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Microbiology — General guidance for the detection of *Enterobacteriaceae* with pre-enrichment

*Microbiologie — Directives générales pour la recherche des
Enterobacteriaceae avec pré-enrichissement*



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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.

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.

International Standard ISO 8523 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Sub-Committee SC 9, *Microbiology*.

Annex A forms an integral part of this International Standard.

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Introduction

This International Standard is intended to provide general guidance for the microbiological examination of food products not dealt with by existing International Standards and for reference for bodies preparing microbiological methods of test for application to foods or to animal feeding stuffs.

Because of the large variety of products within this field of application, these guidelines may not be appropriate for some products in every detail, 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 provided guidelines as far as possible, and that deviations from them will only be made if absolutely necessary for technical reasons.

When this International Standard 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 these guidelines. In cases where International Standards already exist for the product to be tested, they should be followed¹⁾. However, it is hoped that when such standards are reviewed they will be changed to comply with this International Standard so that, eventually, the only remaining departures from these guidelines will be those necessary for well-established technical reasons.

To allow for national practice, the test method given here may be carried out at an incubation temperature of either 35 °C or 37 °C.

NOTE 1 The technique described in this International Standard can be applied for carrying out an enumeration using the most probable number (MPN) technique. For this it is only necessary to carry out the presence/absence test, described in this International Standard, in replicate at several dilutions (as prescribed, for example, in the product specification). For the interpretation of the results obtained, reference should be made to the MPN tables given in ISO 7218.

1) For meat and meat products, see ISO 5552:1979, *Meat and meat products – Detection and enumeration of Enterobacteriaceae (Reference methods)*.

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1 Scope

This International Standard gives general guidelines for the detection of *Enterobacteriaceae* with pre-enrichment in products intended for human consumption or feeding of animals.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 7218:1985, *Microbiology — General guidance for microbiological examinations*.

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 *Enterobacteriaceae*: Micro-organisms which ferment glucose and show a negative oxidase reaction when the test is carried out in accordance with the method specified in this International Standard.

3.2 detection of *Enterobacteriaceae*: Determination of the presence or absence of *Enterobacteriaceae* in a particular mass of product, when tests are carried out in accordance with the method specified in this International Standard.

4 Principle

In general, the detection of *Enterobacteriaceae* requires four successive stages (see also annex A).

4.1 Pre-enrichment in non-selective liquid medium

Inoculation of buffered peptone water (also used as diluent) with the test portion, and incubation at 35 °C or 37 °C for 16 h to 20 h.

The temperature shall be agreed by the parties concerned and shall be indicated in the test report.

4.2 Enrichment in selective liquid medium

Inoculation of enrichment broth with the culture obtained in 4.1.

Incubation at 35 °C or 37 °C (as agreed) for 24 h.

4.3 Plating out and identification

From the cultures obtained in 4.2, inoculation of a selective solid medium (violet red bile glucose agar).

Incubation at 35 °C or 37 °C (as agreed) and examination after 24 h to check for the presence of colonies presumed to be *Enterobacteriaceae* by their characteristics.

4.4 Confirmation

Subculturing of colonies of presumptive *Enterobacteriaceae* (4.3) on non-selective medium, and confirmation by means of appropriate biochemical tests.