IDEXX Summary 6L

Topic:	Colilert® compares well with USFDA methods for detecting coliforms and *E.coli* in food
Title:	Comparison of Commercially Available Kits with Standard Methods for the Detection of Coliforms and *Escherichia coli* in Foods
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Report Highlights:

- Colilert was compared to six other methods, including the USFDA and Japanese standard methods, for detecting coliforms and *E. coli* in food. (p. 2237, paragraph 1)

- Of the methods tested, Colilert had the most comparable results to the USFDA standard method. (p. 2242 and paragraph 2)

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Comparison of Commercially Available Kits with Standard Methods for the Detection of Coliforms and *Escherichia coli* in Foods

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Three commercially available kits that were supplemented with substrates for enzyme reactions were evaluated to determine their abilities to detect coliforms and fecal coliforms in foods. Japanese and U.S. Food and Drug Administration standard methods, as well as two agar plate methods, were compared with the three commercial kits. A total of 50 food samples from various retailers were examined. The levels of detection of coliforms were high with the commercial kits (78 to 98%) compared with the levels of detection with the standard methods (80 to 83%) and the agar plate methods (56 to 83%). Among the kits tested, the Colilert kit had highest level of recovery of coliforms (98%), and the level of recovery of *Escherichia coli* as determined by β-glucuronidase activity with the Colilert kit (83%) was comparable to the level of recovery obtained by the U.S. Food and Drug Administration method (87%). Isolation of *E. coli* on the basis of the β-glucuronidase enzyme reaction was found to be good. Levine's eosine methylene blue agar, which has been widely used in various laboratories to isolate *E. coli*, was compared with 4-methylumbelliferyl-β-D-glucuronide (MUG)-supplemented agar for isolation of *E. coli*. Only 47% of the *E. coli* was detected when eosine methylene blue agar was used; however, when violet red bile (VRB)-MUG agar was used, the *E. coli* detection rate was twice as high. Of the 200 *E. coli* strains isolated, only 2 were found to be MUG negative, and the gene responsible for β-glucuronidase activity (*uidA* gene) was detected by the PCR method in these 2 strains. Of the 90 false-positive strains isolated that exhibited various *E. coli* characteristic features, only 2 non-*E. coli* strains hydrolyzed MUG and produced fluorescent substrate in VRB-MUG agar. However, the PCR did not amplify *uidA* gene products in these VRB-MUG fluorescence-positive strains.

In 1990, a coliform rule to detect coliforms and *Escherichia coli* with fluorogenic substrates was promulgated in the United States (10), and this method was subsequently adopted by the Japanese government for drinking water. In this new procedure, two active substrates, *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG), are combined to simultaneously detect total coliforms and *E. coli*. Total coliforms produce the enzyme β-galactosidase, which hydrolyzes ONPG and releases *o*-nitrophenol, which produces a yellow color. *E. coli* produces the enzyme β-glucuronidase, which hydrolyzes MUG to form a fluorescent compound. This simple and rapid detection method was developed for detecting coliforms in surface water (3, 5, 6, 28), drinking water (8, 30, 31), marine water (27), marine snow (20), wastewater (13), fecal material (31), and food (23, 29). Production of β-glucuronidase was observed in about 97% of the *E. coli* strains examined (18). In the family *Enterobacteriaceae*, the only other microbes that reportedly are able to produce β-glucuronidase are some *Salmonella* spp. (13, 19), some *Shigella* spp. (13, 18), and some *Yersinia* spp. (33). In addition to these organisms, some *Staphylococcus* species are also able to hydrolyze MUG (22).

Following the success of the technology described above (9) and because the assay is rapid and sensitive, many companies have produced chromogenic-fluorogenic substrate systems for enumerating coliforms and fecal coliforms; these systems include the Colilert system (Environetics, Branford, Conn.), the lauryl sulfate tryptose (LST)-MUG system (Hach, Loveland, Colo.), the Colitrack-plus system (BioControl Systems, Bothell, Wash.), the LMX broth system (Merck, Darmstadt, Germany), and the Promedia system (Elmex, Tokyo, Japan). Many of these systems are based on LST enrichment followed by fluorogenic evaluation; one exception is the Colilert system, in which minimal medium is used to promote bacterial growth. Some workers have documented the use of the LST-MUG method for detecting *E. coli* in foods (13, 22, 32, 33) and the use of LMX broth with soft cheeses (16, 20). A collaborative study has been carried out to determine the applicability of the fluorogenic assay for detection of *E. coli* detection in chilled and frozen foods (23). Although Moberg et al. (23) obtained some false-positive results, it has been recommended that the LST-MUG procedure should replace the U.S. Food and Drug Administration (USFDA) method for screening meat and meat products for the presence of *E. coli*.

This study was undertaken to compare three commercial preparations of fluorogenic substrate-based detection media (Colilert, LST-MUG, and Colitrack-plus) with USFDA (14) and Japanese standard methods (15) for detecting total coliforms and *E. coli* in foods. This study was also designed to produce data on the suitability of the MUG-based products for detecting *E. coli* in raw and processed foods of various kinds. In addition, MUG-supplemented agar was compared with eosine methylene blue (EMB) agar for presumptive isolation of *E. coli*.

