

Microbiological examination for dairy purposes —

**Part 3: Methods for detection and/or
enumeration of specific groups of
microorganisms —**

Section 3.9 Detection of *Salmonella* —

Subsection 3.9.1 Reference method

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Foreword

This Subsection of BS 4285 has been prepared under the direction of the Agriculture and Food Standards Policy Committee. The techniques were not given in BS 4285:1968 nor in Supplement No. 1 (1970). The techniques supersede a simpler test applicable only to examination for *Salmonella* in dried milks described in Supplement No. 1 (1970) to BS 4285:1968, which is withdrawn.

Another method for the detection of *Salmonella* using electrical conductance is described in BS 4285-3.9.2. Both Subsections of BS 4285 take into account the isolation and confirmation procedures described in ISO 6785:1985. However, this Subsection is technically different because it allows the use of one of two alternative selective solid media (XLD agar, Hectoen enteric agar) in place of brilliant green/phenol red agar. Subsection 3.9.2 is technically different from ISO 6785:1985 principally because it allows a choice of at least two of the selective solid media from the four described.

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This British Standard, having been prepared under the direction of the Agriculture and Food Standards Policy Committee, was published under the authority of the Board of BSI and comes into effect on 30 June 1987

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The Committees responsible for this British Standard are shown in Part 0.
The following BSI references relate to the work on this standard:
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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.

Amendments issued since publication

Amd. No.	Date of issue	Comments
7209	June 1992	Indicated by a sideline in the margin

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NOTE It is essential that Part 0 and Part 1 and Section 2.1, which are published separately, be read in conjunction with this Section.

1 Scope

This Section of BS 4285 describes a method for the detection of *Salmonella* in milk and dairy products, WARNING. It is essential to observe the safety precautions specified in clause 4.

NOTE The titles of the publications referred to in this standard are listed on the inside back page.

2 Definitions

For the purposes of this Section of BS 4285 the following definitions apply.

2.1

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 standard

2.2

detection of *Salmonella*

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

3 Principle

3.1 General

The detection of *Salmonella* necessitates four successive stages as described in 3.2 to 3.5.

NOTE See also the diagram of procedure in Appendix A.

3.2 Pre-enrichment in liquid medium

The appropriate pre-enrichment medium is inoculated with the test portion and incubated at 37 °C for 16 h to 20 h.

3.3 Enrichment in selective liquid media

A tetrathionate medium and a selenite cystine medium are inoculated with the culture obtained (see 3.1) and the tetrathionate medium is inoculated at 43 °C and the selenite cystine medium is inoculated at 37 °C, for two periods of 18 h to 24 h.

3.4 Plating out and recognition

From the cultures obtained (see 3.2), two selective solid media are inoculated.

The two selective solid media are incubated at 37 °C and examined after 20 h to 24 h and, if necessary, after 40 h to 48 h to check the presence of colonies which, from their characteristics, are considered to be presumptive *Salmonella*.

3.5 Confirmation

Colonies of presumptive *Salmonella* (see 3.3) are subcultured and confirmed by means of appropriate biochemical and serological tests.

4 Safety precautions

4.1 The procedure specified in this Section of BS 4285 shall only be carried out in laboratories with suitable facilities and under the control of a qualified microbiologist.

4.2 These procedures shall not be performed in quality control laboratories, or in food manufacturing or processing premises, where there is a risk of contamination of the environment.

4.3 Full bacteriological precautions shall be taken at all times whilst carrying out the procedure specified in this Section of BS 4285. Particular attention shall be given to the sterilization of used equipment and media after testing suspect samples and prior to disposal or re-use.

5 Culture media, reagents and sera

5.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that dehydrated basic components or complete dehydrated media should be used for the preparation of culture media. The manufacturer's instructions shall be rigorously followed when preparing the culture media.

The chemicals used for preparing the culture media and the reagents shall be of recognized analytical grade.

The water used shall be distilled or deionized water, free from substances that might inhibit growth of microorganisms under the test conditions.

The pH measurements shall be carried out by means of the pH meter (6.7).

If the prepared culture media and reagents are not used immediately, they shall, unless otherwise specified, be stored in the dark at between 0 °C and 5 °C, but for no longer than 1 month, and in conditions that prevent any change in their composition.

5.2 Culture media and reagents

5.2.1 Pre-enrichment medium: buffered peptone water

Composition

peptone	10.0 g
sodium chloride	5.0 g
disodium hydrogenorthophosphate dodecahydrate (Na ₂ HPO ₄ ·12H ₂ O)	9.0 g

potassium dihydrogenorthophosphate (KH_2PO_4)	1.5 g
water	1 000 mL

Preparation

Dissolve the components in the water by boiling.
Adjust the pH, if necessary, so that after sterilization it is 7.0 ± 0.1 at 25°C .

Sterilize the medium by heating in an autoclave for 15 min at $121 \pm 1^\circ\text{C}$.

Distribute the medium aseptically into sterile flasks of suitable capacity to obtain the portions necessary for the test.

