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Development of Methods for the Recovery of *Escherichia coli* O157:H7 and *Salmonella* from Beef Carcass Sponge Samples and Bovine Fecal and Hide Samples†

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ABSTRACT

Culture methods were developed for the concurrent recovery of *Escherichia coli* O157:H7 and *Salmonella* from bovine carcass, hide, and fecal samples. Several enrichment conditions were tested for the overall growth of pure cultures; tryptic soy broth for 2 h at 25°C and then for 6 h at 42°C was the protocol selected for use. Immunomagnetic separation (IMS) was incorporated for sensitivity and selectivity, along with a post-IMS enrichment for the recovery of *Salmonella* as recommended by the manufacturer. Selective agars for plating after IMS were chosen on the basis of ease of target colony identification. Sorbitol MacConkey agar supplemented with cefixime and potassium tellurite and Rainbow agar supplemented with novobiocin and potassium tellurite were chosen for the recovery of *E. coli* O157:H7. Brilliant green agar with sulfadiazine and Hektoen enteric agar supplemented with novobiocin were selected for the recovery of *Salmonella*. The resulting methods were evaluated along with standard or previously used methods for the recovery of *E. coli* O157:H7 and *Salmonella* from bovine hide and fecal samples and carcass sponge samples. The Meats Research Unit (MRU) methods performed at least as well as the established methods, except that a secondary enrichment in tetrathionate (TT) broth prior to IMS was required for the optimal recovery of *Salmonella* from feces. Thus, the MRU and MRU-TT methods are effective in the recovery of both *E. coli* O157:H7 and *Salmonella* from a single bovine carcass, hide, or fecal sample.

Various pathogens are associated with red meat, but the relationships between these pathogens throughout the beef production continuum are unknown. The definitive determination of correlations between organisms requires the recovery of the different pathogens from a single sample, since bacterial loads can differ significantly per animal or carcass and at different, even adjacent, sites on the hide or carcass *(9, 18)*. Furthermore, the recovery of multiple pathogens from a single sample would increase laboratory efficiency during routine monitoring and prevalence studies.

Escherichia coli O157:H7 and *Salmonella* are human pathogens that are carried by cattle and may contaminate beef during the production process. Traditionally, selective enrichments have been used to recover *E. coli* O157:H7 from food, animal, and environmental samples *(3)*. Such enrichments have been necessary to reduce the levels of nontarget organisms during culture, even though the number of positive samples may have been underestimated because of the ineffective recovery of injured cells *(3, 17, 30)*. However, selective enrichments may no longer be necessary for the recovery of *E. coli* O157:H7 and other organisms because of the development and widespread acceptance of immunomagnetic separation (IMS). IMS is a simple, effective tool for the selective concentration of *E. coli* O157:H7 from enrichment cultures. It has been shown to clearly improve the recovery of *E. coli* O157:H7 from bovine fecal samples *(3, 4)*. In fact, enrichment in nonselective broth prior to IMS is recommended for some types of samples by at least one immunomagnetic bead manufacturer (Dynal Inc., Lake Success, N.Y.).

Traditional methods for the recovery of *Salmonella* from food and fecal samples differ from those for the recovery of *E. coli* O157:H7; nonselective preenrichment prior to one or two selective enrichments has generally been recommended *(6, 7, 23, 24, 26)*. These procedures are time-consuming and cumbersome, requiring as much as 72 h of enrichment time and numerous plates because of the need for parallel selective enrichments. Another commonly used method for the recovery of *Salmonella* from animal feces involves two parallel initial selective enrichments, making this method unsuitable for the simultaneous recovery of *E. coli* O157:H7 *(11)*. In addition, this method requires 60 to 72 h of enrichment time. Abbreviated enrichments may be possible with IMS; less overall *Salmonella* growth should be required because the organisms are concentrated during IMS. IMS was used to recover *Salmonella* from environmental and feed samples, but shorter preenrichment and enrichment times were not evaluated *(26)*.

