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17.3.03

AOAC Official Method 989.10
Bacterial and Coliform
Counts in Dairy Products
Dry Rehydratable Film Methods
(Petri-film Aerobic Count Plate and
Petri-film Coliform Count Plate) Methods
First Action 1989
Final Action 1991

Results of the interlaboratory study supporting acceptance of the method:

Aerobic Count

Chocolate milk: $s_r = 0.102$; $s_R = 0.177$; $RSD_r = 4.3\%$; $RSD_R = 7.5\%$

Cheese: $s_r = 0.113$; $s_R = 0.117$; $RSD_r = 3.6\%$; $RSD_R = 3.7\%$

Nonfat dry milk: $s_r = 0.151$; $s_R = 0.230$; $RSD_r = 4.5\%$; $RSD_R = 6.9\%$

Evaporated milk: $s_r = 0.193$; $s_R = 0.198$; $RSD_r = 8.3\%$; $RSD_R = 8.5\%$

Ice cream: $s_r = 0.180$; $s_R = 0.222$; $RSD_r = 6.9\%$; $RSD_R = 8.5\%$

Coliform Count

Chocolate milk: $s_r = 0.164$; $s_R = 0.257$; $RSD_r = 9.2\%$; $RSD_R = 14.4\%$

Cheese: $s_r = 0.221$; $s_R = 0.225$; $RSD_r = 10.4\%$; $RSD_R = 10.6\%$

Nonfat dry milk: $s_r = 0.197$; $s_R = 0.151$; $RSD_r = 8.5\%$; $RSD_R = 4.5\%$

Evaporated milk: $s_r = 0.200$; $s_R = 0.225$; $RSD_r = 13.0\%$; $RSD_R = 13.0\%$

Ice cream: $s_r = 0.081$; $s_R = 0.131$; $RSD_r = 4.1\%$; $RSD_R = 6.6\%$

A. Principle

Method uses bacterial culture plates of dry medium and cold H₂O-soluble gel. Undiluted or diluted test suspensions are added to plates at a rate of 1.0 mL per plate. Pressure, when applied to plastic spreader placed on overlay film, spreads test portion over ca 20 sq. cm growth area. Gelling agent is allowed to solidify and plates are incubated and then counted. Pipet, plate loop continuous pipetting syringe, or automatic pipet can be used for test suspension addition for bacterial count analyses.

B. Apparatus and Reagent

(a) *Petri-film Aerobic Count Plates*.—Plates contain standard methods media nutrients, 940.36A(g) (see 17.1.02), cold H₂O-soluble gelling agent coated onto film base, overlay film coated with gelling agent, and 2,3,5-triphenyltetrazolium chloride indicator. Circular growth area of single plate contains ca twenty 1 cm squares outlined on film base. Petri-film Aerobic Count Plates (Microbiology Products, 3M Center, Bldg 275-5W-05, St. Paul, MN 55144, USA) meet these specifications.

(b) *Petri-film Coliform Count Plates*.—Plates contain violet red bile nutrients conforming to APHA standards as given in *Compendium of Methods for the Microbiological Examination of Foods* (1990) 3rd Ed., American Public Health Association, Washington, DC, USA, cold H₂O-soluble gelling agent, and 2,3,5-triphenyltetrazolium chloride indicator. Petri-film Coliform Count Plates (Microbiological Products, 3M Center) meet these specifications.

(c) *Plastic spreader*.—Provided with Petri-film plates, consists of recessed side and smooth flat side, designed to spread test portion 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*.—Standard apparatus, Quebec model preferred, or one providing equivalent magnification and visibility.

(f) *Dilution water*.—See 940.36A(a) (see 17.1.02).

C. Preparation of Test Suspensions

(a) *For total plate counts*.—Aseptically prepare 1:10 dilution (11 g/99 mL dilution H₂O). Mix well and plate. Prepare additional dilutions as required. Ordinarily, 1:10 and 1:100 dilutions are sufficient.

(b) *For coliform counts*.—(1) *Cream, half-and-half, condensed milk, egg nog, cottage cheese, butter, margarine, and related products*.—Make 1:5 dilution (24.75 g/99 mL dilution H₂O). 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 dilution, adjust pH to 6.6–7.2 with 1.0M NaOH (ca 0.1 mL/g test portion).

(3) *Buttermilk*.—Make 1:10 dilution (11 g/99 mL dilution H₂O). Adjust pH to 6.6–7.2 with 1.0M NaOH (ca 0.1 mL/g test portion). 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, sherbert, and mixes*.—Hydrate dry-film plates with 1 mL sterile dilution H₂O 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 dilution H₂O) onto bottom film of each of 3 plates. Replace top film gently over test suspension. Add counts on the 3 plates to obtain count/g. Alternatively, plate one plate and multiply result by 3 to obtain count/g.

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

(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 test suspension onto center of film base. Carefully roll top film down onto inoculum. Distribute test suspension 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°C.

In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 20 units. Count plates promptly after incubation period. After incubation is complete, plates may be stored frozen (–15°C) up to 7 days. This should be avoided as a routine practice.

Use standard 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 average number of colonies per plate if counting duplicate plates of same dilution) by reciprocal of dilution used. When counting colonies on duplicate plates of consecutive dilutions, compute mean number of colonies for each dilution before determining average bacterial count. Estimated counts can be made on plates with >250 colonies and should be reported as estimated 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 prepared test suspension over plate by using plastic spreader, flat side down. Incubate plates 24 ± 2 h at $32^\circ \pm 1^\circ\text{C}$.

Count as in (a), but count only red colonies that have one or more gas bubbles associated (within one colony diameter) with them. Count all colonies in countable range (15–150 colonies). Red colonies without gas bubbles are not counted as coliform organisms.

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

Revised: March 2002