HS 1006 Moulds and Yeasts Test

1. Purpose

This test method is to determine the moulds and yeasts in the sample by using Pour Plate method for enumeration.

2. Terminology – Nil

3. Apparatus

3.1 Incubator, $27^{o}C \pm 2^{o}C$

3.2 Analytical balance, capable of weighing to 0.1 g.

3.3 Rotary shaker.

3.4 Stomacher.

3.5 Blender.

3.6 Blender jar.

3.7 Laminar flow hood.

3.8 Autoclave for steam sterilisation, capable of operation at 121 °C and 100 kPa.

3.9 Metal spatula and knife or scissors

3.10 Sterile Kraft paper envelopes.

3.11 Glass wide-mouth screw-capped flask with capacity of 500 mL, sterilised by autoclave.

3.12 Petri dishes, made of glass or plastic, of diameter 90 mm to 100 mm.

3.13 Pipettes, 1 mL and 10 mL graduated pipettes and pipette bulb (aspirator).

3.14 Sterile stomacher bag, 7" x 12", use Steward Brand or equivalent.

3.15 Dilution bottles, screw capped, glass or plastic with capacity of approximately 160 mL.

3.16 Water bath for tempering agar

4. Reagents

4.1 Culture medium

Potato-dextrose Agar, the formula is given in Annex A.1.

4.2 Phosphate Buffered Saline, the formula is given in Annex A.2.

4.3 Antibiotic solution(s), the formula is given in Annex A.3.

4.4 Sterilised water.

4.5 70% ethanol.

5. Sample Preparation

5.1 The sample taken for testing should be in its ready-for-use state. The sample should be put into an aseptic container or bag instantly after collection. Obtain a sample in accordance with the requirements of the sampling programme and handle it with care to avoid contamination before testing.

5.2 To avoid contamination, certain precautions are necessary which are common to all sampling procedures for bacteriological examination:

- Scrupulous care should be taken to avoid accidental contamination of the sample during collection and subsequent handling.
- The sample should be collected in a sufficient amount and tested as soon as possible after collection.

5.3 In a laminar flow hood, use sterile scissors to cut the samples into pieces (about 10 mm x 10 mm) and place in the sterile envelope

5.4 Tare petri dishes; and then aseptically weigh about 25 g of test pieces into petri dishes.

5.5 Record the weight of test pieces.

5.6 Replace the cover of the Petri dish.

6. Operating procedure

6.1 A) Under aseptic condition, put 25 g of test pieces in a sterilised conical flask with glass stopper which containing 225 mL sterile water (or Phosphate Buffered Saline). The different mixing methods are listed as follows:

1) Transfer flask contents to a sterile blender jar, cover and blend at low speed for 1 min or high speed for 30 seconds.

or

2) Shake flask on a rotary shaker at about 120 to 150 oscillations/min for about 30 minutes.

or

B) Under aseptic condition, transfer 25 g of test pieces into a sterile stomacher bag which containing 225 mL sterile water (or Phosphate Buffered Saline). Punch the bag for 30 seconds in the stomacher.

6.2 The 10^{-1} (1:10) dilution is obtained.

6.3 Use sterile pipette to transfer 1 mL of the 10^{-1} dilution to 99 mL of sterile Phosphate Buffered Saline, replace the cap and mix by shaking for 3 minutes (result in 10^{-3} dilution).

6.4 Transfer 1mL of the 10^{-3} (1:1000) dilution to a second bottle of 99 mL sterile water (or Phosphate Buffered Saline) and mix (result in 10^{-5} dilution).

6.5 Use the 1 mL pipette and aseptic technique, transfer the proper amount of each dilution to petri dishes as indicated in Table 1.