This report describes the development of effective methods to recover *E. coli* O157:H7 and *Salmonella* from

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† Brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of any product mentioned, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

TABLE 1. *Bacterial strains used in this study*

Strain	Serotype or serovar	Source[a]
E. coli		
55AC1	O157:H7	MARC *(9)*
114AC1	O157:H7	MARC *(9)*
131AC1	O157:H7	MARC *(9)*
237AC1	O157:H7	MARC *(9)*
299AB3	0157:H7	MARC *(9)*
43895	O157:H7	ATCC
MA6	O157:H7 (rough)	P. Feng *(12)*
S. enterica		
95-2876	Enteritidis	NVSL
94-6327	Kentucky	APHIS
94-6529	Montevideo	APHIS
94-3461	Newport	APHIS
94-24085	Typhimurium	NADC
KC	Typhimurium DT104	MARC
564HB1	Poly A serogroup	MARC
574HB1	Poly B serogroup	MARC
575HB1	Poly E serogroup	MARC

[a] MARC, Roman L. Hruska U.S. Meat Animal Research Center; ATCC, American Type Culture Collection (Manassas, Va.); NVSL, National Veterinary Services Laboratory (Ames, Iowa); APHIS, Animal and Plant Health Inspection Service (Fort Collins, Colo.); NADC, National Animal Disease Center (Ames, Iowa).

bovine carcass, hide, and fecal samples. These Meats Research Unit (MRU) methods include a short enrichment in nonselective media, concentration and selection for each organism by IMS, and selective plating.

MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains used in this study are listed in Table 1. Cultures were grown overnight in tryptic soy broth (TSB) at 37°C and diluted in buffered peptone water (BPW) as necessary. To apply a mild acid shock where appropriate, overnight cultures grown in TSB without glucose were diluted 1:100 in brain heart infusion broth adjusted to pH 3.0 with hydrochloric acid and then held at room temperature for 5 min prior to dilution in BPW.

Unless otherwise indicated, culture media, including desoxycholate citrate lactose saccharose agar (DCLS), were of the Difco brand and were obtained from Becton Dickinson (Sparks, Md.). Brilliant green agar with sulfadiazine (BGS agar) was a BBL brand medium and was also obtained from Becton Dickinson. CHROMagar O157 and CHROMagar *Salmonella* were obtained from CHROMagar (Paris, France). Rambach agar was obtained from Merck (Darmstadt, Germany). Biosynth culture medium was obtained from BioSynth International (Naperville, Ill.). Media were prepared according to their manufacturers' directions. Niaproof (tergitol) supplement for XLT4 (xylose lysine tergitol) agar, Tween 20, and phosphate-buffered saline with Tween 20 were obtained from Sigma Chemical Co. (St. Louis, Mo.). Rainbow agar (Biolog, Inc., Hayward, Calif.) was supplemented with 20 mg of novobiocin (Sigma) per liter and 0.8 mg of potassium tellurite (Sigma) per liter (ntRainbow) to improve selectivity as suggested by the manufacturer. Hektoen enteric (HE) agar was supplemented with 15 mg of novobiocin per liter (nHE) to select against *Proteus* and *Citrobacter* species as suggested by the manufacturer. Cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter; Dynal) were added to sorbitol MacConkey agar to prepare ctSMAC agar; this medium was supplemented with 100 mg of 4-methylumbelliferyl-β-D-galactopyranoside (Sigma) per liter and 10 g of salicin (Sigma) per liter to prepare ctSSMAC as previously described *(15)*. Tryptone broth (TB) contains 10 g of Bacto Tryptone (Difco) per liter and 5 g of NaCl per liter *(27)*. Where appropriate, 2 ml of Oxyrase (Oxyrase, Inc., Mansfield, Ohio) per 100 ml of TSB was added just prior to use, as directed by the manufacturer. GNvcc is GN-Hajna broth supplemented with 8 mg of vancomycin (Sigma) per liter, 0.05 mg of cefixime (Dynal) per liter, and 10 mg of cefsuludin (Sigma) per liter *(9)*.

Assessment of selective agars and enrichment conditions. The seven enrichment conditions to be tested were selected on the basis of the use of nonselective media, the potential to increase numbers of injured or uninjured target organisms, and a short incubation time to limit the overgrowth of nontarget organisms (see "Discussion"). To prepare inocula, overnight cultures were diluted, and approximately 10 CFU of each organism was added to 25 ml of BPW–0.1% Tween 20 (BPW-Tween), which was used to sample carcasses (see below). In most cases, one *E. coli* O157:H7 strain and one *Salmonella* strain were added to a single aliquot of BPW-Tween to minimize sample numbers. Seventy-five milliliters of enrichment broth was added to the inoculated samples, and the cultures were incubated as outlined in Table 2. Subsequently, populations of *E. coli* O157:H7 strains were enumerated on ctSMAC and populations of *Salmonella* strains were enumerated on XLT4 using a Spiral Plater (Spiral Biotech, Bethesda, Md.).

