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Enumeration of coliforms and *Escherichia coli* in frozen black tiger shrimp *Penaeus monodon* by conventional and rapid methods

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Abstract

Conventional (most probable number, MPN) and rapid methods—including Chromocult® coliform agar (CCA), Fluorocult® LMX broth (LMX), and Petrifilm™ *Escherichia coli* count plates (PEC) for enumeration of coliforms and *E. coli* in frozen black tiger shrimp from Thailand were compared in order to assess the possibility of using one of the rapid methods for routine analysis. Enumeration of coliforms and *E. coli* from 18 samples of regular frozen black tiger shrimp and 156 samples of frozen black tiger shrimp experimentally contaminated with coliforms or *E. coli* at concentrations of ~ 10, $\sim 10^2$, and $\sim 10^3$ CFU g^{-1} revealed that at the level of ~ 10 CFU g^{-1}, coliform numbers ranked as LMX > CCA > MPN = PEC and *E. coli* as MPN = LMX = PEC = CCA. At the level of $\sim 10^2$ CFU g^{-1}, coliform numbers ranked as LMX > MPN = PEC = CCA and *E. coli* as MPN = LMX > PEC = CCA. At the level of 10^3 CFU g^{-1}, coliforms ranked as LMX > MPN = CCA > PEC and *E. coli* as MPN > LMX > CCA > PEC. Agreements with the conventional MPN method for coliforms were LMX 108%, PEC 87.2%, and CCA 91.2% and agreements for *E. coli* were LMX 101%, PEC 95.7%, and CCA 96.3%. Sensitivities (%) ranked LMX > MPN > CCA = PEC for coliforms and *E. coli*, whereas equal specificities (100%) of all methods for coliforms and *E. coli* were demonstrated. Rankings for the other parameters compared were: convenience, PEC > CCA = LMX > MPN; time to detection, MPN > LMX = PEC = CCA; expense, MPN = PEC > CCA > LMX; labor, MPN > LMX = CCA > PEC; accuracy for coliforms, PEC > CCA > MPN > LMX; and accuracy for *E. coli*, PEC = CCA > LMX > MPN.

Keywords: Coliforms; *Escherichia coli*; Chromocult® coliform agar; Conventional method; Fluorocult® LMX broth; Petrifilm™ *E. coli* count plates; *Penaeus monodon*

1. Introduction

Thailand has annually exported fresh and modified black tiger shrimp, *Penaeus monodon*, at a value of ~ US$1.6–1.7 billion from 1998 till 2000 (Thai Department of Business Economics, 1999; http://www.moc.go.th). Fresh shrimp, after being harvested, are quickly frozen at −20 °C for at least 5 days before exportation as an ice block with a size of 1–2 kg, according to specifications of buyers from different countries, or as individual quick-frozen (IQF). During the frozen storage, shrimp are randomly sampled for chemical and microbiological examination by manu-

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facturers' microbiology tests, the products that pass either Thai specification criteria (under standard analysis of total viable bacterial count $< 1 \times 10^5$ CFU g^{-1}, coliforms $< 10^2$ MPN g^{-1}, *Escherichia coli* < 3 MPN g^{-1}, *Staphylococcus aureus* < 50 CFU g^{-1}, *Bacillus cereus* $< 10^2$ CFU g^{-1}, *Clostridium perfringens* not found in 0.1 g, and *Vibrio parahaemolyticus* and *Salmonella* spp. not found in 25 g) or under the trade policy of individual country as required under Thai government investigators will be certified by the Department of Medical Sciences, Ministry of Public Health, Thailand. Certification information will be attached to the container of frozen shrimp with the same product lot number before shrimp are exported. Since many kinds of bacteria are sought per sample and so many shrimp samples per day have to be tested, using current conventional methods of bacteriological examination and confirmation takes several days of laboratory work and is cumbersome, leading to late reports and customer dissatisfaction. One solution that should be considered is to replace conventional methods with more accurate and less costly rapid methods, or less complex methods, which will shorten analytical times, and possibly return some benefits to customers and laboratory workers.

