
**Microbiology of the food chain —
Horizontal method for the
detection and enumeration of
Enterobacteriaceae —**

**Part 2:
Colony-count technique**

*Microbiologie de la chaîne alimentaire — Méthode horizontale
pour la recherche et le dénombrement des Enterobacteriaceae —
Partie 2: Technique par comptage des colonies*



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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 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 21528-2:2004), which has been technically revised with the following main changes:

- the confirmation step has been changed by replacing glucose agar by glucose OF medium;
- precision data based on the results of an interlaboratory study using the method according to this revised edition has been included in an informative annex.

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

This corrected version of ISO 21528-2:2017 incorporates the following corrections:

- in [6.4](#), a water bath capable of being maintained between 44 °C to 47 °C has been added to the list of equipment;
- in [9.3.2](#), the temperature has been reduced from “47 °C to 50 °C” to “44 °C to 47 °C”.

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 21528-2:2004, are considered as minor changes (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.

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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 for the enumeration 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 technique is intended to be used when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample.

The most probable number (MPN) technique, as included in ISO 21528-1, is generally used when the number sought is expected to be below 100 per millilitre or per gram of test sample.

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*

ISO 18593, *Microbiology of food and animal feeding stuffs — Horizontal methods for sampling techniques from surfaces using contact plates and swabs*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>