Selective agars were evaluated in separate experiments with both pure and mixed cultures. First, pure cultures of *Salmonella* spp. and *E. coli* spp. were streaked for isolation on all selective agars listed in Table 3, including those agars specific for the isolation of the species other than the one plated. Plates were incubated overnight at 37°C, and colony color and morphology were evaluated by several investigators. Second, mixed cultures were used to evaluate the media. *E. coli* O157:H7–specific media were spread with 100 μl of 1:1 and 1:10 mixes of *E. coli* O157:H7 and non-O157 *E. coli* cultures, respectively, diluted to approximately 500 CFU/ml. *Salmonella*-specific media were spread with the final bead suspensions from anti-*Salmonella* IMS of mixed (1:1, 1:10, and 1:1,000) *Salmonella* and *E. coli* cultures diluted to approximately 10 to 10,000 CFU/ml. All plates were incubated overnight at 37°C and examined by multiple investigators to facilitate the differentiation of the target colonies.

Method evaluation assays. The MRU methods were created on the basis of the results of the assays described above (see Fig. 1). To evaluate these methods, hides and carcasses were sampled for natural contamination at a large fed-beef processing plant. One hundred samples of the same type were taken within the shortest possible time frame on the same day to minimize biases. Alternate samples were assigned to each of the methods. Fecal samples were recovered from a local feedlot and inoculated in the laboratory because the natural prevalence of the pathogens was found to be very low (data not shown).

The MRU and MRU-TT methods to recover *E. coli* O157:H7 and *Salmonella* were compared to standard methods for analyzing hide samples. For the MRU and MRU-TT methods, a stack of three 3-in^2 sterile gauze pads was wetted with 15 ml of sterile water and used to swab approximately 1,700 cm^2 of the hide in the brisket area. The pads were then placed in a standard Whirlpak bag (Nasco, Ft. Atkinson, Wis.) and treated as outlined in Figure 1. IMS, with enrichment in Rappaport-Vassiliadis (RV)

TABLE 2. *Growth of* E. coli *O157:H7 and* Salmonella *spp. in nonselective broths*[a]

Organism	Final cell count ($\log_{10}$ CFU/ml) for each enrichment scheme: TSB, 37°C, 6 h	TSB, 42°C, 6 h	Oxyrase, 37°C, 6 h[b]	TB, 37°C, 8 h	TB, 42°C, 8 h	TSB, 25°C, 2 h/37°C, 6 h[c]	TSB, 25°C, 2 h/42°C, 6 h[d]	Inoculum[e]
E. coli O157:H7								
114AC1	3.4	3.0	2.8	5.4	4.6	5.7	5.7	8.8
114AC1 *acid*[f]	3.0	3.6	3.1	4.6	5.2	5.5	5.7	8.5
131AC1	5.4	5.8	5.4	5.4	6.3	5.6	6.2	9.4
131AC1 *acid*	ND[g]	5.4	4.2	5.5	5.5	4.5	5.7	9.3
237AC1	3.1	3.7	2.8	6.0	4.1	5.8	5.6	8.9
237AC1 *acid*	1.9	3.6	1.6	4.2	3.7	4.1	4.2	8.4
299AB3	3.8	6.1	6.4	7.1	5.9	6.5	3.5	9.3
299AB3 *acid*	4.6	6.0	6.0	6.7	5.4	6.5	3.5	9.0
43895	4.9	4.1	4.3	4.1	3.7	4.6	4.6	9.5
43895 *acid*	4.6	4.4	4.0	3.7	3.9	5.4	5.6	ND
MA6	4.3	5.2	3.6	5.5	5.3	3.0	5.3	9.1
MA6 *acid*	4.5	5.5	3.7	5.6	4.4	3.3	5.5	9.2
S. enterica								
Enteritidis	3.0	5.3	4.8	ND	4.1	5.4	5.1	9.2
Enteritidis *acid*	2.3	3.4	2.1	2.9	3.4	4.0	3.0	8.5
Kentucky	2.9	3.1	2.2	ND	2.5	3.4	4.2	9.1
Kentucky *acid*	3.8	3.6	2.7	1.7	2.8	3.1	3.5	8.5
Montevideo	4.0	2.8	3.1	4.3	5.4	4.4	5.4	8.6
Montevideo *acid*	3.2	2.8	3.0	5.3	5.3	4.2	5.2	8.7
Newport	3.3	4.9	3.9	3.6	3.8	4.1	5.2	9.2
Newport *acid*	3.5	4.7	3.3	3.2	2.9	3.5	4.5	8.7
Typhimurium	5.1	5.6	4.9	5.0	4.0	5.7	5.0	9.3
Typhimurium *acid*	4.8	5.0	4.7	5.2	4.8	4.4	5.5	9.1
Typhimurium DT104	5.0	4.2	4.4	4.2	4.5	4.5	6.2	9.2
Typhimurium DT104 *acid*	4.6	4.2	4.6	4.3	4.9	5.5	7.1	ND