Recently, new methods and modifications of methodology for enumeration of coliforms and *E. coli* in food have been developed (Anderson and Baird-Parker, 1975; Feng and Hartman, 1982; Firstenberg-Eden, 1985; Anonymous, 1987). For example, firstly, Chromocult® coliform agar (CCA, Merck, Germany), a selective agar that contains two chromogenic substrates salmon-GAL (red colony for coliforms) and X-glucuronide (dark blue to violet colony for *E. coli*). All plates can be observed within 24- to 48-h incubation at 35 °C. Secondly, Fluorocult® LMX broth (LMX, Merck, Germany) is a modified lauryl sulfate tryptic broth (LST broth) for the simultaneous detection of coliforms and *E. coli*. This selective enrichment broth contains the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-GAL), cleaved by coliforms' β-galactosidase, and the fluorogenic substrate, 4-methylumbelliferyl-β-D-glucuroside (MUG), which is highly specific for *E. coli* β-glucuronidase (GUR) (Curiale et al., 1991) by detection of blue-white light emittance from 4-methylumbelliferone under long-wave ultraviolet light at 366 nm. LMX can be set up as a three-tube most probable number (MPN) series. The tubes can be examined after incubation at 35 °C for 24–48 h. In addition, indole production can be demonstrated. At the end of the incubation period, the broth is examined for growth, blue-green coloration, fluorescence, and indole production. Lastly, Petrifilm™ *E. coli* count plates (PEC, 3M Center, St. Paul, MN, USA) contain growth nutrients, selective agents, indicator dyes, β-glucuronidase indicator, and 5-bromo-4-chloro-3indolyl-β-D-glucuronide (BCIG) in a guar gum base. The medium is laminated to the inside surfaces of two pieces of film, which are hinged. Coliforms produce red colonies on this plate by reduction of triphenyltetrazolium dye, which is incorporated in the gel and gas by fermentation of lactose. The gas is trapped by the film and appears as one or more small gas bubbles associated with the colony. A blue colony associated with gas bubbles, after BCIG is split by β-glucuronidase, will be characteristic of *E. coli*. Plates are incubated at 35 °C and can be checked after 24 h for coliform count and after 24–48 h for *E. coli* count.

Enumeration of coliforms and *E. coli* from foods is of major importance because the determination of these bacteria can be used to assess the sanitary quality of foods and water (Feng and Hartman, 1982; Bredie and de Boer, 1992). There are several methods available for the qualitative determination of *E. coli* in foods; but, as a reference method, the most probable number (MPN) technique is usually employed for routine investigation (Oblinger and Koburger, 1975, 1984). However, the MPN technique is laborious, expensive, and time-consuming (Bredie and de Boer, 1992), so a more practical, rapid method is needed. The purpose of this study is to compare accuracy, agreement, convenience, expenses, labor, sensitivity, specificity, and time of detection of the conventional method to those of rapid methods for enumerating coliforms and *E. coli* in frozen black tiger shrimp.

2. Materials and method

2.1. Media and reagents

Brilliant green bile lactose broth (BGLB), EC broth, Trypticase soy broth, MR-VP broth, eosin

methylene blue agar (EMB), KH_2PO_4, methyl red, Kovacs' reagent, and KOH were obtained from Merck. Lauryl tryptose broth and nutrient agar were purchased from Oxoid (England). Tryptone was obtained from Difco (USA). Standard methods agar and Simmons citrate agar were purchased from BBL (USA).

2.2. Microorganisms

E. coli ATCC 25922, *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes* were provided by the Department of Medical Sciences, Ministry of Public Health. The last three strains were isolated from Thai raw foods and classically identified, employed as reference strains in routine laboratory work, and combined in shrimp samples in a proper ratio as artificial inoculum of coliforms. Each strain was streaked and checked for purity on Trypticase soy agar (TSA) that was incubated at 30 °C for 18 h. All cultures were transferred and maintained on TSA slants, kept at 4 °C with monthly transfers.

