Irish Standard
I.S. EN ISO 6579-1:2017

Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. (ISO 6579-1:2017)

I.S. EN ISO 6579-1:2017

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National Foreword

I.S. EN ISO 6579-1:2017 is the adopted Irish version of the European Document EN ISO 6579-1:2017, Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. (ISO 6579-1:2017)

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 6579-1

March 2017

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Supersedes EN ISO 6579:2002

English Version

Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. (ISO 6579-1:2017)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella - Partie 1: Recherche des Salmonella spp. (ISO 6579-1:2017)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zum Nachweis, zur Zählung und zur Serotypisierung von Salmonellen - Teil 1: Nachweis von Salmonella spp. (ISO 6579-1:2017)

This European Standard was approved by CEN on 3 February 2017.

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European foreword

This document (EN ISO 6579-1:2017) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 "Food products".

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of an identical text or by endorsement, at the latest by September 2017 and conflicting national standards shall be withdrawn at the latest by September 2017.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 6579:2002.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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Endorsement notice

The text of ISO 6579-1:2017 has been approved by CEN as EN ISO 6579-1:2017 without any modification.

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I.S. EN ISO 6579-1:2017

INTERNATIONAL STANDARD

ISO 6579-1

First edition
2017-02

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 1: Detection of *Salmonella* spp.

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella —

Partie 1: 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html

This document was prepared by the European Committee for Standardization (CEN), Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with ISO Technical Committee TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition of ISO 6579-1 cancels and replaces ISO 6579:2002 and ISO 6785:2001, which have been technically revised. It also incorporates ISO 6579:2002/Amd 1:2007 and ISO 6579:2002/Cor 1:2004.

The main changes, compared to ISO 6579:2002, are the following.

— ISO 6785 has been incorporated in this document.

— Samples from the primary production stage have been added to the scope.

— Detection of *Salmonella* Typhi and *Salmonella* Paratyphi is described in Annex D.

— Descriptions of preparations of initial suspensions have been removed and references made to relevant parts of ISO 6887, whenever possible.

— The temperature range for incubation of non-selective media has been extended from 37 °C ± 1 °C to 34 °C to 38 °C without further tolerance.

— For selective enrichment, there is a choice between using the broth or the semi-solid agar of Rappaport Vassiliadis medium (RVS or MSRV) for food, animal feed samples, and for environmental samples from the food production area.

— The inoculation of the isolation medium has become less prescriptive; the objective is to obtain well-isolated colonies after incubation.

— For confirmation, it is acceptable to perform the tests on only one suspect colony (instead of one suspect colony of each medium combination). If this isolate tests negative for *Salmonella,* four more suspect isolates from different media combinations shall be tested.

— It is permitted to perform the biochemical confirmation directly on a suspect, well-isolated colony from the selective plating medium. The purity check on the non-selective agar medium can then be performed in parallel.

— Two confirmation tests have become optional (*ß*-galactosidase test and indole reaction) and one confirmation test has been deleted (Voges-Proskauer reaction).

— In this document, serological confirmation (to serogroup level) is described. For guidance on serotyping (to serovar level), reference is made to ISO/TR 6579-3.

— Table 1 has been improved.

— Performance testing for the quality assurance of the culture media has been added to Annex B.

— Performance characteristics of MSRV have been added to Annex C.

A list of all parts in the ISO 6579 series can be found on the ISO website.

Introduction

This document describes a horizontal method for the detection of *Salmonella* spp. in food (including milk and milk products, originally described in ISO 6785), in animal feed, in animal faeces, and in environmental samples from the primary production stage (the latter two were originally described in ISO 6579:2002/Amd 1:2007).

The main changes, listed in the foreword, introduced in this document compared to ISO 6579:2002, are considered as minor (see ISO 17468[37]).

A procedure for the enumeration of *Salmonella* spp. is described in ISO/TS 6579-2.[3]

Guidance for serotyping of *Salmonella* spp. is described in ISO/TR 6579-3.[24]

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* —

Part 1: Detection of *Salmonella* spp.

