13 Microbiological Methods

Aerobic cell count

(American Association of Cereal Chemists – AACC-Method 42.11)
First approval 10-8-76; revised 10-28-81

1 Scope

This Standard specifies a method for the enumeration of microorganisms by means of the colony count technique at 35°C. The method is applicable to all food products.

2 Apparatus

2.1 Sterile sampling devices suitable for product; scoop, trier, tongue blades, etc.

2.2 Sterile sample jars or containers with suitable covers or sterile plastic bags.

2.3 Autoclave for sterilising app and media.

2.4 Balance; sensitivity 30 mg, with wts.

2.5 Waring Blendor and blending jars of 1000 ml capacity with covers.

2.6 Sterile serological pipettes capable of delivering 1.0, 10.0, and 11.0 ml.

2.7 Sterile cans for pipettes.

2.8 Sterile petri dishes, 100 x 15 mm, preferably disposable plastic.

2.9 Diln bottles with screw caps with capacity of 150-200 ml.

2.10 Sterile weighing papers. Sterilize for minimum of 1 hr at 121°C in suitable container.

2.11 Holding bath adjusted to 45-48°C for tempering agar.

2.12 Bunsen burner.

2.13 Spencer Quebec colony counter or equiv.

2.14 Incubator adjusted to 35±1°C.

3 Reagents

3.1 *Plate count agar:* 5 g tryptone, 2.5 g yeast ext, 1 g glucose, 15 g agar, 1 L distd water. Dissolve ingredients in water by bringing to boil. Dispense into bottles or flasks, and autoclave 15 min at 121°C. Final reaction should be pH 7.0 ± 0.1. In re-melting agar, steam in autoclave or boil in water until medium is completely liquefied. Re-melt only amt sufficient for use within 4 hr period.

3.2 Buffered phosphate diluent

a. Stock soln. Dissolve 34.0 g KH_2PO_4 in 500 ml water, adjust to pH 7.2 with ca 175 ml 1 N NaOH and dil to 1 L.

b. Diluent. Dil 1.25 ml stock soln to 1 L with water. Prep diln blanks with this soln consisting of 450 ml in flasks or bottles and 99 ml in diln bottles Autoclave for 20 min at 121°C.

4 Procedure

4.1 Use sample taken and prepared as directed in "Microbiological Examination"

Note: Steps in the original method describing the preparation of sample for microbiological examination are deleted.

4.2 Pour plates with 12 to 15 ml of plate count agar (cooled to 45°C) within 15 min of time of original diln. Pour agar and diln water control plates for each series of samples.

4.3 Mix sample dilns and agar medium by rotating plates on flat surface, allow agar to solidify, invert petri dishes, and incubate at 35°C for 48 ± 2 hr.

4.4 Count all colonies on those plates contg between 30 and 300 colonies, and multiply by diln factor. Report arithmetic average as Aerobic Plate Count per g (or ml for liquid samples). Report all counts to no more than two significant nos.

5 References

1 Association of Official Analytical Chemists. 1980. Official Methods of Analysis, 13th ed. Sec. 46.013, p. 824; 46.015, p. 825.

2 U.S. Food and Drug Administration. 1978. Bacteriological Analytical Manual for Foods, Chapt. IV. Washington, DC.