MATERIALS AND METHODS

Stock cultures and media. The microorganisms used in this study were *E. coli* ATCC 25922, and *Klebsiella pneumoniae* IFO 13833. All test organisms were grown in Trypticase soy broth (Eiken Co., Tokyo, Japan) at 35°C for 24 h before they were used. The overnight cultures were serially diluted in sterile phosphate-buffered saline (PBS) water.

Food samples. A total of 50 food samples were obtained from retail establishments. These samples represented the following products: finfish (13 samples), shellfish (4 samples), meat and meat products (18 samples), vegetables (12

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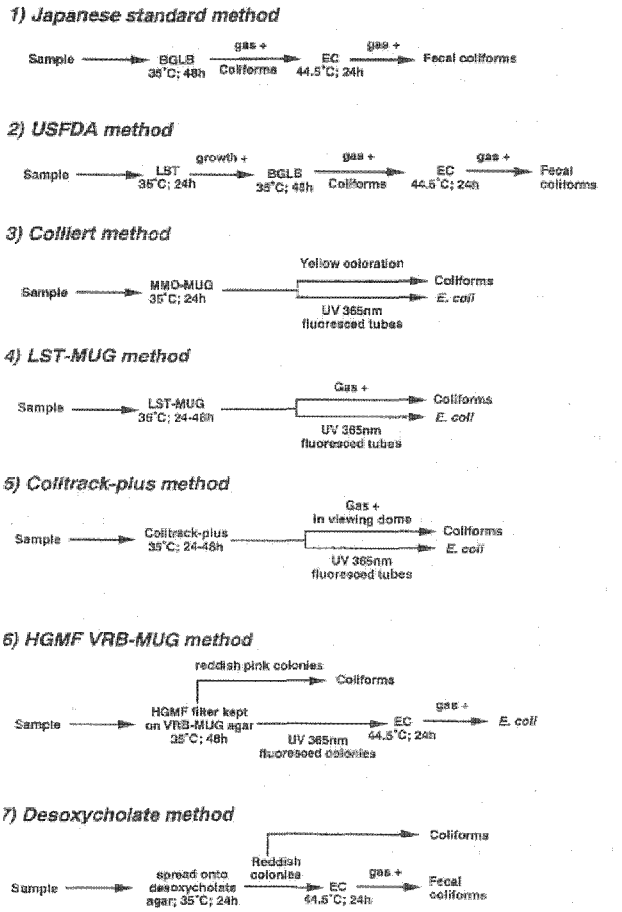

FIG. 1. Various protocols used to detect coliforms and fecal coliforms or *E. coli*. MMO-MUG, ONPG-MUG minimal medium.

samples), and other foods (3 samples). All of the samples were immediately transported to the laboratory in an icebox, and the bacteriological analysis was initiated within 2 to 3 h of purchase. A 25-g analytical unit of each food was homogenized for 1 min with a homogenizer (model SH-001; Elmex) along with 225 ml of sterile PBS, and then serial 10-fold dilutions were prepared with sterile PBS.

Comparison of various methods for detecting total coliforms and fecal coliforms. Two internationally used standard procedures (the Japanese procedure and the USFDA procedure), three commercially available kits, and two simple agar methods were used to recover the total and fecal coliforms in 50 food samples during April 1994 through March 1995, and the results obtained were compared. Figure 1 shows the protocols of the procedures used in this study. Prepared LST broth, brilliant green lactose bile (BGLB) broth, and *E. coli* (EC) broth were used when and where necessary; these media were obtained from Central Kagaku Corp., Tokyo, Japan. A three-tube, three-dilution, most-probable-number (MPN) method was used for the Japanese standard, USFDA, Colilert, LST-MUG, and Colitrack-plus tests. Double-strength media were used when necessary.

(i) Japanese standard method. Three dilutions of food homogenate (10, 1, and 0.1 ml) were inoculated into 9-ml portions of BGLB broth, and the preparations were incubated at 35°C for 48 h. Gas-positive BGLB broth tubes were considered positive for the presence of coliforms, and a portion of the preparation in each gas-positive tube was transferred into EC broth and incubated at 44.5°C. All gas-positive EC broth tubes were considered positive for the presence of fecal coliforms, and the MPN was recorded (15). The contents of the gas-positive tubes were streaked onto Levine's EMB agar (Nissui, Tokyo, Japan) and Fluorocult violet red bile (VRB)-MUG agar (Merck). Three presumptive *E. coli* colonies were picked from each preparation, purified, and identified (see below).

(ii) USFDA method. Three dilutions of food homogenate (10, 1, and 0.1 ml) were inoculated into LST broth, and the preparations were incubated at 35°C for 24 h (14). All growth-positive LST broth tubes were subjected to further analyses with BGLB broth, EC broth, EMB agar, and VRB-MUG agar as described above.