2.3. Conventional method

The three-tube MPN procedure for coliforms and *E. coli* was carried out as specified in FDA (1992). A 25-g sample of naturally or experimentally contaminated shrimp was removed and immediately diluted 1:10 with 225 ml of phosphate buffer, pH 7.2, in a sterile stomacher bag and homogenized for 1 min in a Stomacher (Stomacher Lab-Blender 400, Model BA 7021, Seward, UK). Thereafter, the sample was diluted in serial 10-fold steps used as inoculum for the three-tube MPN procedure for coliforms and *E. coli* as specified in FDA (1992). Suspension: 1 ml from each diluted tube was transferred into lauryl sulfate tryptose broth and incubated at 35 °C for 24–48 h. Inoculum: One loopful from tubes with gas formation within 48 h at 35 °C was transferred to brilliant green lactose broth (BGLB), incubated at 35 °C for 24–48 h, and to EC broth, incubated at 44.5 °C for 24–48 h. The tubes with turbidity and gas formation in the Durham tube of BGLB indicated the presence of coliforms, whereas the tubes of EC broth indicated the presence of faecal coliforms; their numbers per 1 g of shrimp were calculated from the MPN table.

A loopful of culture from EC broth was streaked on EMB agar and incubated at 35 °C for 24 h. Colonies with metallic sheen were picked and transferred to an IMViC set (96–144 h) for confirmation of *E. coli* (FDA, 1992).

2.4. Rapid methods

Chromocult® coliform agar (CCA) and Fluorocult® LMX broth (LMX) were supplied by Merck and Petrifilm™ *E. coli* count plates (PEC) were obtained from 3M Center.

2.4.1. CCA method

Shrimp suspension at a proper dilution (1 ml) was pipetted onto the plate of CCA, then homogeneously mixed with melting agar, and incubated at 35 °C for 24 h. Each dilution was tested on two CCAs, and a plate with 15–150 colonies was selected for counting on a colony counter (Model 3328, Scientific Instrument, USA). Coliform colonies were red and *E. coli* colonies were blue.

2.4.2. LMX method

Suspensions (1 ml) of properly diluted shrimp sample were transferred into LMX broth (three tubes per dilution) and incubated at 35 °C for 24 h. Bromchloroindigo-based blue-green color formation by coliforms or *E. coli* in the broth was observed and reported as MPN per gram. Confirmation of *E. coli* was by detection of fluorescent emission at 366 nm and pink color after adding with Kovacs' solution. *E. coli* was reconfirmed by inoculating the sample into a new set of IMViC.

2.4.3. PEC method

Shrimp suspension (1 ml) at proper dilution was pipetted onto the surface of a Petrifilm™ plate, gently closed with film, and incubated at 35 °C for 24 h. Red colonies surrounded with trapped gas were coliforms, whereas blue colonies with trapped gas were *E. coli*. Duplicate trials were performed per dilution; using this method, plates with 15–150 colonies were recommended for counting.

Coliforms and *E. coli* from all methods were reconfirmed by reisolation on EMB agar and IMViC testing. *E. coli* ATCC 25922 was examined at the same time as a control.

2.5. Shrimp samples and sampling

Frozen whole black tiger shrimp were provided by the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. A total of 192 samples of frozen shrimp were employed. Eighteen samples for examination of natural contamination by coliforms or *E. coli*, and another 18 samples with added *E. coli* ATCC 25922 as a control were randomly selected for coliforms and *E. coli* determination. Another 52 samples each, experimentally inoculated with concentrations of 10, 10^2, or 10^3 CFU g^{-1} of either coliforms or *E. coli*, were carefully prepared for this study.

Six frozen shrimp blocks of either 1 or 2 kg were randomly selected from one lot, and each block was laid on a sterile stainless steel rack. A drill with a shaft ~ 5 cm long connected to a drill bit ~ 8.5 cm long and 2 cm in diameter was used to drill through the frozen block of shrimp. The drill bit was a hollow cylinder with a serrated edge and was removable for flame sterilization after immersion in 70% alcohol before use. Ice-shrimp cores after drilling were transferred into a sterile Erlenmeyer flask with a cotton plug. All steps were performed under aseptic techniques and conditions.

