procedure used in the GENE-TRAK® method. These data do not imply that the GENE-TRAK® assay itself is not sensitive, but rather that the enrichment procedure may need improvement in the GENE-TRAK® protocol.

Most foods (35/73) in which *Listeria* were isolated were raw, but the organisms were isolated from some cooked foods (13/142). High isolation rates were observed for fresh meat samples, such as ground beef and pork sausage, whereas none of the samples of ground pork were found contaminated (Table 4). The Fung-Yu tube and GENE-TRAK® procedures found 47.9 and 39.7% of the raw meat samples to be positive, respectively. Isolation rates for cooked meat samples were 9.2 and 5.6%, respectively.

Fifty-two *Listeria* isolates were obtained from 48 positive samples among the 215 examined (Table 5). *L. innocua* and *L. murrayi* were isolated more frequently than any other species from meats. Six *Listeria* species (*L. denitrificans, L. grayi, L. innocua, L. ivanovii, L. monocytogenes,* and *L. murrayi*) were identified among the isolates. *L. monocytogenes* was isolated from 3 of 142 refrigerated cooked samples and from 3 of 73 raw samples. From the 11 *Listeria*-positive samples obtained with the Fung-Yu tube method but not with GENE-TRAK® system, *L. denitrificans* (one isolate), *L. grayi* (two isolates), *L. innocua* (five isolates), *L. ivanovii* (one isolate), and *L. murrayi* (two isolates) were isolated. All 48 presumptively positive samples identified by the Fung-Yu tube method were confirmed to contain *Listeria* by biochemical tests. No false-negative reactions were observed with the Fung-Yu tube procedure.

DISCUSSION

Listeria contamination of raw meat products is not uncommon (*10,26,27*). Isolation of *L. innocua* from meat is common, and often the incidence of this species is higher than that of *L. monocytogenes* (*7,9,17*). The isolation of *L. denitrificans, L. grayi, L. ivanovii, L. murrayi, L. seeligeri,* and *L. welshimeri* from meats has also been reported (*4,9,25,26*). The prevalence of *Listeria* spp. in ground meats and other products requiring cooking before consumption ranges from 8 to 92%. The additional processing steps and human contact with ground meats may contribute to the number of listeriae present in the final product (*17*). Buchanan et al. (*4*) detected multiple species in 45% of the positive fresh meat samples, and *L. monocytogenes, L. innocua,* and *L. welshimeri* were detected in 82, 55, and 18%, respectively. Recent studies have shown *L. monocyto-*

TABLE 3. *Comparative detection time of Fung-Yu tube and USDA methods to recover uninjured and heat-injured* L. monocytogenes *in culture suspension.*

Strain	Cells/ml in inocula	Time (h)								
		USDA method				Fung-Yu tube method				
		UVM	FB[1]	MOX	Total	FB[2]	tube A	tube B	Total[a]	Total[b]
Uninjured 18 h culture:										
LM 101M	$4.3x10^3$	24	12	24-40	60-76	20	3	13-14	36-37	26
	$4.3x10^2$	24	12	24-40	60-76	22	3	16-17	41-42	28
	$4.3x10^1$	24	12	24-40	60-76	26	4	16-17	46-47	33
	$4.3x10^0$	24	12	24-40	60-76	27	4	16-17	47-48	34
LM 103M	$7.9x10^3$	24	10	24-40	58-74	20	3	11-12	34-35	26
	$7.9x10^2$	24	12	24-40	60-76	21	3	13-14	37-38	27
	$7.9x10^1$	24	12	24-40	60-76	22	3	17-18	42-43	28
	$7.9x10^0$	24	14	24-40	60-76	23	3	18-19	44-45	29
Scott A	$7.0x10^3$	24	12	24-40	60-76	25	3	13-14	41-42	31
	$7.0x10^2$	24	12	24-40	60-76	26	4	17-18	47-48	33
	$7.0x10^1$	24	15	24-40	63-79	27	4	19-20	50-51	34
	$7.0x10^0$	24	15	24-40	63-79	29	4	21-22	54-55	36
Heat-injured culture:										
LM 101M	$1.8x10^3$	24	13	24-40	61-77	24	4	16-17	44-45	31
	$1.8x10^2$	24	13	24-40	61-77	28	3	17-18	48-49	34
	$1.8x10^1$	24	15	24-40	63-79	30	4	17-18	51-52	37
	$1.8x10^0$	24	17	24-40	65-81	30	4	17-18	51-52	37
LM 103M	$4.5x10^3$	24	12	24-40	60-76	20	3	15-16	38-39	26
	$4.5x10^2$	24	13	24-40	61-77	22	3	16-17	41-43	28
	$4.5x10^1$	24	15	24-40	63-79	26	3	17-18	46-47	32
	$4.5x10^0$	24	17	24-40	65-81	27	4	17-18	48-49	34
Scott A	$7.0x10^3$	24	14	24-40	62-78	25	4	16-17	45-46	32
	$7.0x10^2$	24	14	24-40	62-78	27	4	17-18	48-49	34
	$7.0x10^1$	24	15	24-40	63-79	27	5	18-19	50-51	35
	$7.0x10^0$	24	17	24-40	65-81	30	5	20-21	55-56	38

Total[a]: Total time required to complete Fung-Yu tube procedure.
Total[b]: Total time required to obtain blackening of FB in tube A and blackening of half MOX agar column.
FB[1]: Time required to obtain blackening of FB in test tubes.
FB[2]: Time required to obtain blackening of FB in Stomacher bags.

genes to be present in 30-50% of raw minced meat samples (*19,23,25,28*). The reason for the variation in incidence may be due to differences in methodologies (*9*).

