

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

The European Standard EN 12824 : 1997 has the status of a
British Standard

ICS 07.100.30

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Summary of pages

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English version

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

(ISO 6579 : 1993 modified)

Microbiologie des aliments — Méthode horizontale
pour la recherche de *Salmonella*
(ISO 6579 : 1993 modifiée)

Microbiologie von Lebensmitteln und
Futtermitteln — Horizontales Verfahren zum
Nachweis von Salmonellen
(ISO 6579 : 1993 modifiziert)

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CEN

European Committee for Standardization
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Foreword

This European Standard has been prepared by 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of an identical text or by endorsement, at the latest by May 1998, and conflicting national standards shall be withdrawn at the latest by May 1998.

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, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

In June 1995 the Standard ISO 6579 : 1993 was submitted to Questionnaire Procedure of CEN.

In view of the Questionnaire results, the Technical Board of CEN decided not to take over ISO 6579 : 1993 unchanged but to publish this European Standard EN 12824 : 1997 with agreed common modifications as indicated by a vertical line in the margin of the text.

The main modifications are as follows :

- the possibility to incubate the Rappaport-Vassiliadis magnesium chloride/malachite green medium for a further 24 h; and
- the introduction of specific pre-enrichment procedures for some food products.

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Introduction

This European Standard is intended to provide general guidance for the examination of products not dealt with by existing European Standards and to be taken into account by organizations 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, this guidance may not be appropriate in every detail for certain products, and for 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 European Standard is next reviewed, account will be taken of all information then available regarding the extent to which the guidance have been followed and the reasons for deviation from in the case of particular products.

The harmonization of test methods cannot be immediate and, for certain groups of products, European Standards and/or national standards may already exist that do not comply with this guidance. In cases where European 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 European Standard so that, eventually, the only remaining departures from this guidance those necessary for well-established technical reasons.

Warning. In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Salmonella* 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 European Standard specifies a horizontal method for the detection of *Salmonella*.

Subject to the limitations discussed in the Introduction, this European Standard is applicable to products intended for human consumption or feeding of animals.

The incubation temperature (35 °C or 37 °C) is subject to agreement by the parties concerned and is specified in the test report.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

- ISO 6887 *Microbiology — General guidance for the preparation of dilutions for microbiological examination*
- ISO 7218 *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

3 Definitions

For the purposes of this European Standard, the following definitions apply:

3.1 *Salmonella*

Micro-organisms which form typical colonies on solid selective media and which display the biochemical and serological characteristics described when tests are carried out in accordance with this European Standard.

3.2 detection of *Salmonella*

Determination of the presence or absence of these micro-organisms, in a particular mass of product, when tests are carried out in accordance with this European Standard.

4 Principle

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

NOTE. *Salmonella* may be present in small numbers and are often accompanied by considerably larger numbers of other members of *Enterobacteriaceae* or of other families. Therefore, selective enrichment is necessary; furthermore, pre-enrichment is often necessary to permit detection of injured *Salmonella*.

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 (as agreed) for 16 h to 20 h.

For certain foodstuffs, other pre-enrichment procedures shall be used. See 9.1.3.

4.2 Enrichment in selective liquid media

Inoculation of the Rappaport-Vassiliadis magnesium chloride/malachite green medium and of a selenite/cystine medium with the culture obtained in 4.1.

Incubation of the Rappaport-Vassiliadis magnesium chloride/malachite green medium at 42 °C for 24 h and incubation of the selenite/cystine medium at 35 °C or 37 °C (as agreed) for 24 h and a further 24 h.

NOTE. In some cases, the Rappaport-Vassiliadis magnesium chloride/malachite green medium may need to be further incubated for 24 h at 42 °C. This further incubation should, in this case, be noted in the test report.

4.3 Plating out and recognition

From the cultures obtained in 4.2, inoculation of two selective solid media:

- phenol red/brilliant green agar, unless the European Standard appropriate to the product to be examined, or other specific considerations (for example the isolation of lactose-positive *Salmonella*), require substitution of some other medium as the one for obligatory use;
- any other solid selective medium (see 5.2.4.2).

Incubation at 35 °C or 37 °C (as agreed), and examination after 24 h and, if necessary, after 48 h to check for the presence of colonies which are considered to be presumptive *Salmonella*.

4.4 Confirmation

Subculturing of colonies of presumptive *Salmonella*, plated out as described in 4.3, and confirmation 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 has been considered preferable, for the clarity of the text, to give their composition and preparation 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 magnesium chloride/malachite green medium (RV medium)*

See B.2.

5.2.3 Second selective enrichment medium: *Selenite/cystine medium*

See B.3.

5.2.4 Solid selective plating-out media

5.2.4.1 First medium: *Phenol red/brilliant green agar (Edel and Kampelmacher)*

See B.4.

This first medium is compulsory unless otherwise stated (see 4.3).

5.2.4.2 Second medium

The choice of the second medium is left to the discretion of the testing laboratory. Attention is drawn to the fact that a specific European Standard relating to the product to be examined, which specifies the composition of this second medium.

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.

5.2.10.1 VP medium

5.2.10.2 Creatine solution (N-amidininosarcosine)

5.2.10.3 1-Naphthol, ethanolic solution

5.2.10.4 Potassium hydroxide solution

5.2.11 Reagents for indole reaction

See B.11.

5.2.11.1 Tryptone-tryptophan medium

5.2.11.2 Kovacs reagent

(*N, N*-dicyclohexyl-carbodiimide pentachlorophenol complex)

5.2.12 Semi-solid nutrient agar

See B.12.

5.2.13 Saline solution

See B.13.

5.3 Sera

Several types of agglutinant 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 anti-sera prepared by a supplier recognized as competent (for example, by an appropriate government agency).