

AS 4276.3:2021



STANDARDS  
Australia



# Water microbiology

**Method 3: Enumeration of heterotrophic microorganisms — Pour plate, spread plate, membrane filtration and most probable number techniques**



AS 4276.3:2021

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- National Association of Testing Authorities Australia
- National Measurement Institute
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# Water microbiology

## **Method 3: Enumeration of heterotrophic microorganisms — Pour plate, spread plate, membrane filtration and most probable number techniques**

Originated as AS 4276.3.1—1995 and AS/NZS 4276.3.2:2003.  
AS 4276.3.1—1995 revised and redesignated as AS/NZS 4276.3.1:2007.  
Revised and redesignated as AS 4276.3:2021.

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## Preface

This Standard was prepared by the Standards Australia Committee, FT-020, Water Microbiology, to supersede AS/NZS 4276.3.1—2007, *Water microbiology, Method 3.1: Heterotrophic colony count methods — Pour plate method using yeast extract agar*, and AS/NZS 4276.3.2—2003, *Water microbiology, Method 3.2: Heterotrophic colony count methods — Plate count of water containing biocides*.

The objective of this document is to establish a standard method for the examination of water, with or without biocides, for the enumeration of heterotrophic microorganisms that covers a choice of possible techniques. The choice of procedure and medium will depend on the sample to be tested and the application of the results.

The major changes in this edition are as follows:

- (a) Combine the methods of AS/NZS 4276.3.1—2007 with AS/NZS 4276.3.2—2003.
- (b) Update culture media and reagents.
- (c) Update reference cultures.
- (d) Update the normative and informative references.

The term “informative” is used in Standards to define the application of the appendices to which it applies. An “informative” appendix is only for information and guidance.

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## Introduction

Waters of all kinds contain a variety of microorganisms derived from various sources and estimation of their overall number can provide useful information for assessing and monitoring water quality. Microorganisms which are able to survive in water usually grow better in the laboratory at about  $22\text{ °C} \pm 2\text{ °C}$  than at higher temperatures, the results often reflecting the environmental and seasonal conditions prevailing at the time. In contrast, microorganisms which grow well at  $36\text{ °C} \pm 2\text{ °C}$  generally survive with difficulty in water.

Heterotrophic colony counts are useful for assessing the integrity of ground water and the effectiveness of water treatment processes such as coagulation, filtration and disinfection and provide an indication of the cleanliness and integrity of the distribution system. They can also be used to assess the suitability of a supply for the preparation of food and drink, where the water supply should contain few microorganisms to avoid contaminating the product with spoilage organisms.

For these reasons, separate colony counts may be performed at  $22\text{ °C} \pm 2\text{ °C}$  and/or at  $36\text{ °C} \pm 2\text{ °C}$ . For cooling tower waters, plate counts are incubated at  $36\text{ °C} \pm 2\text{ °C}$ .

Waters which may contain biocides, e.g. cooling tower waters, require a modification of procedures to eliminate possible interference with counts.

In samples of water where an unidentified biocide may be present or where effective neutralization is not available it is necessary to use dilution of the sample to minimize interference by the biocide with counts. This will increase the lower detection limit.

With regard to *Legionella* control legislation in most states and territories, the heterotrophic colony count (HCC) is used as a surrogate measure for the cleanliness of cooling towers, and the procedure of choice is listed in AS/NZS 3666.3, *Air-handling and water systems of buildings — Microbial control, Part 3: Performance-based maintenance of cooling water systems*.

Heterotrophs are broadly defined as microorganisms that require organic carbon for growth. They include bacteria and fungi. The heterotrophic count (HC) refers to the number of organisms grown in non-specific culture media without inhibitory or selective agents. Even though the test is non-selective, there will be a proportion of the microorganisms present in the water sample that may not be recovered. The types of organisms detected by HC tests can vary widely depending on the source of water, and time of year. It is important to note that the heterotrophic count results may differ for each of the procedures described below due to different incubation temperatures and times, the methods/techniques used and, to a lesser extent, differences in the media formulation. This means that equivalence of results derived from a single sample using different techniques and procedures is not to be expected.

Where HC limits exist in standards, or are referred to in guidelines, it is essential to prescribe and adhere to the technique, medium and incubation conditions where these have been prescribed.

Where there are no limits for HC set out in water standards or guidelines, the absolute HC result is not relevant. The main purpose of HC is in detecting changes in the cleanliness of a water system. Consequently, it is important not to change the technique when looking for temporal or locational changes within a water system.

