

---

---

**Microbiology of food and animal feeding  
stuffs — Horizontal methods for the  
detection and enumeration of  
Enterobacteriaceae —**

Part 2:

**Colony-count method**

iTeh STANDARD PREVIEW  
(standards.iteh.ai)

*Microbiologie des aliments — Méthodes horizontales pour la recherche  
et le dénombrement des Enterobacteriaceae —*

*Partie 2: Méthode par comptage des colonies*

<https://standards.iteh.ai/catalog/standards/sist/62421689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004>



**PDF disclaimer**

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[ISO 21528-2:2004](https://standards.iteh.ai/catalog/standards/sist/6242f689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004)

<https://standards.iteh.ai/catalog/standards/sist/6242f689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004>

© ISO 2004

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
Case postale 56 • CH-1211 Geneva 20  
Tel. + 41 22 749 01 11  
Fax + 41 22 749 09 47  
E-mail [copyright@iso.org](mailto:copyright@iso.org)  
Web [www.iso.org](http://www.iso.org)

Published in Switzerland

## Contents

Page

Foreword .....	iv
Introduction .....	v
1 Scope .....	1
2 Normative references .....	1
3 Terms and definitions .....	2
4 Principle .....	2
4.1 Preparation of initial suspension and decimal dilutions .....	2
4.2 Isolation .....	2
4.3 Confirmation .....	2
4.4 Calculation .....	2
5 Diluent, culture media and reagent .....	3
6 Apparatus and glassware .....	5
7 Sampling .....	5
8 Preparation of test sample .....	6
9 Procedure .....	6
9.1 General .....	6
9.2 Test portion, initial suspension and dilutions .....	6
9.3 Inoculation and incubation .....	6
9.4 Counting and selection of colonies for confirmation .....	6
9.5 Subculturing selected colonies .....	7
9.6 Biochemical confirmation tests .....	7
10 Expression of results .....	7
11 Test report .....	8
Annex A (normative) Confidence limits for the estimation of small numbers of colonies .....	9
Bibliography .....	10

## 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 21528-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This first edition of ISO 21528-2, together with ISO 21528-1:2004, cancels and replaces the following standards:

- ISO 5552:1997, *Meat and meat products — Detection and enumeration of Enterobacteriaceae without resuscitation — MPN technique and colony-count technique*,  
<https://standards.iteh.ai/catalog/standards/sis/6242f689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004>
- ISO 7402:1993, *Microbiology — General guidance for the enumeration of Enterobacteriaceae without resuscitation — MPN technique and colony-count technique*;
- ISO 8523:1991, *Microbiology — General guidance for the detection of Enterobacteriaceae with pre-enrichment*.

ISO 21528 consists of the following parts, under the general title *Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae*:

- *Part 1: Detection and enumeration by MPN technique with pre-enrichment*
- *Part 2: Colony-count method*

## Introduction

This part of ISO 21528 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.

When this part of ISO 21528 is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons for deviation from them in the case of particular products.

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 part of ISO 21528 so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

## iTeh STANDARD PREVIEW (standards.iteh.ai)

[ISO 21528-2:2004](https://standards.iteh.ai/catalog/standards/sist/6242f689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004)

<https://standards.iteh.ai/catalog/standards/sist/6242f689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

ISO 21528-2:2004

<https://standards.iteh.ai/catalog/standards/sist/6242f689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004>

# Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae —

## Part 2: Colony-count method

### 1 Scope

This part of ISO 21528 specifies a method, without pre-enrichment, 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 food production and food handling.

Enumeration is carried out by counting colonies in a solid medium after incubation at 37 °C (or 30 °C)<sup>1</sup>.

This technique is recommended when the number of colonies sought is expected to be more than 100 per millilitre or per gram of the test sample.

### 2 Normative references

The following referenced documents are indispensable for the application 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 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1 : General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products*

1) The temperature of 37 °C is generally used when the enumeration of Enterobacteriaceae is for a hygienic indicator. Alternatively, a temperature of 30 °C can be chosen when the enumeration of Enterobacteriaceae is conducted for technological purposes and includes psychrotrophic Enterobacteriaceae.

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*, and Amendment 1:2001.

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2:2003, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

### 3 Terms and definitions

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

**3.1 Enterobacteriaceae**  
microorganisms that form characteristic colonies on violet red bile glucose agar and that ferment glucose and show a negative oxidase reaction when the tests are carried out in accordance with the methods specified in this part of ISO 21528

**3.2 count of Enterobacteriaceae**  
number of Enterobacteriaceae found per millilitre or per gram of the test sample when the test is carried out according to the method specified in this part of ISO 21528

iTeh STANDARD PREVIEW  
(standards.iteh.ai)  
ISO 21528-2:2004  
<https://standards.iteh.ai/catalog/standards/sist/6242f689-2aa1-47b6-bc89-a4276131247f/iso-21528-2-2004>

### 4 Principle

#### 4.1 Preparation of initial suspension and decimal dilutions

An initial suspension and decimal dilutions are prepared from the test sample.

#### 4.2 Isolation

Violet red bile glucose agar contained in two Petri dishes (poured-plate technique) is inoculated with a specified quantity of the test sample if the product is liquid, or of the initial suspension in the case of other products. An overlay of the same medium is added.

Other pairs of plates are prepared under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

The dishes are incubated at 37 °C (or 30 °C)<sup>1)</sup> for 24 h ± 2 h.

#### 4.3 Confirmation

Subculture of colonies of presumptive Enterobacteriaceae on non-selective medium, and confirmation by means of tests for fermentation of glucose and presence of oxidase.

#### 4.4 Calculation

The number of Enterobacteriaceae per millilitre or gram of the test sample is calculated from the number of confirmed typical colonies per dish.



## 5 Diluent, culture media and reagent

For current laboratory practice, see ISO 7218, ISO/TS 11133-1 and ISO/TS 11133-2.

### 5.1 Diluent

See ISO 6887-1.

### 5.2 Culture media

#### 5.2.1 Violet red bile glucose (VRBG) agar

##### 5.2.1.1 Composition

Enzymatic digest of animal tissues	7,0 g
Yeast extract	3,0 g
Bile salts No. 3	1,5 g
Glucose	10,0 g
Sodium chloride	5,0 g
Neutral red	0,03 g
Crystal violet	0,002 g
Agar	9 g to 18 g <sup>a</sup>
Water	1 000 ml
<sup>a</sup> Depending on the gel strength of the agar.	

iTeh STANDARD PREVIEW  
(standards.iteh.ai)

ISO 21528-2:2004

##### 5.2.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling.

Adjust the pH, if necessary, so that after boiling it is  $7,4 \pm 0,2$  at 25 °C.

Dispense the culture medium into sterile tubes or flasks (6.5) of capacity not more than 500 ml.

Do not sterilize the medium.

Prepare the medium just before use. Use the molten medium within 4 h of its preparation.

##### 5.2.1.3 Performance testing for the quality assurance of the culture medium

For the definition of selectivity and productivity refer to ISO/TS 11133-1. For the performance criteria, refer to ISO/TS 11133-2:2003, Table B.1.

### 5.2.2 Nutrient agar

#### 5.2.2.1 Composition

Meat extract	3,0 g
Enzymatic digest of animal tissues	5,0 g
Sodium chloride	5,0 g
Agar	9 g to 18 g <sup>a</sup>
Water	1 000 ml
<sup>a</sup> Depending on the gel strength of the agar.	