

Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp

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National foreword

This British Standard is the UK implementation of EN ISO 6579:2002+A1:2007. It is identical with ISO 6579:2002, incorporating amendment 1:2007 and corrigendum April 2004. It supersedes BS EN ISO 6579:2002, BS EN 12824:1998 and BS 4285-3.9.1:1987, which are withdrawn.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 secretary.

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Microbiologie des aliments - Méthode horizontale pour la
recherche des *Salmonella* spp (ISO 6579:2002)

Mikrobiologie von Lebensmitteln und Futtermitteln -
Horizontales Verfahren zum Nachweis von Salmonellen
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Foreword

This document (ISO 6579:2002) has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2003, and conflicting national standards shall be withdrawn at the latest by January 2003.

This document supersedes EN 12824:1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of ISO 6579:2002 has been approved by CEN as EN ISO 6579:2002 without any modifications.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

Foreword to amendment A1

This document (EN ISO 6579:2002/A1:2007) has been prepared by Technical Committee ISO/TC 34 "Agricultural food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

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According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Endorsement notice

The text of ISO 6579:2002 has been approved by CEN as EN ISO 6579:2002/A1:2007 without any modifications.

INTERNATIONAL STANDARD

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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

*Microbiologie des aliments — Méthode horizontale pour la recherche des
Salmonella spp.*



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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 6579 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This fourth edition cancels and replaces the third edition (ISO 6579:1993), which has been technically revised.

Annexes A and B form a normative part of this International Standard. Annex C is for information only.

Introduction

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products, may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this method 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 International Standard so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Salmonella*, and especially *Salmonella* Typhi and *Salmonella* Paratyphi, 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.

1 Scope

This International Standard specifies a horizontal method for the detection of *Salmonella*, including *Salmonella* Typhi and *Salmonella* Paratyphi.

Subject to the limitations discussed in the Introduction, this International Standard is applicable to

- products intended for human consumption and the feeding of animals;
- environmental samples in the area of food production and food handling.

WARNING — The method may not recover all *Salmonella* Typhi and Paratyphi.

2 Normative references

[A1] 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. **[A1]**

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 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*

[A1] 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* **[A1]**

3 Terms and definitions

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

3.1

Salmonella

microorganisms which form typical or less typical colonies on solid selective media and which display the biochemical and serological characteristics described when tests are carried out in accordance with this International Standard

3.2

detection of *Salmonella*

determination of the presence or absence of *Salmonella* (3.1), in a particular mass or volume of product, when tests are carried out in accordance with this International Standard

4 Principle

4.1 General

The detection of *Salmonella* necessitates four successive stages (see also annex A).

NOTE The *Salmonella* may be present in small numbers and are often accompanied by considerably larger numbers of other *Enterobacteriaceae* or other families. Furthermore, pre-enrichment is necessary to permit the detection of low numbers of *Salmonella* or injured *Salmonella*.

4.2 Pre-enrichment in non-selective liquid medium

Buffered peptone water is inoculated at ambient temperature with the test portion, then incubated at $37\text{ °C} \pm 1\text{ °C}$ for $18\text{ h} \pm 2\text{ h}$.

For certain foodstuffs the use of other pre-enrichment procedures is necessary. See 9.1.2.

For large quantities, the buffered peptone water should be heated to $37\text{ °C} \pm 1\text{ °C}$ before inoculation with the test portion.

4.3 Enrichment in selective liquid media

Rappaport-Vassiliadis medium with soya (RVS broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTn) are inoculated with the culture obtained in 4.2.

The RVS broth is incubated at $41,5\text{ °C} \pm 1\text{ °C}$ for $24\text{ h} \pm 3\text{ h}$, and the MKTn broth at $37\text{ °C} \pm 1\text{ °C}$ for $24\text{ h} \pm 3\text{ h}$.