## NOTES

# Australian Standard<sup>®</sup>

## Water microbiology

### Method 3: Enumeration of heterotrophic microorganisms — Pour plate, spread plate, membrane filtration and most probable number techniques

## 1 Scope and general

### 1.1 Scope

This Standard specifies methods for enumeration of heterotrophic microorganisms in water using the following techniques:

- (a) Pour plate.
- (b) Spread plate.
- (c) Spiral plate.
- (d) Membrane filtration.
- (e) Most probable number (MPN).

These techniques are intended to measure the operational effectiveness of treatment processes and be used for the general examination of many types of water to evaluate and/or note changes in water quality.

This document does not cover the comparison of results obtained from different techniques.

### 1.2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document:

NOTE Documents for informative purposes are listed in the Bibliography.

AS 2031, *Water quality — Sampling for microbiological analysis (ISO 19458:2006, MOD)*

AS 5140, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133:2014, MOD)*

AS 4276.1, *Water microbiology, Method 1: Water quality — General requirements and guidance for microbiological examinations by culture (ISO 8199:2018, MOD)*

### 1.3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

#### 1.3.1 count

observed number of objects, such as colonies, determined by direct counting, or most probable number (MPN) estimation based on statistical calculation using the number of positive units in a dilution series of a test sample

#### 1.3.2 may

indicates the existence of an option

**1.3.3****shall**

indicates that a statement is mandatory

**1.3.4****should**

indicates a recommendation

**2 Principle**

Techniques used to enumerate heterotrophic microorganisms are summarized in the table below:

Technique	Typical volume of water tested	Examples of water types	Media (refer to specific technique)
Pour plate	≤ 1 mL	Potable, non-potable	Non-selective agar (YEA, R2A, NA, TSA, PCA) <sup>a</sup>
Spread plate and spiral plate	0.1 mL to 0.5 mL	Potable, non-potable	
Membrane filtration	Any volume	Reuse medical devices washer-disinfector, dialysis RO, potable, non-potable (low turbidity)	Non-selective agar (YEA, R2A, TSA, PCA, m-HPC, NA) <sup>a</sup>
MPN	≤ 100 mL	Potable, non-potable (high or low turbidity)	Non-selective liquid medium (tryptone soy/soya broth, nutrient broth) or as per manufacturer

<sup>a</sup> R2A is recommended as the recovery medium of choice for stressed and chlorine tolerant microorganisms: see Bibliography.[1]

**3 Samples****3.1 Laboratory samples**

Laboratory samples shall conform to AS 2031.

**3.2 Dilutions**

Dilutions of the laboratory sample shall be prepared, as required, in accordance with the procedure in AS 4276.1.

**4 Apparatus**

The applicable apparatus is required depending on the technique used:

- (a) *Apparatus for preparation of dilutions* — Refer to AS 4276.1.
- (b) *Petri dishes* — 50 mm to 90 mm or up to 150 mm in diameter.
- (c) *Pipettes, volume measuring devices.*
- (d) *Colony counter, microscope* — Provide with a lens for magnification and a means for the illumination of the prepared plates.
- (e) *Tally counter, automated spiral plate counters, automated counters.*
- (f) *Incubators, water baths.*
- (g) *Membrane filtration apparatus* — Refer to AS 4276.1.

- (h) *Membrane filters* — 0.45 µm pore size, refer to AS 4276.1.
- (i) *Sterile filter funnels.*
- (j) *Forceps.*
- (k) *Spiral plater and associated equipment.*
- (l) *Sterile spreaders.*
- (m) *Circulating water bath* — Able to maintain temperature of 45 °C ± 1 °C.
- (n) *MPN* — Sealers and trays in accordance with manufacturer's instructions or requirements.
- (o) *Apparatus for media preparation* — Include an autoclave, see [Appendix B](#).
- (p) *Refrigerator* — Able to maintain temperatures of 2 °C to 8 °C for media storage.

## 5 Culture media and diluents

The culture medium shall be chosen from the following:

- (a) Plate count agar (PCA).
- (b) Reasoner's 2A agar (R2A).
- (c) m-Heterotrophic plate count agar (m-HPC).
- (d) Tryptone soya agar (TSA).
- (e) Yeast extract agar (YEA).
- (f) Nutrient agar (NA).

NOTE For formulations and procedures for preparing the culture media, see [Appendix B](#).

Dilutions of the laboratory sample shall be prepared as required, using the procedure given in AS 4276.1.

