nutrients conforming to APHA standards as given in Compendium of Methods for the Microbiological Examination of Foods, 2nd ed., 1984 (American Public Health Association, 1015 18th St, NW, Washington, DC 20005), cold H_2O-sol. gelling agent, and 2,3,5-triphenyltetrazolium chloride indicator. Petrifilm Coliform Count Plates™ (available from Medical-Surgical Division/3M) meet these specifications.

(c) *Plastic spreader.*—Provided with Petrifilm plates, consists of recessed side and smooth flat side, designed to spread sample evenly over plate growth area.

(d) *Pipets.*—Calibrated for bacteriological use, or plate loop continuous pipetting syringe to deliver 1.0 mL. Automatic pipet to deliver 1.0 mL may be used.

(e) *Colony counter.*—Std app., Quebec model preferred, or one providing equiv. magnification and visibility.

(f) *Dilution water.*—*See* **940.36A(a)**.

C. Sample Preparation

(a) For total plate counts: Aseptically prep. 1:10 diln (11 g/99 mL diln H_2O). Mix well and plate. Prep. addnl dilns as required. Ordinarily, 1:10 and 1:100 dilns are sufficient.

(b) For coliform counts:

(1) Cream, half-and-half, condensed mild, egg nog, cottage cheese, butter, margarine, and related products.—Make 1:5 diln (24.75 g/99 mL diln H_2O). Mix well and plate 1 mL on each of 2 plates. Multiply total of counts on 2 plates by 2.5 to obtain count/g.

(2) Sour cream, dips, and yogurt.—Proceed as in (*1*) except after diln, adjust pH to 6.6–7.2 with 1.0N NaOH (ca 0.1 mL/g sample).

(3) Buttermilk.—Make 1:10 diln (11 g/99 mL diln H_2O). Adjust pH to 6.6–7.2 with 1.0N NaOH (ca 0.1 mL/g samples). Mix well and plate 1 mL on each of 2 plates. Multiply total of counts on 2 plates by 5 to obtain count/g.

(4) Ice cream, sherbet, and mixes.—Hydrate dry-film plates with 1 mL sterile diln H_2O and allow at least 1 h for gel to solidify. Then, lift top film of prehydrated dry-film coliform count plate (gel will adhere to top film) and dispense 0.5 mL of 2:3 homogenate (10 g/5 mL diln H_2O) onto bottom film of each of 3 plates. Replace top film gently over sample. Add counts on the 3 plates to obtain count/g. Alternatively, plate 1 plate and multiply result by 3 to obtain count/g.

(5) Cheese.—Proceed as in (*1*). Do not use citrate buffer to homogenize sample.

(6) Chocolate milk.—Proceed as in (*1*).

D. Analysis

(a) *Bacterial colony count.*—Use dry-film aerobic count plates. Place plate on flat surface. Lift top film and inoculate 1 mL sample onto center of film base. Carefully roll top film down onto inoculum. Distribute sample over prescribed growth area with downward pressure on center of plastic spreader device (recessed side down). Leave plate undisturbed 1 min to permit gel to solidify. Incubate plates 48 ± 3 h at 32 ± 1°.

In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 20 units. Count plates promptly after incubation period. If impossible to count at once after required incubation, store plates at 0–4.4° for not >24 h. This should be avoided as a routine practice.

Use std colony counter for counting purposes. Magnifier-illuminator may also be used to facilitate counting. Colonies stain in various shades of red. Count all colonies in countable range (25–250 colonies).

To compute bacterial count, multiply total number of colonies per plate (or av. number of colonies per plate if counting duplicate plates of same diln) by reciprocal of diln used. When counting colonies on duplicate plates of consecutive dilns, compute mean number of colonies for each diln before detg av. bacterial count. Estd counts can be made on plates with >250 colonies and should be reported as estd counts. In making such counts, circular growth area can be considered to contain ca twenty 1 cm squares. To isolate colonies for further identification, lift top film and pick colony from gel.

(b) *Coliform count.*—Use dry-film coliform count plates. Proceed as in (a), but distribute prepd sample over plate by using plastic spreader, flat side down. Incubate plates 24 ± 2 h at 32 ± 1°. Count as in (a), but count only red colonies that have one or more gas bubbles associated (within 1 colony diam.) with them. Count all colonies in countable range (15–150 colonies). Red colonies without gas bubbles are not counted as coliform organisms.

Ref.: JAOAC **72,** 312(1989).

978.23 Fecal Coliforms in Shellfish Growing Waters

Medium A-1 Method

First Action 1978
Final Action 1979

(Applicable to enumeration of fecal coliforms and also as presumptive test for *Escherichia coli* in shellfish growing waters)

A. Apparatus

(a) *Pipets.*—1.0 mL serological with 0.1 mL graduations and 10.0 mL with 0.1 mL graduations. Pipets conforming to APHA stds as given in "Standard Methods for the Examination of Dairy Products," 15th ed., 1985, American Public Health Association, 1015 15th St, NW, Washington, DC 20005, may also be used.