(iii) Colilert method. Each Colilert kit contains two active substrates, ONPG and MUG, to simultaneously detect total coliforms and *E. coli*, respectively. Three dilutions of 10 ml of a food homogenate were inoculated into three ONPG-MUG minimal medium (Colilert) (Aska Diagnostics, Inc., Tokyo, Japan) tubes that contained concentrated powder of the chromogenic-fluorogenic substrate-supplemented media, and the preparations were incubated at 35°C for 24 h. The contents of the tubes that fluoresced were streaked onto EMB agar and VRB-MUG agar, and presumptive *E. coli* colonies were purified and identified. A change of color from transparent to yellow was considered a positive reaction for the presence of coliforms, and bluish fluorescence under 365-nm UV light was considered a positive reaction for the presence of *E. coli*, and the MPN was recorded.

(iv) LST-MUG method. Three dilutions of 10 ml of a food homogenate were inoculated into three LST-MUG tubes (Central Kagaku), and the preparations were incubated at 35°C for 48 h. Each LST-MUG kit contains LST broth supplemented with MUG, in which total coliforms ferment lactose and produce gas within 48 h. *E. coli* was detected by the β-glucuronidase reaction after 24 h as described above for the Colilert method, and the MPN was recorded.

(v) Colitrack-plus method. Each Colitrack-plus kit (Gunze Sangyo, Inc., Tokyo, Japan) contains nine wells so that three sets of three dilutions of 1-ml samples can be inoculated. Each inoculated kit was incubated at 35°C for 24 h. The presence of gas bubbles in the viewing dome after 48 h of incubation indicated that a test was presumptively positive for coliforms. *E. coli* results were determined after 24 h of incubation by determining whether fluorescence was present in the chambers.

(vi) HGMF VRB-MUG agar method. Samples (10 ml) of food homogenate were filtered through hydrophobic grid membrane filter (HGMF) units (pore size, 0.45 μm; Gunze Sangyo), and the HGMF filters were placed on VRB-MUG agar and incubated for 48 h at 35°C. Reddish pink colonies were considered coliform colonies, and colonies that fluoresced bluish under 365-nm UV light were considered *E. coli* colonies. The colonies that fluoresced were transferred into EC broth, and gas production at 44.5°C after 24 h of incubation was checked.

(vii) Desoxycholate agar method. Desoxycholate agar (Nissui) has been widely used to count coliforms in chilled foods. Pour-plated desoxycholate agar plates were incubated at 35°C for 24 h. Red colonies were considered coliform colonies, and typical large, dark red colonies were transferred into EC broth and incubated at 44.5°C to confirm that they were *E. coli* colonies.

Determination of fluorescence in MUG-based kits. Foods that exhibited autofluorescence under 365-nm UV light before incubation were centrifuged at 9,000 × *g* for 10 min at 4°C, and the resulting precipitates were resuspended in sterile PBS before they were analyzed. In this analysis tubes inoculated with *E. coli* (1 CFU/ml) were used as positive controls (analyzed by the Colilert and LST-MUG methods), and fluorescence intensity that was as great as the fluorescence intensity of the controls after 24 h of incubation at 35°C was considered a positive result.

Isolation of *E. coli*. Initially, EMB agar plates were used to isolate *E. coli* from food samples 1 to 29, along with VRB-MUG agar. In addition to colonies with a green metallic sheen, colonies with violet centers and violet colonies were also picked from the EMB agar plates and purified on trypto-soy agar (Eiken) plates before they were identified. As the rate of *E. coli* isolation in EMB agar was very low, *E. coli* isolation was carried out on VRB-MUG agar plates for subsequent food samples (samples 30 to 50). Colonies on VRB-MUG agar plates that exhibited fluorescence at 365 nm were picked and purified before they were identified.

Identification of *E. coli*. All of the strains isolated were examined to determine their characteristics on EMB agar, whether they produced gas at 44.5°C in EC medium, and whether they exhibited β-glucuronidase activity. β-Glucuronidase activity was checked by using Bactident E. coli (Merck) as recommended by the manufacturer. Biochemical characteristics of the bacterial isolates were determined by using the EB-20 system (Nissui) and the Biolog identification system (Biolog, Hayward, Calif.) as recommended by the manufacturers (35).

PCR assays. *E. coli* strains were grown on trypto-soy agar plates overnight at 37°C. The bacterial cells in a well-isolated colony were resuspended in sterile PBS to a concentration of 10^5 CFU/ml and used as a DNA template. Oligonucleotide primers based on previously published nucleotide sequence data for *uidA* genes (2) were synthesized with an apparatus (Beckman Instruments, Inc., Fullerton, Calif.) as recommended by the manufacturer.

Bacterial DNA was amplified by using 100-μl volumes. A 10-μl portion of a 10^5-CFU/ml bacterial suspension prepared in sterile PBS was added to a PCR mixture containing 2 to 4 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dCTP, 0.2 mM dTTP, each primer at a concentration of 1 μM, and 2.5 U of *Thermus aquaticus* (*Taq*) DNA polymerase. Amplification was performed with a DNA thermal cycler (Perkin-Elmer Cetus) by using 30 cycles consisting of 30 s at 94°C, 1.5 min at 58°C, and 2.5 min at 72°C, with a final extension step at 72°C for 7 min. Following DNA amplification, 15-μl aliquots of each PCR mixture were analyzed by submarine gel electrophoresis on 3% agarose (Nusieve) gels. The samples were electrophoresed for 50 min at 100 V and then stained with ethidium bromide for 15 min. The stained bands were visualized by UV transillumination and photographed. Molecular size markers (100-bp ladder; Gibco-BRL) were included in each gel.