## 6 Quality assurance

### 6.1 General

The laboratory shall have a clearly defined quality control system to ensure that the apparatus, culture media, reagents and technique are suitable for the test.

NOTE The use of controls and blanks, as appropriate, is part of the quality control system.

### 6.2 Performance testing of culture media

The performance of the culture medium shall be tested in accordance with the method and criteria in AS 5140. [Table 6.1](#) provides a summary of expected performance results.

NOTE Refer to AS 5140 for the following:

- (a) Definitions of productivity.
- (b) Preparation of the working cultures and test suspensions.

**Table 6.1 — Summary of expected performance results**

Media	Type	Microorganisms	Function	Incubation	Control strains	AS 5140 quality control strains	Reference media	Method of control	Criteria
All	Solid and liquid	Heterotrophic plate count	Productivity	36 °C ± 2 °C for 44 h ± 4 h	<i>Escherichia coli</i>	WDCM <sup>(a)</sup> 00090 or WDCM 00012 or WDCM 00013	Tryptone soya agar (TSA) or previously validated batch when testing TSA	Quantitative	PR ≥ 0.7

(a) WDCM — World Data Centre for Microorganisms.

## 6.3 Reference cultures

### 6.3.1 General

The reference cultures are used in media quality control and analytical quality control procedures relevant to this method. The purpose of the reference cultures is to demonstrate and ensure that typical growth characteristics are exhibited by the cultures on the media used.

As a minimum, the reference cultures shall be used to ensure that the method has been used properly. Each day that samples are processed by this method, suspensions of the reference cultures shall be submitted to the test procedure.

If the reference cultures do not give appropriate results, then an investigation shall be undertaken. Test results shall be invalid unless the investigation reveals reasons for this failure that invalidate the quality control procedures (e.g. prepared suspensions shown not to contain target bacteria).

### 6.3.2 Positive culture

*Escherichia coli*, WDCM 00090, WDCM 00012 or WDCM 00013 or a culture traceable to any of these strains.

NOTE World Data Centre for Microorganisms (WDCM).

## 7 Techniques

### 7.1 Pour plate

#### 7.1.1 General

This technique is commonly used for the enumeration of HCC when water samples may vary widely in microbial loads. There are limitations, however, when water samples contain particulates that can interfere with colony development or the counting process.

NOTE A flow diagram of the procedure is shown in [Figure A.1](#).

#### 7.1.2 Procedure

The procedure for pour plate shall be in accordance with AS 4276.1.

During the preparation of the pour plates the following precautions shall be taken:

- (a) Keep the lids of the plates in place except when they are partially lifted for adding the inoculum and the medium.
- (b) Do not exceed a time interval of 10 min between pipetting into the plate and pouring the medium.
- (c) Do not exceed a total time interval of 20 min between the preparation of the first dilution and the pouring of the last plate.

### 7.1.3 Inoculation and pouring of plates

Plates shall be inoculated and poured as follows:

- (a) Prepare plates by adding 1 mL of sample or appropriate dilution to each plate.  
When dilution is necessary, plate out appropriate dilutions.
- (b) To each plate add molten agar, which has been cooled to 44 °C to 46 °C, to a depth of 3 mm to 5 mm.

NOTE Some organisms may suffer heat-shock above 47 °C.

- (c) Immediately after pouring, keep the plate horizontal and, taking care not to wet the lid, thoroughly mix the medium and the inoculum by to-and-fro movements, followed by circular clockwise movements, followed by to-and-fro movements at right-angles to the first set, followed by circular anti-clockwise movements.
- (d) Allow the plates to stand on a horizontal surface until the medium has set.

### 7.1.4 Incubation

Incubation shall be carried out as described in AS 4276.1. The plates should be incubated at 36 °C ± 2 °C for 44 ± 4 h and/or 22 °C ± 2 °C for 68 ± 4 h, as required.

For cooling tower waters, the incubation used shall be 36 °C ± 2 °C for 44 ± 4 h.

### 7.1.5 Counting of colonies

The total number of colonies shall be counted as follows:

- (a) Register the count.  
NOTE Use of a counter is recommended. If manually counting it is recommended to mark the counted colonies to avoid recounting. Use of an illuminated magnification source can also assist in counting.
- (b) Count each spreading colony as a single colony.

Plates shall be rejected where 25 % or more of the medium is occupied by spreading organisms.

### 7.1.6 Calculation of results

The calculation of results shall be carried out in accordance with AS 4276.1.

