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Coliform Behavior in Frozen Foods

I. Rapid Test for the Recovery of *Escherichia coli* from Frozen Foods

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An assortment of 496 samples of frozen foods consisting of fish or marine products, variety types, and cream pie desserts were subjected to four parallel examinations for the recovery of *Escherichia coli*. The test procedures consisted of two low-temperature (35 C) and two high-temperature (44 C) presumptive tests, followed by an E C confirmatory test at 45.5 C. Of all the test methods examined, a single Lauryl Sulfate Tryptose (LST) presumptive test at 44 C gave best *E. coli* recovery (425). This recovery compared favorably with the lengthier Association of Official Analytical Chemists test with which only 420 *E. coli* cells were recovered. The LST (44 C) test saves much time, since it renders a follow-up 48-hr confirmatory test unnecessary. Moreover, since 96% of all the *E. coli* are recovered within 24 hr by LST (44 C), it is essentially a 24-hr test. The results of this study also confirmed earlier findings, in that it is possible to describe a specific coliform bacteriological test method by simple reproducible productivity ratios. *E. coli* recovery dilution data and coliform group behavior were also examined.

Members of the coliform group have historically been used to demonstrate pollution or unsanitary production conditions in the preparation of a variety of foods and beverages. Numerous enrichment media and elevated temperatures have been employed in the isolation of these organisms.

MacConkey (7) and Eijkman (2) originally established elevated-temperature incubations for the recovery of fecal coliforms. Wilson et al. (13) demonstrated the value of this technique in detecting the presence of the coliform group. More recently, Mackenzie et al. (8) suggested a rapid test for water analysis consisting of a MacConkey presumptive at 37 C followed by a confirmatory Brilliant Green Lactose Bile (BGLB) and peptone broth (for indole determinations) both at 44 C. The British Food Hygiene Laboratory uses this procedure for the isolation of "faecal coliform bacilli" (*Escherichia coli*) in the analysis of food products. On the continent, Mossel (9), in the examination of human milk and ice cream, utilized BGLB at 44 C for both primary and secondary tests supplemented with a test in tryptone-water at 44 C.

In the United States, Williams et al. (12), Vaughn and Levine (11), and Hajna and Perry (5) used elevated incubation temperatures to recover fecal indicator organisms. Levine et al. (6), Geldreich et al. (4), and Raj and Liston (10) have described continuing experience with temperature variations, different types of media, and various foods.

Studies in our laboratories (3) demonstrated the overall advantages of E C Medium (Difco) at 45.5 C as a confirmatory test in the recovery of *E. coli*. This method, when preceded by Lauryl Sulfate Tryptose (LST) medium at 35 C as a presumptive step, has been adopted by the Association of Official Analytical Chemists (AOAC) as official, first action (1).

The present investigation is basically a comparison of the AOAC method with three other procedures for the recovery of *E. coli* from a variety of frozen foods.

MATERIALS AND METHODS

Types of foods. The frozen foods represented three categories: marine products, variety foods, and cream pies. The marine products consisted of shrimp croquettes, codfish cakes, breaded fish, breaded shrimp, shrimp rolls, stuffed flounder, deviled crab, and fried fish steaks. The variety foods consisted of cheese pizza, barbeque beef, cheese blintz, instant mashed potatoes, breaded onion rings, potato salads, macaroni salads, and hash brown potatoes. The cream pie group consisted of coconut, chocolate, strawberry, and lemon cream pies.

Sample preparation. A 50-g portion of each food

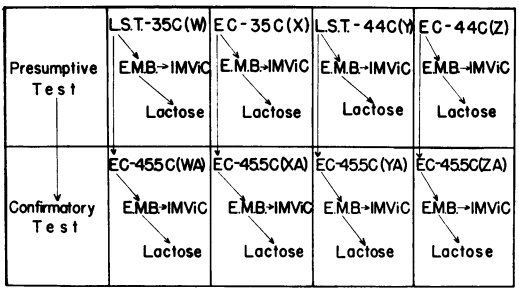

FIG. 1. *Analytical procedure for recovery of Escherichia coli.*

was weighed into a sterile blending container, and 450 ml of sterile Butterfield's phosphate buffer diluent was added. The mixture was blended for 2 min. The resulting 1:10 dilution was added to 90 ml of diluent to obtain the 1:100 dilution, and the latter dilution was processed in a similar manner to obtain the 1:1,000 dilution (0.1, 0.01, 0.001 g).