5.2.2 First selective enrichment medium: tetrathionate medium (Muller-Kauffmann)**5.2.2.1 Base****Composition**

meat extract	5.0 g
peptone	10.0 g
sodium chloride	3.0 g
calcium carbonate	45.0 g
water	1 000 mL

Preparation

Add the dehydrated base components or the dehydrated complete base to the water and boil until complete dissolution of the soluble components.

Adjust the pH, if necessary, so that after sterilization it is 7.0 ± 0.1 at 25°C .

Sterilize the base by heating in an autoclave for 15 min at $121 \pm 1^\circ\text{C}$.

5.2.2.2 Sodium thiosulphate solution**Composition**

sodium thiosulphate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$)	50.0 g
water to a final volume of	100 mL

Preparation

Dissolve the sodium thiosulphate in a part of the water.

Dilute to the final volume.

Sterilize the solution by heating in an autoclave for 15 min at $121 \pm 1^\circ\text{C}$.

5.2.2.3 Iodine solution**Composition**

iodine	20.0 g
potassium iodide	25.0 g
water to a final volume of	100 mL

Preparation

Dissolve the potassium iodide in a minimal volume of water and add the iodine.

Shake until dissolution is complete.

Dilute to the final volume.

Store the solution in a tightly closed opaque container.

5.2.2.4 Brilliant green solution**Composition**

brilliant green (see Appendix B)	0.5 g (approximately)
water	100 mL

Preparation

Add the brilliant green to the water.

Store the solution for at least 1 day in the dark to allow auto-sterilization to occur.

5.2.2.5 Ox bile solution**Composition**

ox bile, desiccated	10.0 g
water	100 mL

Preparation

Dissolve the desiccated ox bile in the water by boiling.

Sterilize the solution by heating in an autoclave for 15 min at $121 \pm 1^\circ\text{C}$.

5.2.2.6 Complete medium**Composition**

base	900 mL
sodium thiosulphate solution	100 mL
iodine solution	20 mL
brilliant green solution	2 mL
ox bile solution	50 mL

Preparation

Add to the base, under aseptic conditions, the other ingredients, in the above-mentioned order.

Mix the liquids well after each addition.

Distribute the medium aseptically into sterile flasks of suitable capacity to obtain the portions necessary for the test.

Use within 1 week of preparation.

5.2.3 Second selective enrichment medium: selenite cystine medium

WARNING. Extreme care should be taken with the laboratory use of selenite solutions because of their potentially toxic effect. Do not pipette by mouth under any circumstances.

5.2.3.1 Base

Composition

tryptone ^a	5.0 g
lactose	4.0 g
disodium hydrogenorthophosphate dodecahydrate (Na ₂ HPO ₄ ·12H ₂ O)	10.0 g
sodium hydrogenselenite	4.0 g
water	1 000 mL

^a This term is used at present only by certain producers of media. Any other casein digest giving comparable results may be used.

Preparation

Dissolve the first three ingredients in the water by boiling for 5 min. After cooling, add the sodium hydrogenselenite.

Adjust the pH, if necessary, so that it is 7.0 ± 0.1 at 25 °C.

Do not autoclave.

5.2.3.2 L-Cystine solution

Composition

L-cystine	0.1 g
sodium hydroxide 40 g/L solution	15 mL
water	85 mL

Preparation

Dissolve the L-cystine in the sodium hydroxide solution.

Dilute to 100 mL with sterile water in a sterile flask.

Do not autoclave.

5.2.3.3 Complete medium

Composition

base	1 000 mL
L-cystine solution	10 mL

Preparation

Cool the base and add the L-cystine solution aseptically.

Adjust the pH, if necessary, so that it is 7.0 ± 0.1 at 25 °C.

Do not autoclave.

Distribute the medium aseptically into sterile flasks of suitable capacity to obtain the portions necessary for the test.

Use the medium on the day of preparation.

5.2.4 First selective solid medium: bismuth sulphite agar

Composition

peptone	10.0 g
beef extract	5.0 g
glucose	5.0 g
disodium hydrogenorthophosphate dodecahydrate (Na ₂ HPO ₄ ·12H ₂ O)	4.0 g
iron (II) sulphate	0.3 g
ammonium bismuth citrate	1.85 g
sodium sulphite	6.15 g
agar	15 g to 25 g (according to manufacturer's instructions)
brilliant green (see Appendix B)	0.025 g
water	1 000 mL

Preparation

Dissolve the ingredients in the water by boiling for approximately 1 min.

Adjust the pH to 7.7 ± 0.1 .

Cool to 45 °C to 50 °C, suspending the precipitate with gentle agitation.

Do not autoclave.

Transfer the medium in quantities of 20 mL to sterile Petri dishes and allow to solidify.

Immediately before use, dry the plates carefully, preferably with the lids off and the agar surfaces downwards, in an oven or incubator (6.2) controlled at 50 ± 5 °C for 30 min.

NOTE The plates lose their selectivity after 72 h.

5.2.5 Second selective solid medium

5.2.5.1 General. The second selective solid medium shall be one of those specified in 5.2.5.2 to 5.2.5.4, to be chosen either by the person carrying out the test or by the person commissioning the test.

5.2.5.2 Brilliant green/phenol red agar (Edel and Kampelmacher)