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. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This document specifies a horizontal method for the detection of *Salmonella*. It is applicable to the following:

— products intended for human consumption and the feeding of animals;

— environmental samples in the area of food production and food handling;

— samples from the primary production stage such as animal faeces, dust, and swabs.

With this horizontal method, most of the *Salmonella* serovars are intended to be detected. For the detection of some specific serovars, additional culture steps may be needed. For *Salmonella* Typhi and *Salmonella* Paratyphi, the procedure is described in Annex D.

The selective enrichment medium modified semi-solid Rappaport-Vassiliadis (MSRV) agar is intended for the detection of motile *Salmonella* and is not appropriate for the detection of non-motile *Salmonella* strains.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 (all parts), *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133:2014, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
Salmonella
microorganism which forms typical or less typical colonies on solid selective media and which displays the characteristics described when confirmation tests are carried out in accordance with this document

3.2
detection of *Salmonella*
determination of *Salmonella* (3.1), in a particular mass or volume of product or surface area or object (e.g. boot socks), when tests are carried out in accordance with this document

4 Principle

4.1 General

The detection of *Salmonella* requires four successive stages as specified in Annex A.

NOTE *Salmonella* can be present in small numbers and is often accompanied by considerably larger numbers of other *Enterobacteriaceae* or bacteria of other families. Pre-enrichment is used to permit the detection of low numbers of *Salmonella* or injured *Salmonella*.

4.2 Pre-enrichment in non-selective liquid medium

Buffered peptone water at ambient temperature is inoculated with the test portion, then incubated between 34 °C and 38 °C for 18 h.

For large quantities (e.g. 1 l or more), it is recommended to pre-warm the BPW to 34 °C to 38 °C before mixing it with the test portion.

4.3 Enrichment in/on selective media

Rappaport-Vassiliadis medium with soya (RVS broth) or Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar and Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn broth) are inoculated with the culture obtained in 4.2.

The RVS broth or the MSRV agar is incubated at 41,5 °C for 24 h and the MKTTn broth at 37 °C for 24 h.

For some products, it may be necessary to incubate the selective enrichment medium/media for an additional 24 h.

NOTE MSRV agar is intended for the detection of motile *Salmonella* strains and is not appropriate for the detection of non-motile *Salmonella* strains.

4.4 Plating out on selective solid media

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

— Xylose Lysine Deoxycholate agar (XLD agar);

— any other solid selective medium complementary to XLD agar (for examples, see Annex E).

The XLD agar is incubated at 37 °C and examined after 24 h. The second selective agar is incubated according to the manufacturer's instructions.

4.5 Confirmation

Colonies of presumptive *Salmonella* are subcultured and their identity is confirmed by means of appropriate biochemical and serological tests.

5 Culture media, reagents, and antisera

For current laboratory practice, see ISO 7218 and ISO 11133.

Composition of culture media and reagents and their preparation are described in Annex B.

6 Equipment and consumables

Disposable equipment 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)**.

As specified in ISO 7218.

6.2 Drying cabinet or **oven**, capable of operating between 25 °C and 50 °C.

6.3 Incubator(s), capable of operating in the range 34 °C to 38 °C and at 37 °C ± 1 °C.

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

6.5 Water bath, capable of operating at 47 °C to 50 °C.

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

6.7 Water bath, capable of operating at 45 °C ± 1 °C.

It is recommended to use a water bath (6.4 to 6.7) containing an antibacterial agent because of the low infective dose of *Salmonella*.

6.8 Refrigerator, capable of operating at 5 °C ± 3 °C.

6.9 Freezer, capable of operating at -20 °C ± 5 °C.

6.10 Sterile loops, of approximate diameter, 3 mm (10 µl volume), and of 1 µl volume and **inoculation needle** or **wire**.

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

6.12 Sterile tubes, **bottles**, or **flasks** with caps of appropriate capacity.

6.13 Sterile graduated pipettes or **automatic pipettes**, of nominal capacities of 25 ml, 10 ml, 1 ml, and 0,1 ml.

6.14 Sterile Petri dishes, with a diameter of approximately 90 mm and (optional) large size (diameter approximately 140 mm).