## 7.2 Spread plate

### 7.2.1 General

Surface spread plating is an alternative technique for enumerating microorganisms in water samples. This technique avoids heat shock and all colonies are on the agar surface where they can be readily

distinguished from particles and bubbles. Spread plates allow for easily discernible colonies to be sub-cultured.

The major disadvantage of the spread plate technique is that the lower level of detection is increased due to the reduction in the volume of the inoculum able to be applied to the surface of the plate.

NOTE A flow diagram of the procedure is shown in [Figure A.2](#).

### **7.2.2 Procedure**

This procedure describes the technique for enumerating microorganisms in water by inoculating a volume of sample onto the surface of an agar plate. Record the volume of sample inoculated.

The procedure for spread plating shall be in accordance with AS 4276.1.

### **7.2.3 Incubation**

Plates should be incubated at  $36\text{ °C} \pm 2\text{ °C}$  for  $44\text{ h} \pm 4\text{ h}$  and/or  $22\text{ °C} \pm 2\text{ °C}$  for  $68\text{ h} \pm 4\text{ h}$ , as required.

### **7.2.4 Counting of colonies**

Colonies shall be counted and recorded after the incubation period.

### **7.2.5 Calculation of results**

The calculation of results shall be carried out in accordance with AS 4276.1.

## **7.3 Spiral plate**

### **7.3.1 General**

This is a variation of the spread plate technique.

The spiral plater dispenses a known volume of the sample on the surface of a rotating agar plate in an Archimedes spiral. The volume of sample deposited is decreased as the dispensing stylus moves from the centre to the edge of the plate, so that an exponential relationship exists between the volume deposited and the radius of the spiral. Following incubation, microorganisms present will develop on the surface of the agar. Using a counting grid, the number of colonies in a known area is calculated by referring to manufacturer's instructions.

NOTE A flow diagram of the procedure is shown in [Figure A.2](#).

### **7.3.2 Procedure**

The procedure shall be in accordance with the manufacturer's instructions.

### **7.3.3 Incubation**

Plates should be incubated at  $36\text{ °C} \pm 2\text{ °C}$  for  $44\text{ h} \pm 4\text{ h}$  and/or  $22\text{ °C} \pm 2\text{ °C}$  for  $68\text{ h} \pm 4\text{ h}$ , as required.

### **7.3.4 Reading of results**

After the required incubation period, the reading of results shall be in accordance with the manufacturer's instructions.

### **7.3.5 Calculation of results**

The calculation of results shall be performed using the manufacturer's instructions.

## 7.4 Membrane filtration

### 7.4.1 General

Membrane filtration is the preferred technique for water expected to contain low microbial counts. This technique is suitable for analysing large volumes of water with low turbidity, including analysis of ultra-pure dialysis fluid and water used to process re-useable medical devices. This method is not suitable for turbid samples.

NOTE A flow diagram of the procedure is shown in [Figure A.3](#).

### 7.4.2 Procedure

The procedure for membrane filtration shall be in accordance with AS 4276.1.

### 7.4.3 Incubation

Plates should be incubated at  $36\text{ °C} \pm 2\text{ °C}$  for  $44\text{ h} \pm 4\text{ h}$  and/or  $22\text{ °C} \pm 2\text{ °C}$  for  $68\text{ h} \pm 4\text{ h}$ , as required.

### 7.4.4 Counting of colonies

After incubation, counting of colonies shall be performed in accordance with the procedure specified in AS 4276.1.

### 7.4.5 Calculation of results

The calculation of results shall be performed in accordance with AS 4276.1.

## 7.5 Most probable number (MPN)

### 7.5.1 General

A series of test portions and dilutions are inoculated into a liquid growth medium, and the resulting growth pattern is used to determine the MPN of microorganisms.

The precision of this technique increases with an increasing number of replicates at each dilution and relies on dilution to extinction to obtain a count. Therefore, this technique is particularly suitable for samples expected to have a low microbial load.

NOTE 1 This technique may not be suitable for water samples containing biocides or high levels of salts.

NOTE 2 A flow diagram of the procedure is shown in [Figure A.4](#).

### 7.5.2 Procedure

The MPN procedure shall be in accordance with AS 4276.1.

NOTE Commercial kits are available as an alternative to traditional laboratory cultures, in which case, follow the manufacturer's instructions.

### 7.5.3 Reading of results

After the required incubation period, read and record the number and arrangement of growth chambers, or follow the manufacturer's instructions.

#### **7.5.4 Calculation of results**

The calculation of results shall be performed in accordance with the appropriate MPN tables in AS 4276.1.

