Australian Standard®

Food microbiology

Method 14.2: General procedures and techniques—Colony count—Membrane filtration method

AS 5013.14.2—2009

PREFACE

This Standard was prepared by the Standards Australia Committee FT-024, Food Products and Subcommittee FT-024-01, Food Microbiology (Constituted), to supersede AS 1766.1.5—1991, *Food microbiology*, Method 1.5: *General procedures and techniques—Colony count—Membrane filtration method.*

The objective of this revision is to update the references and to transfer the procedure to AS 5013 series. It also incorporates minor technical variations on the apparatus used in the test technique.

METHOD

1 SCOPE

This Standard sets out a method for estimating the number of colony-forming units (CFUs) in suitable liquids using a membrane filtration technique.

The method is applicable only to liquids that can be efficiently filtered without causing a build-up on the filter.

NOTE: The membrane filtration method is most suitable when the microorganisms are in low concentration, e.g. in rinse waters collected from cleaned and sanitized pipelines or tanks. The method is suitable for microbiological assessment of water supplies to processing plants.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS	
5013	Food microbiology
5013.14	Method 14: Microbiology of food and animal feeding stuffs—General rules for microbiological examinations

3 PRINCIPLE

The method involves passing a liquid sample through a membrane of known physical properties. Microorganisms in the sample are retained on the membrane which is then placed on a filter pad saturated with liquid medium, or on solid medium, and incubated. Colonies, corresponding to the viable organisms collected on the filter, are then counted.

4 DILUENTS AND CULTURE MEDIA

The diluents and culture media shall be as specified in the relevant methods of AS 5013 according to the product under examination and the microorganisms to be enumerated.

Where the medium does not give rise to a good contrast for the colonies developed, a stain comprising 0.01 percent aqueous solution of malachite green oxalate is required.

5 APPARATUS

The following apparatus is required:

(a) Membrane filtration apparatus.

NOTE: Various types of apparatus suitable for the membrane filtration method are available commercially in Australia.

(b) Grid-mark membrane filters of 47 mm to 50 mm diameter to fit the apparatus and having a pore size appropriate to the organism or organisms to be assessed. The usual size for water is 0.45 μm.

NOTE: Sterile membrane filters are commercially available.

(c) A filter flask of capacity appropriate to the volume of liquid to be filtered.

(d) A source of vacuum.

(e) Forceps that will not damage the membrane.

(f) Petri dishes.

(g) Graduated measuring cylinders, where required, suitable for measuring the volume of sample to be filtered.

(h) Graduated pipettes.

(i) A tally counter.

(j) A suitable incubator.

(k) Non-toxic absorbent filter pads of diameter either equal to or slightly greater than the diameter of the membrane filters with which they are to be used.

(l) Pasteur pipettes.

NOTE: Items (k) and (l) above are only required where the nutrient pad technique is to be used.

6 PREPARATION OF APPARATUS AND MATERIALS

6.1 Sterilization

The apparatus and materials shall be sterilized as follows:

(a) Wrap the base of the membrane filtration apparatus, with the membrane support in position, in kraft paper or other suitable material. Wrap the filter funnel separately and sterilize the contents of both parcels by autoclaving.

NOTE: Some commercial unit can be sterilized fully assembled.

(b) Sterilize the remainder of the glassware as described in AS 5013.14.

(c) Sterilize the membrane filters according to the supplier's instructions.

NOTES:

1 The filtration characteristics of the membranes can be adversely affected by overheating.

2 Sterile membrane filters are available commercially.

6.2 Medium

6.2.1 *Liquid medium*

Where used, a filter pad shall be soaked with liquid medium as follows:

(a) Using forceps, place a filter pad in a Petri dish.

(b) Add sufficient liquid medium to saturate the pad and to result in a small excess.

NOTE: A 5 cm (in diameter) filter pad will require 2.0 mL to 2.5 mL of medium.

6.2.2 *Solid medium*

Where a solid medium is used, the plates should be prepared as follows:

(a) Pour sufficient volume of molten medium into a Petri dish to give a depth of approximately 5 mm. Allow to set.

(b) As soon as the medium has set, remove excess moisture from the plates by one of the following methods:

(i) Incubate the plates open with the internal surface of the base facing downwards and with the base resting on the lid, for the minimum time necessary to obtain plates free from condensate, e.g. at 37°C for about 2 h or at 45°C for about 1 h. Do not dry plates at temperatures above 45°C.

(ii) Incubate at 37°C for 16 h in the inverted position with the lids on. If the plates are not then free of condensate, open them and incubate as described in Step (i) above until dry.

(iii) Other suitable time/temperature regimes. For example, on bench over night.