TABLE 1. Comparison of various methods for detecting food-borne coliforms, fecal coliforms, and *E. coli*

Method	No. (%) of samples positive for[a]:		No. of food samples from which *E. coli* was isolated in the following categories[b]:				
	Coliforms (n = 50)	Fecal coliforms (n = 50)	Finfish (n = 13)	Shellfish (n = 4)	Meat and meat products (n = 18)	Vegetables (n = 12)	Others (n = 3)
Japanese standard	32 (80.0)	12 (52.2)	0	0	8	3	0
USFDA	33 (82.5)	20 (87.0)	2	0	12	6	0
Colilert	39 (97.5)	19 (82.6)	1	1	13	3	0
LST-MUG	37 (92.5)	15 (65.2)	2	1	9	1	0
Colitrack-plus	31 (77.5)	12 (52.2)	0	1	9	2	0
HGMF VRB-MUG	33 (82.5)	8 (34.8)	0	0	5	1	0
Desoxycholate agar	22 (55.0)	6 (26.1)	0	0	6	0	0

[a] The numbers of coliform and fecal coliform samples positive for coliforms, fecal coliforms, or *E. coli* were 40 and 23, respectively.
[b] The numbers of finfish, shellfish, meat and meat product, vegetable, and other samples positive for coliforms, fecal coliforms, or *E. coli* were 3, 1, 13, 6, and 0, respectively.

RESULTS

Comparison of various methods for isolating coliforms and fecal coliforms. The results obtained with various methods for detecting coliforms and fecal coliforms are shown in Table 1. Of the 50 food samples tested, 40 contained coliforms and 23 harbored fecal coliforms. For coliform detection, two commercial kits, the Colilert and LST-MUG kits, performed well (levels of detection, more than 90%) compared with both the Japanese standard method (level of detection, 80%) and the USFDA method (level of detection, 82.5%). One of the agar plate methods, the desoxycholate agar method (level of detection, 55%), was found to be harmful for some coliforms compared with VRB-MUG agar method (level of detection, 82.5%). The incidence of *E. coli* in the 50 food samples that were analyzed by all seven method is also shown in Table 1. Of the 50 samples tested, 23 were found to harbor *E. coli*; 72% of the meat and meat product samples were contaminated, and 55% of the vegetable samples were contaminated. Two finfish samples and one shellfish sample also contained this organism. Identification of the presumptively positive *E. coli* strains showed that the Colilert test detected the organism in all 13 meat and meat product samples that harbored *E. coli*. The USFDA test failed to detect *E. coli* in one meat sample. Similarly, the Japanese standard, LST-MUG, and Colitrack-plus methods detected *E. coli* in 62 to 69% of the samples when meat and meat products were tested. Four samples that were *E. coli* negative as determined by the Colilert test but positive as determined by the USFDA method had an *E. coli* MPN range of 1 to 7 CFU/g. Three of the four Colilert *E. coli*-negative samples detected by the USFDA method were vegetable samples. The chromogenic-fluorogenic system did not perform well when it was used to detect *E. coli* in vegetables; however, this organism was detected by the USFDA method in all samples. *E. coli* that was isolated from a shrimp (Black Tiger) was correctly identified by all three fluorogenic substrate-based methods used, but *E. coli* was not isolated by the USFDA method.

Isolation of *E. coli*. Isolation and recovery of *E. coli* were checked by using EMB agar and MUG-supplemented VRB agar (Fig. 2). Initially, EMB agar and VRB-MUG agar were used to isolate *E. coli* from 29 food samples. Although colonies that had a metallic sheen, violet coloration, or violet center coloration were picked from EMB agar and identified, *E. coli* could be detected in 44% of the presumptive fecal coliform-positive tubes. *E. coli* was isolated from EMB agar when the fecal coliform concentration was >9 CFU/g (44% of the samples), whereas most *E. coli*-negative samples on EMB agar contained <9 CFU of fecal coliforms per g (56% of the samples); the exceptions were food sample 25 (from fried garlic chicken; 1.5×10^1 CFU/g) and food sample 28 (from boiled pumpkin; 4.3×10^1 CFU/g). Only the following three food samples (Fig. 2) contained *E. coli* when EMB agar was used: food samples 1 (from chicken meat), 21 (from boiled chicken meat and egg boiled with vegetable and fish paste), and 23 (from hackberry-grown mushroom and spinach salad). Fecal coliform concentrations of $>2.4 \times 10^3$ CFU/g were found in samples 1 and 23, whereas sample 21 contained only 9 CFU/g. However, *E. coli* was isolated from all of the fecal coliform-positive tubes when VRB-MUG agar was used as the isolation medium.

On the basis of the results described above, it could be concluded that the MUG-supplemented, less selective VRB-MUG agar was superior to EMB agar. Hence, VRB-MUG agar was used to isolate *E. coli* from food samples 30 through 50. The results obtained showed that *E. coli* was isolated from 90% of the 59 fecal coliform-positive tubes when VRB-MUG was used. Only six tubes that were presumptively positive for fecal coliforms did not yield *E. coli*. With LST-MUG agar there were two false-positive samples (both from pork meat), with the Japanese standard method there was one false-positive sample (from pork), and with the HGMF VRB-MUG method there were three false-positive samples (two from pork and one from sweet potato). *E. coli* was detected in all presumptively fecal coliform-positive tubes by the Colilert and USFDA methods when VRB-MUG was used as the *E. coli* isolation medium.