4.4 Plating out and identification

From the cultures obtained in 4.3, two selective solid media are inoculated:

- xylose lysine deoxycholate agar (XLD agar);
- any other solid selective medium complementary to XLD agar and especially appropriate for the isolation of lactose-positive *Salmonella* and *Salmonella* Typhi and *Salmonella* Paratyphi strains; the laboratory may choose which medium to use.

The XLD agar is incubated at $37\text{ °C} \pm 1\text{ °C}$ and examined after $24\text{ h} \pm 3\text{ h}$. The second selective agar is incubated according to the manufacturer's recommendations.

NOTE For information, Brilliant green agar (BGA), bismuth sulfite agar, etc., could be used as the second plating-out medium.

4.5 Confirmation of identity

Colonies of presumptive *Salmonella* are subcultured, then plated out as described in 4.4, and their identity is confirmed by means of appropriate biochemical and serological tests.

5 Culture media, reagents and sera

5.1 General

For current laboratory practice, see ISO 7218.

5.2 Culture media and reagents

NOTE Because of the large number of culture media and reagents, it is considered preferable, for clarity, to give their compositions and preparations in annex B.

5.2.1 Non-selective pre-enrichment medium: Buffered peptone water

See B.1.

5.2.2 First selective enrichment medium: Rappaport-Vassiliadis medium with soya (RVS broth)

See B.2.

5.2.3 Second selective enrichment medium: Muller-Kauffmann tetrathionate novobiocin broth (MKTn broth)

See B.3.

5.2.4 Solid selective plating-out media

5.2.4.1 First medium: Xylose lysine deoxycholate agar (XLD agar)

See B.4.

5.2.4.2 Second medium

The choice of the second appropriate medium is left to the discretion of the testing laboratory. The manufacturer's instructions should be precisely followed regarding its preparation for use.

5.2.5 Nutrient agar

See B.5.

5.2.6 Triple sugar/iron agar (TSI agar)

See B.6.

5.2.7 Urea agar (Christensen)

See B.7.

5.2.8 L-Lysine decarboxylation medium

See B.8.

5.2.9 Reagent for detection of β -galactosidase (or prepared paper discs used in accordance with the manufacturer's instructions)

See B.9.

5.2.10 Reagents for Voges-Proskauer (VP) reaction

See B.10.

5.2.11 Reagents for indole reaction

See B.11.

5.2.12 Semi-solid nutrient agar

See B.12.

5.2.13 Physiological saline solution

See B.13.

5.3 Sera

Several types of agglutinating sera containing antibodies for one or several O-antigens are available commercially; i.e. anti-sera containing one or more "O" groups (called monovalent or polyvalent anti-O sera), anti-Vi sera, and anti-sera containing antibodies for one or several H-factors (called monovalent or polyvalent anti-H sera).

Every attempt should be made to ensure that the anti-sera used are adequate to provide for the detection of all *Salmonella* serotypes. Assistance towards this objective may be obtained by using only anti-sera prepared by a supplier recognized as competent (for example, by an appropriate government agency).

6 Apparatus and glassware

Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)

See ISO 7218.

6.2 Drying cabinet or oven, ventilated by convection, capable of operating between 37 °C and 55 °C.

6.3 Incubator, capable of operating at 37 °C ± 1 °C.

6.4 Water bath, capable of operating at 41,5 °C ± 1 °C, or **incubator**, capable of operating at 41,5 °C ± 1 °C.

6.5 Water baths, capable of operating at 44 °C to 47 °C.

6.6 Water bath, capable of operating at 37 °C ± 1 °C.

It is recommended to use a water bath (6.4, 6.5 and 6.6) containing an antibacterial agent because of the low infective dose of *Salmonella*.

6.7 Sterile loops, of diameter approximately 3 mm or 10 µl, or **sterile pipettes**.

6.8 pH-meter, having an accuracy of calibration of ± 0,1 pH unit at 20 °C to 25 °C.

6.9 Test tubes or flasks, of appropriate capacity.

Bottles or flasks with non-toxic metallic or plastic screw-caps may be used.