# 13 Microbiological Methods

# Mould and yeast counts

(American Association of Cereal Chemists – AACC-Method 42-50)
First approval 10-8-76; reviewed 10-27-82

## 1 Scope

This Standard specifies a method for the detection and enumeration of viable yeasts and moulds in food products by means of the colony count technique at 22-25°C. The method is applicable to all food products.

## 2 Apparatus

2.1 Sterile sampling devices suitable for product; scoop, trier, 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 22-25°C.

## 3 Reagents

3.1 *Potato dextrose agar:* 200 g potato infusion, 20 g dextrose, 15 g agar, 1 L distd water. Mix thoroughly and heat to boiling to dissolve the ingredients. Dispense in flasks and sterilise at 121°C for 15 min.

3.2 *Standard 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 a boil. Dispense into flasks and autoclave 15 min at 121°C.

3.3 *Mycophil agar:* 10 g phytone peptone, 10 g dextrose, 16 g agar, 1 L distd water. Dissolve ingredients in water by bringing to boil. Dispense into flasks and autoclave 15 min at 118°C (12 lb pressure).

3.4 *Malt agar:* 30 g malt ext, 15 g agar, 1 L distd water. For firmer gel, addnl 5 g agar can be added. Dissolve ingredients in water by bringing to a boil. Dispense into flasks and autoclave 15 min at 118-121°C.

3.5 Tartaric acid soln, sterile 10%.

3.6 Antibiotic soln: 500 g chlortetracycline HCl or chloramphenicol, 50 ml distd water, sterile. Dissolve antibiotic in water. Sterilize by filtration, preferably with 0.45µ plain membrane disposable filter unti. (Catalogue 245-0045 from Nalge Sybron Corp.)

3.7 Diln blanks. Dissolve 34.0 g $KH_2PO_4$ in 500 ml distd water. Adjust pH to 7.2 with 1 N NaOH and make vol to 1 L. Add 1.25 ml of this soln per L distd water to make up diluent. Dispense into bottles so that they contain 99 ml after sterilisation. Cover and autoclave 20 min at 121°C.

## 4 Procedure

4.1 Prep medium using either potato dextrose agar, std plate count agar, Mycophil agar, or malt agar by either acidifying it or supplementing it with antibiotic soln after it has been sterilised.

a. To acidify agar, titrate small, measured portion to ph 3.5 ± 0.1 with 10% tartaric acid soln. From amt of tartaric acid used, calculate amt needed for vol of tempered agar to be used for pouring plates. Add tartaric acid soln immediately before pouring plates. Mix thoroughly. Do not reheat agar once it has been acidified.

b. To supplement agar with antibiotic, add 1 ml of either one or both chlortetracycline HCl and chloramphenicol solns to 250 ml of tempered agar (40 or 80 ppm). Mix thoroughly.

4.2 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.

4.3 Pour plates with ca 15 ml of acidified or antibiotic-supplemented agar (cooled to 44-46°C) within 15 min of time of original diln. Mix well and let solidify before inverting plates.

4.4 Incubate at 22-25°C.

4.5 Count those plates that contain less than 50 colonies at 3 and 5 days' incubation.

4.6 Check smears of typical colonies by observation under microscope if necessary.

**5 Reference**

1 U.S. Food and Drug Administration. 1978. Bacteriological Analytical Manual for Foods, chapt. XVII. Washington, DC

# Yeast and mould count – alternative method

**1 Scope**

The method is a direct plating method which makes it possible both to enumerate the yeasts and moulds and to identify individual mould species.

**2 Apparatus**

2.1 Work area: Preferably within a laminar flow-hood, well-lighted (100 foot-candles at the working surface), and well-ventilated room that is reasonably free from dust and drafts. The microbial density measured in fallout pour plates taken during plating, of the air of the working area should not exceed 5 colonies/plate during a 60 min exposure.

2.2 Storage space, free of dust and insects and adequate for protection of equipment and supplies.

2.3 Petri dishes, glass (15 x 100 mm) or plastic (15 x 90 mm).

2.4 Water bath for tempering agar; thermostatically controlled at 46°C.

2.5 Incubator, 22-25°C.

2.6 Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate.

2.7 Stereomicroscope (magnification 10-100x).

2.8 Thermometer, Mercury, appropriate range, accuracy checked with a National Bureau of Standards – certified thermometer.

**3 Reagents**

*Dichloran-glycerol agar (DG18):* 5.0 g Peptone, 10.0 g Dextrose, 1.0 g Potassium dihydrogen phosphate, 0.5 g Magnesium sulphate, 0.002 g Dichloran, 15.0 g Agar, 1 L distilled water, pH 5.6 ± 0.2.

*Chloramphenicol -- vial content:* 50 mg Chloramphenicol, 200 g Glycerol.

**4 Procedure**

4.1 Suspend 15.75 g DG18 in 500 ml distilled water and heat to dissolve completely. Add 100 g of glycerol. Re-hydrate 1 vial of chloramphenicol in 3 ml of acetone and add the supplement. Sterilise by autoclaving at 121°C for 15 min. Cool to 50°C. Mix well and pour into sterile petri dishes.

4.2 Weigh out aseptically 1 g of the product.

4.3 Distribute the sample equally into 4 petri dishes already prepared with DG18.

4.4 Incubate the plates at 25°C.

4.5 Count the yeast and mould colonies after 5 days of incubation. The total count = cfu/g of sample.

4.6 The individual mould species (on above medium) can be identified directly by experienced analysts with low power (10-30x) magnification.

Modified method of the following reference:

Bacteriological Analytical Manual FDA, August 1990.