Characterization of *E. coli* isolates. Table 2 shows the characteristic features of *E. coli* strains that were isolated from EMB agar and VRB-MUG agar. The strains that were isolated on VRB-MUG agar from samples 1 to 29 were not compared because at least one isolate from a fecal coliform-positive tube was positive for *E. coli*. The isolates that fell into three categories, categories I, II, and III, were defined as follows: category I strains produced typical colonies on EMB agar (green metallic sheen, violet centers, violet); category II strains produced gas from lactose in EC medium at 44.5°C; and category III strains exhibited β-glucuronidase activity. Strains that had these characteristics but were not identified as *E. coli* were considered "false positive" isolates, and strains that did not exhibit *E. coli* characteristics but were identified as *E. coli* were referred to as "false negative" isolates. A total of 91 strains that had the characteristics of all three categories were isolated on EMB agar, and 43 of these strains were identified as *E. coli* strains. Only 27.9% of the colonies that exhibited characteristics typical of *E. coli* on EMB agar were identified as *E. coli* colonies. This finding reaffirmed the fact that EMB agar is not

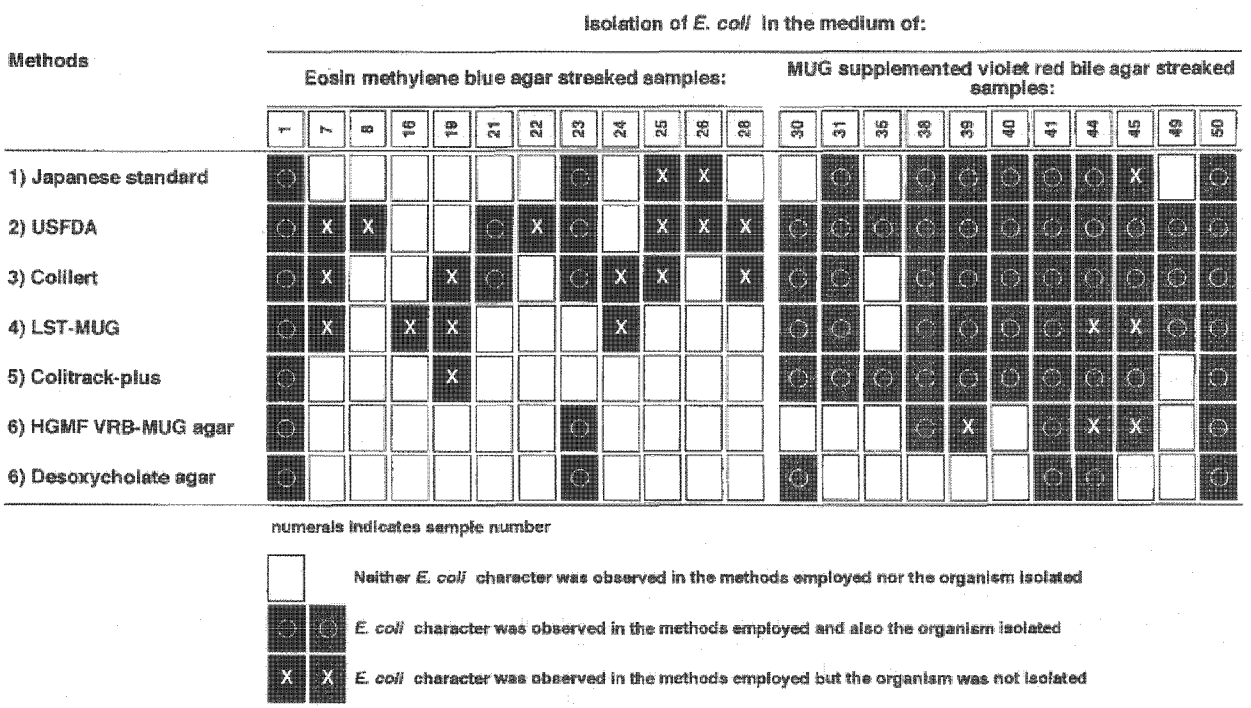

FIG. 2. Sensitivity of EMB agar and VRB-MUG agar for differential isolation of *E. coli* from presumptively positive fecal coliform tubes or colonies.

a suitable substrate for isolating *E. coli*. A total of 95% of the strains produced gas from lactose at 44.5°C, and all 43 strains that were identified as *E. coli* strains had β-glucuronidase activity. A total of 23% of the fecal coliforms (12 strains) produced gas at 44.5°C in EC medium, and 11 of these strains were identified as *K. pneumoniae* strains (Table 3).

