FOOD BIOLOGICAL CONTAMINANTS

Comparison of the Compact Dry EC with the Most Probable Number Method (AOAC Official Method 966.24) for Enumeration of *Escherichia coli* and Coliform Bacteria in Raw Meats

***Performance-Tested Method*℠ 110402**

Abstract

Compact Dry *E. coli*/Coliform Count (EC) is a ready-to-use test method for the enumeration of *Escherichia coli* and coliform bacteria in food. The plates are presterilized and contain culture medium and a cold water-soluble gelling agent. The medium should be rehydrated with 1 mL diluted sample inoculated onto the center of the self-diffusible medium, allowing the solution to diffuse by capillary action. The plate can be incubated at 35°C for 20–24 h and the colonies counted without any further working steps. The Compact Dry EC medium plates were validated as an analysis tool for determining colony-forming units (CFU) of *E. coli* and coliform bacteria from a variety of raw meats using 5 different types of raw meats. The performance tests were conducted at 35°C. In all studies performed, no apparent differences were observed between the Compact Dry EC method and the AOAC Official Method **966.24** results. For the accuracy claim ($n = 75$), a correlation factor of $r^2 = 0.93$ (*E. coli*) and $r^2 = 0.93$ (coliform bacteria) could be assigned, as stated in the application for "*Performance-Tested Method*℠."

1 Scope of Method

Prefabricated counting plates for enumeration of *Escherichia coli* and coliform bacteria in 5 specific raw meats (raw ground pork, raw pork, raw lamb, raw veal, and raw ground beef) were used in the validation study.

1.1 Target Organisms

E. coli and coliform bacteria occurring in 5 specific raw meats (raw ground pork, raw pork, raw lamb, raw veal, and raw ground beef).

1.2 Matrixes

Five specific raw meats (raw ground pork, raw pork, raw lamb, raw veal, and raw ground beef).

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1.3 Summary of Validated Performance Claims

In all studies performed, no apparent differences were observed between the Compact Dry EC method and the AOAC Official Method **966.24** results.

2 Participants

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3 Introduction

3.1 Principle

The test method is a plate count unit facilitating the rapid determination of *E. coli* and coliform bacterial loads of raw meats. The plates are presterilized, and contain nutrients supplemented with selective substances, 2 chromogenic enzyme substrates, and a cold water-soluble gelling agent. The medium should be rehydrated with 1 mL (diluted) sample material that diffuses naturally by capillary action and can be incubated. Full medium size in the plate is 20 cm^2. Gelling agent is allowed to solidify, plates are incubated, and *E. coli* and coliforms are then counted.

3.2 General Information

The ability to rapidly and accurately detect *E. coli* and coliform bacteria is important to any food safety program. These 2 microorganisms are referred to as "indicator organisms." *E. coli* and coliform bacteria are important indicators of the safety of processed foods (1), raw foods such as ground beef (2, 3) and water quality (4, 5). The presence of indicator organisms such as *E. coli* and coliforms can be an indication of: (*a*) fecal contamination arising from within the slaughter facility or post slaughter; (*b*) inadequate processing; (*c*) post-processing contamination; or (*d*) a combination of any or all of the above. The rapid, reliable, and sensitive enumeration of *E. coli* and coliform bacteria for use as a sanitation and safety tool is needed.

Current classical methodologies for the detection of *E. coli* and coliforms (6) are labor intensive, time consuming, and costly with respect to equipment, media, and personnel. The 3M™ Petrifilm™ *E. coli*/Coliform Count (EC) Plates method is the AOAC Official Method **998.08** for poultry, meats, and seafood. This 3M Petrifilm EC Plates contain Violet Red Bile nutrients and a β-glucuronidase substrate (5-bromo-4-chloro-3-indoxyl-β-D-glucuronide: X-Gluc) for the differentiation of *E. coli*. *E. coli* colonies growing on the Petrifilm EC plate produce gas from lactose and β-glucuronidase which yields a blue precipitate. Finally, the colonies of *E. coli* are blue to red-blue with gas on the Petrifilm EC plate. The colonies of coliform bacteria are darker red than the plate with gas for conformed coliforms.

The Compact Dry EC is an enumeration method for *E. coli* and coliform bacteria that can yield a result in 20–24 h. The sample is diluted, plated, and incubated at 35°C. Compact Dry EC using the Compact Dry system (7) contains the nutrients developed primarily for rapid growth of coliform bacteria (8), 5-bromo-6-chloro-3-indoxyl-β-D-galactopyranoside (Magenta-Gal) for coliform bacteria, and 5-bromo-4-chrolo-3-indoxyl-β-D-glucuronic acid, cyclohexylammonium salt (X-Gluc) for *E. coli*. A single medium containing 2 chromogenic substrates, such as Chromocult Coliform Agar, is a useful method for enumerating *E. coli* and coliforms in meat samples (9).

The colonies of *E. coli* and coliforms on Compact Dry EC are blue to blue-purple and red to pink, respectively. The combined total number of red/pink with blue/blue purple colonies results in the total number of coliforms. The inoculum is diffused naturally by capillary action. The selective system or its concentration in the Compact Dry EC may be less inhibitory than that of the Petrifilm EC plate, allowing for greater recovery of *E. coli* and coliforms. Gram-positive bacteria are inhibited by bile salts, and Gram-negative bacteria other than coliforms do not form the typical blue to blue-purple or red to pink color colonies.

3.3 Summary of Results

This is a report for Compact Dry EC according to protocol instructed by AOAC Research Institute (RI) on August 22, 2003, for matrixes of raw meats. The Compact Dry EC medium plates were validated as an analysis tool for determining CFU of *E. coli* and coliform bacteria from a variety of raw meats. The studies compared the Compact Dry EC methodology with AOAC Official Method **966.24** in raw meats for enumeration of *E. coli* and coliform bacteria. The validation procedure was performed using 5 different types of raw meats. For Compact Dry EC, 35°C is the recommended temperature as specified in standard method defined conditions. Therefore, the performance tests were conducted at 35°C. In all studies performed, no apparent differences were observed between the Compact Dry EC method and the AOAC Official Method **966.24** results. For the accuracy claim made, a correlation between the 2 enumeration methods was investigated. For all pooled sample data (n = 75), a correlation factor of $r^2 = 0.93$ (*E. coli*) and $r^2 = 0.93$ (coliform bacteria) could be assigned, as stated in the application for "*Performance-Tested Method*℠." The consistency of the quality and storage robustness of the dehydrated film plates could be demonstrated using the claimed food matrixes. No significant variations in *E. coli* and coliform bacterial counts were observed with different production lots or plates of diverse storage age (3 lots; expiry before 11, 10, and 7 months). However, significant differences in *E. coli* and coliform bacterial counts were observed with plates stored for 40 months, well beyond the expiry date of 18 months determined for the Compact Dry EC.

The Compact Dry EC plates can be used for the estimation of *E. coli* and coliform bacterial counts for a broad spectrum of raw meats. However, due to microbial physiology, the recommended optimized incubation parameters of 35°C and 20–24 h should be kept constant. A sample volume deviation of 0.9–1.1 mL can be tolerated. An incubation temperature deviation from 33 to 37°C can be tolerated. The colony counts were equivalent for the whole plate count and the grid count (1 × 1 cm count × 20 and 0.5 × 0.5 cm count × 80).

4 Materials and Methods

4.1 Test Kit Information

(**4.1.1**) *Kit name.*—Compact Dry EC.

