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17.3.02

AOAC Official Method 986.33
Bacterial and Coliform Counts in Milk
Dry Rehydratable Film Methods
(Petrifilm Aerobic Count Plate
and Petrifilm Coliform Count Plate) Methods
First Action 1986
Final Action 1988

A. Principle

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

B. Apparatus

(a) *Petrifilm 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. Petrifilm Aerobic Count Plates (Microbiology Products, 3M Center, Bldg 275-5W-05, St. Paul, MN 55144, USA) or equivalent meet these specifications.

(b) *Petrifilm 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. Petrifilm Coliform Count Plates (Microbiology Products, 3M Center), or equivalent meet these specifications.

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

(d) *Pipets*.—Calibrated for bacteriological use of plate loop continuous pipetting syringe to deliver 1.0 mL.

(e) *Colony counter*.—Standard apparatus, Quebec model preferred, or one providing equivalent magnification and visibility.

C. Analysis

(a) *Bacterial colony count*.—Use Petrifilm Aerobic Count Plates or equivalent. Place plate on flat surface. Lift top film and inoculate 1 mL test portion onto center of film base. Carefully roll top film down onto inoculum. Distribute test portion 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 $32^\circ \pm 1^\circ\text{C}$.

In incubator, place plates in horizontal position, clear side up, in stacks not exceeding 10 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 (30–300 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 >300 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 Petrifilm Coliform Count Plates or equivalent. Proceed as in (a), but distribute test portion 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* **69**, 527(1986).

Revised: March 2002