Shrimp (400 g) from one frozen sample was cut and homogenized under sterile conditions. Experimental inoculation of shrimp with coliforms or *E. coli* was performed in order to study the efficacy of enumeration methods. Inocula were prepared in Trypticase soy broth (24 h, 35 °C), adjusted to an absorbency of 0.58–0.62 at 600 nm in a UV–visible spectrophotometer (Model EB 3412, Bausch and Lomb, Germany), and suspended with one volume of phosphate buffer, pH 7.2. Cells were then added to the 400-g portion of shrimp sample to produce test samples containing approximately 10, 10^2, or 10^3 CFU g^{-1} of variable coliforms or *E. coli*. Samples were stabilized by storage for 5 days at −20 °C (Curiale et al., 1991).

2.6. Percentage agreement, sensitivity, and specificity

Percentage agreement was defined as accuracy or efficacy of one method compared to standard one that has universal acceptance (Jinayon, 1998), or a percentage of the competency ratio of detecting a microorganism between two methods (Bolderdijk and Milas, 1996; Keith, 1997).

Sensitivity was defined as the percentage of accuracy for examining contaminated samples, or 100% − percentage of false negative results (Feldsine et al., 1997). Specificity was defined as the percentage of accuracy for examining uncontaminated samples or 100% − percentage of false positive results (Feldsine et al., 1997).

2.7. Data analysis

All data were analyzed at $p < 0.05$ for significant values by ANOVA and Duncan's multiple range tests (Statistical Analysis System, 1983).

3. Results and discussion

Two kinds of observations were obtained from our studies. Firstly, quantitative data for coliform and *E.*

Table 1
Comparison of methods for enumerating coliforms in frozen black tiger shrimp

Coliform contamination	Expected CFU g^{-1}	Number of samples	Mean[a] log (CFU g^{-1}) ± S.D. coliforms detected			
			MPN[b]	LMX[c]	PEC[d]	CCA[e]
Natural	unknown	18	0.20 ± 0.37^{A}	0.35 ± 0.48^{A}	0^{B}	0.06 ± 0.24^{B}
Experimental	~ 10	52	1.17 ± 0.41^{B}	1.47 ± 0.30^{A}	1.34 ± 0.35^{B}	1.34 ± 0.44^{AB}
	~ 10^2	52	2.33 ± 0.25^{B}	2.43 ± 0.34^{A}	2.38 ± 0.11^{B}	2.38 ± 0.10^{B}
	~ 10^3	52	3.37 ± 0.26^{AB}	3.45 ± 0.24^{A}	3.37 ± 0.16^{B}	3.37 ± 0.09^{AB}

[a] Among values in a row, means not sharing a common superscript capital letter differ significantly ($p < 0.05$).
[b] MPN = most probable number, three tubes per dilution.
[c] LMX = Fluorocult® LMX broth, three tubes per dilution.
[d] PEC = Petrifilm™ *E. coli* count plates, two per dilution.
[e] CCA = Chromocult® coliform agar, two plates per dilution.

Table 2
Comparison of methods for enumerating *E. coli* in frozen black tiger shrimp

E. coli contamination	Expected CFU g^{-1}	Number of samples	Mean[a] log (CFU g^{-1}) ± S.D. *E. coli* detected			
			MPN[b]	LMX[c]	PEC[d]	CCA[e]
Natural	unknown	18	0^{A}	0^{A}	0^{A}	0^{A}
Experimental	~ 10	52	0.86 ± 0.42^{A}	0.82 ± 0.37^{A}	0.79 ± 0.52^{A}	0.84 ± 0.49^{A}
	~ 10^2	52	1.93 ± 0.36^{A}	1.98 ± 0.39^{A}	1.91 ± 0.27^{B}	1.91 ± 0.43^{B}
	~ 10^3	52	3.08 ± 0.36^{A}	3.04 ± 0.30^{AB}	2.99 ± 0.10^{C}	3.04 ± 0.14^{BC}
	~ 10^{2f}	18	2.36 ± 0.31^{A}	2.39 ± 0.30^{A}	2.38 ± 0.20^{A}	2.42 ± 0.22^{A}

