



BSI Standards Publication

**Microbiology of food and animal feeding
stuffs — Horizontal method for the detection
and enumeration of presumptive *Escherichia
coli* — Most probable number technique**

National foreword

This British Standard is the UK implementation of ISO 7251:2005+A1:2023. It supersedes [BS ISO 7251:2005](#), which is withdrawn.

The start and finish of text introduced or altered by amendment is indicated in the text by tags. Tags indicating changes to ISO text carry the number of the ISO amendment. For example, text altered by ISO amendment 1 is indicated by A1 A1.

The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology.

A list of organizations represented on this committee can be obtained on request to its committee manager.

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient’s own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

© The British Standards Institution 2023
Published by BSI Standards Limited 2023

ISBN 978 0 539 18287 3

ICS 07.100.30

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 5 May 2005.

Amendments/corrigenda issued since publication

Date	Text affected
31 October 2023	Implementation of ISO amendment 1:2023

INTERNATIONAL STANDARD

ISO
7251

Third edition
2023-10-12

Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *Escherichia coli* — Most probable number technique

*Microbiologie des aliments — Méthode horizontale pour la recherche
et le dénombrement d'*Escherichia coli* présumés — Technique du
nombre le plus probable*



Reference number
ISO 7251:2023(E)
Documents
Document Sharing Hub
© ISO 2023



COPYRIGHT PROTECTED DOCUMENT

© ISO 2023, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle	2
4.1 Detection method	2
4.2 Enumeration method	2
5 Diluent, culture media and reagent	3
5.1 Diluent	3
5.2 Selective enrichment medium (lauryl sulfate broth)	3
5.2.1 Composition	3
5.2.2 Preparation	4
5.3 EC broth (selective medium)	4
5.3.1 Composition	4
5.3.2 Preparation	4
5.3.3 Performance testing and quality assurance of the culture medium	4
5.4 Peptone water, indole free	4
5.4.1 Composition	4
5.4.2 Preparation	4
5.5 Indole reagent (Kovacs' reagent)	5
5.5.1 Composition	5
5.5.2 Preparation	5
A) 5.5 Performance testing	5
6 Apparatus and glassware	6
6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)	6
7 Sampling	7
8 Preparation of test sample	7
9 Procedure	7
9.1 Detection method	7
9.1.1 Test portion and initial suspension	7
9.1.2 Incubation of the selective enrichment medium (lauryl sulfate broth)	7
9.1.3 Inoculation and incubation of the selective medium (EC broth)	7
9.1.4 Inoculation and incubation of the peptone water	8
9.1.5 Examination for indole production	8
9.1.6 Interpretation	8
9.2 Enumeration method	8
9.2.1 Test portion, initial suspension and dilutions	8
9.2.2 Inoculation and incubation of the selective enrichment medium (lauryl sulfate broth)	8
9.2.3 Subculturing and incubation of the selective medium (EC broth)	9
9.2.4 Inoculation and incubation of the peptone water	9
9.2.5 Examination for indole production	9
9.2.6 Interpretation	9
10 Expression of results	9
10.1 Detection method	9
10.2 Enumeration method	9
11 Test report	9
Annex A (normative) MPN table	11

Bibliography	14
---------------------------	-----------

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 7251](#) was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This third edition cancels and replaces the second edition (ISO 7251:1993), and also [ISO 11866-1:1997](#) and IDF 170-1:1999.

Clauses 4, 9 and 10 of ISO 7251:1993 have been technically revised. The main changes are as follows:

- the temperature of the second incubation is now $44\text{ °C} \pm 1\text{ °C}$ (see [4.2.5](#));
- detection ([9.1](#)) and enumeration ([9.2](#)) of presumptive *E. coli* are covered;
- the use of five tubes per dilution is allowed for some specific products (see [9.2.2.1](#));
- some products (dairy products) may hinder the collection of gas (see [9.1.2](#) and [9.2.2.5](#));
- the MPN calculation refers to ISO 7218 (see [Clause 10](#)).

Introduction

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 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 deviations 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 followed, but 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.