Three replicate tubes of each dilution were inoculated in the presumptive test. Since 496 samples were examined, a total of 4,464 tubes were inoculated in the presumptive test.

Incubations. The tubes were incubated at 35 C in a walk-in air incubator. Incubations at 44 and 45.5 ± 0.03 C took place in a water bath. Thermometers were calibrated against a National Bureau of Standards certified thermometer. Dehydrated media were purchased either from Difco or BBL.

The inoculated presumptive media were incubated for 24 hr, and all gas-positive tubes were subcultured by loop onto Eosin Methylene Blue (EMB) plates. After 48-hr incubation, all remaining gas-positive and gas-negative tubes were subcultured into the confirmatory media and streaked onto EMB plates.

The confirmatory media were treated in similar fashion. Tubes gas-positive after 24 hr were streaked onto EMB plates. After 48 hr of incubation, all remaining tubes were streaked onto EMB plates. The EMB plates from the presumptive and confirmatory tests were picked for *E. coli.* The isolated organisms were subcultured into lactose broth and subjected to indole, methyl red, Voges-Proskauer, citrate (IMViC) determinations at 35 C.

Protocol. Figure 1 outlines the experimental design. Presumptive tests W and Y consisted of LST medium incubated at 35 and 44 C, respectively. Presumptive tests X and Z employed E C medium at the same respective temperatures. The confirmatory tests were conducted at 45.5 C in E C medium in all instances. Thus, a combination of four parallel series was inoculated and tested simultaneously.

All presumptive tests, regardless of the presence or absence of gas, were subcultured into confirmatory media and streaked onto EMB. Similarly, all confirmatory tubes were streaked onto EMB. With the exception that all of the gas-negative presumptive tubes were subcultured into confirmatory tubes, series W duplicated the method adopted by the AOAC.

Terminology. The terms, which will be subsequently used, are defined as follows: positive tube (LST^+, $E\ C^+$), a tube that produces gas within 24 or 48 hr; negative tube, a tube that produces no gas after 48 hr of incubation; *E. coli*$^+$, a tube from which *E. coli* (++−−, −+−−) is recovered (these are also gas-producing tubes); false-negative tube, a tube that produces no gas in 48 hr but from which *E. coli* is recovered; false-positive tube, a tube that gasses within 24 or 48 hr but from which *E. coli* is not recovered; false-positive (group), a tube which gasses within 24 or 48 hr and from which a coliform organism other than *E. coli* is recovered; false positive (synergistic), a tube which gasses within 24 or 48 hr but from which neither *E. coli* nor any coliform group member is recovered.

TABLE 1. *Positive tube reactions*

Test	No. of positive tubes[a]	Percentage of positive tubes[b]
W[c] (LST, 35 C, presumptive)	1,717	38.46
WA (E C, 45.5 C, confirmatory)	481	10.78
X (E C, 35 C, presumptive)	1,451	32.50
XA (E C, 45.5 C, confirmatory)	406	9.09
Y (LST, 44 C, presumptive)	628	14.07
YA (E C, 45.5 C, confirmatory)	417	9.34
Z (E C, 44 C, presumptive)	492	11.02
ZA (E C, 45.5 C, confirmatory)	328	7.35

[a] All tubes that produced gas in 24 or 48 hr.

[b] These are based on the total number of tubes inoculated.

[c] Every test included a total of 4,464 inoculated tubes.

RESULTS AND DISCUSSION

Table 1 presents a summation of the positive tubes occurring in the four parallel series. A total of 4,464 tubes were inoculated in every test. The low-temperature primaries produced a greater number of positive tubes (1,717 and 1,451) than the elevated-temperature primaries of the Y and Z tests (628 and 492). Also, the elevated-temperature primary tests in Y and Z produced a range of positive tubes similar to that produced by the secondary WA and XA tests. Furthermore, in all four series the secondary test always produced fewer positive tubes than the primary test.