[a] Among values in a row, means not sharing a common superscript capital letter differ significantly ($p < 0.05$).
[b] MPN = most probable number, three tubes per dilution.
[c] LMX = Fluorocult® LMX broth, three tubes per dilution.
[d] PEC = Petrifilm™ *E. coli* count plates, two per dilution.
[e] CCA = Chromocult® coliform agar, two plates per dilution.
[f] Inoculum was *E coli* ATCC 25922.

coli counts in shrimp samples were obtained by different methods. Positive tubes in the MPN and LMX were used to estimate the most probable number (MPN g^{-1}) per gram at 95% of statistical significance, whereas values from plate count techniques (PEC and CCA) were recorded as colony forming units per gram (CFU g^{-1}). Both plate and MPN counts were transformed to logarithm function in order to distribute data normally and decrease the variation somewhat (Smith et al., 1989) and finally reported as log (CFU g^{-1}). Secondly, qualitative data that characterized the properties of different methods in terms of sensitivity and specificity, as well as convenience and cost, of various techniques for detecting coliforms and *E. coli* were compiled.

3.1. Enumerative comparison among methods

The quantitative analyses revealed significant differences at $p < 0.05$ among the methods tested (Tables 1 and 2). Coliforms from naturally contaminated shrimp were enumerated by these methods as: LMX = MPN > CCA = PEC (Table 1). *E. coli* was not found as a natural contaminant in shrimp by any of the methods (Table 2). The coliform numbers recorded were all < 10 CFU g^{-1}, and thus meet the criterion stated earlier of $< 10^2$ CFU g^{-1}. Generally, shrimp exported from Thailand are guaranteed to be premium products, since all manufacturers operate under HACCP regulations and are very concerned with the wholesomeness of food for human consumption.

Table 3
Agreement of LMX[a], PEC[b], and CCA[c] methods with results of MPN[d] method for detecting coliforms in frozen black tiger shrimp

Contamination	Expected CFU g^{-1}	Number of samples	Positive results				Negative results			Agreement with MPN (%)		
			MPN	LMX	PEC	CCA	LMX	PEC	CCA	LMX	PEC	CCA
Natural	unknown	18	5	7	2	3	0	5	4	140	40	60
Experimental	10	52	52	52	50	50	0	2	2	100	96.2	96.2
	10^2	52	52	52	52	52	0	0	0	100	100	100
	10^3	52	52	52	52	52	0	0	0	100	100	100
Totals	–	174[e]	179	181	174	175	0	7	6	108	87.2	91.2

[a] LMX = Fluorocult® LMX broth, three tubes per dilution.
[b] PEC = Petrifilm™ *E. coli* count plates, two per dilution.
[c] CCA = Chromocult® coliform agar, two plates per dilution.
[d] MPN = most probable number, three tubes per dilution.
[e] Of the 174 samples, 156 were experimentally inoculated.

Table 4
Agreement of LMX[a], PEC[b], and CCA[c] methods with results of MPN[d] method for detecting *E. coli* in frozen black tiger shrimp

Contamination	Expected CFU g^{-1}	Number of samples	Positive results				Negative results			Agreement with MPN (%)		
			MPN	LMX	PEC	CCA	LMX	PEC	CCA	LMX	PEC	CCA
Natural	unknown	18	0	0	0	0	0	0	0	100	100	100
Experimental	10	52	47	49	37	40	3	15	12	104	78.7	85.1
	10^2	52	52	52	52	50	0	0	2	100	100	96.2
	10^3	52	52	52	52	52	0	0	0	100	100	100
	10^{2e}	18	18	18	18	18	0	0	0	100	100	100
Totals	–	192[f]	169	171	159	160	3	15	14	101	95.7	96.3

[a] LMX = Fluorocult® LMX broth, three tubes per dilution.
[b] PEC = Petrifilm™ *E. coli* count plates, two per dilution.
[c] CCA = Chromocult® coliform agar, two plates per dilution.
[d] MPN = most probable number, three tubes per dilution.
[e] Inoculum was *E coli* ATCC 25922.
[f] Of the 192 samples, 174 were experimentally inoculated.

