

# TAPPI/ANSI T 449 om-14

SUGGESTED METHOD – 1940  
OFFICIAL STANDARD – 1956  
OFFICIAL TEST METHOD – 1979  
REVISED – 1984  
REVISED – 1990  
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## CAUTION:

This Test Method may include safety precautions which are believed to be appropriate at the time of publication of the method. The intent of these is to alert the user of the method to safety issues related to such use. The user is responsible for determining that the safety precautions are complete and are appropriate to their use of the method, and for ensuring that suitable safety practices have not changed since publication of the method. This method may require the use, disposal, or both, of chemicals which may present serious health hazards to humans. Procedures for the handling of such substances are set forth on Material Safety Data Sheets which must be developed by all manufacturers and importers of potentially hazardous chemicals and maintained by all distributors of potentially hazardous chemicals. Prior to the use of this method, the user must determine whether any of the chemicals to be used or disposed of are potentially hazardous and, if so, must follow strictly the procedures specified by both the manufacturer, as well as local, state, and federal authorities for safe use and disposal of these chemicals.

## Bacteriological examination of paper and paperboard

### 1. Scope

The following procedure is recommended for the bacteriological examination of paper and paperboard intended for use as single service containers and closures for dairy products. Because of the exacting technique required in bacteriological procedures, reproducible results can be obtained only by a trained technician. All tests should be performed under the appropriate laboratory conditions to ensure quality assurance and safety.

### 2. Significance

This method is important because it provides a procedure for determining the number of colony-forming units (CFU's) per gram of paper or paperboard in accordance with the requirements of the Dairymen's Standard as published by the U. S. Department of Health and Human Services, Public Health Service, Food and Drug Administration.

### 3. Apparatus and materials

- 3.1 *Alcohol*, 70% ethyl alcohol for sterilizing instruments. Alternatively, methyl or isopropyl alcohol may be substituted.
- 3.2 *Balance*, sensitive to 0.1 g, with a pan large enough to hold the petri dishes.
- 3.3 *Dilution blanks*, commercially available, prefilled, presterilized phosphate buffered dilution blanks are recommended. Alternatively milk dilution bottles scribed at the 99 mL level (150 mL capacity) of borosilicate glass or other autoclavable plastic material, fitted with either screw caps or Escher rubber stoppers may be used.
- 3.4 *Colony counter*, any one of several types. A hand tally for recording the count is recommended.

Approved by the Standard Specific Interest Group for this Test Method



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3.5 *Envelopes*, two pressure-sensitive sealing envelopes are needed per sample, one smaller 16.5 × 24.1 cm (6.5 × 9.5 in.) and one larger 22.9 × 30.5 cm (9 × 12 in.).

3.6 *Disintegrator*, high speed electric blender with a sterile metal (stainless steel preferred) cup or glass cup of 500 mL capacity.

3.7 *Flaming equipment*, an alcohol lamp, or a Bunsen burner, to flame tongs, scissors, knives, and the mouths of sterile containers.

3.8 *Incubator*, capable of maintaining a temperature of  $32 \pm 1^\circ\text{C}$ .

3.9 *Knife*, preferably one with an inserted blade of adjustable length to cut paper and board.

3.10 *Petri dishes*, commercially available, presterilized, disposable (90 × 15 mm) plates are recommended.

3.11 *Pipets*, commercially available presterilized 10 or 20 mL pipets with large or breakaway tips (3 mm opening) are recommended. If dilution of the suspension is necessary, the presterilized, 1.1 mL dilution pipets will be needed.

3.12 *Scissors*, preferably with 10.2-cm (4-in.) cutting edges.

3.13 *Nutrient substrate*, Standard Methods Agar (SMA), commercially available as a premixed powder.  
Composition per liter:

|                                                                                                                                |       |
|--------------------------------------------------------------------------------------------------------------------------------|-------|
| Agar                                                                                                                           | 15.0g |
| Pancreatic digest of casein                                                                                                    | 5.0g  |
| Yeast extract                                                                                                                  | 2.5g  |
| Glucose                                                                                                                        | 1.0g  |
| pH $7.0 \pm 0.2$ at $25^\circ\text{C}$ , sterilize by autoclaving for 20 minutes at a minimum of $121^\circ\text{C}$ (15 psi). |       |

3.14 *Sterilizing equipment*, two types of suitable size: an autoclave for steam sterilization; a hot-air oven at  $165^\circ\text{C}$ , with thermometer.

3.15 *Sterile tongs or forceps*.

3.16 *Laminar flow hood*, optional equipment.

#### 4. Sterilization of equipment and media

4.1 Depending upon the nature of the equipment to be sterilized, use one of three methods as follows:

4.1.1 *Steam sterilizer* (autoclave). Sterilize the following by autoclaving for 20 min at a minimum of  $121^\circ\text{C}$ , corresponding to 103 kPa (15 psi) steam pressure: (a) disintegration jars; (b) media (unless other conditions are specified by the manufacturer of the medium); (c) sample bottles; (d) corkborers; and (e) water blanks for dilution. Include a biological or chemical indicator strip to evaluate autoclave efficacy.

4.1.2 *Dry heat*. Sterilize the following by heating for at least 2 h at a minimum temperature of  $165^\circ\text{C}$ : (a) heavy kraft envelopes and folders; (b) tongs; (c) knives; (d) pipets; and (e) scissors. Take care to dry glassware completely before heating and avoid scorching any paper containers or wrapper used for the instruments being sterilized. Since some kraft envelopes vary in their heat resistance, it is recommended that heat embrittlement be determined prior to selection.

4.1.3 *Alcohol*. Immerse the contact portion of scissors, tongs, knives, and similar instruments in alcohol. When needed for cutting or handling samples, remove; allow to drain for a few moments, then burn off the excess alcohol. Use extreme care when using an open flame around alcohol.

#### 5. Sampling

5.1 Please refer to TAPPI T 400 "Sampling and Accepting a Single Lot of Paper, Paperboard, Containerboard or Related Product."

5.2 Cut each sample from the roll of paper with a clean, sharp knife placing each test unit in a different envelope. For each test unit cut into the roll two laps deep and discard these first two layers. Then cut three sides of a square approximately 10 × 10 inches (25 × 25 cm) into the roll six laps deep, forming a flap. Raise the flap and tape the three sides of the flap to prevent undue exposure and then cut the fourth (hinge) side of the flap. Place the sample into a clean paper envelope, seal and insert it into a manila or brown kraft envelope. Preferably the envelopes should seal with a pressure-sensitive adhesive rather than one that must be moistened.