[a] Various broths were inoculated with overnight cultures diluted to $1:1 \times 10^7$ and then incubated as indicated.
[b] Oxyrase was added to TSB as described in "Materials and Methods."
[c] Cultures were incubated at 25°C for 2 h, then at 37°C for 6 h.
[d] Cultures were incubated at 25°C for 2 h, then at 42°C for 6 h.
[e] Inoculum levels ($\log_{10}$ CFU/ml) prior to dilution.
[f] *acid,* inocula were subjected to a mild acid shock (see "Materials and Methods").
[g] ND, not determined.

TABLE 3. *Comparison of selective plating agars*[a]

Selective agar	Rating
E. coli O157:H7	
ctSMAC	++
BCM	++
CHROMagar O157	+
Rainbow	+++
ctSSMAC	++
Salmonella	
Rambach	+
CHROMagar *Salmonella*	+
BGS	++
DCLS	++
HE	+++
XLT4	++

[a] Ratings are based on subjective observations of multiple investigators. Both mixed and pure cultures were examined as described in "Materials and Methods." Arbitrary units range from +++ (easy to use) to + (harder to use).

broth (Oxoid, Ogdensburg, N.Y.) for *Salmonella,* was performed according to the directions provided with the immunomagnetic beads (Dynal), except that protamine (100 μl of a 50-μg/ml filter-sterilized solution; Sigma) was added to the enrichment aliquots prior to the beads *(22).* For *E. coli* O157:H7, hide samples also were recovered and tested according to the standard method described by Elder et al. *(9).* Briefly, swab samples taken with water-wetted gauze pads were enriched in 20 ml of 1.5-fold-concentrated brilliant green bile 2% (BGB) at 37°C for 6 h and then held at 4°C overnight. Aliquots (1 ml) were subjected to anti–*E. coli* O157 IMS, and 50 μl of the final bead suspensions were spread onto ctSMAC agar. After 18 h of incubation at 37°C, up to three suspected *E. coli* O157:H7 colonies were picked and tested as described below. For *Salmonella,* hide samples also were tested by the standard method of the Food Safety and Inspection Service (FSIS) *(23).* Briefly, 50 ml of BPW was added to gauze swab samples, and these preenrichment cultures were incubated at 35°C for 20 to 24 h. Subsequently, 0.5 ml of culture was transferred to 10 ml of TT broth, and 0.1 ml was transferred to 10 ml of RV broth. These selective enrichments were incubated at 42°C for 22 to 24 h, and then loopfuls were streaked separately onto double

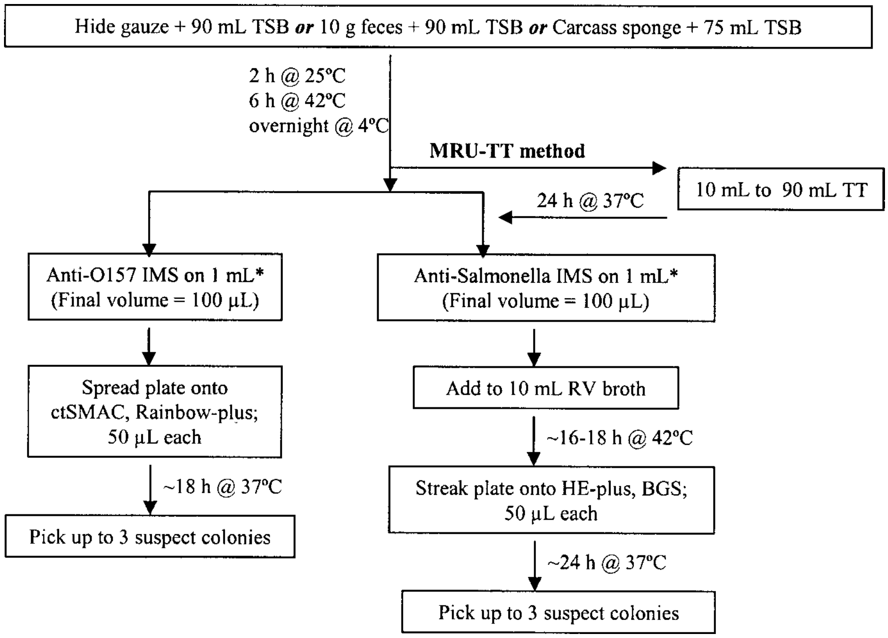

FIGURE 1. *Outline of the MRU and MRU-TT methods for the recovery of* E. coli *O157:H7 and* Salmonella *from bovine fecal, hide, and carcass samples. * One hundred microliters of a protamine solution (50 μg/ml) was added to the 1-ml hide and carcass enrichment aliquots prior to the addition of immunomagnetic beads. The RV enrichment step for* Salmonella *recovery was incorporated per the immunomagnetic bead manufacturer's recommendations.*

modified lysine iron agar *(23)* and BGS agar (BBL brand, Becton Dickinson). Plates were incubated at 37°C for 22 to 24 h; negative plates were returned to the incubator and reexamined the next day. Up to three suspected *Salmonella* colonies were picked from plates from each selective enrichment and tested as described below.

