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Australian Standard®

Food microbiology

Method 5: Preparation of culture media, diluents and reagents

PREFACE

This Standard was prepared by the Standards Australia Committee on Food Microbiology as part of a program to issue all Standards pertaining to the microbiological examination of foods (including dairy products) in the series AS 1766, *Food microbiology*.

This Standard supersedes the following Standards:

AS	
1095	*Methods of microbiological examination of dairy products and for dairy purposes*
1095.3A—1976	*Methods for the detection of specific groups of micro-organisms—Preparation of media and diluents*
1766	*Methods for the microbiological examination of food*
1766.5—1982	*Preparation of media, diluents and reagents*

This Standard is intended to provide formulations of culture media, diluents and reagents specified in methods set out in AS 1766. As methods are revised and new methods introduced that specify culture media, diluents and reagents not listed in this edition of AS 1766.5, the required formulations will be provided in the revised or new methods and will be published in the next edition of this Standard.

The formulae of the media, diluents and reagents set out in this Standard have been drawn from many sources and where possible a literature reference is given to provide more detailed information about each medium and its use.

METHOD

1 SCOPE This Standard sets out the formulations and procedures, and precautions to be followed, for the preparation of the culture media, diluents and reagents specified in this series of standard methods for the microbiological examination of foods.

2 REFERENCED DOCUMENT The following document is referred to in this Standard:

AS
2243 Safety in laboratories

3 HAZARDOUS CHEMICALS Many chemicals are hazardous and care shall be taken when handling all chemicals used to make up culture media, diluents and reagents. Safety precautions and practices as detailed in AS 2243 shall be observed at all times.

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4 REQUIREMENTS FOR INGREDIENTS

4.1 Agar

4.1.1 *Description* Agar for microbiological purposes shall be prepared especially for this use and shall be identified as bacteriological agar. Such agar, when used at an appropriate concentration in media, shall give satisfactory biological performance and shall not cause significant inhibition to the growth of microorganisms. Information on the gel strength, maximum moisture content and maximum inorganic matter content expressed as ash shall be available from the manufacturer.

4.1.2 *Analysis and specifications* Bacteriological agar shall be subject to the following tests and shall comply with the specifications indicated:

(a) *Gel strength* Gel strength of agar used for microbiological purposes is determined by means of the FIRA gel tester* or an instrument giving similar results. Results of such analyses shall be available from the manufacturer.

In most of the media formulations which follow, a satisfactory gel strength with 15 g/L agar has been assumed but this concentration may have to be varied according to the quality of the agar available.

(b) *Gelling* Prepare a suspension of agar in water at the concentration indicated by the stated gel strength and dissolve by autoclaving at 121°C for 15 min. Cool the solution with agitation to approximately 46°C, adjust to pH 7.0 ±0.1 and allow to stand in a water bath, capable of being controlled to ±1°C, adjusted to the temperature at which the test is to be carried out in the range 39°C to 46°C. After 3 h, examine the solution for gelation by pouring through a 200 mm length tube of 5 mm internal diameter which has been preheated by immersion in water at the temperature selected for the test. The agar solution shall flow smoothly through the tube without any irregularity of flow indicative of gelling.

(c) *pH* Prepare a 15 g/L solution of agar in water by autoclaving at 121°C for 15 min and cool to 45 ±1°C. Retain this solution at this temperature for the tests described in Items (d) and (e). The pH of the solution when cooled to 45 ±1°C shall be in the range 6.0 to 7.5.

(d) *Insoluble sediment* Pour 100 mL of the solution from (c) into a beaker with a base diameter of 65 mm, cover and allow to cool to 22.5 ±2.5°C. The amount of sediment in the beaker shall be such that, when the agar solution is used to prepare pour plates, particulate sediment will not significantly affect the counting of microbial colonies.

(e) *Opacity* Pour some of the solution described in Item (c) into a 10 mm spectrophotometer cell, cool to 22.5 ±2.5°C and allow to stand at this temperature for 1 h. Measure the absorbance in a spectrophotometer at 40 nm using distilled water as the blank. The optical density shall not exceed 1.00.

NOTES:

1 Measurements of opacity made by any other method, including the use of Brown's opacity tubes, should be related to the above method.

2 Microbiological grades of agar usually remain sufficiently clear for most media. However, if greater clarity is essential, filtration of the media through cellulose pulp may be necessary.

