

Australian Standard®

AS 5013.29—2009

Food microbiology

Method 29: Examination for specific organisms— Colony count of yeasts and moulds

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 1766.2.2—1997, *Food microbiology*, Method 2.2: *Examination for specific organisms—Colony count of yeasts and moulds*.

This edition confirms the Method without technical change but updates the referenced documents and reflects current editorial style.

It is one of a series of methods for the microbiological examination of foods for quality control and investigative purposes and, where applicable, for checking that foods comply with regulatory specifications.

The laboratory should have a clearly defined quality control system to ensure that the apparatus, culture media, reagents and technique are suitable for the test. The use of positive controls is part of this system.

The term ‘normative’ has been used in this Standard to define the application of the appendix to which it applies. A ‘normative’ appendix is an integral part of a Standard.

METHOD

1 SCOPE

This Standard sets out procedures for determining the colony count of yeasts and moulds in foods.

2 REFERENCED DOCUMENTS

AS

5013 Food microbiology

5013.11 Method 11: Microbiology of food and animal feeding stuffs—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination

3 CULTURE MEDIA AND DILUENTS

3.1 General

Formulations and instructions for preparing culture media and diluents are given in the Appendix A.

3.2 Diluents

NOTE: The diluent SHOULD NOT CONTAIN NaCl, as this is deleterious to yeasts.

3.2.1 Peptone solution

0.1% or specified diluent as detailed in AS 5013 series.

3.2.2 Osmophilic diluent

20% to 30% (w/v) glucose in 0.1% peptone is recommended (see Clause 6, Reference 5).

3.3 Dichloran Rose Bengal Chloramphenicol Agar

3.4 Dichloran 18% Glycerol Agar (DG18)

4 PROCEDURE

The procedure shall be as follows:

- (a) Prepare dilutions of the sample as described in AS 5013.11, using Peptone Solution 0.1%. Use osmophilic diluent if enumerating yeasts in concentrates, syrups and other low water activity samples (3.2). Several dilutions may be required.
 - (b) Using the procedures described for the Surface Spread Method in AS 5013, prepare and inoculate spread plates according to the following:
 - (i) For high water activity foods ($a_w > 0.95$), use Dichloran Rose Bengal Chloramphenicol (DRBC) agar.
 - (ii) For reduced water activity foods ($a_w < 0.95$) use Dichloran 18% Glycerol (DG18) agar.
- NOTE: A reference to a Table on water activity of foods is given in Clause 6 (see reference 6).
- (c) Incubate the plates, upright at $25 \pm 1^\circ\text{C}$ for 5 d, unless specified otherwise in the AS 5013 series. DG18 plates should be incubated for 7 d. Examine the plates after 68 ± 4 h and then at 24 h intervals, and count the colonies on any plates likely to be overgrown before the full incubation period.
 - (d) Confirm presumptive yeast colonies by microscopic examination of each colony type, as some bacteria are capable of growth on DRBC agar.

5 TEST REPORT

The following information shall be reported:

- (a) All details necessary for the complete identification of the sample.
- (b) Reference to this Australian Standard, i.e. AS 5013.29.
- (c) Date of testing.
- (d) Any abnormality observed in the physical condition of the container or product.
- (e) Number of yeasts per millilitre or gram of material under test.
- (f) Number of moulds per millilitre or gram of material under test.
- (g) Any circumstances or conditions that may have influenced the test results.

6 BIBLIOGRAPHY

Scientific findings published in the following papers were taken into account in the preparation of this Standard:

- 1 KING, A.D., HOCKING, A.D. and PITT, J.I. Dichloran-rose bengal medium for enumeration and isolation of moulds from foods. *Appl. Environ. Microbiol.* 1979., Vol. 37, pp. 959–964.
- 2 HOCKING, A.D. and PITT, J.I. Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. *Appl. Environ. Microbiol.* 1980, Vol. 39, pp. 488–492.
- 3 BEUCHAT, L.R. Media for detecting and enumerating yeasts and moulds. *Int. J. Food Microbiol.* 1992. Vol. 17, pp. 145–158.
- 4 SAMSON, R.A., HOCKING, A.D., PITT, J.I. and KING, A.D. (eds) 1992, *Modern Methods in Food Mycology*. Elsevier, Amsterdam.
- 5 PITT, J.I., HOCKING, A.D., SAMSON, R.A. and KING, A.D. 1992. Recommended methods for mycological examination of food, 1992. In SAMSON, R.A., HOCKING, A.D., PITT, J.I. and KING, A.D. (eds) 1992. *Modern Methods in Food Mycology*, Elsevier, Amsterdam, pp. 365–368.
- 6 TROLLER, J.A., CHRISTIAN, J.H.B. *Water Activity and Foods* Academic Press, New York, San Francisco, London, 1978, p. 214.