The MRU method to recover *E. coli* O157:H7 and *Salmonella* was compared to standard methods for analyzing carcass samples. For the MRU method, samples were taken immediately after hide removal and prior to evisceration. The perineal-hock area was swabbed as described previously (ca. 2,500 cm^2) *(9)* with a Speci-Sponge (NASCO) wetted with 25 ml of BPW-Tween. The samples were then treated as outlined in Figure 1, with protamine being added prior to IMS (see above). To determine the effects of extended storage, the TSB enrichments were also stored for an additional 24 h at 4°C and then reanalyzed with IMS and plating as described. For *E. coli* O157:H7, samples also were tested by the method of Elder et al. *(9)*. Briefly, perineal-hock samples were taken prior to evisceration with Speci-Sponges wetted with 25 ml of Butterfield's phosphate diluent–0.1% Tween 20, and then 90 ml of brilliant green bile was added prior to incubation at 37°C for 10 h. Enrichments were stored overnight at 4°C prior to anti–*E. coli* O157 IMS; 50-μl aliquots of the final bead suspensions were spread onto ctSMAC and BCM (Biosynth International) agars. Plates were incubated overnight at 37°C, and three to six suspected *E. coli* O157:H7 colonies per sample were examined. For *Salmonella,* carcass samples also were tested by the method of the FSIS as described above for hide samples *(23)*.

Fresh fecal samples were collected separately from 10 individual animals at a local feedlot. Each fecal sample was divided into five 25-g aliquots. Five *E. coli* O157 strains were used to inoculate the feces at 1 to 10 CFU/10 g (75 μl of culture per 25 g of feces); strains 55AC1, 114AC1, 131AC1, 237AC1, and 299AB3 had previously been recovered from beef carcasses (see Table 1). The aliquots were then split and assayed by each method, so all 50 samples were assayed by the methods described below. The experiment was repeated with five different *Salmonella* strains at 0.4 to 7 CFU/10 g of feces (15 μl of culture per 15 g of feces). The *Salmonella* strains used were 95-2876 (Enteritidis), 94-6327 (Kentucky), 564HB1, 574 HB1, and 575HB1 (see Table 1). The latter three *Salmonella* strains had previously been recovered from bovine hides (unpublished data). For the MRU and MRU-TT methods, 10-g aliquots of inoculated fecal samples were placed in stomacher bags with side filters (Spiral Biotech) and treated as outlined in Figure 1; protamine was not added prior to IMS. For *E. coli* O157:H7, 10-g aliquots of inoculated fecal samples were incubated into 90 ml of GNvcc broth at 37°C for 6 h as previously described *(9)*. *E. coli* O157:H7 was recovered with IMS and plating as described above for the analysis of hide samples by the Elder et al. *(9)* method. For *Salmonella,* inoculated feces were analyzed by the method developed in the Fedorka-Cray laboratory *(11)*. Briefly, separate 1-g fecal aliquots were placed in 9 ml of GN-Hajna broth and 9 ml of TT broth. The GN-Hajna broth cultures were incubated at 37°C for 18 to 24 h, and the TT broth cultures were incubated at 37°C for 48 h. One hundred microliters of each enrichment was then transferred to separate tubes containing 10 ml of RV broth. These secondary cultures were incubated at 37°C overnight. Loopfuls of each RV culture were streaked onto XLT4 and BGS plates, which were incubated for approximately 24 h at 37°C. Up to three suspected *Salmonella* colonies per sample were selected and tested as described below.