Table 2 presents the *E. coli* recovery data. The LST presumptive medium (column 2) produced more *E. coli* than E C at 35 C (337 versus 277), although the productivity of both tests was about 19%. On the other hand, the presumptive LST at 44 C (Y) yielded a greater number of recoveries of *E. coli* (425) than any of the other presumptive

TABLE 2. *Escherichia coli recovery*

(1) Test	(2) *E. coli*+ tubes[a]	(3) *E. coli*− tubes[b]	(4) *E. coli*+/(total + gassing tubes)	(5) *E. coli*+/(total no. of tubes)	(6) *E. coli*+, 24 hr/(total *E. coli*+)
			%	%	%
W (LST, 35 C, presumptive)....	337	1	19.63	7.55	98.21
WA (E C, 45.5 C, confirmatory).	420	11	87.31	9.41	92.86
X (E C, 35 C, presumptive).....	277	1	19.09	6.21	99.64
XA (E C, 45.5 C, confirmatory).	354	3	87.19	7.93	96.33
Y (LST, 44 C, presumptive).....	425	3	67.67	9.52	96.24
YA (E C, 45.5 C, confirmatory).	398	5	95.44	8.92	91.71
Z (E C, 44 C, presumptive).....	355	0	72.15	7.95	95.21
ZA (E C, 45.5 C, confirmatory).	308	12	93.90	6.90	88.64

[a] The number of gassing tubes from which *E. coli* was recovered.
[b] The number of nongassing tubes from which *E. coli* was recovered.

TABLE 3. *Coliform group[a] recoveries*

(1) Test	(2) Total no. of FP tubes[b] (group + synergistic)[c]	(3) No. FP (group)	(4) No. FP (synergistic)	(5) FP tubes/total no. of tubes[d]	(6) FP tubes/total positive tubes[e]	(7) FP (group)/total FP tubes
				%	%	%
W (LST, 35 C, presumptive).....	1,380	995	385	30.91	80.37	72.10
WA (E C, 45.5 C, confirmatory).....	61	45	16	1.37	12.68	73.78
X (E C, 35 C, presumptive).....	1,174	819	355	26.30	80.90	69.76
XA (E C, 45.5 C, confirmatory).....	52	49	3	1.17	12.81	94.23
Y (LST, 44 C, presumptive).....	203	165	38	4.55	32.32	81.28
YA (E C, 45.5 C, confirmatory).....	19	18	1	0.43	4.56	94.74
Z (E C, 44 C, presumptive).....	137	121	16	3.07	27.84	88.32
ZA (E C, 45.5. C, confirmatory).....	20	12	8	0.45	6.10	60.00

[a] All *Aerobacter* and *Escherichia* species excluding *E. coli* types I and II. FP = false positive.
[b] Gas-producing tubes which were negative for *E. coli*.
[c] Gas-producing tubes containing neither *E. coli* nor coliform group members.
[d] Every test consisted of 4,464 inoculated tubes.
[e] All gas-producing tubes, including *E. coli*, coliform group, and synergistic types.

or confirmatory tests. The Y test by itself was thus more rapid and yielded more *E. coli* than any of the other series or individual tests.

Since the subcultured negative tubes in the W test gave rise to only negative tubes in the WA test, the *E. coli* recovery results were identical with those of the AOAC procedure. Thus, the AOAC procedure (W series) produced a total of 420 *E. coli*+ in the confirmatory test. In comparison, the Y series, at the presumptive stage alone, produced 425 *E. coli*+.

The incidence of false-negative tubes (column 3) was surprisingly low in view of the fact that elevated temperatures would be expected to suppress the aerogenic function of *E. coli*. Column 4 shows the relationship of *E. coli*+ to the total number of gassing tubes on a percentage basis. The low-temperature primary tests were poorly productive, but the elevated primaries and secondaries were much more productive. The tendency towards false-positive tubes was always greater at lower temperatures.

The interpretation of the presence or absence of *E. coli* on the basis of gas formation alone without the supportive IMViC reaction could lead to an incorrect evaluation of a food product. Thus, in the AOAC (WA) confirmatory test, 87.31% of the gassing tubes would have contained *E. coli*, but in the Y presumptive test alone only 67.67% of the gassing tubes would have contained *E. coli*. The value of the Y test by itself must be weighed against the overall savings in time, labor, and media.