### **8 Test report**

The following shall be reported:

- (a) All details necessary for the identification of the sample.
- (b) Reference to this Australian Standard, i.e. AS 4276.3 and technique used.
- (c) Date of sampling and testing.
- (d) Heterotrophic count (colony forming units, CFU or most probable number, MPN) per volume of sample, refer to AS 4276.1.
- (e) Incubation time, incubation temperature and medium used.
- (f) Presence of spreaders.
- (g) Any circumstances or unusual observations made during sampling and the course of the tests that may have had an effect on the result.

## Appendix A (informative)

### Flow diagrams

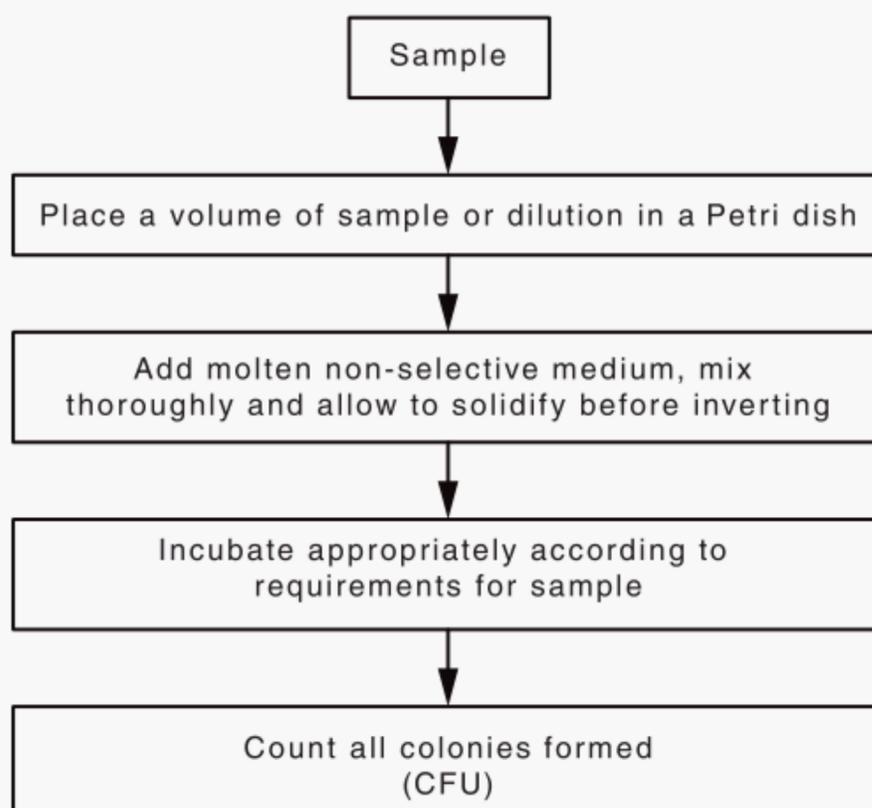