(b) *Incubator.*—Air, 35 ± 0.5°.

(c) *Water bath.*—Covered, circulating, 44.5 ± 0.2°.

(d) *Dilution bottles or tubes.*—Borosilicate glass, with glass or rubber stoppers or polyethylene screw caps equipped with Teflon liners.

B. Media

Note: Because geographical differences may affect performance of Medium A-1 method, det. comparability with LST-EC tube method prior to using Medium A-1. Moreover, this medium must be made from individual ingredients. Preformulated Medium A-1 is unacceptable.

(a) *Butterfield's buffered phosphate diluent.*—*See* **966.23A(m)**.

(b) *Medium A-1 broth.*—Dissolve 5 g lactose, 20 g tryptone, 5 g NaCl, and 0.5 g salicin in 1 L H_2O. Heat to dissolve ingredients, pipet in 1 mL Triton X-100 (Rohm & Haas Co.), and adjust pH to 6.9 ± 0.1. For 10 mL sample aliquots, prep. and use double strength medium. To achieve approx. same level of medium and inoculum in all tubes, dispense 10 mL portions of single strength broth into 150 × 18 mm tubes contg inverted fermentation vials; use 175 × 22 mm tubes contg inverted fermentation vials for double strength broth. Autoclave 10 min at 121°. Formation of flocculent ppt, particularly in double strength medium, is common and does not impair performance. Store in dark at room temp. and use within 7 days. Store dehydrated ingredients and/or medium under conditions that will prevent absorption of moisture.

C. Determination

Shake sample and each successive diln bottle vigorously using 25 complete up and down movements of ca 30 cm in 7 sec. Inoculate H_2O sample directly into tubes contg A-1 Medium in suitable decimal dilns using 3 or 5 tubes/diln with

Butterfield's buffered phosphate diluent. Place inoculated tubes into air incubator and incubate 3 hr at 35 ± 0.5°. Transfer tubes to H_2O bath and incubate 21 ± 2 hr at 44.5 ± 0.2°. Maintain H_2O level in bath above level of liq. in inoculated tubes. Presence of gas in inverted vial or of dissolved gas which can be removed by slight agitation of tube constitutes pos. test. Use std Most Probable Number (MPN) tables, Table **966.24** or Table **978.23**, to det. MPN values. Report results as fecal coliform MPN/100 mL sample.

Ref.: JAOAC **61,** 1317(1978).

983.25 Total Coliforms, Fecal Coliforms, and *Escherichia coli* in Foods

Hydrophobic Grid Membrane Filter Method

First Action 1983
Final Action 1985

A. Principle

Hydrophobic grid membrane filter (HGMF) uses membrane filter imprinted with hydrophobic material in grid pattern. Hydrophobic lines act as barriers to spread of colonies, thereby dividing membrane filter surface into sep. compartments of equal and known size. Number of squares occupied by colonies is enumerated and converted to most probable number value of organisms by using formula given below.

B. Apparatus, Culture Media and Reagents

(a) *Hydrophobic grid membrane filter (HGMF).*—Membrane filter has pore size of 0.45 μm and is imprinted with nontoxic hydrophobic material in grid pattern. ISO-GRID (available from QA Laboratories Ltd, 135 The West Mall, Toronto, Ontario, Canada M9C 1C2) or equiv. meets these specifications.

(b) *Filtration units for HGMF.*—Equipped with 5 μm mesh prefilter to remove food particles during filtration. One unit is required for each sample. ISO-GRID (available from QA Laboratories Ltd.) or equiv. meets these specifications.

(c) *Pipets.*—1.0 mL serological with 0.1 mL graduations; 1.1 mL or 2.2 mL milk pipets are satisfactory. 5.0 mL serological with 0.1 mL graduations.

(d) *Blender.*—Waring Blendor, or equiv., multispeed model, with low-speed operation at 10 000–12 000 rpm, and 250 mL glass or metal blender jars with covers. One jar is required for each sample.

(e) *Vac. pump.*—Water aspirator vac. source is satisfactory.

(f) *Manifold or vac. flask.*

(g) *Filter paper.*—Whatman No. 1 or No. 4, or equiv.

(h) *Peptone/Tween 80 diluent.*—Dissolve 1.0 g peptone (Difco 0118) and 10.0 g Tween 80 in 1 L H_2O. Dispense enough vol. into diln bottles to give 90 ± 1 mL after autoclaving 15 min at 121°.