(**4.1.2**) *Cat. No.* -Nissui Pharmaceutical Co., Ltd., 06742 (40 plates), 06743 (240 plates); HyServe, 1 000168 (40 plates), 1 000169 (240 plates), 1 002878 (880 plates).

(**4.1.3**) *Ordering information.—HyServe.*—Seeshaupter Str. 27, 82393 Iffeldorf, Germany, Tel: +49 (0) 8856 8020588, Fax: +49 (0) 8856 8020339, Website: www.hyserve.com. *Nissui Pharmaceutical Co., Ltd.*—3-23-9, Ueno, Taito-ku, Tokyo 110-8736, Japan, Tel: +81 (0)3-5846-5707, Fax: +81(0)3-5846-5629, Website: www.nissui-pharm.co.jp.

(**4.1.4**) *Test kit reagents.*—The Compact Dry EC plates are selective media consisting of complex nutrients such as peptone, KNO_3, NaCl, and sodium pyruvate, phosphate-buffered and supplemented with selective substances for Gram-positive bacteria. Additionally, 2 chromogenic enzyme substrates, Magenta-GAL and X-Gluc, are present in the plates and responsible for the color

change. The medium is solidified by algae polysaccharides and lyophilized in a net structure.

4.2 Additional Supplies and Reagents

Dilution buffer.—Because a maximum of 250 colonies can be resolved and counted on one Compact Dry EC plate and some food matrixes can harbor up to 10^8 CFU/g, (decimal-) dilution of the sample material is necessary. For this purpose, sterile Butterfield's buffered phosphate diluent is used.

4.3 Apparatus

Incubator.—The only equipment needed for the whole plate count testing method with Compact Dry EC is an incubator. Any commercially available apparatus keeping the adjustable temperature ±1°C of the target temperature (35°C) is applicable.

4.4 Standard Reference Materials

The product was performance tested with the following strains: *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Klebsiella oxytoca* ATCC 13182, *Pseudomonas aeruginosa* ATCC 9027, and *Staphylococcus aureus* ATCC 6538. Good growth for *E. coli* ATCC 8739 (blue/blue purple colonies), *K. oxytoca* ATCC 13182 (red/pink colonies), and *P. aeruginosa* ATCC 9027 (white colonies) should be observed after incubation at 35°C for 20–24 h. The inhibition for *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538 should be observed after incubation at 35°C for 20–24 h.

4.5 Standard Solutions

None.

4.6 General Preparation

None.

4.7 Sample Preparation

Samples for inoculation should be prepared following the specific national and international standards for enumeration techniques, e.g., AOAC Official Method **966.23**: Microbiological Methods.

Sample preparation procedure completely followed AOAC Official Method **966.23**. Each 50 g test portion was weighed aseptically (using sterile forceps or spatulas) into a sterile high-speed blender cup. Then 450 mL sterile Butterfield's phosphate buffer diluent was added into the cup and blended for 2 min at 14 000 rpm in a blender to disperse the material.

Butterfield's buffered phosphate diluent: 34.0 g KH_2PO_4, ca 175 mL 1.0M NaOH, pH 7.2.

Dilution for use.—To 1.25 mL stock, add 1 L H_2O. Autoclave 15 min at 121°C.

Sample preparation procedure for this test completely follows AOAC Official Method **966.23**.

4.8 Analysis

(4.8.1) *Inoculation of the Compact Dry EC plates.*—After removing the cap of the plate, inoculate 1 mL sample solution onto the center of the Compact Dry EC plate. The liquid is diffused evenly into the sheet (total medium of 20 cm^2) by capillary action to transform the dried sheet into a gel within seconds. Place the cap back onto the plate, and record the information on the memorandum section. Then incubate the plate.

The recommended incubation time and temperature for the Compact Dry EC plates are 20–24 h at 35°C.

4.9 Interpretation and Test Result Report

The Compact Dry EC is an enumeration method for *E. coli* and coliform bacteria that can yield a result in 20–24 h. The sample is diluted, plated, and incubated at 35°C. The plate is then counted by observing red/pink colonies indicative of coliforms and blue/blue purple colonies indicative of *E. coli*. The total number of red/pink with blue/blue purple colonies is the total number of coliforms. When the number of colonies is high, it is convenient to use the grids carved on the back of the container consisting of 1 × 1 cm, or 0.5 × 0.5 cm squares. Count the colonies of one square and multiply by 20 or 80, respectively.

5 Safety Precautions

5.1 Precautions for Danger

When medium or reagent comes into contact with eyes or mouth, wash immediately with plenty of water and consult a physician. Manipulations of microorganisms always involve certain risks of laboratory-acquired infections. Therefore, manipulations should be performed under the supervision of key specialists with biohazard protection measures. Any laboratory equipment and medium that have been in contact with specimen should be regarded as infectious.

5.2 Precautions for Disposal of Waste

Any medium, reagent, and materials must be sterilized by autoclaving after use and then disposed as industrial waste according to the national regulations on laboratory waste disposal.

6 Summary of Results

6.1 Validation Studies

The validation studies were performed in the laboratories of Nissui Pharmaceutical Co., Ltd.

(6.1.1) *Inclusivity study.*—The inclusivity study was performed according to protocol instructed by AOAC RI on August 22, 2003.

(6.1.1.1) *Methodology.*—The 54 isolates of *E. coli* and coliforms were suspended in nonselective broth such as Tryptic Soy Broth (TSB). The bacterial suspensions were diluted to <250 CFU/mL as per package insert instructions

Table 1. Inclusivity study using *E. coli* and other coliforms for Compact Dry EC

Organism	Designation[a]	Test No.	No. of positive strains	Color reaction[b]	No. of negative strains	Color reaction[b]
Citrobacter amalonaticus	ATCC 25405	1	1	R/Pi	0	
Citrobacter freundii	ATCC 8090, NIHJ 97, NHIJ 98, NIHJ 99	4	4	R/Pi	0	
Citrobacter koseri	ATCC 27156, ATCC 27028	2	2	R/Pi	0	
Enterobacter aerogenes	ATCC 13048, NIHJ 227, NIHJ 228, NIHJ 229	4	4	Pi	0	
Enterobacter amnigenus	ATCC33072, NIHJ 247, NIHJ 248, NIHJ 249	4	4	R/Pi	0	
Enterobacter asburiae	ATCC 35953	1	1	R/Pi	0	
Enterobacter cancerogenus	ATCC 35317	1	1	R	0	
Enterobacter cloacae	ATCC 13047, NI HJ 207, NIHJ 208,NIHJ 209	4	4	Pi	0	
Enterobacter gergoviae	ATCC 33028	1	1	R/Pi	0	
Enterobacter intermedium	NIHJ 260, NIHJ 264	2	2	R/Pi	0	
Enterobacter sakazakii	ATCC 29544	1	1	R/Pi	0	
Escherichia blattae	JCM 1650	1	0		1	W
Escherichia coli	ATCC 9637, ATCC 12036, ATCC 12435, ATCC 15224, ATCC 15939, ATCC 27662, ATCC 29194, ATCC 33582, ATCC 35218, ATCC 11775, ATCC 25922	11	11	B/BPu	0	
Escherichia coli O157:H7	ATCC 35150, ATCC 43888	2	0		2	R/Pi
Escherichia coli O111	ATCC 33780, ATCC 43887	2	2	B	0	
Escherichia fergusonii	JCM 5897, JCM 5899	2	2	R/Pi	0	
Escherichia hermanii	ATCC 33650	1	1	Pi	0	
Klebsiella oxytoca	ATCC 13182, NIHJ 182, NIHJ 183, NIHJ 184	4	4	R/Pi	0	
Klebsiella ozaenae	ATCC 11296, NIHJ 195	2	2	R/Pi	0	
Klebsiella pneumoniae	ATCC 13883, ATCC 700603	2	2	R/Pi	0	
Klebsiella terrigena	ATCC 33257	1	1	R/Pi	0	
Serratia marcescens	ATCC 13880	1	1	R/Pi	0	
Total		54	51	B, BPu, Pi, Pu, R	3	R/Pi, W

[a] ATCC = American Type Culture Collection; JCM = Japan Collection of Microorganisms; NIHJ = National Institute of Health, Japan.
[b] B = Blue; BPu = blue purple; Pi = pink; Pu = purple; R = red; W = white.

and then inoculated onto the Compact Dry EC medium and analyzed as per package insert instructions.