A total of 184 strains that exhibited at least one of the three characteristic features mentioned above were isolated from MUG-supplemented VRB agar and identified. Of the 157 strains that exhibited typical *E. coli* characteristics on EMB agar, 86% were identified as *E. coli* strains. This value is very high compared with the percentage of *E. coli* strains that were isolated from EMB agar during this study (27.9%). A total of 92% of the strains isolated from VRB-MUG agar produced gas from lactose, and 99% of the strains exhibited β-glucuronidase activity, findings which were consistent with the data obtained for strains isolated on EMB agar. Among the false-positive isolates (Table 2), 10% of the strains picked from VRB-MUG agar produced gas at 44.5°C in EC medium, compared with 23% of the strains isolated from EMB agar. Furthermore, two strains that were not identified as *E. coli* strains hydrolyzed MUG and produced the fluorescence substrate. One strain was identified as an *Escherichia vulneris* strain, and the other strain could not be identified to the genus level. These two strains exhibited only one-fifth of the fluorescence intensity (as determined with a spectrophotometer at 365 nm) that was recorded for type strain *E. coli* ATCC 25922. Further-

TABLE 2. Specificity of EMB agar and VRB-MUG agar for isolating *E. coli* from foods[a]

Category	Characteristic	Medium used to isolate *E. coli*	No. of isolates that had characteristic	No. of *E. coli* isolates that had characteristic	% of *E. coli* isolates that had characteristic	No. of false-negative isolates	No. (%) of false-positive isolates[b]
I	Typical colonies on EMB agar	EMB agar	50	12	28	31	38 (76)
		VRB-MUG agar	157	135	86	22	22 (14)
II	Gas production in EC medium at 44.5°C	EMB agar	53	41	95	2	12 (23)
		VRB-MUG agar	159	143	91	12	16 (10)
III	β-glucuronidase activity	EMB agar	43	43	100	0	0
		VRB-MUG agar	157	155	99	2	2 (1)

[a] A total of 91 strains were isolated from EMB agar, and 43 of these strains were identified as *E. coli*; 184 strains were isolated from VRB-MUG agar, and 157 of these strains were identified as *E. coli*. Every isolate that exhibited at least one of the characteristics was included.

[b] Percentages were calculated as follows: (number of isolates that had characteristic−number of *E. coli* isolates that had characteristic)/(number of isolates that had characteristic) × 100.

TABLE 3. Species of false-positive isolates that exhibited *E. coli* characteristic features

Taxon	No. of colonies isolated from EMB agar that showed:		No. of colonies isolated from VRB-MUG agar that showed:		
	Typical colonies on EMB agar	Gas production in EC medium	Typical colonies on EMB agar	Gas production in EC medium	β-glucuronidase activity
Citrobacter freundii	1		1		
Enterobacter agglomerans	2				
Enterobacter amnigenus	1				
Enterobacter cloacae	1				
Escherichia vulneris					1
Hafnia alvei			1		
Klebsiella oxytoca	5		4		
Klebsiella ozaenae	1		10	6	
Klebsiella pneumoniae	12	11	3	4	
Kluyvera cryocrescens			1		
Leclercia adecarboxylata	2				
Rahnella aquatilis	3				
Serratia liquefaciens	4				
Unidentified	6	1	2	6	1

more, the gene responsible for β-glucuronidase activity could not be detected by the PCR method (2) in these two strains.

Figure 3 shows various biovars of *E. coli* that were isolated during this study. A total of 200 strains of *E. coli* were characterized, and these strains were divided into five biovar groups. The group A strains were predominant when all three categories of *E. coli* characters were considered (67%), followed by the group B strains (24.5%), and the group C strains (6.5%). A total of 1% of the *E. coli* strains exhibited only β-glucuronidase activity. Furthermore, only two *E. coli* strains (1%) did not exhibit β-glucuronidase activity on VRB-MUG agar. If conventional methods had been used to detect *E. coli*, the *E. coli* group C and D strains (7.5% of the strains) could have been neglected. Five group A strains, five group B strains, all group C, D, and E strains and the type strain of *E. coli* (ATCC 25922) were examined for the presence of the *uidA* gene, which is responsible for β-glucuronidase activity. *K. pneumoniae* IFO 13833 was included as a negative control. All of the *E. coli* strains, including two isolates that did not exhibit β-glucuronidase activity, produced a clear band at 147 bp.

The false-positive strains isolated in this study were identified (Table 3). The *K. pneumoniae* strains produced colonies with a metallic sheen on EMB agar and produced gas from lactose at 44.5°C. Production of gas from lactose by this organism is not uncommon (24). The false-positive strains isolated from VRB-MUG agar fluoresced weakly, and only the colonies fluoresced. These fluorescence-positive strains were all *Klebsiella* strains (data not shown). All false-positive isolates from VRB-MUG agar were examined by the test to detect the *uidA* gene, and no *E. coli*-specific band was detected by PCR methods.