For isolate identification, presumptive *E. coli* O157:H7 colonies were screened with DrySpot *E. coli* O157 latex tests (Oxoid). Agglutination-positive colonies were streaked for isolation on ctSMAC and then stored in nutrient agar stabs *(27)* for further characterization. Isolates were confirmed to be *E. coli* O157:H7 by an enzyme-linked immunosorbent assay (ELISA) to detect the O157 and H7 antigens *(9)*. The presence of the H7 gene was confirmed by polymerase chain reaction–restriction fragment length polymorphism in the few isolates that were O157-positive and H7-negative by ELISA *(13)*. For *Salmonella,* suspected positive colonies were streaked for isolation on nHE agar. Black or grayish green colonies were considered potentially positive and stored in nutrient agar stabs for further characterization. Isolates were confirmed to be *Salmonella* on the basis of a combination of colony color on nHE agar, latex agglutination (Oxoid), and biochemical assay (Sensititre gram-negative AutoIdentification AP80 System, Accumed International, Westlake, Ohio). In all cases, only samples with isolates confirmed to be the target species were considered positive.

For statistical analyses, the chi-square distribution was used to compare the results for all methods except for *Salmonella* re-

TABLE 4. *Comparison of standard or previously used methods and the MRU and MRU-TT methods*[a]

Sample type	No. of positive samples for *E. coli* O157:H7 recovery method			No. of positive samples for *Salmonella* recovery method			
	Elder et al. *(9)*	MRU	MRU24[b]	Standard[c]	MRU	MRU-TT	MRU24[b]
Carcass	13 A[d]	24 B	24 B	21 A	48 B	ND[e]	29 A
Hide	6 A	37 B	42 B	49 A	45 A	49 A	ND
Feces	5 A	12 A	11 A	15 A	22 A	41 B	47 B

[a] Fifty samples were analyzed by each method; the numbers of samples identified as positive are indicated. Naturally contaminated samples were used to evaluate carcass and hide samples. Carcasses were sampled immediately after hide removal. Fecal samples were inoculated with very low levels of target bacteria (see "Materials and Methods").

[b] Samples were analyzed by the MRU method (or by the MRU-TT method for the recovery of *Salmonella* from fecal samples), except that the TSB enrichments were stored at 4°C for an additional 24 h prior to further processing.

[c] Methods currently widely in use: the method used by the FSIS for carcass and hide samples *(23)* and the method used in the Fedorka-Cray laboratory for fecal samples *(11)*.

[d] Within a row, values for an organism with the same letter are not significantly different ($P > 0.05$).

[e] ND, not determined.

covery from hides, for which the results were evaluated by Fisher's exact test because of the small numbers of negative samples. Note that these statistical methods are specifically designed to evaluate results from independent samples. A modified chi-square test was used for the analysis of nonindependent samples, that is, inoculated fecal samples by all methods and MRU–MRU-TT or MRU-MRU24 analyses from the same TSB enrichments *(28)*. Differences were considered statistically significant at the $P \leq 0.05$ level.

RESULTS

Selection of enrichment conditions and plating media. Nonselective enrichment conditions were evaluated on the basis of the final populations of several *E. coli* O157:H7 and *Salmonella* strains. Inocula were prepared both with and without a mild acid shock to evaluate the recovery of injured cells (Table 2). Incubation periods were kept relatively short to limit the potential overgrowth of competing species in actual samples. More of the strains were found to grow to high levels with TSB incubated at 25°C for 2 h and then at 42°C for 6 h than with any of the other enrichment schemes examined. Therefore, this enrichment scheme was selected for further use.

Several selective plating media were evaluated for their efficiency in the identification of *E. coli* O157:H7 or *Salmonella* colonies (Table 3). The media were compared on the basis of ease of distinction between positive colonies and other colonies by phenotype. *E. coli* O157:H7 colonies on crowded plates were more difficult to distinguish on BCM and CHROMagar O157 than on Rainbow agar, and the fluorogenic substrate in ctSSMAC was too diffuse to aid in colony identification on crowded plates. Thus, ct-SMAC and Rainbow agar were selected for further use. Rainbow agar was subsequently supplemented with novobiocin and potassium tellurite (ntRainbow) to improve selectivity, as recommended by the manufacturer.

Salmonella colonies were more difficult to distinguish from other colonies on Rambach agar and CHROMagar *Salmonella* than on the other agars tested (Table 3). While it also was somewhat difficult to distinguish positive colonies on BGS agar, this medium provided excellent recovery of *Salmonella* after direct plating following IMS. DCLS agar also provided excellent recovery of *Salmonella* after IMS, but *Salmonella* colonies were much harder to identify on this agar. HE agar provided the second best recovery of *Salmonella* after IMS, and *Salmonella* colonies were very distinctive on this medium. In addition, novobiocin could be added to HE agar (as recommended by the manufacturer) to eliminate the growth of many organisms that can be confused with *Salmonella* on both HE and XLT4 agar. XLT4 agar provided the poorest recovery of *Salmonella* after IMS, at least in part because *Salmonella* colonies did not always exhibit the typical black phenotype on this medium after IMS. Thus, BGS agar and nHE agar were selected for further use.