(6.1.1.2) *Results.*—The results are shown in Table 1. The percentage of target strains producing a positive result by the test was: Positive isolates/tested isolates × 100 = 51/54 × 100 = 94.4%.

(6.1.2) *Exclusivity study.*—The exclusivity study was performed according to protocol instructed by AOAC RI on August 22, 2003.

(6.1.2.1) *Methodology.*—The 50 isolates of noncoliform bacteria were suspended in nonselective broth such as TSB. The bacterial suspensions were diluted to <250 CFU/mL as per package insert instructions and then inoculated onto the Compact Dry EC medium and analyzed as per package insert instructions.

(6.1.2.2) *Results.*—The results are shown in Table 2. The percentage of nontarget strains producing negative results, which are recognized either no growth or growth of white colonies, by the test was: Negative isolates/tested isolates × 100 = 48/50 × 100 = 96.0%.

(6.1.3) *Method comparison study.*—The method comparison study was performed according to protocol instructed by AOAC RI on August 22, 2003, for 4 kinds of raw meat, including raw ground pork, raw pork, raw lamb, and raw veal. Reference method is AOAC Official Method **966.24**.

(6.1.3.1) *Methodology.*—Sample preparation procedure completely followed AOAC Official Method **966.23**. Each 50 g test portion was weighed aseptically (using sterile forceps or spatulas) into a sterile high-speed blender cup. Then 450 mL sterile Butterfield's phosphate buffer diluent was added into the cup and blended for 2 min at 14 000 rpm by a blender of CELL MASTER CM-100 (AZ ONE CORP.) to disperse the material. The blended 1:10 dilution sample was

Table 2. Exclusivity study using noncoliform bacteria for Compact Dry EC

Organism	Designations[a]	Test No.	No. of positive strains	Color reaction[b]	No. of negative strain	Color reaction[b]
Achromobacter xylosoxidans subsp. denitrificans	JCM 5490	1	0		1	sW
Achromobacter xylosoxidans subsp. xylosoxidans	JCM 9659	1	0		1	sW
Acinetobacter baumanii	ATCC 19606	1	0		1	W
Acinetobacter calcoaceticus	ATCC 23055, JCM 6842	2	0		2	W/–
Aeromonas hydrophila	ATCC 7966, ATCC 15468	2	1	LRPi	1	–
Alcaligenes faecalis	JCM 1474	1	0		1	sW
Bacillus cereus	ATCC 14579, ATCC 11778	2	0		2	–
Edwardsiella tarda	ATCC 15947, NIHJ 127, NIHJ 128	3	0		3	W
Lactobacillus lactis	JCM 6123	1	0		1	–
Micrococcus luteus	ATCC 4698	1	0		1	–
Micrococcus lylae	ATCC 27566	1	0		1	–
Moraxella nonliquefaciens	ATCC 19975	1	0		1	–
Moraxella ovis	ATCC 33078	1	0		1	–
Proteus mirabilis	ATCCC 29906	1	0		1	br
Proteus vulgaris	ATCC 13315, ATCC 6380	2	0		2	br
Providencia alcalifaciens	ATCC 9886, ATCC 51902	2	0		2	br
Pseudomonas aeruginosa	ATCC 27853	1	0		1	W
Pseudomonas alcaligenes	ATCC 14909	1	0		1	–
Pseudomonas diminuta	JCM 2790, JCM 2792	2	0		2	–
Pseudomonas mendocina	ATCC 25411	1	0		1	W
Pseudomonas pseudoalcaligenes	ATCC 17440	1	0		1	sW
Pseudomonas putida	ATCC 12633	1	0		1	–
Pseudomonas stutzeri	ATCC 17588	1	0		1	W
Pseudomonas vesicularis	ATCC 11426	1	0		1	–
Salmonella Choleraesuis	NHIJ 54, NHIJ 55	2	0		2	W
Salmonella Typhimurium	ATCC 14028	1	0		1	W
Shigella flexineri	NHIJ 17, NHIJ 18	2	0		2	W
Shigella boydii	NHIJ 25	1	1	B	0	
Staphylococcus aureus	ATCC 12600, ATCC 25923, ATCC 6538P	3	0		3	–
Streptococcus agalactiae	ATCC 12927	1	0		1	–
Streptococcus bovis	ATCC 9809	1	0		1	–
Streptococcus canis	ATCC 43496	1	0		1	–
Streptococcus equines	ATCC 9812		0		1	–
Streptococcus pneumoniae	ATCC 33400	1	0		1	–
Streptococcus pyogenes	ATCC 12383	1	0		1	–
Streptococcus salivarius	ATCC 7073	1	0		1	–
Streptococcus sanguis	ATCC 10556	1	0		1	–
Streptococcus uberis	ATCC 19436	1	0		1	–
Total		50	2	B, LRPi	48	

[a] ATCC = American Type Culture Collection; JCM = Japan Collection of Microorganisms; NIHJ = National Institute of Health, Japan.
[b] B = Blue; LRPi = light red pink; br = brown; W = white, sW = small white; – = no growth.

Table 3. AOAC method comparison for raw ground pork[a]

		Compact Dry EC		AOAC **966.24**	
E. coli/coliform level		*E. coli*, $\log_{10}$ CFU/g	*Coliform*, $\log_{10}$ CFU/g	*E. coli*, $\log_{10}$ MPN/g	*Coliform*, $\log_{10}$ MPN/g
10–100 CFU/g	1	1.00	1.00	1.36	1.36
	2	1.00	1.00	0.96	0.96
	3	0.70	1.18	0.96	0.96
	4	1.00	1.00	1.63	1.63
	5	1.00	1.00	1.36	1.59
	Mean	0.94	1.04	1.26	1.30
	s_r	0.13	0.08	0.29	0.33
	RSD_r, %	14.32	7.61	23.27	25.27
100–1000 CFU/g	1	2.71	2.76	2.81	2.81
	2	2.66	2.70	2.59	2.72
	3	2.54	2.62	2.66	2.66
	4	2.54	2.59	2.56	2.56
	5	2.57	2.66	2.45	2.54
	Mean	2.61	2.67	2.61	2.66
	s_r	0.08	0.07	0.13	0.11
	RSD_r, %	2.89	2.52	5.10	4.19
1000–10000 CFU/g	1	3.88	3.90	3.66	3.66
	2	3.79	3.84	3.04	3.66
	3	3.11	3.08	2.97	3.38
	4	3.95	3.98	3.38	3.66
	5	3.77	3.84	3.38	3.66
	Mean	3.70	3.73	3.29	3.61
	s_r	0.34	0.37	0.28	0.13
	RSD_r, %	9.07	9.86	8.61	3.50
Uninoculated	Mean	<1	NT[b]	<1	NT

[a] The numerical data were based on 5 replicates.