Column 5 lists the percentage of *E. coli*+ in the total inoculated tubes. The Y test alone gave the highest productivity (9.52%), but the total AOAC procedure (W series) was almost as good with 9.41% in the WA test. Column 6 displays the percentage of *E. coli* within the first 24 hr of incubation. All tests showed a fairly high degree

TABLE 4. *Escherichia coli recoveries by dilution*

(1) Test	(2) No. of *E. coli*+ tubes[a]				(3) No. of positive dilutions[b] containing *E. coli*				(4) Column 2/column 3 = avg no. of *E. coli*/positive dilution[c]			
	0.1 g/ml	0.01 g/ml	0.001 g/ml	Combined dilutions	0.1 g/ml	0.01 g/ml	0.001 g/ml	Combined dilutions	0.1 g/ml	0.01 g/ml	0.001 g/ml	Combined dilutions
W (LST, 35 C, presumptive)....	180	105	52	337	105	66	30	201	1.71	1.59	1.73	1.68
WA (E C, 45.5 C, confirmatory).	246	119	55	420	113	59	26	198	2.18	2.02	2.12	2.12
X (E C, 35 C, presumptive).....	176	74	27	277	101	44	21	166	1.74	1.68	1.29	1.67
XA (E C, 45.5 C, confirmatory)..	221	90	43	354	105	42	21	168	2.11	2.14	2.05	2.11
Y (LST, 44 C, presumptive).....	262	109	54	425	118	56	27	201	2.22	1.95	2.00	2.11
YA (E C, 45.5 C, confirmatory)..	246	100	52	398	112	47	24	183	2.20	2.13	2.17	2.18
Z (E C, 44 C, presumptive).....	224	86	45	355	98	41	21	160	2.29	2.10	2.14	2.22
ZA (E C, 45.5 C, confirmatory)..	192	80	36	308	84	39	19	142	2.29	2.05	1.90	2.17

[a] The total number of tubes containing *E. coli*.
[b] The total number of positive dilutions containing *E. coli*.
[c] The average number of *E. coli* cells recovered from a positive dilution.

TABLE 5. *Productivity ratios of the various tests*

Productivity ratio	Method employed			
	W W—LST, 35 C, presumptive WA—E C, 45.5 C, confirmatory	X X—E C, 35 C, presumptive XA—E C, 45.5 C, confirmatory	Y Y—LST, 44 C, presumptive YA—E C, 45.5 C, confirmatory	Z Z—E C, 44 C, presumptive ZA—E C, 45.5 C, confirmatory
(1) *Escherichia coli*+ (E C, 45.5 C, confirmatory)[a]/E C+ (E C, 45.5 C, confirmatory)[b]	420/481 = 87.31%	354/406 = 87.19%	398/417 = 95.44%	308/328 = 93.90%
(2) *E. coli*+ (LST, 35/44 C; E C, 35/44 C, presumptive)[c]/Total no. of tubes (presumptive)[d]	337/4,464 = 7.55%	277/4,464 = 6.21%	425/4,464 = 9.52%	355/4,464 = 7.95%
(3) *E. coli*+ (E C, 45.5 C, confirmatory)/Total no. of tubes (E C, 45.5 C, confirmatory)	420/4,464 = 9.41%	354/4,464 = 7.93%	398/4,464 = 8.92%	308/4,464 = 6.90%
(4) *E. coli*+ (LST, 35/44 C; E C, 35/44 C, presumptive)/No. of positive tubes (presumptive)[e]	337/1,717 = 19.63%	277/1,451 = 19.09%	425/628 = 67.68%	355/492 = 72.15%
(5) *E. coli*+ (E C, 45.5 C, confirmatory)/No. of positive tubes (LST, 35/44 C; E C, 35/44 C, presumptive)	420/1,717 = 24.46%	354/1,451 = 24.40%	398/628 = 63.37%	308/492 = 62.60%
(6) E C+ (E C, 45.5 C, confirmatory)/No. of positive tubes (LST, 35/44 C; E C, 35/44 C, presumptive)	481/1,717 = 28.01%	406/1,451 = 27.98%	417/628 = 66.40%	328/492 = 66.66%
(7) E C+ (E C, 45.5 C, confirmatory)/Total no. of tubes (E C, 45.5 C, confirmatory)	481/4,464 = 10.78%	406/4,464 = 9.10%	417/4,464 = 9.34%	328/4,464 = 7.35%
(8) No. of positive tubes (LST, 35/44 C; E C, 35/44 C, presumptive)/Total no. of tubes (LST, 35/44 C; E C, 35/44 C, presumptive)	1,717/4,464 = 38.46%	1,451/4,464 = 32.50%	628/4,464 = 14.07%	492/4,464 = 11.02%