Comparison of methods for the recovery of *E. coli* O157:H7 and *Salmonella* from carcass, hide, and fecal samples. The chosen enrichment conditions and selective plating media were incorporated into the MRU and MRU-TT methods (Fig. 1). These methods are based on the use of IMS to selectively concentrate *E. coli* O157:H7 and *Salmonella* after enrichment and prior to plating. A preliminary evaluation of the MRU and MRU-TT methods was conducted in parallel with previously established protocols for the recovery of *E. coli* O157:H7 and *Salmonella* from bovine carcass, hide, and fecal samples (Table 4). In addition, the effect of storing the initial MRU enrichments for an additional 24 h at 4°C prior to IMS was examined.

E. coli O157:H7 was recovered from naturally contaminated carcass and hide samples by the MRU methods and by the method of Elder et al. *(9)* (Table 4). More positive samples were identified by the MRU methods than by the previously described methods ($P \leq 0.05$). Inoculated fecal samples were also analyzed by the MRU methods and by the method of Elder et al. *(9)*. Twice as many positive samples were identified with the MRU method, but the difference was not statistically significant ($P > 0.05$). For all three sample types, storage of the MRU enrichment for a longer period (24 h at 4°C) prior to IMS did not significantly change the number of positive samples.

The MRU methods were evaluated along with the method used by the FSIS for the recovery of *Salmonella* from naturally contaminated carcass and hide samples *(23)* (Table 4). More positive carcass samples were identified by the MRU method than by the established method ($P \leq 0.05$). However, the number of positive carcass samples identified by the MRU method dropped significantly when the TSB enrichments were stored for an additional 24 h at 4°C. There were no statistically significant differences between the MRU, MRU-TT, and standard methods for the recovery of *Salmonella* from hide samples ($P > 0.05$).

Preliminary experiments indicated that the MRU method was inadequate for the recovery of *Salmonella* from bovine feces (data not shown), so the MRU-TT method was developed. Inoculated feces were then used to compare both of these methods with the established method of Fedorka-Cray et al. *(11)* (Table 4). The number of positive samples identified by the MRU-TT method was significantly larger than the number identified by either the established method or the unmodified MRU method. Extended storage of the TSB enrichments prior to the secondary TT enrichment had no statistically significant effect on the identification of positive samples by the MRU-TT method.

DISCUSSION

The MRU and MRU-TT culture methods presented here can be used to recover both *E. coli* O157:H7 and *Salmonella* from bovine carcass, hide, and fecal samples. The initial, nonselective enrichment in TSB also permits the recovery of non-O157 Shiga toxin–producing *E. coli* (data not shown). Since at least two pathogens can be recovered from one sample, fewer samples may be required for testing and/or monitoring procedures, and relationships between the presence of different organisms can be evaluated.

A few methods for the detection, but not the recovery, of both *E. coli* O157:H7 and *Salmonella* in food and environmental samples have previously been described *(1, 10, 14, 25)*. These methods rely on 18- to 24-h enrichments and nonculture assays to identify positive samples. The MRU and MRU-TT methods were designed for the recovery of the actual organisms, which is still considered the "gold standard" and provides isolates for further characterization or tracking studies. However, the same enrichment can also be used for detection by polymerase chain reaction (data not shown), suggesting that alternative screening and detection technologies could be employed if desired. Alternative detection technologies may be desirable for faster results.

Our method development efforts were focused on the use of a nonselective enrichment, because direct inoculation of selective media can prohibit the recovery of injured bacteria and could have limited our ability to recover all target organisms *(3, 20)*. Fukushima and Gomyoda *(16)* suggested that enrichment in TSB for 6 h at 42°C was optimal for the recovery of *E. coli* O157:H7 prior to an acid treatment for selectivity. A preliminary 2-h incubation at 25°C was added to allow for the resuscitation of any injured *E. coli* O157:H7 cells *(17)*. The preincubation period did improve the growth of *E. coli* spp. and *Salmonella* spp. in our assay, but the general increase in growth may simply have been due to the increase in incubation time. Alternative strategies to recover injured cells—for example, the addition of Oxyrase to TSB or the use of a medium lacking sugars (TB)—appeared to limit growth even though the incubation period was extended for the TB cultures. Oxyrase had previously been shown to decrease oxidative stress during enrichment in media containing sugars (contributed by peptone in BPW) and to enhance the recovery of *E. coli* O157:H7 and *Salmonella (3, 29)*. A recent report suggests that universal preenrichment broth may provide a suitable alternative for the recovery of injured pathogens, but this broth was not included in our assay *(31)*.