[b] NT = Not tested. Coliform bacterial counts were tested using naturally contaminated raw ground pork.

used for inoculation and for making 1:100 and 1:1000 dilutions with sterile Butterfield's phosphate buffer diluent.

Because *E. coli* contamination levels on raw ground pork, raw pork, and raw lamb were quite low (nearly 0 CFU/g), *E. coli* ATCC 9637, as representative bacteria, was spiked into meat samples to yield bacteria contamination levels of about 10–100, 100–1000, and 1000–10 000 CFU/g. Contaminated meats were kept at refrigerated temperatures for 3 days before testing. There were quite low contaminated levels (nearly 0 CFU/g) for *E. coli* and coliform bacteria on raw veal. Therefore, *E. coli* ATCC 9637 and *E. cloacae* ATCC 13047, as representative bacteria, were spiked into meat samples to yield bacteria contamination levels of about 10–100, 100–1000, and 1000–10 000 CFU/g. Contaminated meats were kept at refrigerated temperatures for 3 days before testing. The test procedures followed were as described in AOAC Official Method **966.24** and in the instruction on package insert of Compact Dry EC, respectively.

*AOAC Official Method **966.24***. —Each 1 mL from samples of 1:10, 1:100, and 1:1000 dilutions was inoculated into a 3-tube most probable number (MPN) series in triplicate tubes of lauryl sulfate tryptose broth. They were incubated 48 ± 2 h at 35°C for gas formation as evidenced by displacement of liquid in an insert tube. The tubes were examined for gas formation at 24 and 48 h intervals. Cultures producing gas were transferred to brilliant green lactose bile (BGLB) broth and EC broth by using a 3 mm loop. Each BGLB broth was incubated 48 ± 2 h at 35°C. The MPN Table **966.24A** was used to compute MPN on basis of number of tubes of BGLB broth producing gas by the end of the incubation period. Data of coliform bacteria/g from MPN were compared with the data from Compact Dry EC. Each EC broth was incubated 48 ± 2 h at 45.5 ± 0.05°C in a covered water-bath. The water level was above highest level of medium. The tubes were examined for gas formation at 24 and 48 h intervals. Cultures producing gas were streaked on Levine eosin methylene blue agar (EMB)

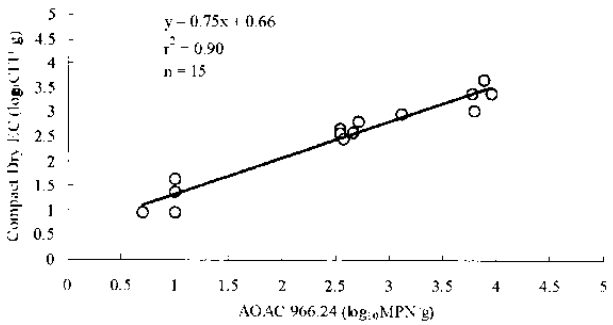

Figure 1. Correlation between Compact Dry EC and AOAC 966.24 for *E. coli* in raw ground pork.

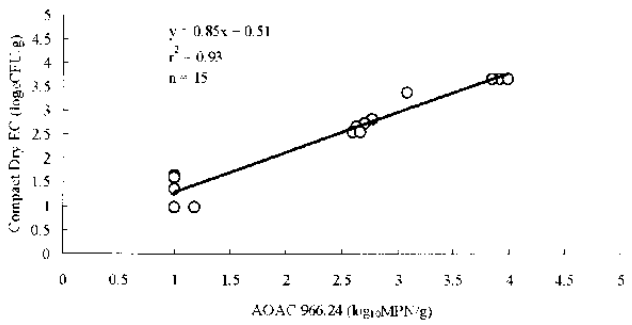

Figure 2. Correlation between Compact Dry EC and AOAC 966.24 for coliform bacteria in raw ground pork.

plates and incubated 24 ± 2 h at 35°C. Two or more well-isolated typical colonies from Levine EMB plates were picked and transferred to plate count agar slants. The slants were incubated 18–24 h at 35°C. The cultures were identified by Gram stain and biochemical tests. The biochemical tests included an oxidase test, IMViC test, and API 20E. Data of *E. coli*/g from MPN were compared with the data from Compact Dry EC.

Compact Dry EC.—Each 1 mL from samples of 1:10, 1:100, and 1:1000 dilutions was inoculated onto Compact Dry EC, with duplicate plates at each dilution. The plates were incubated for 20–24 h at 35°C. Each 5 or more well-isolated typical blue/blue purple colonies and typical red/pink colonies were picked and restreaked on plate count agar plates to confirm pure cultures. The pure cultures on plate count agar plates were transferred to plate count agar slants for identification by Gram stain and biochemical tests. The biochemical tests included oxidase test, IMViC test, and API 20E.

Data analysis.—The bacterial counts were converted into logarithms for data analysis. The difference of mean $\log_{10}$ CFU was compared by using a one-way analysis of variance (ANOVA). The MS Excel 2000 was used for performing ANOVA.

(6.1.3.2) *Results.*—One-way ANOVA for data obtained from 3 levels of *E. coli* and coliforms in raw ground pork (Table 3) showed comparable results for both methods ($P > 0.05$) and r^2 correlation coefficient for *E. coli* and coliform counts by both methods were 0.90 (Figure 1) and 0.93 (Figure 2), respectively, indicating good correlation between the methods. The range of variance within each of the 3 levels by Compact Dry EC for raw ground pork was narrower than that from each of the results for those samples tested according to the AOAC methodology, with the exception of the high level of coliform bacteria. The Compact Dry EC yielded coliform results ranging from 3.08 to 3.98 $\log_{10}$ CFU/g. Coliform results by AOAC methodology ranged from 3.38 to 3.66 $\log_{10}$ MPN/g. The MPN values have a range associated with them and calculations for confidence limits (6) associated with them. The 95% confidence intervals of 3.38 and 3.66 $\log_{10}$ MPN/g are in the range of 2.62–4.00 and 2.95–4.30 $\log_{10}$ MPN/g, respectively. The results of Compact Dry EC fall well within these confidence limits.

One-way ANOVA for data on 3 levels of *E. coli* and coliforms in raw pork (Table 4) showed comparable results on both methods ($P > 0.05$) and r^2 as correlation coefficient for *E. coli* and coliform counts by both methods were 0.96 (Figure 3) and 0.93 (Figure 4), respectively, indicating good correlation between the 2 methods. The range of variance within each of the 3 levels by Compact Dry EC for raw pork was narrower than that from each of the results for those samples tested according to the AOAC methodology, with the exception of the medium level of *E. coli* and the high level of coliform bacteria. The Compact Dry EC results for the medium level of *E. coli* ranged from 1.30 to 1.88 $\log_{10}$ CFU/g. *E. coli* results by AOAC methodology ranged from 1.36 to 1.63 $\log_{10}$ MPN/g. The 95% confidence intervals of 1.36 and 1.63 $\log_{10}$ MPN/g are in the range of 0.66–1.97 and 0.95–2.26 $\log_{10}$ MPN/g, respectively. The *E. coli* results of Compact Dry EC fall well within these confidence limits. The Compact Dry EC yielded the high level of coliform results ranging from 3.49 to 4.15 $\log_{10}$ CFU/g. Coliform results by AOAC methodology ranged from 3.38 to 4.04 $\log_{10}$ MPN/g. The 95% confidence intervals of 3.38 and 4.04 $\log_{10}$ MPN/g are in the range of 2.62–4.00 and 3.26–4.61 $\log_{10}$ MPN/g, respectively. The results of Compact Dry EC fall well within these confidence limits.