Table 978.23 Most Probable Numbers per 100 mL of Sample, Planting 5 Portions in Each of 3 Dilutions in Geometric Series

Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes				Number of Positive Tubes			
10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN	10 mL	1 mL	0.1 mL	MPN
0	0	0		1	0	0	2.0	2	0	0	4.5	3	0	0	7.8	4	0	0	13	5	0	0	23
0	0	1	1.8	1	0	1	4.0	2	0	1	6.8	3	0	1	11	4	0	1	17	5	0	1	31
0	0	2	3.6	1	0	2	6.0	2	0	2	9.1	3	0	2	13	4	0	2	21	5	0	2	43
0	0	3	5.4	1	0	3	8.0	2	0	3	12	3	0	3	16	4	0	3	25	5	0	3	58
0	0	4	7.2	1	0	4	10	2	0	4	14	3	0	4	20	4	0	4	30	5	0	4	76
0	0	5	9.0	1	0	5	12	2	0	5	16	3	0	5	23	4	0	5	36	5	0	5	95
0	1	0	1.8	1	1	0	4.0	2	1	0	6.8	3	1	0	11	4	1	0	17	5	1	0	33
0	1	1	3.6	1	1	1	6.1	2	1	1	9.2	3	1	1	14	4	1	1	21	5	1	1	46
0	1	2	5.5	1	1	2	8.1	2	1	2	12	3	1	2	17	4	1	2	26	5	1	2	64
0	1	3	7.3	1	1	3	10	2	1	3	14	3	1	3	20	4	1	3	31	5	1	3	84
0	1	4	9.1	1	1	4	12	2	1	4	17	3	1	4	23	4	1	4	36	5	1	4	110
0	1	5	11	1	1	5	14	2	1	5	19	3	1	5	27	4	1	5	42	5	1	5	130
0	2	0	3.7	1	2	0	6.1	2	2	0	9.3	3	2	0	14	4	2	0	22	5	2	0	49
0	2	1	5.5	1	2	1	8.2	2	2	1	12	3	2	1	17	4	2	1	26	5	2	1	70
0	2	2	7.4	1	2	2	10	2	2	2	14	3	2	2	20	4	2	2	32	5	2	2	95
0	2	3	9.2	1	2	3	12	2	2	3	17	3	2	3	24	4	2	3	38	5	2	3	120
0	2	4	11	1	2	4	15	2	2	4	19	3	2	4	27	4	2	4	44	5	2	4	150
0	2	5	13	1	2	5	17	2	2	5	22	3	2	5	31	4	2	5	50	5	2	5	180
0	3	0	5.6	1	3	0	8.3	2	3	0	12	3	3	0	17	4	3	0	27	5	3	0	79
0	3	1	7.4	1	3	1	10	2	3	1	14	3	3	1	21	4	3	1	33	5	3	1	110
0	3	2	9.3	1	3	2	13	2	3	2	17	3	3	2	24	4	3	2	39	5	3	2	140
0	3	3	11	1	3	3	15	2	3	3	20	3	3	3	28	4	3	3	45	5	3	3	180
0	3	4	13	1	3	4	17	2	3	4	22	3	3	4	31	4	3	4	52	5	3	4	210
0	3	5	15	1	3	5	19	2	3	5	25	3	3	5	35	4	3	5	59	5	3	5	250
0	4	0	7.5	1	4	0	11	2	4	0	15	3	4	0	21	4	4	0	34	5	4	0	130
0	4	1	9.4	1	4	1	13	2	4	1	17	3	4	1	24	4	4	1	40	5	4	1	170
0	4	2	11	1	4	2	15	2	4	2	20	3	4	2	28	4	4	2	47	5	4	2	220
0	4	3	13	1	4	3	17	2	4	3	23	3	4	3	32	4	4	3	54	5	4	3	280
0	4	4	15	1	4	4	19	2	4	4	25	3	4	4	36	4	4	4	62	5	4	4	350
0	4	5	17	1	4	5	22	2	4	5	28	3	4	5	40	4	4	5	69	5	4	5	430
0	5	0	9.4	1	5	0	13	2	5	0	17	3	5	0	25	4	5	0	41	5	5	0	240
0	5	1	11	1	5	1	15	2	5	1	20	3	5	1	29	4	5	1	48	5	5	1	350
0	5	2	13	1	5	2	17	2	5	2	23	3	5	2	32	4	5	2	56	5	5	2	540
0	5	3	15	1	5	3	19	2	5	3	26	3	5	3	37	4	5	3	64	5	5	3	920
0	5	4	17	1	5	4	22	2	5	4	29	3	5	4	41	4	5	4	72	5	5	4	1,600
0	5	5	19	1	5	5	24	2	5	5	32	3	5	5	45	4	5	5	81				