Figure A.1 — Flow diagram for heterotrophic colony count pour plate technique

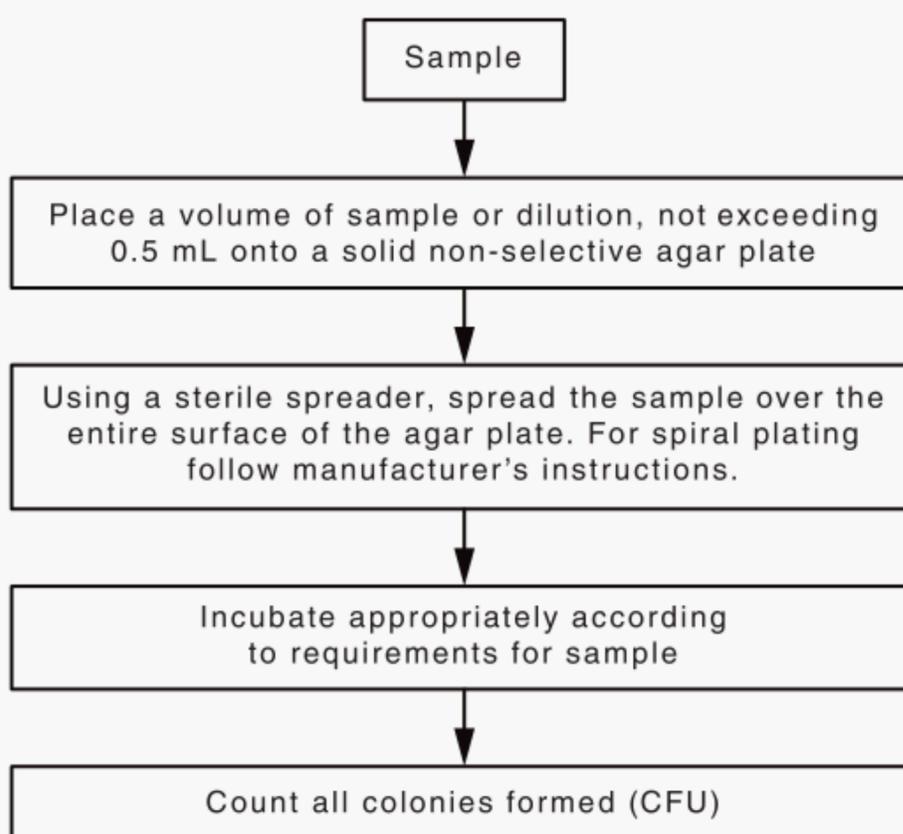
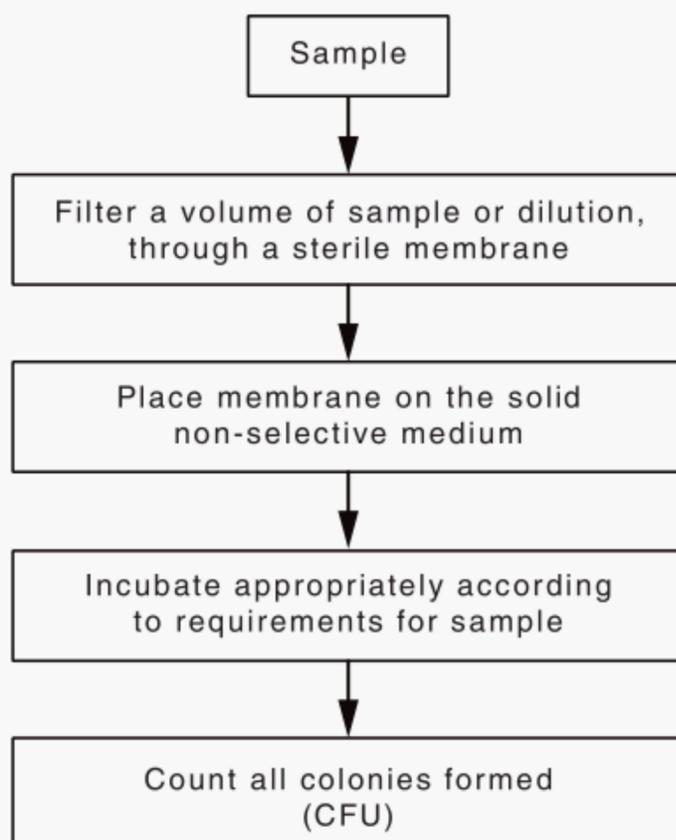
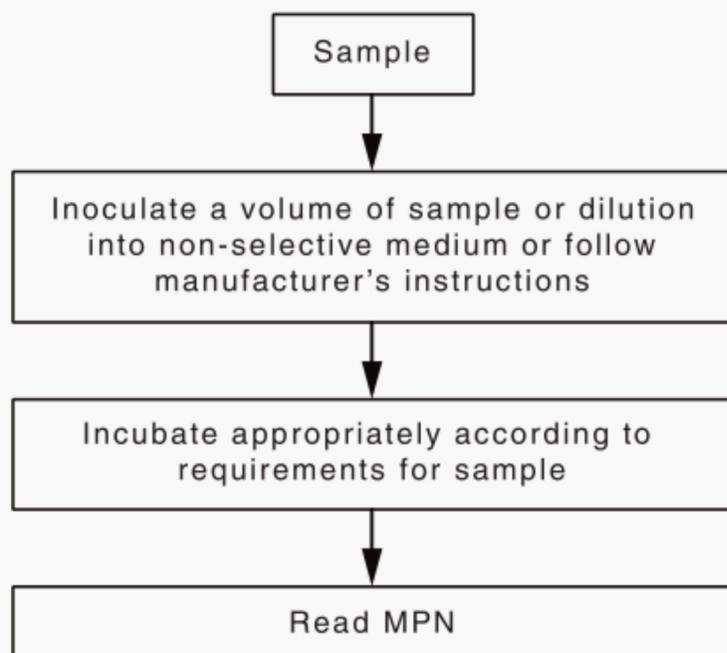


Figure A.2 — Flow diagram for heterotrophic colony count spread plate and spiral plate techniques



**Figure A.3 — Flow diagram for heterotrophic colony count membrane filtration technique**



**Figure A.4 — Flow diagram for heterotrophic count MPN technique**

## Appendix B (informative)

### Culture media

#### B.1 Scope

This appendix provides the formulations and procedures for preparing the culture media specified in this document.