One-way ANOVA for data on 3 levels of *E. coli* and coliforms in raw lamb (Table 5) showed comparable results on both methods and r^2 as correlation coefficient for *E. coli* and coliform counts by both methods ($P > 0.05$) were 0.99 (Figure 5) and 0.94 (Figure 6), respectively, indicating good correlation between the 2 methods. The range of variance within each of the 3 levels by Compact Dry EC for raw lamb was narrower than that from each of the results for those samples tested according to the AOAC methodology with the exception of the low and medium levels of *E. coli*. The Compact Dry EC yielded the low and medium levels of *E. coli* results ranging from 0.70 to 1.00 and 1.90–1.65 $\log_{10}$ CFU/g, respectively. *E. coli* results by AOAC methodology ranged from 0.86 to 0.91 $\log_{10}$ MPN/g for low level and 1.98 to 2.20 $\log_{10}$ MPN/g for medium level. The 95% confidence intervals of 0.86, 0.96, 1.98, and 2.20 $\log_{10}$ MPN/g are in the

Table 4. AOAC method comparison for raw pork[a]

		Compact Dry EC		AOAC **966.24**	
E. coli/coliform level		*E. coli*, $\log_{10}$ CFU/g	Coliform, $\log_{10}$ CFU/g	*E. coli*, $\log_{10}$ MPN/g	Coliform, $\log_{10}$ MPN/g
10–100 CFU/g	1	1.00	2.00	0.56	1.63
	2	0.70	1.89	0.96	1.88
	3	1.18	1.95	1.18	1.59
	4	1.00	1.65	0.56	1.97
	5	1.00	1.96	0.48	1.66
	Mean	0.98	1.89	0.74	1.75
	s_r	0.17	0.14	0.31	0.17
	RSD_r, %	17.65	7.37	41.08	9.49
100–1000 CFU/g	1	1.65	2.86	1.63	2.66
	2	1.40	2.96	1.36	2.66
	3	1.88	2.98	1.63	2.88
	4	1.30	2.89	1.63	2.88
	5	1.74	2.82	1.63	2.81
	Mean	1.59	2.90	1.58	2.78
	s_r	0.24	0.07	0.12	0.11
	RSD_r, %	14.99	2.37	7.70	3.89
1000–10000 CFU/g	1	3.48	3.69	3.63	3.63
	2	3.52	3.82	3.38	3.38
	3	3.59	4.00	4.04	4.04
	4	3.46	4.15	4.04	3.66
	5	3.43	3.49	3.66	3.63
	Mean	3.50	3.83	3.75	3.67
	s_r	0.06	0.26	0.29	0.24
	RSD_r, %	1.79	6.68	7.63	6.46
Uninoculated	Mean	<1	NT[b]	<1	NT

[a] The numerical data were based on 5 replicates.

[b] NT = Not tested. Coliform bacterial counts were tested using naturally contaminated raw pork.

range of 0.11–1.26, 0.65–1.58, 1.26–2.62, and 1.60–2.62 $\log_{10}$ MPN/g, respectively. The low and medium levels of *E. coli* results of Compact Dry EC fall well within these confidence limits.

One-way ANOVA for data on 3 levels of *E. coli* and coliforms in raw veal (Table 6) showed comparable results on both methods ($P > 0.05$) and r^2 as correlation coefficient for *E. coli* and coliform counts by both methods were 0.85 (Figure 7) and 0.93 (Figure 8), respectively, indicating good correlation between the 2 methods. The range of variance within each of the 3 levels by Compact Dry EC for raw veal was narrower than that from each of the results for those samples tested according to the AOAC methodology. A lower correlation coefficient of 0.85 was obtained here, compared to the other correlation coefficients that were >0.90. Only raw veal was spiked with *E. coli* and *E. cloacae* as representative bacteria, because coliform bacteria contamination levels on raw veal were nearly 0 CFU/g.

(6.1.4) *Lot-to-lot and stability study.*—The lot-to-lot and stability study was performed according to protocol instructed by AOAC RI on August 22, 2003.

(6.1.4.1) *Methodology.*

(6.1.4.1.1) *Lot-to-lot study.*—For the lot-to-lot consistency study, 3 lots of test kits must be tested and must show consistent results. This part of the testing should have at least 10 positive and 5 negative results for each test kit lot.

(6.1.4.1.2) *Stability study.*—Stability data can be generated through accelerated time or real time (preferred) testing. Data must support the expiration term. If accelerated stability data are used, the sponsor must provide real time data supporting the entire expiration term of the kit prior to renewal at the end of the first year of certification.

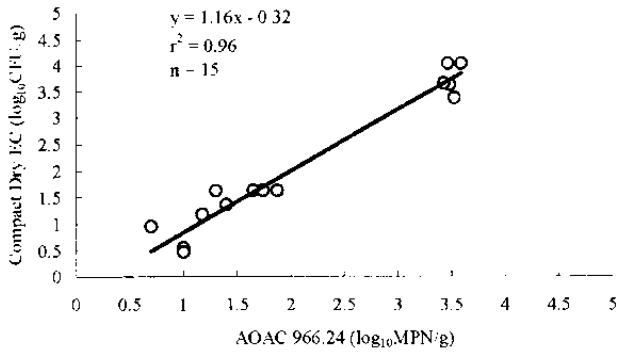

Figure 3. Correlation between Compact Dry EC and AOAC 966.24 for *E. coli* in raw pork.

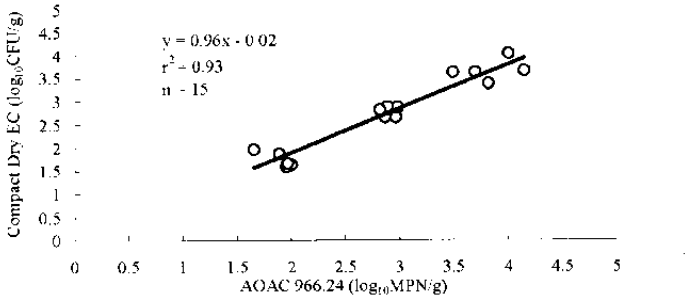

Figure 4. Correlation between Compact Dry EC and AOAC 966.24 for coliform bacteria in raw pork.

(6.1.4.1.3) *Combined lot-to-lot and stability studies.*—The studies can be performed concurrently by testing 3 lots (expiry before 11, 10, 7 months) of kits: one newly manufactured lot, one lot at the middle of the expiration term, and one lot at or near expiration.

(6.1.4.1.4) *Bacterial suspensions and test procedure.*—*E. coli* ATCC 15224 as *E. coli* strain and *E. cloacae* ATCC 13047 as non-*E. coli* coliform strain were suspended in nonselective broth such as TSB and diluted to a high level and low level within the quantitative range of the Compact Dry EC method. *P. aeruginosa* ATCC 27853 as non-*E. coli* and noncoliform bacterial strain was suspended in nonselective broth such as TSB and diluted to a high level within the quantitative range of the Compact Dry EC method. Five replicates of each level of each culture were tested on each lot of kits. A one-way ANOVA was performed to compare means of replicates. The repeatability of replicates for each lot was compared.