DISCUSSION

Detection of coliforms and *E. coli* by an enzymatic analysis in which fluorogenic substrates are used has several advantages. First, only one medium containing two substrates is required to detect both coliforms and *E. coli*, instead of the three liquid media (LST medium, BGLB broth, and EC broth) and one solid medium (VRB agar) required by the conventional method. Moreover, the total time needed to accomplish the analysis is reduced to 24 h, and the confirmation step for *E. coli* is not necessary, with some exceptions (such as shellfish samples). Although β-glucuronidase-negative *E. coli* strains are found, only 1% of the *E. coli* strains isolated in this study were MUG negative on MUG-supplemented selective agar. About 94% of *E. coli* strains, including the members of many pathogenic serogroups, produce β-glucuronidase and are positive as determined by the MUG assay (13, 17). However, the exceptions are isolates that belong to enterohemorrhagic *E. coli* serogroup O157:H7, which are consistently negative as determined by the MUG assay (11). Although the *E. coli* O157:H7 strains were not β-glucuronidase positive, these strains could be detected by targeting the β-glucuronidase gene by a molecular method (*uidA*). The absence of β-glucuronidase activity in *E. coli* is not due to a lack of enzyme induction (12), but is due to catabolic repression by lactose (4); shifting the isolates to a lactose-free minimal medium restored β-glucuronidase activity (12). Two isolates that did not exhibit β-glucuronidase activity in VRB-MUG agar exhibited β-glucuronidase activity when they were grown in Bactident E. coli kits. Lactose was the carbohydrate substrate in VRB-MUG agar, and this sugar was not present in Bactident E. coli kits.

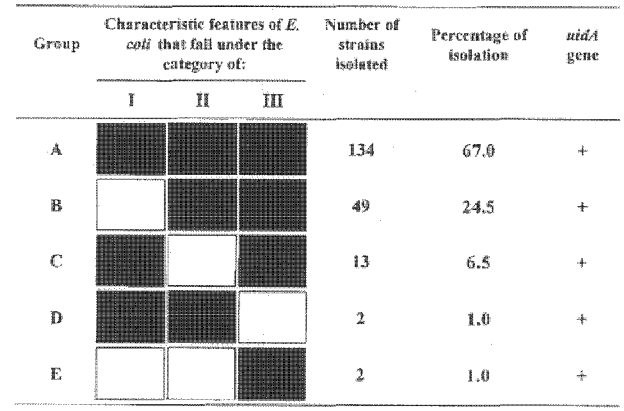

FIG. 3. Characterization of various biogroups of *E. coli* and their *uidA* gene expression. Isolates that fell into categories I, II, and III were defined as follows: category I, colonies with a green metallic sheen produced on EMB agar; category II, gas produced from lactose in EC medium at 44.5°C; and category III, β-glucuronidase activity.

TABLE 4. Comparison of MUG-supplemented media with USFDA and Japanese standard methods for detecting *E. coli* in various foods

USFDA test result	No. of strains (%)	No. (%) of samples isolated by:							
		Japanese standard method		Colilert method		LST-MUG method		Colitrack-plus method	
		Positive test	Negative test	Positive test	Negative test	Positive test	Negative test	Positive test	Negative test
Positive	20 (40)	11 (22)	9 (18)	16 (32)	4 (8)	10 (20)	10 (20)	11 (22)	9 (18)
Negative	30 (60)	0	30 (60)	2 (4)	28 (56)	3 (6)	27 (54)	1 (2)	29 (58)

Because of this, it could be concluded that lactose-based kits may not provide optimal conditions for isolation of *E. coli*.

The lower incidence of coliforms as determined by the Colitrack-plus method (level of detection, 77.5%) and the desoxycholate method (level of detection, 55%) compared with other methods (levels of detection, >80%) might have been due to the limitations of the methods. Only 1 ml of a test sample could be inoculated in both of these methods, and this could have led to the lower incidence of coliforms. The MPN counts of coliforms also supported this finding. The agreement between the USFDA method and the MUG-based kits for *E. coli* detection varied (Table 4). Significant differences were noticed between the results obtained with the USFDA method and the results obtained with the other methods used to detect *E. coli* in food samples. The greatest agreement was found between the USFDA method (level of detection, 40%) and the Colilert method (level of detection, 36%). The levels of *E. coli* detection were 26, 24, and 22% for the LST-MUG, Colitrack-plus, and Japanese standard methods, respectively. Similarly, Araujo et al. (1) reported that they underestimated fecal coliform counts when they used the Spanish official method compared with the United States standard method for shellfish-growing waters. There were three samples (from cuttlefish, shrimp, and fried chicken) in which the MUG-based kits were positive but the USFDA method was negative for *E. coli*. In these cases, the LST-MUG method was positive for all three samples and the Colilert method isolated *E. coli* from the shrimp and fried chicken samples. A possible explanation for the absence of *E. coli* in these samples when they were evaluated by the USFDA method was not found, except that the samples had low MPN values for *E. coli* (<2.3 CFU/g). In general, *E. coli* could be detected by the fluorogenic methods in meat and meat products, whereas some problems were encountered with vegetable samples. Further study is warranted. In addition, some finfish and shellfish product samples exhibited autofluorescence when *E. coli* detection based on a fluorescent substrate was used.