Table 1. Volume of serial dilutions required to achieve the final dilution (mL)

Final dilution	Serial dilutions		
	10^{-1}	10^{-3}	10^{-5}
10^{-1}	1	---	---
10^{-2}	0.1	---	---
10^{-3}	---	1	---
10^{-4}	---	0.1	---
10^{-5}	---	---	1

6.6 Choose the appropriate range of final dilution to give a 10 to 150 colonies formed per plate.

6.7 Inoculate three plates for each of the diluted solution.

6.8 Prepare a suitable volume of molten potato dextrose agar.

6.9 Remove the agar bottle from the water bath (45 °C) and pour in about 20mL of agar to each petri dishes.

6.10 Mix thoroughly by swirling and allow to set.

6.11 Invert the plates and incubate at 27^{o}C for 5 days.

6.12 Count plates with between 25 and 250 colonies.

7. Results

7.1 Enumeration method:

Examine the incubated plates for the presence of moulds and yeasts colonies. Count the number of colonies with a suitable colony counter. Record the number of the colony counted per plate and level of dilution that is used for the plate.

7.2 Expression of results

The result is expressed as the number of moulds and yeasts colonies formed per gram of the undiluted sample. To obtain this value, multiply the number of colonies at each dilution by the dilution factor. Take the average colonies number of the same concentration samples.

Total Moulds and Yeasts count per 1 g sample = colonies count x dilution factor

Note: For example, if 1mL of the 1:100 diluted solution of sample is used, after incubation of the plates, 2 colonies were counted on the plate, then the result is 2 × 100 = 200 colonies per gram of the undiluted sample.

8. References

1. ISO 8199-1988, Water quality – General guide to the enumeration of micro-organisms by culture, International Organization for Standardization.
2. GB 4789.15-94, Microbiological examination of food hygiene – Enumeration of moulds and yeasts, China National Standards, Ministry of Health, P.R. china.
3. GB 4789.28-84, Microbiological examination of food hygiene – Staining methods, culture mediums and reagents, China National Standards, Ministry of Health, P.R. China.
4. ISO 8784-1, Paper and board – Determination of microbiological properties – Part 1: Total bacterial count, International Organization for Standardization.
5. Bacteriological Analytical Manual, 7th Ed (1992)-FDA.
6. Recommended Methods for the Microbiological Examination of Foods – A.P.H.A. New-York, 1958.

Annex A

A.1 Potato Dextrose Agar

1. Ingredient:

Potato (infusion form)	200 g
Dextrose	20 g
Agar	15 g
Distilled water	1000 mL

2. Preparation

   Suspend 39 g in 1 L of distilled water and boil to dissolve. Sterilise by autoclaving at 121^{o}C for 15 minutes. Cool in water bath (45^{o}C), then add antibiotics solution before pouring plates.

3. Usage

   Potato dextrose agar is recommended by the American Public Health Association for counts of moulds and yeasts in dairy products, soft drinks and frozen foods.

A.2 Phosphate Buffered Saline

1. Ingredient:

Sodium chloride	8.0 g
Potassium chloride	0.2 g
Disodium hydrogen phosphate	1.15 g
Potassium dihydrogen phosphate	0.2 g

2. Preparation

   Dissolve all ingredients in 900 mL of distilled water and adjust pH to 7.2 (with 1M of Sodium hydroxide or 1M of Hydrochloric acid), bring final volume to 1000 mL with distilled water. Autoclave at 121^{o}C for 15 minutes.

A.3 Antibiotics

Two choices of antibiotics are listed as follow:

A) Penicillin-Streptomycin mix (Gibco BRL Products, USA)

Commercially available solution in bottles of 20 mL
Contains 5000 μg of penicillin (base) and 5000 μg of streptomycin (base)/mL utilising penicillin G (sodium salt) and streptomycin sulfate in 0.85% saline.
Spectrum: Gram-positive and Gram-negative bacteria

1. Preparation
Add 8 mL of the stock penicillin-streptomycin mix to 1 liter of medium to obtain 40 ppm concentration.

or

B) Chlortetracycline-HCl

1. Preparation:
Dissolve 1 g of antibiotic in 100 mL of sterile distilled water and filter through a 0.45 μm membrane to prepare stock solution. Store stock solutions in dark at 4-8 °C. Shelf life must be up to 1 month. Equilibrate stock solutions at room temperature immediately before use. If agar medium is in 250 mL aliquots, add 1 mL stock solution to obtain 40 ppm concentration.