(6.1.4.2) *Results.—E. coli strain.*—One-way ANOVA for data on the high level of *E. coli* ATCC 15224 showed comparable results from 4 lots of Compact Dry EC. One-way ANOVA for data on the low level of *E. coli* ATCC 15224 showed comparable results from 4 lots of Compact Dry EC.

Coliform strain.—One-way ANOVA for data on the high level of *E. cloacae* ATCC 13047 showed comparable results from Lots 2–4. However, the results from the expired Compact Dry EC, Lot 1 (>18 months), were significantly less ($P < 0.05$) than the result of Lot 2. One-way ANOVA for data on the low level of *E. cloacae* ATCC 13047 showed comparable results from 4 lots of Compact Dry EC ($P > 0.05$).

Non-E. coli and noncoliform bacterial strain.—One-way ANOVA for data on the high level of *P. aeruginosa* ATCC 27853 showed comparable results from Lots 2–4. However, the results from the expired Compact Dry EC, Lot 1 (>18 months), were significantly different ($P < 0.05$) from the result of Lot 4.

(6.1.5) *Ruggedness study.*—The method comparison study was performed according to protocol instructed by AOAC RI on August 22, 2003. The AOAC RI recommended testing the following parameters: (*1*) sample volume: 1.0 ± 0.1 mL; (*2*) incubation temperature: 35 ± 2°C; and (*3*) counting colonies: whole plate count vs grid count (1 cm × 1 cm count × 20, and 0.5 cm × 0.5 cm count × 80).

(6.1.5.1) *Methodology.*—One *E. coli* strain (*E. coli* ATCC 15224) and one non-*E. coli* coliform strain (*E. cloacae* ATCC 13047) were suspended in nonselective broth such as TSB and diluted to a high level and low level within the quantitative range of the Compact Dry EC method. One non-*E. coli* noncoliform bacterial strain (*P. aeruginosa* ATCC 27853) was suspended in nonselective broth such as TSB and diluted to a high level within the quantitative range of the Compact Dry EC method. Five replicates of each level of each culture for each parameter varied in the Compact Dry method were tested. A one-way ANOVA was performed to compare means of replicates. The repeatability of replicates for each lot was compared.

(6.1.5.2) *Results.*—The one-way ANOVA for high levels of *E. coli* and *Pseudomonas* showed a P value of <0.05, indicating significant difference, because the standard deviation logs were very narrow for *E. coli* and *Pseudomonas*: 0.9, 1.0, and 1.1 mL inoculation level; 0.02, 0.04, and 0.03; and 0.03, 0.02, and 0.02, respectively (Table 7).

One-way ANOVA for data from the variations of the incubation temperature and the counting methods showed comparable results ($P > 0.05$) for *E. coli*, *E. cloacae*, and *P. aeruginosa* (Tables 8 and 9).

6.2 Independent Validation Studies

The validation studies were conducted by Q Laboratories, Inc., under the direction of the AOAC RI.

(6.2.1) *Method comparison study.*—A study of method comparison conducted at Q Laboratories, Inc., Research Center (Cincinnati, OH) compared the analytical results of the Compact Dry EC enumeration method for *E. coli* and coliform bacteria with those of AOAC Official Method **966.24** in raw ground beef.

(6.2.1.1) *Methodology.*—The methodology for this study was followed as outlined in the AOAC RI Independent Validation Protocol: Nissui Compact Dry EC for Enumeration of *E. coli* and Coliform Bacteria. This study was conducted at 4 levels of inoculum, and compared to the AOAC Official Method **966.24**. Each of the 4 levels consisted of 5 samples: 5

Table 5. AOAC method comparison for raw lamb[a]

E. coli/coliform level		Compact Dry EC		AOAC **966.24**	
		E. coli, $\log_{10}$ CFU/g	Coliform, $\log_{10}$ CFU/g	*E. coli*, $\log_{10}$ MPN/g	Coliform, $\log_{10}$ MPN/g
10–100 CFU/g	1	0.70	0.70	0.86	1.63
	2	1.00	1.18	0.96	1.36
	3	1.00	1.18	0.96	0.96
	4	1.00	1.00	0.96	0.96
	5	1.00	1.30	0.96	1.36
	Mean	0.94	1.07	0.94	1.26
	s_r	0.13	0.23	0.05	0.29
	RSD_r, %	14.32	21.83	4.85	23.27
100–1000 CFU/g	1	1.95	2.56	1.98	2.63
	2	2.08	2.41	2.08	2.38
	3	2.00	2.29	2.08	2.38
	4	1.90	2.11	1.98	2.63
	5	2.16	2.41	2.20	2.18
	Mean	2.02	2.35	2.06	2.44
	s_r	0.10	0.16	0.09	0.19
	RSD_r, %	5.07	6.98	4.53	7.98
1000–10000 CFU/g	1	3.93	3.93	3.97	3.97
	2	3.49	3.93	3.97	3.97
	3	3.46	3.70	3.38	3.63
	4	3.42	3.74	3.66	3.66
	5	3.50	3.69	3.66	3.66
	Mean	3.56	3.80	3.73	3.78
	s_r	0.21	0.12	0.25	0.17
	RSD_r, %	5.84	3.14	6.64	4.58
Uninoculated	Mean	<1	NT[b]	<1	NT

[a] The numerical data were based on 5 replicates.

[b] NT = Not tested. Coliform bacterial counts were tested using naturally contaminated raw lamb.

uninoculated samples, 5 low-level (1.45 $\log_{10}$ CFU/g coliform; 1.63 $\log_{10}$ CFU/g *E. coli*) inoculated samples, 5 intermediate-level (2.67 $\log_{10}$ CFU/g coliform; 2.67 $\log_{10}$ CFU/g *E. coli*) inoculated samples, and 5 high-level (3.66 $\log_{10}$ CFU/g coliform; 3.66 $\log_{10}$ CFU/g *E. coli*) inoculated samples. The target levels of the organism used for challenging the different broths and incubation times for both organisms were as follows: the target (*a*) for the low level inoculation was 1.00–2.00 $\log_{10}$ CFU/g, (*b*) for the intermediate-level inoculation was 2.00–3.00 $\log_{10}$ CFU/g, (*c*) for the high-level inoculation was 3.00–4.00 $\log_{10}$ CFU/g, and (*d*) for the uninoculated samples was 0 CFU/g.

Desired levels were achieved and were within the ranges dictated by the AOAC RI Independent Validation Protocol. The actual levels for coliform were (*a*) 1.45 $\log_{10}$ CFU/g, (*b*) 2.66 $\log_{10}$ CFU/g, (*c*) 3.66 $\log_{10}$ CFU/g, and (*d*) 0 CFU/g. The actual levels for *E. coli* were (*a*) 1.63 $\log_{10}$ CFU/g,

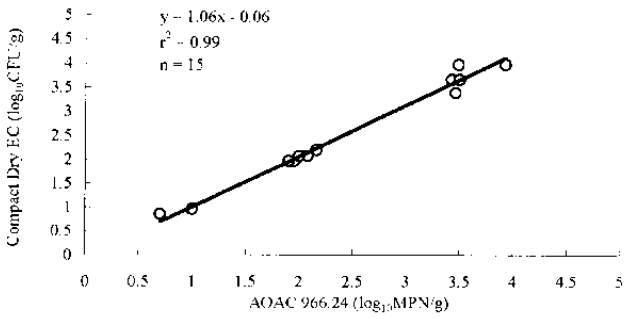

Figure 5. Correlation between Compact Dry EC and AOAC 966.24 for *E. coli* in raw lamb.