[a] The number of *E. coli* cells recovered from the E C (45.5 C) confirmatory test.
[b] The number of gas-positive tubes in the E C (45.5 C) confirmatory test.
[c] The number of *E. coli* cells recovered from the LST (35/44 C) or E C (35/44 C) presumptive tests.
[d] The total number of inoculated tubes was 4,464 for each presumptive test.
[e] The number of gas-positive tubes from the presumptive LST (35/44 C) or E C (35/44 C) tests.

TABLE 6. *Comparison of earlier[d] and current productivity ratios[a]*

No.[b]	Productivity ratio	Current data adjusted to earlier test conditions[c]	Earlier data[d]
		%	%
6	E C+, 45.5 C (confirmatory)[e]/LST+, 35 C (presumptive)[f]	28.0	27.1
5	*Escherichia coli*+ (E C, 45.5 C, confirmatory)[g]/LST+, 35 C (presumptive)	24.5	24.4
1	*E. coli*+ (E C, 45.5 C, confirmatory)[g]/E C+, 45.5 C (confirmatory)	87.3	89.8

[a] W series—LST, 35 C, presumptive; E C, 45.5 C, confirmatory.

[b] Numbers are from Table 5.

[c] Under the earlier test conditions, nongassing, LST (35 C) presumptive tubes were not subcultured into E C medium.

[d] Fishbein and Surkiewicz (3).

[e] The number of positive tubes in the E C (45.5 C) confirmatory test.

[f] The number of positive tubes in the LST (35 C) presumptive test.

[g] The number of *E. coli* cells recovered from the E C (45.5 C) confirmatory test.

of recovery. In the Y test (LST at 44 C), this represented 409 *E. coli*+ cells in the first 24 hr of incubation.

The coliform group data (Table 3) include all the *Aerobacter* and *Escherichia* organisms recovered except *E. coli* types I and II. Column 2 indicates that the greater number of false positives usually occur in the primaries (low temperatures) as contrasted to the homologous secondaries. Also, 6.79 times as many false-positive tubes occurred in the W test as in the Y test. In the recovery of *E. coli*, this would represent unnecessary subculture work.

In columns 3 and 4, the false-positive tubes are further subdivided into those tubes containing the coliform group (excluding *E. coli*), and synergistic types (tubes producing gas but containing neither group organisms nor *E. coli*). The synergistic false positive occurs chiefly at low incubation temperatures and more frequently in the primary than in the corresponding secondary tests. The effect of temperature is seen in the W test, where fully 10 times as many of these types were produced as in the Y test.

In columns 5 and 6, the relationships of false-positive tubes to the total number of tubes inoculated (4,464) and to the total number of positive tubes produced are given on a percentage basis. Likewise the relationships of the false-positive group reaction to the total number of false-positive tubes produced are given in column 7. In the Y series, only 4.55% of all the inoculated tubes were false-positive types, compared with 30.91% in the W test. Also in the Y test, 32.32% of the total positive tubes were false positives, as contrasted to 80.37% for the W test.

Table 4 presents the *E. coli*+ dilution data. The original sample is represented in decimal dilutions of 0.1-, 0.01-, and 0.001-g portions. Column 2 details the total number of positive tubes from which *E. coli* was recovered for each dilution. The lowest dilutions produced the highest numbers of *E. coli*+. These numbers decreased as the dilutions increased within the same test. Also, in the W and X series, the confirmatory tests yielded greater recoveries of *E. coli*+ than the presumptive tests.

