

BS EN ISO 19250:2013



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Water quality — Detection of *Salmonella* spp.

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National foreword

This British Standard is the UK implementation of EN ISO 19250:2013. It supersedes BS ISO 19250:2010, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee EH/3, Water quality.

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

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English Version

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Qualité de l'eau - Recherche de *Salmonella* spp. (ISO 19250:2010)

Wasserbeschaffenheit - Bestimmung von *Salmonella* spp. (ISO 19250:2010)

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Foreword

The text of ISO 19250:2010 has been prepared by Technical Committee ISO/TC 147 “Water quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 19250:2013 by Technical Committee CEN/TC 230 “Water analysis” 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of an identical text or by endorsement, at the latest by October 2013, and conflicting national standards shall be withdrawn at the latest by October 2013.

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Endorsement notice

The text of ISO 19250:2010 has been approved by CEN as EN ISO 19250:2013 without any modification.

Contents

Page

Introduction.....	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle.....	2
4.1 General	2
4.2 Pre-enrichment in non-selective liquid medium	2
4.3 Enrichment in selective liquid media	2
4.4 Plating out and recognition	3
4.5 Confirmation	3
5 Apparatus	3
6 Sampling.....	4
7 Culture media and reagents	4
8 Procedure	5
8.1 Preparation of the sample	5
8.2 Non-selective pre-enrichment.....	5
8.3 Selective enrichment.....	5
8.4 Plating out	6
8.5 Confirmation	6
9 Expression of results	9
10 Test report.....	9
Annex A (normative) Diagram of procedure	10
Annex B (normative) Composition and preparation of culture media and reagents.....	11
Annex C (informative) Results of the interlaboratory trial.....	18
Bibliography.....	23

Introduction

Salmonella species are bacteria which are widely distributed all over the world. They are usually classified as pathogens, although their virulence and pathogenesis vary widely. The natural hosts of *Salmonella* include humans, agricultural and domestic livestock, and wild animals including birds. Humans and animals can excrete these bacteria while carrying them asymptotically as well as during disease. It is therefore impossible to eliminate them from the environment. Following the infection of humans, the transmission of *Salmonella* can cause severe disease.

Since water is a recognized vehicle of infection, the presence or absence of *Salmonella* is monitored in water where there is perceived to be a risk of infection. *Salmonella* can be present in all types of domestic and agricultural waste water, freshwaters, including ground and drinking waters, as well as sea water.

The detection of *Salmonella* in water usually requires a concentration step. Since *Salmonella* cells can be present in low numbers and injured in the aqueous environment, their detection in water usually requires a pre-enrichment step.

Water quality — Detection of *Salmonella* spp.

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Salmonella*, and especially *S. enterica* subsp. *enterica* ser. Typhi (*Salmonella* ser. Typhi) and *S. enterica* subsp. *enterica* ser. Paratyphi (*Salmonella* ser. Paratyphi), be undertaken only in properly equipped laboratories, under the control of a skilled microbiologist, and that great care be taken in the disposal of all incubated materials.

Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, 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.

IMPORTANT — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

1 Scope

This International Standard specifies a method for the detection of *Salmonella* spp. (presumptive or confirmed) in water samples. It is possible that, for epidemiological purposes or during outbreak investigations, other media are also required.

WARNING — It is possible that the method does not recover all *Salmonella* ser. Typhi and ser. Paratyphi.

NOTE For a semi-quantitative approach, most probable number (MPN) tests can be performed using appropriate sample volumes. For these cases, the volume of the buffered peptone water is adjusted accordingly.

2 Normative references

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.

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

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

ISO 7704, *Water quality — Evaluation of membrane filters used for microbiological analyses*

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

ISO 19458, *Water quality — Sampling for microbiological analysis*

3 Terms and definitions

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

3.1
presumptive *Salmonella* spp.
bacteria which grow in the selective enrichment medium specified, and form typical or atypical colonies on the solid selective media

3.2
confirmed *Salmonella* spp.
bacteria which grow in the selective enrichment medium specified, and form typical and suspicious colonies on the solid selective media, and which display specific biochemical and serological characteristics

NOTE The specific biochemical and serological characteristics are determined by tests specified in this International Standard.

3.3
***Salmonella* detection**
determination of the presence or absence of *Salmonella* (3.4)

3.4
***Salmonella* spp.**
Salmonella
microorganisms which form typical or atypical colonies on solid selective media and which display specific biochemical and serological characteristics

4 Principle

4.1 General

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

Pre-enrichment is often necessary to permit detection of low numbers of *Salmonella* or injured *Salmonella*. Some *Salmonella* and those which are sublethally injured may require additional incubation time (4.3). Furthermore, *Salmonella* can 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.