Equivalent formulations of the culture medium listed in this appendix are commercially available in dehydrated form and should be made up according to the manufacturer's instructions. For the MPN technique, liquid media is prepared by omitting the agar ingredient in the formulations given in [Clauses B.6](#) and [B.7](#).

#### B.2 Reasoner's 2A agar (R2A)

##### B.2.1 Reference

NOTE For further reading, see Bibliography.<sup>[1]</sup>

##### B.2.2 Materials

The following are required:

- (a) *Yeast extract* — 0.5 g.
- (b) *Proteose peptone No. 3 or polypeptone* — 0.5 g.
- (c) *Casamino acids* — 0.5 g.
- (d) *Glucose* — 0.5 g.
- (e) *Soluble starch* — 0.5 g.
- (f) *Dipotassium hydrogen phosphate, K<sub>2</sub>HPO<sub>4</sub>* — 0.3 g.
- (g) *Magnesium sulfate heptahydrate, MgSO<sub>4</sub>·7H<sub>2</sub>O* — 0.05 g.
- (h) *Sodium pyruvate* — 0.3 g.
- (i) *Agar* — 15.0 g.
- (j) *Reagent grade water* — 1 L.

##### B.2.3 Apparatus

The following are required:

- (a) Glassware/vessel.
- (b) Hot plate and magnetic stirrer.
- (c) Thermometer.
- (d) pH meter.

- (e) Autoclave.
- (f) Petri dishes, tubes or bottles.
- (g) Refrigerator able to maintain a temperature at 2 °C to 8 °C.
- (h) Balance.

#### **B.2.4 Procedure**

The procedure is as follows:

- (a) For a medium made from basic ingredients, adjust to pH 7.2 with solid  $K_2HPO_4$  or  $KH_2PO_4$  before adding agar.
- (b) Dissolve the ingredients in the water, heat to boiling until solution is complete.
- (c) Autoclave at 121 °C for 15 min.
- (d) Cool to 50 °C and dispense as required.  
NOTE Final pH of the medium should be  $7.2 \pm 0.2$ .
- (e) Store the prepared medium at 2 °C to 8 °C.

### **B.3 Plate count agar (PCA)**

#### **B.3.1 Reference**

NOTE For further reading, see Bibliography.[\[2\]](#)

#### **B.3.2 Materials**

The following are required:

- (a) *Tryptone* — 5.0 g.
- (b) *Yeast extract* — 2.5 g.
- (c) *Glucose* — 1.0 g.
- (d) *Agar* — 15.0 g.
- (e) *Reagent grade water* — 1 L.

#### **B.3.3 Apparatus**

The following are required:

- (a) Glassware/vessel.
- (b) Hot plate and magnetic stirrer.
- (c) Thermometer.
- (d) pH meter.
- (e) Autoclave.
- (f) Petri dishes, tubes or bottles.
- (g) Refrigerator able to maintain a temperature at 2 °C to 8 °C.

- (h) Balance.

### **B.3.4 Procedure**

The procedure is as follows:

- (a) Dissolve the ingredients in the water, heat to boiling until solution is complete.
- (b) Autoclave at 121 °C for 15 min.
- (c) Cool to 50 °C and dispense as required.
- NOTE Final pH of the medium should be  $7.0 \pm 0.2$ .
- (d) Store the prepared medium at 2 °C to 8 °C.

## **B.4 m-HPC agar**

### **B.4.1 Reference**

NOTE For further reading, see Bibliography.<sup>[2]</sup>

### **B.4.2 Materials**

The following are required:

- (a) *Peptone* — 20.0 g.
- (b) *Gelatin* — 25.0 g.
- (c) *Glycerol* — 10.0 mL.
- (d) *Agar* — 15.0 g.
- (e) *Reagent grade water* — 1 L.

### **B.4.3 Apparatus**

The following are required:

- (a) Glassware/vessel.
- (b) Hot plate and magnetic stirrer
- (c) Thermometer.
- (d) pH meter.
- (e) Autoclave.
- (f) Petri dishes, tubes or bottles.
- (g) Refrigerator able to maintain a temperature at 2 °C to 8 °C.
- (h) Balance.