Column 3 indicates the total number of positive dilutions which contained *E. coli*+. Comparing this with the previous data shows no special difference between the low- and high-temperature tests.

The number of *E. coli* recovered per dilution is recorded in column 4. This value is obtained by dividing the number of *E. coli*+ (column 2) by the number of positive dilutions (column 3). Regardless of the type of test, all three dilutions gave similar results. The significant differences occurred only between the low- and high-temperature tests. Recoveries averaged approximately 1.68 *E. coli*+ per positive dilution for the low-temperature test and approximately 2.12 *E. coli*+ per positive dilution for the high-temperature tests. These productivity ratios describing behavior are fairly similar for all three dilutions of the same or different media. They are also similar for the different media at the same temperature (W/X and Y/Z), for similar media in different tests (WA, XA, YA, ZA), and for different test sequences involving the same or different media (WA, XA, Y, Z).

Table 5 lists some selected productivity ratios. Such values as *E. coli*+, gas-positive tubes, and the total number of inoculated tubes are related to each other in the form of fractions. Examination reveals that there is basically a temperature-related, paired response of the various ratios in the WX and YZ series. Generally, the temperature differences between LST and E C media are significant.

Fishbein and Surkiewicz (3) recorded certain productivity ratios based on such measured quantities as gassing tubes in the primary and secondary tests and *E. coli* recoveries from the confirmed E C test. These earlier data were from a series of LST at 35 C followed by an E C at 45.5

C; only the gassing primaries were subcultured. Since in the current study the subcultured primary negative gassing tubes produced neither gassing tubes nor *E. coli* in the secondary, the data of the two methods can be compared.

Table 6 lists the productivity ratios of the earlier work and also the current data adjusted to the specifications of the earlier work (subcultured primary positive tubes only). The identity numbers in the first column refer to the productivity ratio notation in Table 5. A comparison of the earlier and the current data in Table 6 indicates that the method used in the AOAC procedure and in the present W series is highly reproducible when applied to a number of different foods by different microbiologists.

The productivity ratios are also inter-related in the form of multiplicative identities. On the basis of the specifications of the present study, the WA (E C, 45.5 C, confirmatory) identities 1, 7, and 3 (from Table 5) can be written as follows: *E. coli*$^+$/E C$^+$ $\times$ E C$^+$/total number of tubes = *E. coli*$^+$/total number of tubes; i.e., 0.8731 $\times$ 0.1078 = 0.0941.

The LST, 35 C, presumptive (W) identities 4, 8, and 2 from Table 5 can be written as follows: *E. coli*$^+$/LST$^+$ $\times$ LST$^+$/total number of tubes = *E. coli*$^+$/total number of tubes; i.e., 0.1963 $\times$ 0.3846 = 0.0755.

These equations indicate that, when a large and varied sampling of frozen food products is examined for *E. coli* under the conditions indicated, it is possible to describe the results of the confirmatory and presumptive tests in a mathematical manner. Thus, in the E C, 45.5 C, confirmatory (WA) test, a total of 9.41% of the total number of inoculated tubes (4,464) contained *E. coli*. This value is in turn related to the left-hand members of the equation as shown. Thus, the number of inoculated tubes, the number of gassing tubes, and the number of gassing tubes containing *E. coli* are all related mathematically. The same is true for the LST, 35 C, presumptive (W) test.

One can compare productivities of the two tests. For example, the gassing tubes in the E C, 45.5 C, confirmatory test were 87.31% positive for *E. coli*, whereas in the presumptive test (LST, 35 C) the gassing tubes yielded only 19.63% *E. coli*, indicating the production of a large number of false-positive tubes. Further, the presumptive test yields 38.46% gassing tubes out of 4,464 inoculated tubes, whereas the confirmatory test produced only 10.78% gassing tubes from a similar number of inoculated tubes. This shows that fewer gassing tubes have to be processed to produce a greater number of *E. coli* recoveries (9.41%) in the confirmatory test than in the presumptive test (7.55%). The comparison shows that the confirmatory test is likely to be more productive in the recovery of *E. coli* than is the presumptive test. That some of these values are reproducible and perhaps fixed is also likely.

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