

Australian Standard®

Food microbiology

Method 8.1: Microbiology of the food chain— Horizontal method for the detection and enumeration of *Enterobacteriaceae*—Detection of *Enterobacteriaceae* (ISO 21528-1:2017, MOD)

AS 5013.8.1:2018

PREFACE

This Standard was prepared by the Standards Australia Committee FT-035, Food Microbiology, to supersede AS 5013.8—2004, *Food microbiology, Method 8: Meat and meat products—Detection and enumeration of Enterobacteriaceae without resuscitation—MPN technique and colony-count technique*.

This Standard is an adoption with national modifications and has been reproduced from ISO 21528-1:2017, *Microbiology of the food chain — Horizontal method for the detection and enumeration of Enterobacteriaceae, Part 1: Detection of Enterobacteriaceae* and has been varied as indicated to take account of Australian conditions. The modifications are specified in Appendix ZZ.

As this Standard is reproduced from an International Standard, the following applies:

- (a) A full point substitutes for a comma when referring to a decimal marker.
- (b) Substitute ‘mL’ for ‘ml’ wherever it appears.

Australian or Australian/New Zealand Standards that are identical adoptions of international normative references may be used interchangeably. Refer to the online catalogue for information on specific Standards.

The terms ‘normative’ and ‘informative’ have been used in this Standard to define the application of the annex or appendix to which they apply. A ‘normative’ annex or appendix is an integral part of a Standard. An ‘informative’ annex or appendix is only for information and guidance.

CONTENTS

	Page
Foreword	iii
Introduction	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
4.1 Enrichment in non-selective medium	2
4.2 Isolation and selection for confirmation	2
4.3 Confirmation	2
5 Diluent, culture media and reagent	2
6 Equipment and consumables	2
7 Sampling	3
8 Preparation of test sample	3
9 Procedure	3
9.1 General	3
9.2 Test portion and initial suspension	4
9.3 Enrichment	4
9.4 Isolation and selection for confirmation	4
9.4.1 Isolation	4
9.4.2 Selection of colonies for confirmation	4
9.5 Subculturing selected colonies	4
9.6 Biochemical confirmation tests	4
9.6.1 Oxidase reaction	4
9.6.2 Fermentation test	5
10 Expression of results	5
11 Precision	5
11.1 Interlaboratory study	5
11.2 Sensitivity	5
11.3 Specificity	5
12 Test report	5
13 Quality assurance	6
Annex A (informative) Enumeration by MPN technique	7
Annex B (normative) Culture media and reagents	10
Annex C (informative) Method validation studies and performance characteristics	15
Bibliography	17

FOREWORD

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 21528-1:2004), which has been technically revised with the following main changes:

- the MPN method has become an informative [Annex A](#);
- the pre-enrichment step in BPW followed by enrichment in EE broth has been changed to enrichment in BPW^[Z] and confirmation now takes place in Glucose OF medium instead of using glucose agar;
- performance testing for the quality assurance of the culture media has been added;
- performance characteristics for this method have been added to [Annex C](#).

A list of all the parts in the ISO 21528 series can be found on the ISO website.

INTRODUCTION

This document is intended to provide general guidance for the examination of products not dealt with by existing International Standards and to be taken into account by organizations preparing microbiological test methods for application to foods or animal feeding stuffs. Because of the large variety of products within this field of application, these guidelines may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases, every attempt will be made to apply the guidelines provided as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

The main changes, listed in the foreword, introduced in this document compared to ISO 21528-1:2004 are considered as major (see ISO 17468).

The harmonization of test methods cannot be immediate, and for certain groups of products, International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed, they will be changed to comply with this document so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

AUSTRALIAN STANDARD

Food microbiology

Microbiology of the food chain—Horizontal method for the detection and enumeration of *Enterobacteriaceae*—Detection of *Enterobacteriaceae* (ISO 21528-1:2017, MOD)

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Enterobacteriaceae* are only undertaken in properly equipped laboratories under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

1 Scope

This document specifies a method, with enrichment, for the detection of *Enterobacteriaceae*. It is applicable to

- products intended for human consumption and the feeding of animals, and
- environmental samples in the area of primary production, food production and food handling.

This method is applicable

- when the microorganisms sought are expected to need resuscitation by enrichment, and
- when the number sought is expected to be below 100 per millilitre or per gram of test sample.

A limitation on the applicability of this document is imposed by the susceptibility of the method to a large degree of variability (see [Clause 11](#)).

NOTE Enumeration can be carried out by calculation of the most probable number (MPN) after incubation in liquid medium. See [Annex A](#).

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. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

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