4.2 Pre-enrichment in non-selective liquid medium

Buffered peptone water (B.1) is inoculated at ambient temperature with a known volume of the sample or its dilutions, then incubated at $(36 \pm 2) ^\circ\text{C}$ for (18 ± 2) h. Larger volumes can be concentrated using membrane filtration and the membrane filter is then added to buffered peptone water.

NOTE For waste water it has been shown that shorter incubation times or direct inoculation of the sample in selective medium (4.3) produce better results.

For a semi-quantitative approach, MPN tests can be performed using appropriate sample volumes. In these cases, adjust the volumes of the buffered peptone water accordingly.

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 $(41,5 \pm 1) ^\circ\text{C}$ for (24 ± 3) h and the MKTn broth at $(37 \pm 1) ^\circ\text{C}$ for (24 ± 3) h.

To detect slow-growing *Salmonella* spp., incubate the enrichment broth for a further (24 ± 3) h to a total of (48 ± 4) h at $(41,5 \pm 1,0)$ °C.

NOTE *Salmonella* Typhi and *Salmonella* Paratyphi A are usually not important in routine water quality monitoring, but can be relevant in epidemiological investigations. MKTTn broth is used for enrichment with incubation at (36 ± 2) °C for up to (24 ± 3) h and recovers most strains of *Salmonella*, including some strains of *Salmonella* Paratyphi, but is not thought to be able to recover strains of *Salmonella* Paratyphi C. MKTTn broth is not used if *Salmonella* Typhi is suspected after the use of selenite cystine broth.

4.4 Plating out and recognition

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

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

Incubate the XLD agar at (36 ± 2) °C and examine after (24 ± 3) h to check for the presence of colonies which are considered to be presumptive *Salmonella*. Incubate the second selective agar according to the manufacturer's recommendations.

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

4.5 Confirmation

Subculture colonies of presumptive *Salmonella*, then plate out as described in 4.4 and confirm their identity by means of appropriate biochemical (8.5.3) and serological (8.5.4) tests.

5 Apparatus

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

5.1 General. Except for disposable glassware which is delivered sterile, sterilize glassware as specified in ISO 8199. Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

5.2 Autoclave, capable of being maintained at (121 ± 3) °C and at (115 ± 3) °C.

5.3 Water bath or incubator, capable of being maintained at (36 ± 2) °C.

5.4 Water bath or incubator, capable of being maintained at $(41,5 \pm 1,0)$ °C.

5.5 Water baths, capable of operating at (70 ± 1) °C and at 50 °C to 55 °C.

5.6 Membrane filtration apparatus, as specified in ISO 8199.

5.7 Sterile membrane filters, with a nominal pore size of 0,45 µm.

The quality of membrane filters may vary from brand to brand or even from batch to batch. It is therefore advisable to check the quality on a regular basis, as specified in ISO 7704.

5.8 pH-meter, with an accuracy of calibration of $\pm 0,1$ pH at 20 °C to 25 °C.

5.9 Sterile forceps.

5.10 Sterile loops, approximate diameter 3 mm (10 µl volume), and inoculation needle or wire.

6 Sampling

Sampling is not part of the method specified in this International Standard. Samples should be taken in accordance with ISO 19458.

It is important the laboratory receive a truly representative sample which has not been damaged or changed during transport or storage.

7 Culture media and reagents

NOTE For guidelines on quality assurance and performance testing, see ISO/TS 11133-1^[2] and ISO/TS 11133-2^[3].

7.1 Basic materials. For uniformity of results, in the preparation of media, either use a dehydrated complete medium or use constituents of uniform quality and reagents of recognized analytical grade.

Other grades of reagents may be used provided they can be shown to produce comparable results.

7.2 Water, ISO 3696^[1], grade 3.

7.3 Culture media, prepared in accordance with Annex B.

7.3.1 Buffered peptone water, non-selective pre-enrichment medium buffered peptone water (BPW, B.1).

7.3.2 Rappaport-Vassiliadis broth with soya (RVS broth, B.2), selective enrichment medium.

7.3.3 Xylose lysine deoxycholate agar (XLD agar, B.3).

7.3.4 Second solid selective plating-out medium, whose choice is left to the discretion of the testing laboratory. Follow the manufacturer's instructions precisely regarding its preparation for use.

7.3.5 Nutrient agar (B.4), or other appropriate non-selective agar.

7.3.6 Triple sugar and iron agar (TSI agar, B.5).

As an alternative, iron and two sugar agar may be used.

7.3.7 Urea agar, Christensen (B.6).

7.3.8 L-Lysine decarboxylation medium (B.7).

7.3.9 Selenite cystine broth (B.8).

7.3.10 Muller-Kauffmann tetrathionate-novobiocin broth (MKTn, B.9).

7.3.11 Filter aid (B.10).