Rippey et al. (32) reported that LST-MUG agar is not suitable for enumerating *E. coli* in molluscan shellfish and that the shellfish tissue itself appeared to be β-glucuronidase positive. For both oysters and hard clams, fluorescence was observed when as little as 0.1g of homogenate was inoculated into LST-MUG broth after tubes had been incubated at 35°C for 24 h. This problem was also encountered with all three fluorogenic assays used. Some of the finfish and shellfish products exhibited autofluorescence under 366-nm UV light during this study. The dissolved materials of the fish tissue were found to autofluoresce, and this problem could be solved by centrifuging the samples before they were inoculated (34a). The shrimp, tuna fish, and oyster samples tested in the present study also exhibited autofluorescence, and centrifugation at 9,000 × *g* for 10 min at 4°C removed the fluorescing material from the samples. However, the oyster sample still posed a problem and needed further study. This problem was solved by using a Colilert standard tube that was inoculated with 1 CFU of *E. coli* per ml and incubating this tube in parallel with the test material. Sample fluorescence intensity that matched or was greater than the fluorescence intensity of the *E. coli*-inoculated Colilert standard tube was considered a positive reaction for *E. coli*. In addition to the autofluorescence of some foods, the presence of β-glucuronidase activity in some other bacterial species (*Shigella* spp. [34]) should also be considered when the *E. coli* population in a given sample is estimated.

The sensitivities of MUG-based kits for detecting *E. coli* are shown in Table 5. The number of *E. coli* CFU was determined by identifying colonies from positive plates derived by any method that were positive for fecal coliforms. It appears that the USFDA method exhibited the greatest detection sensitivity (level of detection, 87%), followed by the Colilert method (level of detection, 78%), the LST-MUG method (level of detection, 56%), the Colitrack-plus method (level of detection, 52%), and the Japanese standard method (level of detection, 48%), for most ranges of *E. coli* concentrations. When the *E. coli* concentration was 1 to 9 CFU/g, all of the methods used had some difficulty in detecting this organism. When the *E. coli* concentration was 1 to 9 CFU/g, the Japanese standard

TABLE 5. Sensitivity of MUG-based kits for detecting *E. coli* in foods and percentages of false-negatives when *E. coli* is present in samples[a]

E. coli concn (CFU/g)	No. of samples	No. (%) of samples exhibiting negative reaction or MUG reaction with:				
		Japanese standard method	USFDA method	Colilert method	LST-MUG method	Colitrack-plus method
1	11	11 (100)	3 (27)	5 (45)	5 (46)	8 (73)
10^1	4	0	0	0	3 (75)	2 (50)
10^2	3	0	0	0	0	0
10^3	2	1 (50)	0	0	1 (50)	0
$>10^3$	3	0	0	0	1 (33)	1 (33)

[a] A total of 23 samples contained *E. coli* as determined by at least one of the methods used; 12 (52%), 3 (13%), 5 (22%), 10 (44%), and 11 (48%) of these samples exhibited false-negative results with the Japanese standard, USFDA, Colilert, LST-MUG, and Colitrack-plus tests, respectively.

method did not detect *E. coli* in all 11 samples. Two samples (from fried garlic chicken and boiled pumpkin) contained 10^1 CFU of *E. coli* per g, and the LST-MUG and Colitrack-plus methods failed to detect this microorganism in these samples. One mushroom sample containing more than 2.4×10^3 CFU of *E. coli* per g was found, but at this concentration *E. coli* was not detected by the LST-MUG and Colitrack-plus methods. On the basis of these observations, the Colilert method seems to be superior to the other two MUG-based methods. Both the LST-MUG medium and the Colitrack-plus medium contained lauryl sulfate to suppress the growth of gram-positive bacteria, and perhaps this inhibited the proliferation of injured *E. coli* cells, if any were present. In addition, catabolic repression of fluorescence by lactose (4) might also have led to the false-negative results obtained with these two methods.

In summary, our data indicated that the MUG assay was comparable to the USFDA method for detecting coliforms and *E. coli* in foods. The Colilert and USFDA method results showed good agreement. Perhaps brilliant green is lethal to injured *E. coli* cells and a preenrichment step (e.g., lactose broth or LST broth) would resuscitate the organism in the Japanese standard method. Catabolic repression by lactose would have led to the false-negative results obtained with the LST-MUG and Colitrack-plus methods when they were used to detect *E. coli*.

Olson et al. (25) compared the MUG-based fecal coliform assay with the membrane filter fecal coliform assay for treated drinking water and untreated drinking water and found that the MUG assay was not effective in determining the *E. coli* counts in treated drinking water. Too many false-negative results were obtained with the commercially available kits for chlorinated drinking water that include Colilert ONPG-MUG minimal medium tubes. However, Covert et al. (7) showed that MUG-supplemented media were capable of detecting 1 CFU of *E. coli* per 100 ml and found no significant differences in detection of chlorine-exposed *E. coli* between the Colilert test and EC medium supplemented with MUG. Likewise, McCarthy et al. (21) obtained a significant number of positive reactions with the Colilert test for chlorine-treated coliform bacteria and *E. coli* when the disinfectant was chloramine. Furthermore, when free chlorine was used during chlorine treatment, the levels of recovery of *E. coli* were found to be equal with the Colilert test and the USFDA method.

Many researchers have encountered false-positive results (32) because of the presence of β-glucuronidase in some foods, including fish and shellfish products. Poelma et al. (29) observed the highest number of false-positive results when the LST-MUG method was used to detect *E. coli* in pork sausage, ground beef, and turkey. To solve this problem, some pretreatment procedure should be used to eliminate the materials that exhibit autofluorescence before the sample is analyzed enzymatically for *E. coli*. Further studies to deal with this problem are in progress in our laboratory.

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