Recoveries of coliforms inoculated experimentally at levels of ~ 10, ~ 10^2, and ~ 10^3 CFU g^{-1} ranked as: LMX>CCA>MPN = PEC, LMX>MPN = PEC = CCA, and LMX> MPN = CCA>PEC, respectively (Table 1). Comparable recoveries of experimentally added *E. coli* cells were: MPN = LMX = PEC = CCA, MPN = LMX>PEC = CCA, and MPN>LMX>CCA> PEC, respectively (Table 2). The numbers of coliforms and *E. coli* recorded tended to rank higher for the LMX and MPN techniques than for the plate count techniques (Tables 1 and 2). The LMX and MPN techniques use fluid media, which may allow more random distribution of organisms among the tubes, provide a better environment for injured cells to revive, and thus provide more chance of detecting the organisms (Frampton et al., 1990; Bredie and de Boer, 1992). In contrast, plate count techniques may subject coliforms and *E. coli* to drying that inhibits their growth on agar or gel medium. Our results are consistent with those of McCarthy et al. (1958) and Frampton et al. (1988), indicating that MPN techniques yielded higher counts of coliform and *E. coli* cells than those from plate count techniques. McCarthy et al. (1958) reported that observed numbers of coliforms were ~ 10–29% higher by MPN techniques than those from plate count techniques, depending on food types and the specificity of organisms for the culture medium used. In addition, 1-isopropyl-1-β-D-thiogalactopyranoside (IPTG), a

Table 5
Sensitivity and specificity of LMX,[a] PEC,[b] and CCA[c] compared to MPN[d] for detecting coliforms and *E. coli* in frozen black tiger shrimp

Test	Coliforms			*E. coli*		
	Total positive results[e]	Sensitivity[f] (%)	Specificity[g] (%)	Total positive results[h]	Sensitivity (%)	Specificity (%)
MPN	179	98.89	100	169	97.13	100
LMX	181	100	100	171	98.28	100
PEC	174	96.13	100	159	91.38	100
CCA	175	96.69	100	160	91.95	100

[a] LMX = Fluorocult® LMX broth, three tubes per dilution.
[b] PEC = Petrifilm™ *E. coli* count plates, two per dilution.
[c] CCA = Chromocult® coliform agar, two plates per dilution.
[d] MPN = most probable number, three tubes per dilution.
[e] Total number of coliform-contaminated samples = 181.
[f] Sensitivity (%) = 100% – percentage of false negative results.
[g] Specificity (%) = 100% – percentage of false positive results.
[h] Total number of *E. coli*-contaminated samples = 174.

Table 6
Biochemical test confirmation of coliforms and *E. coli* detected by various methods

Method	Coliforms			*E. coli*		
	Number of positive tubes or isolated colonies	Biochemical confirmation result		Number of positive tubes or isolated colonies	Biochemical confirmation result	
		Positive	Negative		Positive	Negative
MPN[a]	1003	963 (96.0%)	40 (4%)	987	913 (92.5%)	74 (7.5%)
LMX[b]	1260	1067 (92.0%)	93 (8%)	919	864 (94.0%)	55 (6.0%)
PEC[c]	843	829 (98.3%)	14 (1.7%)	668	663 (99.3%)	5 (0.7%)
CCA[d]	857	832 (97.1%)	25 (2.9%)	665	661 (99.4%)	4 (0.6%)

[a] MPN = most probable number, three tubes per dilution.
[b] LMX = Fluorocult® LMX broth, three tubes per dilution.
[c] PEC = Petrifilm™ *E. coli* count plates, two per dilution.
[d] CCA = Chromocult® coliform agar, two plates per dilution.

