INTERNATIONAL STANDARD

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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

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

Partie 1: Recherche et dénombrement à l'aide de la technique NPP avec préenrichissement

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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
Web www.iso.org

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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.

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.

This first edition of ISO 21528-1, together with ISO 21528-2, 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;*

— 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 21528-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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.

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

1 Scope

This part of ISO 21528 specifies a method, with pre-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 food production and food handling.

Enumeration is carried out by calculation of the most probable number (MPN) after incubation at 37 °C (or 30 °C)[1] in liquid medium.

This method is applicable

— when the microorganisms sought are expected to need resuscitation before enrichment, and

— when the number sought is expected to be in the range 1 to 100 per millilitre or per gram of test sample.

A limitation on the applicability of this part of ISO 21528 is imposed by the susceptibility of the method to a large degree of variability (see Clause 11).

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 6887-1:1999, *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*

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 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*

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

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
detection of Enterobacteriaceae
determination of the presence or absence of these bacteria, in a particular quantity of product, when tests are carried out in accordance with this part of ISO 21528

3.3
enumeration of Enterobacteriaceae
most probable 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

4 Principle

4.1 Detection of Enterobacteriaceae (see Annex A)

4.1.1 Pre-enrichment in non-selective medium

Buffered peptone water (BPW) is inoculated with the test portion, then incubated at 37 °C (or 30 °C)[1)] for 18 h ± 2 h.

4.1.2 Enrichment in selective liquid medium

The enrichment broth [buffered brilliant green bile glucose broth (EE broth)] is inoculated with the culture obtained after pre-enrichment, then incubated at 37 °C (or 30 °C)[1)] for 24 h ± 2 h.

4.1.3 Isolation and selection for confirmation

A selective solid medium (violet red bile glucose agar) is inoculated with the culture obtained after enrichment in EE broth, then incubated at 37 °C (or 30 °C)[1)]. It is examined after 24 h ± 2 h to check for the presence of colonies presumed by their characteristics to be Enterobacteriaceae.

4.1.4 Confirmation

Colonies of presumptive Enterobacteriaceae are subcultured onto non-selective medium, and confirmed by means of tests for the fermentation of glucose and the presence of oxidase.

4.2 Enumeration by the MPN technique (see Annex B)

4.2.1 Pre-enrichment in non-selective medium

A test portion of x g is added to 9 x ml of buffered peptone water (BPW) and homogenized. One or more 10-fold dilutions (according to the expected level of contamination) are prepared in BPW. Aliquots (10 ml) of this initial dilution are transferred to three tubes. Then 3 × 1 ml of the initial dilution are added to 9 ml of BPW and 3 × 1 ml of each further dilution are added to 9 ml of BPW. These tubes are incubated 37 °C (or 30 °C)[1)] for 18 h ± 2 h.

4.2.2 Enrichment in selective liquid medium

Tubes of liquid enrichment broth (EE broth) are inoculated with each tube of culture obtained after pre-enrichment (at least 3 × 3). The tubes are incubated at 37 °C (or 30 °C)[1)] for 24 h ± 2 h.

4.2.3 Isolation and selection for confirmation

A selective solid medium (violet red bile glucose agar) is inoculated with a loop from each of the incubated cultures obtained after enrichment in EE broth, then incubated at 37 °C (or 30 °C)[1)]. It is examined after 24 h ± 2 h to check for the presence of colonies presumed by their characteristics to be Enterobacteriaceae.

4.2.4 Confirmation

Colonies of presumptive Enterobacteriaceae are subcultured on non-selective medium, then confirmed by means of tests for the fermentation of glucose and the presence of oxidase.

4.2.5 Calculation

The most probable number of Enterobacteriaceae per millilitre or per gram of the test sample is calculated from the number of confirmed positive tubes using the MPN table (see ISO 7218).

5 Diluent, culture media and reagent

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

5.1 Diluent: buffered peptone water (BPW)

See ISO 6887-1:1999, 5.2.2.

BPW is used as the non-selective pre-enrichment medium for the enumeration method.

5.2 Culture media

5.2.1 Enrichment medium: Buffered brilliant green bile glucose broth (EE broth)

5.2.1.1 Composition

Enzymatic digest of animal tissues	10,0 g
Glucose	5,0 g
Disodium hydrogen phosphate (Na_2HPO_4)	6,45 g
Potassium dihydrogen phosphate (KH_2PO_4)	2,0 g
Beef bile for bacteriological use	20,0 g
Brilliant green (bacteriological quality)	0,0135 g
Water	1 000 ml

5.2.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by boiling. Do not heat the medium for longer than 30 min. Cool the medium rapidly.

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

Dispense the medium in 10 ml amounts into sterile tubes of appropriate capacity (6.5).

Do not sterilize the medium.

The medium may be stored for up to 1 month at 5 °C ± 3 °C.

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.3.

5.2.2 Violet red bile glucose (VRBG) agar

5.2.2.1 Components

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[a)]
Water	1 000 ml

[a)] Depending on the gel strength of the agar.

5.2.2.2 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 ± 0,2 at 25 °C.

Dispense the culture medium into sterile tubes or flasks (6.5) of appropriate capacity.

Do not sterilize the medium.

Use the molten medium within 4 h of its preparation.

5.2.2.3 Preparation of agar plates

Immediately transfer approximately 15 ml of the culture medium, cooled to between 44 °C and 47 °C (6.4), to Petri dishes (6.7) and allow to solidify.

Just before use, dry the plates, preferably with the lids off and the agar surface downwards, in a drying cabinet (6.3) until the agar is dry.

If prepared in advance, the undried plates may be stored in conditions that do not change their composition for up to 2 weeks at 5 °C ± 3 °C.

5.2.2.4 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.

5.2.3 Nutrient agar

5.2.3.1 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 [a)]
Water	1 000 ml

[a)] Depending on the gel strength of the agar.

5.2.3.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 7,3 ± 0,2 at 25 °C.

Dispense the culture medium into sterile tubes or flasks (6.5) of appropriate capacity.

Sterilize for 15 min in an autoclave (6.1) set at 121 °C.

5.2.3.3 Preparation of agar plates

Transfer portions of about 15 ml of the culture medium, melted and cooled to approximately 47 °C, to Petri dishes (6.7) and allow to solidify.