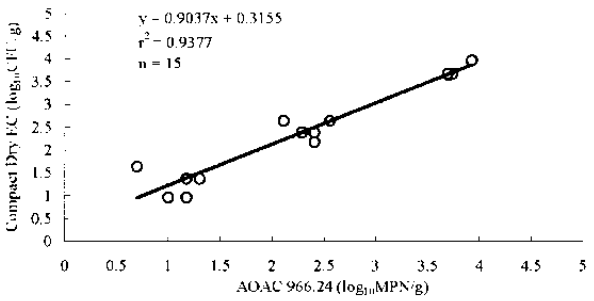

Figure 6. Correlation between Compact Dry EC and AOAC 966.24 for coliform bacteria in raw lamb.

(*b*) 2.66 $\log_{10}$ CFU/g, (*c*) 3.66 $\log_{10}$ CFU/g, and (*d*) 0 CFU/g. Replicates for each level were taken from a singularly inoculated lot of raw ground beef. The organisms used in the study were obtained directly from the American Type Culture Collection (Nos. 8090 and 8739).

(6.2.1.2) *Results.*—Detailed results can be seen for each individual sample in Table 10. The Compact Dry EC plates yielded a consistent result for each of the 5 replicates at each of the 3 inoculum levels. The range of variance within each of the 3 levels was also narrower than that from each of the results from those samples studied according to the AOAC methodology, with the exception of the intermediate level.

(6.2.1.3) *Discussion.*—The intermediate levels showed *P* values of <0.05, indicating significant difference because, the standard deviation log of coliform levels was very narrow for both methods (0.041) and the mean log of *E. coli* levels was exactly the same for both methods (2.66). All 5 replicates for both coliform and *E. coli* yielded a result consistent with the inoculum levels expected for all 5 data points (2.66 $\log_{10}$ CFU/g). The Compact Dry EC plates yielded coliform results ranging from 2.72 to 2.85 $\log_{10}$ CFU/g. *E. coli* results ranged from 2.60 to 2.72 $\log_{10}$ CFU/g.

Table 6. AOAC method comparison for raw veal[a]

		Compact Dry EC		AOAC 966.24	
E. coli/coliform level		*E. coli*, $\log_{10}$ CFU/g	Coliform, $\log_{10}$ CFU/g	*E. coli*, $\log_{10}$ MPN/g	Coliform, $\log_{10}$ MPN/g
10–100 CFU/g	1	1.00	1.18	0.56	0.96
	2	1.00	1.00	0.96	1.36
	3	1.00	1.18	0.96	1.63
	4	1.00	1.65	0.96	1.36
	5	0.70	0.70	0.56	0.56
	Mean	0.94	1.14	0.80	1.17
	s_r	0.13	0.35	0.22	0.42
	RSD_r, %	14.32	30.37	27.64	35.86
100–1000 CFU/g	1	1.90	2.28	1.32	2.36
	2	1.81	2.23	1.45	2.63
	3	1.81	2.32	2.36	2.36
	4	1.40	2.15	1.63	2.18
	5	1.93	2.28	1.36	1.97
	Mean	1.77	2.25	1.63	2.30
	s_r	0.22	0.07	0.43	0.25
	RSD_r, %	12.15	2.98	26.38	10.74
1000–10000 CFU/g	1	2.82	3.45	2.97	3.36
	2	2.81	3.45	2.32	3.63
	3	2.86	3.30	3.36	3.63
	4	2.85	3.32	2.63	3.38
	5	2.71	3.15	2.63	3.38
	Mean	2.81	3.33	2.78	3.48
	s_r	0.06	0.12	0.40	0.14
	RSD_r, %	2.15	3.74	14.21	4.09
Uninoculated	Mean	<1	<1	<1	<1

[a] The numerical data were based on 5 replicates.

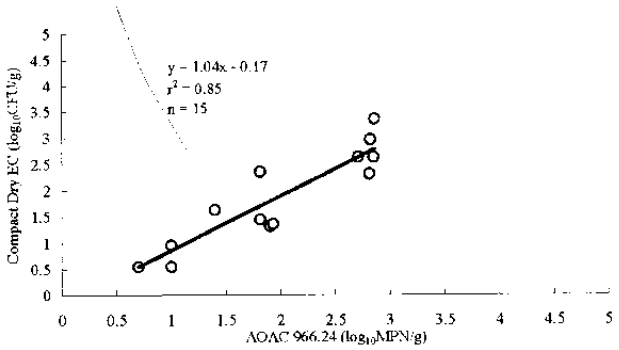

Figure 7. Correlation between Compact Dry EC and AOAC 966.24 for *E. coli* in raw veal.

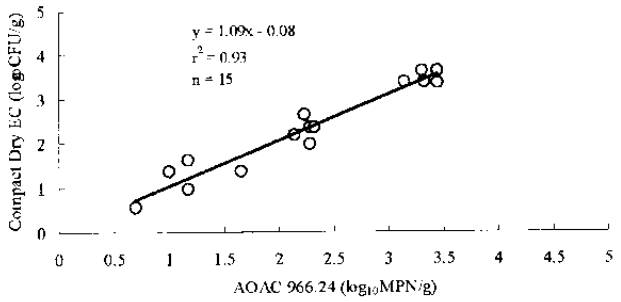

Figure 8. Correlation between Compact Dry EC and AOAC 966.24 for coliform bacteria in raw veal.

7 Discussion

The Compact Dry EC plates yielded consistent results within each inoculum level by internal testing and independent testing.

Only raw veal was spiked with *E. coli* and *E. cloacae* as representative bacteria because coliform bacteria contamination levels on raw veal were nearly 0 CFU/g. Others were spiked only with *E. coli*. We have recognized this as the only differentiation between raw veal and others; however, we do not know how the presence of *E. cloacae* would influence *E. coli* detection for both methods. The results obtained from the Compact Dry EC method were higher than those with AOAC Official Method **966.24** and the correlation coefficient of 0.85 was not significantly different ($P > 0.05$) by one-tailed value of the F-distribution. Although these MPN tables are as accurate as they can be, this range still lends itself to some difficulty in doing statistical analysis between 2 methods, one of which gives a precise number and the other which yields an MPN with a range attached.

It should be noted that all of the results for the Compact Dry EC fall well within these confidence limits. This can become more troublesome as the inoculum reaches higher levels. This can be shown with an inoculum at 2.85 $\log_{10}$ CFU/g. Although the Compact Dry EC could potentially get the correct result, statistically it is more difficult to achieve this result through the MPN table, as the 2 results nearest the 2.85 $\log_{10}$ CFU/g are a 3-3-1 (2.66 $\log_{10}$ MPN/g) and 3-2-0 (3.04 $\log_{10}$ MPN/g), respectively. The nearest possible result would be 3-3-1-1 (2.88 $\log_{10}$ MPN/g), which is still over by 1.70 $\log_{10}$ CFU/g. Taking this into account, and observing that

Table 7. Ruggedness study (sample volume) for Compact Dry EC[a]

Microbe	Level	Sample volume, mL	Mean, $\log_{10}$ CFU	SD, $\log_{10}$ CFU	ANOVA, P value
E. coli	Low	0.9	0.16	0.15	0.76
		1.0	0.25	0.23	
		1.1	0.25	0.23	
	High	0.9	2.05	0.02	0.002
		1.0	2.09	0.04	
		1.1	2.14	0.03	
Enterobacter	Low	0.9	0.22	0.24	0.96
		1.0	0.24	0.15	
		1.1	0.25	0.24	
	High	0.9	2.21	0.02	0.07
		1.0	2.24	0.02	
		1.1	2.24	0.02	
Pseudomonas	High	0.9	2.23	0.03	0.0001
		1.0	2.27	0.02	
		1.1	2.32	0.02	

[a] The data for mean and SD were based on 5 replicates.