compound in LMX that functions as an inducer of β-D-galactosidase (GAL), a key enzyme for lactose utilization by coliforms and *E. coli*, may enhance the total coliform count from naturally and experimentally contaminated shrimp samples, compared with those from MPN and plate count techniques. Recovery of coliforms by the MPN method from shrimp experimentally contaminated with 10 CFU g^{-1} was significantly lower than recoveries by the CCA and PEC methods, possibly because the sample contained natural contaminants such as *Proteus vulgaris*, which can interfere with catabolism of lactose in MPN broth, leading to lower recorded numbers, as reported previously by Olson (1978). In our study, shrimp samples contaminated experimentally with $\sim 10^2$ CFU g^{-1} of *E. coli* ATCC 25922 were used in a control treatment, with no significant difference in cell numbers recovered among all methods (MPN = LMX = PEC = CCA), as shown in Table 2.

3.2. Qualitative comparisons among methods

The degree to which the qualitative results obtained with the alternate methods agreed with those from the MPN test was reasonably high both for coliforms (Table 3) and for *E. coli* (Table 4). In particular, however, naturally occurring coliforms were more likely to be detected by the LMX test, and less likely to be detected by the PEC and CCA methods, respectively. No similar comparison was possible for *E. coli*, in that none was detected by any method in the shrimp that had not been experimentally inoculated. However, low levels of inoculated *E. coli* were less likely to be detected by the plating methods.

Under the conditions of these experiments, the specificities of all methods were considered to be 100% for either coliform or *E coli* counts from shrimp samples (Table 5). There were presumed to be no false positive results, although not all of the positive tests

Table 7
Summary of compared parameters between conventional and rapid methods for enumerating coliforms and *E. coli* from frozen black tiger shrimp

Method	Convenience	Days to detection	Cost per replicate (US$)	Labor	Sensitivity	Specificity	Accuracy for		Agreement to MPN for	
							Coliforms	*E. coli*	Coliforms	*E. coli*
MPN[a]	*[b]	7–10	3.50	***	***	***	**	*	–	–
LMX[c]	**	1–2	0.70	**	***	***	*	**	***	***
PEC[d]	***	1–2	3.50	*	**	***	****	***	*	**
CCA[e]	**	1–2	1.00	**	**	***	***	***	**	**

[a] MPN = most probable number, three tubes per dilution.
[b] Subjective evaluations more positive with increasing number of asterisks.
[c] LMX = Fluorocult® LMX broth, three tubes per dilution.
[d] PEC = Petrifilm™ *E. coli* count plates, two per dilution.
[e] CCA = Chromocult® coliform agar, two plates per dilution.

could be confirmed by biochemical criteria (Table 6). Sensitivities were somewhat lower for the plate-based tests than for those performed in tubes of liquid medium, both for coliforms and *E. coli* (Table 5) detection from shrimp samples. The sensitivities of the tested methods for coliform or *E. coli* detection from shrimp ranked as: LMX>MPN>CCA = PEC. In contrast, the accuracies of these methods for coliform and *E. coli* detection were PEC>CCA>MPN>LMX and PEC = CCA>LMX>MPN, respectively, based on biochemical confirmation. Both the CCA and PEC methods seem to be reliable and rapid techniques for coliforms and *E. coli* enumeration, as reported from the previous studies of Chung et al. (2000) and Turner et al. (2000) with meat products.

Additional information regarding convenience, time of detection, expense per one replication in US dollars, and labor of all methods is summarized in Table 7 in order to provide further bases for selecting a test method. Thus far, only the PEC method has been tested in a collaborative study by the Association of Official Analytical Chemists and approved for application to six types of foods (AOAC, 1995). The results of our study indicate that the PEC, CCA, and LMX methods are appropriate alternatives to MPN for the enumeration of both coliforms and *E. coli* in black tiger shrimp, with more accurate results. In addition to the sensitivity and specificity of a test, convenience, cost, available laboratory equipment, and other considerations are important in selecting the most useful method for a given situation.

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