After enrichment and IMS, selective plating media are needed to identify target colonies. Many chromogenic media have been developed for the identification of *E. coli* O157:H7 or *Salmonella* colonies *(5, 20)*. Each of these media does offer distinctive target colony phenotypes, but nontarget background growth can still complicate the identification of the correct colonies. For the MRU methods for carcass and hide samples, background carryover to the plates was reduced with the addition of protamine to the cultures during the first IMS step. Protamine has been shown to reduce background from IMS by inhibiting nonspecific bacterial binding *(22)*. Protamine was not added to fecal enrichments because background growth was much less of a problem for these samples; we did not investigate the reasons for this difference. Background microflora carryover can also be effectively reduced by changing microcentrifuge tubes between wash steps during IMS *(22)*, but this procedure is impractical when large numbers of samples must be analyzed.

The use of two types of media with different selective mechanisms enhanced the identification of positive samples for both *E. coli* O157:H7 and *Salmonella*. The media chosen for the recovery of *E. coli* O157:H7, ntRainbow agar and ctSMAC, have also performed well in other studies *(3, 19, 21)*. For the recovery of *Salmonella,* nHE agar was superior to XLT4 agar and complemented BGS agar in our study. XLT4 agar is frequently used for the recovery of *Salmonella* but may not be suitable for use after IMS. We found that *Salmonella* colonies often appeared yellow instead of typically black when IMS beads were plated directly on XLT4 agar (data not shown). The biochemical basis for this observation was not investigated, but it raised sufficient concerns that we chose not to use XLT4 and to incorporate the secondary RV enrichment recommended for heavily contaminated samples by the immunomagnetic bead manufacturer (Dynal). The elimination of this secondary enrichment would result in additional time savings and warrants further investigation.

The data presented here suggest that the MRU methods effectively identify *E. coli* O157:H7 in bovine carcass, hide, and fecal samples. These methods appear to represent improvements over the methods used by Elder et al. *(9)*, which may be hampered by the presence of selective agents that can limit the recovery of injured *E. coli* O157:H7 cells *(2, 30)*. Additional studies are needed to confirm these observations. Even so, the Elder et al. *(9)* method for the recovery of *E. coli* O157:H7 from bovine feces represented a substantial improvement over previous methods, such as direct plating *(9).* It was therefore surprising that both this method and the MRU method were only moderately successful in recovering *E. coli* O157:H7 from inoculated fecal samples. Sufficient numbers of naturally contaminated samples were not available for comparison, and it is possible that both methods would have been more successful at identifying such samples. In fact, all five of the samples identified as positive by the Elder et al. *(9)* method and five of the samples identified as positive by the MRU method came from the feces of one animal, suggesting that natural contamination may have been detected. Naturally contaminated samples may contain higher levels of *E. coli* O157:H7, and laboratory-cultured *E. coli* O157:H7 directly inoculated into bovine feces may not survive well.

The MRU methods appear to provide excellent recovery of *Salmonella* from carcass and hide samples and may be at least as successful as the method established by the FSIS *(23).* In addition, the data suggest that *Salmonella* may be recovered more effectively from bovine feces by the MRU-TT method than by the established method developed in the Fedorka-Cray laboratory *(11).* However, as was the case for *E. coli* O157:H7, inoculated feces were used, and the recovery differences may not be reflected in the analysis of naturally contaminated samples. Most likely, the smaller number of positive samples identified by the established method is due to the very low inoculum levels used, since this method employs 1-g fecal samples instead of 10-g fecal samples. A pilot experiment suggested that this method was as effective as the MRU-TT method when 10-g fecal samples were employed (data not shown). Interestingly, the Fedorka-Cray et al. *(11)* method has been shown to be as effective as the method of Davies et al. *(8),* which analyzes 10-g fecal samples and includes a nonselective preenrichment.

In summary, the MRU and the MRU-TT methods were developed for the recovery of both *E. coli* O157:H7 and *Salmonella* from bovine carcass, hide, and fecal samples. These methods employ a brief, nonselective two-temperature enrichment to recover the organisms, with a secondary enrichment (for the MRU-TT method) for the optimal recovery of *Salmonella* from bovine feces. Specificity is achieved with IMS and selective plating media, with a supplemental selective enrichment for *Salmonella.* These methods appear to be at least as effective as or more effective than established methods for the independent recovery of *E. coli* O157:H7 and *Salmonella,* although additional studies are needed to further establish their reproducibility and sensitivity.

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