4.2 Beef extract Any standardized brand of beef extract especially manufactured for the preparation of microbiological culture media may be used.

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* JONES, N.R., 1956, *Analyst*, Vol. 83, p. 243.

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4.3 Bile salts Bile salts are a standardized mixture of sodium glycocholate and sodium taurocholate, prepared from fresh ox bile, for use as a selectively inhibitory agent in media. Only standardized, commercially available dehydrated preparations especially prepared for microbiological work shall be used.

4.4 Bile salts No. 3 This is a refined bile salt used as a selectively inhibitory agent in bacteriological culture media in amounts less than one-third of the concentration of bile salts normally quoted in formulae for bile salt media. Use only commercially available dehydrated preparations especially prepared for bacteriological work.

4.5 Sugars All sugars used in the preparation of culture media shall be chemically pure and known to be suitable for bacteriological purposes.

4.6 Salts and general chemicals All salts and general chemicals used as ingredients shall be AR (analytical reagent) grade or equivalent. The nomenclature used in this document is in accordance with the Merck Index.

4.7 Dyes All dyes used in the preparation of culture media shall be certified suitable for microbiological purposes. This particularly applies to brilliant green used in Salmonella media.

4.8 Peptone This is a general purpose peptone recommended for the preparation of routine microbiological media. Any peptone which comparative tests have shown to give satisfactory results may be used.

4.9 Proteose peptone This is a specialized peptone prepared by papain digestion of selected fresh meat for use in media mainly for the production of bacterial toxins. Any proteose peptone preparation which comparative tests have shown to give satisfactory results may be used.

4.10 Skim milk powder Any standardized brand of thermophile-free, antibiotic-free powdered skim milk especially manufactured for the preparation of microbiological culture media may be used. Any readily soluble spray dried skim milk powder free from antibiotics may be used.

4.11 Yeast extract Yeast extract is the water soluble extract of lysed yeast cells. Any yeast extract shown to yield satisfactory results may be used.

4.12 Water Unless otherwise specified, glass-distilled water, deionized water or water purified by reverse osmosis shall be used. The water shall be free from toxic substances.

NOTES:

1 Where appropriate in these methods, water of suitable quality may be referred to as ‘laboratory-grade water’.

2 Where deionized water is used, precautions should be taken against microbial build-up in the deionizing equipment.

3 Routine conductivity determinations are recommended for checking the purity of laboratory-grade water.

4 Attention is drawn to the possibility of distillate from chlorinated water containing chlorine.

5 FILTERING

5.1 Clearing agar media For quantities greater than 500 mL, filter through paper pulp. The filter shall be preheated with warm water when hot agar is to be filtered.

5.2 Sterilization by filtration Where sterilization by filtration is specified, a Seitz filter or a membrane filter of 0.2 μm pore size or a Seitz-type filter with a sterilizing pad shall be used.

6 pH OF MEDIA

The pH of media shall be read when the temperature of the medium is below 50°C. The pH can be adjusted with 1 mol/L NaOH or 1 mol/L HCL, or other appropriate base or acid.

NOTE: When adjusting the pH before autoclaving, take into account that the pH of most media falls by about 0.2 units during autoclaving.

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7 STERILIZATION It is most important that the specified time-temperature conditions of sterilization are strictly observed to ensure sterility. Overheating can also have adverse effects on culture media.

8 COMMERCIAL MEDIA In most cases dehydrated media of similar performance to those described here are available commercially, although the concentration of some ingredients may differ slightly. These commercial media should be prepared for use in accordance with the manufacturers' instructions. Several of the media may be prepared by supplementing commercial products; for example, mannitol selenite cystine broth may be prepared by the addition of cystine to commercially prepared mannitol selenite broth.

9 QUALITY CONTROL OF CULTURE MEDIA Because of batch-to-batch variation, the properties of each batch of each medium shall be verified by inoculation with the appropriate microorganisms. Selective media shall be checked for their growth-promoting and growth-inhibiting properties with standard reference cultures known to give positive and negative results. In addition, all batches of prepared media shall be checked for sterility and pH.