Table 8. Ruggedness study (incubation temperature) for Compact Dry EC[a]

Microbe	Level	Incubation temp., °C	Mean, $\log_{10}$ CFU	SD, $\log_{10}$ CFU	ANOVA, *P* value
E. coli	Low	33	0.16	0.22	0.88
		35	0.22	0.21	
		37	0.22	0.21	
	High	33	2.17	0.03	0.10
		35	2.12	0.03	
		37	2.13	0.03	
Enterobacter	Low	33	0.06	0.13	0.69
		35	0.16	0.22	
		37	0.16	0.22	
	High	33	2.25	0.02	0.59
		35	2.25	0.03	
		37	2.26	0.02	
Pseudomonas	High	33	2.20	0.03	0.12
		35	2.17	0.01	
		37	2.17	0.02	

[a] The data for mean and SD were based on 5 replicates.

the results within each level of inoculum were within close proximity to one another, it is important to keep this in mind while analyzing the data.

These results obtained from tests using AOAC Official Method **966.23** sample preparation procedure were comparable for both Compact Dry EC method and the AOAC Official Method **966.24**, as evidenced by data obtained from 5 replicates and 3 levels of 5 types of raw meat (n = 75) through internal and independent validation studies. The correlation coefficient (r^2) was 0.93 (Figure 9) for *E. coli* and 0.93 (Figure 10) for coliform bacteria, indicating good correlation between the 2 methods. Confidence in the data among the 3 levels is observed when the mean of each of the 3 groups is plotted and the slope is calculated. This yielded a slope very close to 1.0 for both *E. coli* (0.99) and coliforms (0.91).

The big advantages of the Compact Dry EC system are to reduce hands-on time and to increase saving or cost benefit, as confirmed by the independent laboratory. In terms of plate preparation, inoculation, and reading the result, the Compact Dry EC system was easier and quicker than the conventional technique. Reading the plates was faster with the Compact Dry EC system, with the chromogenic substrates speeding up counting. Two chromogenic enzyme substrates, Magenta-GAL and X-Gluc, are present in the plates and responsible for the color change. The sensitivities and specificities of the chromogenic substrate for β-galactosidase and the chromogenic substrate for β-glucronidase were

Table 9. Ruggedness study (counting colonies) for Compact Dry EC[a]

Microbe	Level	Counting colonies	Mean, $\log_{10}$ CFU	SD, $\log_{10}$ CFU	ANOVA, *P* value
E. coli	High	Whole plate	2.14	0.03	0.06
		1cm × 1cm × 20	2.17	0.11	
		0.5 cm × 0.5 cm × 80	2.30	0.14	
Enterobacter	High	Whole plate	2.26	0.03	0.98
		1 cm × 1 cm × 20	2.26	0.05	
		0.5 cm × 0.5 cm × 80	2.25	0.21	
Pseudomonas	High	Whole plate	2.19	0.04	0.73
		1 cm × 1 cm × 20	2.20	0.05	
		0.5 cm × 0.5 cm × 80	2.25	0.21	

[a] The data for mean and SD were based on 5 replicates.

Table 10. Comparison of Compact Dry and AOAC 966.24 coliform and *E. coli* counts

Level	Nissui	AOAC	SD, $\log_{10}$ CFU	Paired *t*-test, *P* value
	E. coli counts, mean $\log_{10}$ CFU[a]			
High	3.65	3.55	0.110	0.120
Intermediate	2.66	2.66	0.147	0.039
Low	1.87	1.75	0.125	0.138
	Coliform counts, mean $\log_{10}$ CFU			
High	3.72	3.61	0.103	0.128
Intermediate	2.78	2.66	0.041	0.002
Low	1.92	1.88	0.133	0.662

[a] Number shown is mean log of actual counts of *E. coli* and coliform samples out of a total of 5 replicates for each category, SD and *P* values from paired *t*-test.

98.5 and 100%, and 99.2 and 99.5%, respectively (8). There were no non-*E. coli* and noncoliform colonies showing false-positive color reaction on Compact Dry EC during the method validation studies.

For the Compact Dry EC system, less training is required than for the conventional technique. The Compact Dry EC system would also bring advantages in reduced storage space, waste disposal, and required incubator space. The long shelf life of the product also has benefits compared to ready-prepared agar, which has a limited shelf life and therefore requires more logistical planning. It was the general consensus at Q Laboratories that the Compact Dry EC plates are easy to use, with a minimal amount of analyst time needed for a single quantitative test.

Overall, the Compact Dry EC is a very quick and easy screening method for the enumeration of *E. coli* and coliform bacteria in raw meats.

8 Conclusions

This method validation study demonstrated that the Compact Dry EC methodology and the reference conventional culture method produced comparable *E. coli* and coliform bacteria count results. Therefore, Compact Dry EC plates could be a convenient alternative for routine microbiological food testing. Observations during this study have shown that the Compact Dry EC plates are easy to use, with a minimal amount of analyst time needed for a single quantitative test. The time from preparation of media to setting up was 250 min per 20 samples for AOAC methodology. The time for the same procedures was 150 min per 20 samples for Compact Dry EC methodology. The equipment requirement and overall cost of the assay is less than that of AOAC methodology. There is no secondary transfer into multiple tubes or additional biochemical analyses. The amount of time required to read the Compact Dry EC is a bit longer than reading successive tubes and biochemicals from the AOAC methodology, but the plates are read anywhere from 1 to 9 days sooner than with the AOAC methodology, allowing for faster reaction time and less down time for the producer or originator of the sample. There are economical and safety advantages to having a more rapid response time. In conclusion, the Compact Dry EC, using the newly developed dry sheet medium technology, is a convenient alternative for routine microbiological food testing.

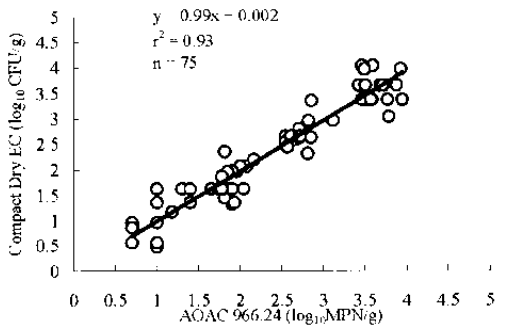

Figure 9. Correlation between Comapct Dry EC and AOAC 966.24 for *E. coli* in raw meats.

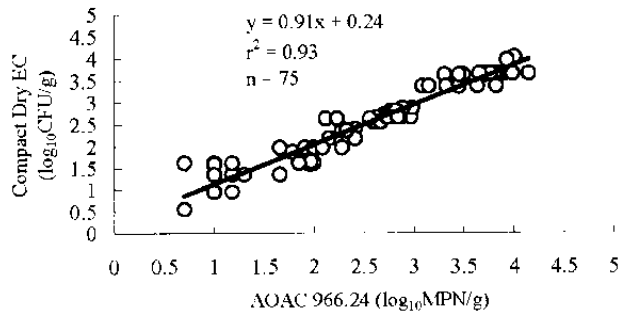

Figure 10. Correlation between Compact Dry EC and AOAC 966.24 for coliform bacteria in raw meats.

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