

Methods for

# Microbiological examination of food and animal feeding stuffs —

Part 4: Detection of *Salmonella*

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- Association of Public Analysts
- BLWA Ltd. (Association of the Laboratory Supply Industry)
- British Food Manufacturing Industries Research Association
- Campden Food and Drink Research Association
- Department of Agriculture Northern Ireland
- Department of Health
- Department of Trade and Industry (Laboratory of the Government Chemist)
- Flour Milling and Baking Research Association
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- Fruit and Vegetable Canners' Association
- Institute of Brewing
- Joint Committee of the Milk Marketing Board and the Dairy Trade Federation
- Ministry of Agriculture, Fisheries and Food
- Natural Resources Institute
- Public Health Laboratory Service
- Society for Applied Bacteriology
- United Kingdom Dairy Association
- University of Reading

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# National foreword

This Part of BS 5763 has been prepared under the direction of the Agriculture and Food Standards Policy Committee. It is identical with ISO 6579:1993 *Microbiology — General guidance on methods for the detection of Salmonella*, published by the International Organization for Standardization (ISO), and in the preparation of which the United Kingdom played a full part. This revision supersedes BS 5763-4:1990 which is withdrawn and from which it differs in that some errors in the description of the method have been corrected.

**Cross-references**

| International Standard | Corresponding British Standard                                                                                                                             |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ISO 6887:1983          | BS 5763 <i>Methods for microbiological examination of food and animal feeding stuffs</i><br>Part 6:1983 <i>Preparation of dilutions</i><br>(Identical)     |
| ISO 7218:1985          | BS 5763 <i>Methods for microbiological examination of food and animal feeding stuffs</i><br>Part 0:1986 <i>General laboratory practices</i><br>(Identical) |

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**Summary of pages**

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 16, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

## Introduction

This International Standard is intended to provide general guidance for the examination of products not dealt with by existing International 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, these guidelines 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 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 deviation 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.

**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 International Standard gives general guidance on methods for the detection of *Salmonella*.

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

The incubation temperature (35 °C or 37 °C) shall be agreed by the parties concerned and shall be specified in the test report.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 6887:1983, *Microbiology — General guidance for the preparation of dilutions for microbiological examination*.

ISO 7218:1985, *Microbiology — General guidance for microbiological examinations*.

## 3 Definitions

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

### 3.1

*salmonella*

microorganisms 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 International Standard

### 3.2

**detection of *salmonella***

determination of the presence or absence of these microorganisms, in a particular mass of product, when tests are carried out in accordance with this International Standard

## 4 Principle

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

NOTE 1 *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.

### 4.2 Enrichment in selective liquid media

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

Incubation of the 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.

#### 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 International 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, from their characteristics, 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 2 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 clause B.1.

##### 5.2.2 First selective enrichment medium: *Rappaport-Vassiliadis magnesium chloride/malachite green medium* (RV medium)

See clause B.2.

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

See clause 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 clause 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, unless there is a specific International Standard relating to the product to be examined, which specifies the composition of this second medium.

###### 5.2.5 Nutrient agar

See clause B.5.

###### 5.2.6 Triple sugar/iron agar (*TSI agar*)

See clause B.6.

###### 5.2.7 Urea agar (Christensen)

See clause B.7.

###### 5.2.8 L-Lysine decarboxylation medium

See clause B.8.

###### 5.2.9 Reagent for detection of $\beta$ -galactosidase (or prepared paper discs, used in accordance with the manufacturer's instructions)

See clause B.9.

###### 5.2.10 Reagents for Voges-Proskauer (*VP reaction*)

See clause B.10.

###### 5.2.10.1 VP medium

###### 5.2.10.2 Creatine solution (*N*-amidinosarcosine)

###### 5.2.10.3 1-Naphthol, ethanolic solution

###### 5.2.10.4 Potassium hydroxide solution

###### 5.2.11 Reagents for indole reaction

See clause 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 clause B.12.

###### 5.2.13 Saline solution

See clause 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).

## 6 Apparatus and glassware

NOTE 3 Disposable apparatus is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment 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  $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  and  $55\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ .

**6.3 Incubator**, capable of operating at  $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  or  $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ , depending on the temperature agreed.

**6.4 Water bath**, capable of operating at  $42,0\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  or *incubator*, capable of operating at  $42,0\text{ }^{\circ}\text{C} \pm 0,5\text{ }^{\circ}\text{C}$ .

**6.5 Water baths**, capable of operating at  $45\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ ,  $55\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  and  $70\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ .

**6.6 Water bath**, capable of operating at  $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$  or  $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ , depending on the temperature agreed.

**6.7 Loops**, made of platinum/iridium or nickel/chromium, of diameter approximately 3 mm.

**6.8 pH-meter**, having an accuracy of calibration of  $\pm 0,1\text{ pH unit}$  at  $25\text{ }^{\circ}\text{C}$ .

**6.9 Culture bottles or flasks**

NOTE 4 Bottles or flasks with non-toxic metallic or plastic screw-caps may be used.

**6.10 Culture tubes**, 8 mm in diameter and 160 mm in length.

**6.11 Measuring cylinders**

**6.12 Graduated pipettes**, of nominal capacities 10 ml and 1 ml, graduated respectively in 0,5 ml and 0,1 ml divisions.

**6.13 Petri dishes**, of small size (diameter 90 mm to 100 mm) and/or large size (diameter 140 mm).

## 7 Sampling

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

Sampling is not part of the method specified in this International Standard. See the specific International Standard dealing with the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

## 8 Preparation of the test sample

Prepare the test sample in accordance with the specific International Standard dealing with the product concerned. If there is no specific International Standard, it is recommended that the parties concerned come to an agreement on this subject.

## 9 Procedure

(See diagram in Annex A.)

### 9.1 Test portion and initial suspension

**9.1.1** See ISO 6887 and the specific International Standard dealing with the product concerned.

For preparation of the initial suspension, use as dilution fluid the pre-enrichment medium specified in **5.2.1**.

**9.1.2** In general, to prepare the initial suspension, add a 25 g test portion to 225 ml of pre-enrichment medium (**5.2.1**), which is the ratio of test portion to pre-enrichment medium specified in this method.

If the prescribed test portion is other than 25 g, use the necessary quantity of pre-enrichment medium to yield approximately a 1/10 dilution (mass to volume).

NOTE 5 To reduce the examination workload when more than one 25 g test portion from a specified lot of food has to be examined, and when evidence is available that compositing (pooling the test portions) does not affect the result for that particular food, the test portions may be composited. For example, if 10 test portions of 25 g are to be examined, combine the 10 units to form a composite test portion of 250 g and add 2,25 litres of pre-enrichment broth. Alternatively, the 0,1 ml (RV medium) and 10 ml (selenite/cystine medium) portions of the pre-enrichment broths from the 10 separate test portions (**9.3.1**) may be composited for enrichment in 0,1 litre and 1 litre respectively of selective enrichment medium.

NOTE 6 Dried or powdered food products may need a special rehydration procedure to enhance the recovery of *Salmonella*. Two techniques may be used for this purpose, that of immersion and that of agitation. Refer for this purpose to the specific International Standard dealing with the product under examination. If such a standard is not available, it is recommended that the parties concerned come to an agreement on this subject.

### 9.2 Non-selective pre-enrichment

Incubate the initial suspension at  $35\text{ }^{\circ}\text{C}$  or  $37\text{ }^{\circ}\text{C}$  (as agreed) for not less than 16 h and not more than 20 h.