### **B.4.4 Procedure**

The procedure is as follows:

- (a) Dissolve the solid ingredients in the water, heat to boiling until solution is complete.

- (b) Add glycerol.
- (c) Autoclave at 121 °C for 15 min.
- (d) Cool to 50 °C and dispense as required.  
NOTE Final pH of the medium should be  $7.1 \pm 0.2$ .
- (e) Store the prepared medium at 2 °C to 8 °C.

## **B.5 Yeast extract agar (YEA)**

### **B.5.1 Reference**

NOTE For further reading, see Bibliography.<sup>[3]</sup>

### **B.5.2 Materials**

The following are required:

- (a) *Tryptone (peptone from casein, pancreatic)* — 6.0 g.
- (b) *Dehydrated yeast extract* — 3.0 g.
- (c) *Agar, powdered or in pellets* — 10 g to 20 g.
- (d) *Reagent grade water* — 1 L.

### **B.5.3 Apparatus**

The following are required:

- (a) Glassware/vessel.
- (b) Hot plate and magnetic stirrer.
- (c) Thermometer.
- (d) pH meter.
- (e) Autoclave.
- (f) Bottles or tubes.
- (g) Refrigerator able to maintain a temperature at 2 °C to 8 °C.
- (h) Balance.

### **B.5.4 Procedure**

The procedure is as follows:

- (a) Add the ingredients, or the complete dehydrated medium, to the water and dissolve by heating.
- (b) Adjust the pH if necessary so that after sterilization it will be  $7.2 \pm 0.2$  at 25 °C.
- (c) Distribute volumes as required into bottles or tubes.
- (d) Sterilize in the autoclave at 121 °C for 15 min.
- (e) Store the prepared medium at 2 °C to 8 °C.

## **B.6 Tryptone soy/soya agar (casein soya bean digest agar) (TSA)**

### **B.6.1 Reference**

NOTE For further reading, see Bibliography.<sup>[4]</sup>

### **B.6.2 Materials**

The following are required:

- (a) *Pancreatic digest of casein* — 15.0 g.
- (b) *Enzymatic\* digest of soya bean (\*contains papain)* — 5.0 g.
- (c) *Sodium chloride* — 5.0 g.
- (d) *Agar* — 15.0 g.
- (e) *Reagent grade water* — 1 L.

### **B.6.3 Apparatus**

The following are required:

- (a) Glassware/vessel.
- (b) Hot plate and magnetic stirrer.
- (c) Thermometer.
- (d) pH meter.
- (e) Autoclave.
- (f) Bottles or tubes.
- (g) Refrigerator able to maintain a temperature at 2 °C to 8 °C.
- (h) Balance.

### **B.6.4 Procedure**

The procedure is as follows:

- (a) Add the ingredients, or the complete dehydrated medium, to the water and dissolve by heating.
- (b) Adjust the pH if necessary so that after sterilization it will be  $7.3 \pm 0.2$  at 25 °C.
- (c) Distribute volumes as required into bottles or tubes.
- (d) Sterilize in the autoclave at 121 °C for 15 min.
- (e) Store the prepared medium at 2 °C to 8 °C.

## **B.7 Nutrient agar**

### **B.7.1 Reference**

NOTE For further reading, see Bibliography.<sup>[5]</sup>

### B.7.2 Materials

The following are required:

- (a) *Beef extract* — 3.0 g.
- (b) *Peptone* — 5.0 g.
- (c) *Sodium chloride* — 5.0 g.
- (d) *Agar* — 15.0 g.
- (e) *Water* — 1.0 L.

### B.7.3 Apparatus

The following are required:

- (a) Glassware/vessel.
- (b) Hot plate and magnetic stirrer.
- (c) Thermometer.
- (d) pH meter.
- (e) Autoclave.
- (f) Bottles or tubes.
- (g) Refrigerator able to maintain a temperature at 2 °C to 8 °C.
- (h) Balance.

### B.7.4 Procedure

The procedure is as follows:

- (a) Add ingredients to water, mix thoroughly and heat to dissolve.
- (b) Distribute volumes as required into bottles or tubes.
- (c) Sterilize in the autoclave at 121 °C for 15 min.  
NOTE Final pH of the medium should be  $7.1 \pm 0.2$ .
- (d) Store the prepared medium at 2 °C to 8 °C.

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## NOTES

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