NOTE: In humid climates either extend drying time or place a tray of desiccant, such as silica gel, in the base of the drying oven.

7 FILTRATION PROCEDURE

7.1 General

The optimum volume of liquid to be used will depend upon the amount of undissolved solids in the sample and the expected count.

If the expected count is high, suitable dilutions of the sample should be made (see Note). Where the expected count is uncertain, it is recommended that two determinations be made using two different volumes. In this way, the probability that at least one determination will be within the range 20 to 80 will be increased.

NOTE: The optimum number of colonies on the filter is about 50 and the volume of sample filtered should be such that the number of colonies to be counted on the membrane is not greater than 80. For example, samples expected to contain less than 80 organisms per 100 mL require the filtration of at least 100 mL of sample for each test.

7.2 Procedure

The procedure shall be as follows:

NOTE: Where a pre-assembled filtration unit is used, Steps (a) to (c) below are not required.

(a) Assemble the funnel base in the filter flask and connect the flask to the source of vacuum. Apply a slight vacuum.

(b) Using sterile forceps, centre a membrane filter on the membrane support with the grid-marked side upwards.

(c) Place the funnel in position and having tightened it, turn off the vacuum.

(d) Pour a measured volume of sample into the filter funnel. Gently apply vacuum and gradually increase it to that recommended by the supplier of the membrane. Where no such value is indicated, adjust the vacuum to about –40 kPa.

NOTE: When the volume to be filtered is less than 10 mL, add at least 20 mL of sterile diluent to the funnel before addition of the sample to aid uniform dispersion of the bacteria over the entire surface of the membrane during filtration.

(e) When the level of the sample has fallen to within about 6 mm of the membrane, reduce the vacuum to approximately –10 kPa. Rinse the sides of the funnel with 20 mL to 30 mL of diluent, added from the graduated cylinder used to measure the volume of the sample.

(f) Remove any liquid medium in the Petri dish in excess of that required to saturate the filter pad.

NOTE: A sterile Pasteur pipette is convenient for this operation.

(g) Immediately filtration has ceased, turn off the vacuum at the control tap, disconnect the funnel and remove the membrane using sterile forceps. Roll the membrane grid-marked side upwards, on the filter pad or on the solid medium, taking care to avoid entrapping air bubbles between the membrane and the substrate. Replace the lid on the Petri dish.

NOTE: Where there is no control tap directly under the funnel, it may be necessary to release the vacuum just before the completion of filtration, to avoid excessive drying of the membrane filter.

8 INCUBATION

The dishes shall be incubated as follows:

(a) Transfer the Petri dishes to the incubator. Place the plates in either an upright or an inverted position, according to the instructions for the organisms under test.

(b) Distribute the dishes in such a manner that overcrowding is avoided and there is no contact with the sides of the incubator.

(c) Incubate the dishes at the temperature and for the period specified for the organism to be estimated.

9 STAINING, COUNTING AND IDENTIFICATION (See Note 1)

Where the medium that has been used does not give rise to a good contrast for the colonies developed, staining of the membrane may be needed. If this is the case, stain the membrane by gently flooding the surface with a 0.01 percent aqueous solution of malachite green oxalate, and after 5 s to 6 s contact, pouring off the excess dye.

NOTES:

1 If required, subculturing of colonies should be carried out before any staining operation.

2 Colonies normally remain unstained, and the filter area not covered by colonies is stained a light green.

Using a tally counter, count the presumptively identified colonies and confirm their identification. Where spreaders occur, count each as a single colony provided that the outer edge of each spreader can be defined.

If the count is greater than 80, repeat the test where possible, using either a smaller volume or a dilution designed to produce a count in the range of 20 to 80.

10 CALCULATION

From the actual count, the number of organisms per unit volume or per unit mass of the sample shall be calculated taking dilution factors into account.

Where tests have been carried out on two different volumes or dilutions (see Clause 7.1) and each membrane has a count within the range 20 to 80, the two counts shall be calculated separately and the mean of the two reported as the result (see AS 5013.14).

11 REPORT

The report shall contain the following information:

(a) Reference to this Australian Standard, i.e. AS 5013.14.2.

(b) The number, and identity if confirmed, of colony-forming units (CFUs) per unit volume or per unit mass sample, stating that the count was determined by the membrane filtration method.

(c) The presence of spreading organisms, if encountered.

(d) The membrane filters used and the supplier's specification of pore size.

(e) The culture medium used.

(f) The conditions of incubation.

(g) Details of confirmation used.

NOTES

NOTES

This Australian Standard® was prepared by Committee FT-024, Food Products. It was approved on behalf of the Council of Standards Australia on 5 June 2009 and published on 14 July 2009.

The following are represented on Committee FT-024:

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