When 306 inoculated food samples were tested, the colorimetric GENE-TRAK® hybridization assay had a false-negative rate of approximately 0.8-4.7% (*18*). The enrichment approach consisted of 24 h in modified Food and Drug Administration (FDA's) *Listeria* enrichment broth buffered by the addition of MOPS [3(N-morpholino) propanesulfonic acid], followed by a period of growth on the surface of LiCl-phenylethanol-moxalactam agar plates. King et al. (*18*) found this procedure to be superior to other enrichments. The different false-negative rate observed in our study probably relates to the different enrichment approaches. The detectability may depend on the initial *Listeria* spp. count and condition (injured or uninjured), as well as the levels and types of background microorganisms present (*14*). Heisick et al. (*14*) compared the FDA culture procedure, the FDA probe procedure, and the enzyme-linked immunosorbent assay (ELISA) of Organon Teknika Co. with the DNA probe procedure of GENE-TRAK® Systems. With the GENE-TRAK® method, 14 false-negatives occurred among the 309 sample tests, apparently because the *Listeria* contamination in milk and vegetable samples had not after enrichment, reached the levels necessary for detection by the GENE-TRAK® procedure. In contrast, the motility enrichment Fung-Yu tube procedure detected low levels of *Listeria* spp. in meat products with the aid of Oxyrase™.

Epidemiological studies have associated human listeriosis with consumption of uncooked hot dogs and undercooked chicken (*3,23*). Johnson et al. (*17*) reported that 13-50% of ready-to-eat meat products sampled in Europe and Canada may be contaminated with *Listeria* spp., with about a third of such products contaminated with *L. monocytogenes*. In our study, a substantial proportion of

TABLE 4. *Comparison of the number of positive* Listeria *spp. isolated with Fung-Yu tube and GENE-TRAK® methods.*

Sample	Numbers of samples examined	Numbers of samples positive for *Listeria* Fung-Yu tube	GENE-TRAK®
Fresh raw meats:			
Ground beef	36	26 (72.2%)	20 (55.6%)
Beef breakfast strips	4	1 (25%)	1 (25%)
Ground pork	16	0	0
Pork sausage	10	8 (80%)	8 (80%)
Bacon	2	0	0
Bratwurst	5	0	0
Subtotal	73	35 (47.9%)	29 (39.7%)
Processed meats:			
Salami	6	0	0
Bologna	15	0	0
Pepperoni	2	0	0
Hams	25	3 (12%)	2 (8%)
Sliced, roast beef	17	0	0
Loaf (pickled M&C)	8	2 (25%)	2 (25%)
Franks, hot dogs, wieners, links	39	6 (15.4%)	4 (10.3%)
Braunschweiger	5	1 (20%)	0
Summer sausage	10	0	0
Smoked roast turkey/chicken slice	13	1 (7.7%)	0
Sandwich spread	2	0	0
Subtotal	142	13 (9.2%)	8 (5.6%)
Total	215	48 (22.3%)	37 (17.2%)

TABLE 5. *Numbers of* Listeria *spp. isolated from meat products.*

Listeria spp.	No. of isolates (%)	Sample description (No. of positive sample)
L. innocua	22 (42.3%)	Ground beef (6), braunschweiger (1) cheese hot dogs (2), sliced ham (4) pork sausage (7), M & C loaf (2)
L. monocytogenes	6 (11.5%)	Ground beef (2), smokie link (1) cheese hot dog (2), pork sausage (1)
L. grayi	8 (15.4%)	Ground beef (8)
L. murrayi	12 (23.1%)	Ground beef (11), beef breakfast strip (1)
L. ivanovii	2 (3.8%)	Ground beef (2)
L. denitrificans	2 (3.8%)	Cheese hot dog (1), roast turkey (1)
Total	52	

cheese hot dogs and M (macaroni) & C (cheese) loaf were found to be contaminated (Table 4). Two packaged cheese hot dog samples contained *L. innocua* and *L. monocytogenes*. Two packages of M&C loaf samples yielded *L. innocua*. The reasons for contamination are not clear; however, we speculate that it may have been due to the incorporation of underpasteurized, contaminated cheese into the finished meat products. This survey has again indicated that *L. monocytogenes* is frequently present in raw meat products. Therefore, the postprocessing cross-contamination of finished products with raw materials should be eliminated to ensure a low bacterial population. The Fung-Yu tube motility enrichment is a simplified culture method for rapid identification of the species of *Listeria* (i.e., not only *L. monocytogenes*), which may be served as indicator organisms of *Listeria* contamination in food. This new Fung-Yu tube system, using the combination of motility of *Listeria*, selectivity of culture media, and growth-enhancing effect of Oxyrase™ may reduce the time, media, labor, and cost requirements for *Listeria* detection.

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