10 FORMULARY OF CULTURE MEDIA, DILUENTS AND REAGENTS

ALKALINE PEPTONE WATER

Ref. FURNISS, A.L, LEE, J.V. and DONOVAN, T.J. *Public Health Laboratory Service Monograph*, Series 11, The Vibrios. London: Her Majesty's Stationery Office, 1978.

Peptone	10.0 g
Sodium chloride	10.0 g
Water	1.0 L

Dissolve the ingredients in the water and adjust the pH so that after sterilization the pH is 8.6 ±0.1. Dispense as required and sterilize at 121°C for 15 min.

ANAEROBIC AGAR WITHOUT GLUCOSE OR INDICATOR

Ref. VERA, H.D. *J. Bacteriol.* 1942, Vol. 44, p. 507.

Tryptone	20.0 g
Sodium chloride	5.0 g
Sodium thioglycollate	2.0 g
Sodium formaldehyde sulfoxylate	1.0 g
Agar	15.0 g
Water	1.0 L

Dissolve the ingredients in the water and adjust the pH so that after autoclaving the pH is 7.2 ±0.1. Dispense as required and autoclave at 121°C for 15 min.

BAIRD-PARKER AGAR

Ref. BAIRD-PARKER, A.C. *J. Appl. Bacteriol*. 1962, Vol. 25, p. 12.

Preparation of complete medium

Basal medium (see below)	90.0 mL
Concentrated egg yolk emulsion (see below)	5.0 mL
Glycine solution (see below)	6.3 mL
Potassium tellurite solution (see below)	1.0 mL
Sulfamethazine solution (see below)	2.25 mL
Sodium pyruvate solution (see below)	see Note 2 below

Melt the basal medium, cool to 45°C to 50°C. Prewarm all other ingredients except sodium pyruvate solution, add to basal medium, mix thoroughly and pour plates (10 mL to 15 mL per plate).

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NOTES:

1 These plates may be stored at 4°C for up to 1 month, provided that 15 mL to 20 mL per plate is used and care is taken to prevent desiccation during storage, but it is preferable to use them as soon as possible after preparation.

2 Immediately before use, spread 0.5 mL of the sodium pyruvate solution over the agar surface of each plate and dry at 50°C in a horizontal position with the agar surface uppermost for 0.5 h to 1 h.

3 The storage life of the complete medium is 24 h to 48 h.

Basal medium

Tryptone	10.0 g
Beef extract	5.0 g
Yeast extract	1.0 g
Lithium chloride hexahydrate	5.0 g
Agar	20.0 g

Dissolve the ingredients in 1 L water by steaming and adjust to pH 6.8. Dispense the medium in 90 mL amounts, and autoclave at 121°C for 15 min. The pH after autoclaving should be 6.9 ±0.1. This basal medium can be stored indefinitely at room temperature in screw-capped bottles.

Concentrated egg yolk emulsion Prepare as follows:

(a) Select clean, fresh, antibiotic-free hen eggs (less than 1 week old). Prepare by brushing in 5% soap solution, then wash well in running tap water and place in 70% ethanol for 10 min.

(b) Remove each egg, flame, break and aseptically separate the yolk from the white. Break yolk sacs and collect contents in a sterile wide-mouthed container. To one part of egg yolk add four parts of sterile water and mix thoroughly using sterile glass beads.

(c) Dispense 5 mL aliquots into sterile vials and store at not more than 5°C. For long periods store at –20°C or lower.

Solutions

Prepare the following aqueous solutions:

(a)	Glycine solution	20 g per 100 mL
(b)	Potassium tellurite solution	1 g per 100 mL
(c)	Sodium pyruvate solution	20 g per 100 mL
(d)	Sulfamethazine solution	0.2 g per 100 mL

To prepare Solution (d), dissolve 0.5 g of pure sulfamethazine in 25 mL of 0.1 mol/L sodium hydroxide solution and dilute to 250 mL with water.

Sterilize all solutions by filtration.

NOTES:

4 The literature reference states that experiments with various brands of potassium tellurite showed wide variations in the toxicity of commercially available tellurites, but different batches of BDH potassium tellurite gave consistent results.

5 The sulfamethazine is included to suppress growth of Proteus spp. and is not an essential constituent of the medium.

6 Stock solutions of 20% glycine and 1% potassium tellurite can be stored for at least 6 months at room temperature, but the 20% sodium pyruvate solution and sulfamethazine solution are best stored at 5°C and renewed monthly.

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