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Milk and milk products — Detection of *Salmonella* spp.

Lait et produits laitiers — Recherche de 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.

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Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 6785 | IDF 93 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

This second edition cancels and replaces the first edition (ISO 6785:1985), which has been technically revised.

Annexes A and B form a normative part of this International Standard. Annex C is for information only.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO and AOAC International in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team on *Harmonization*, of the Standing Committee on *Microbial methods of analysis*, under the aegis of its project leader, Mr. H. Becker (DE).

This fourth edition cancels and replaces the third edition (IDF 93:1995).

Milk and milk products — Detection of *Salmonella* spp.

1 Scope

This International Standard specifies a method for the detection of *Salmonella* spp. in milk and milk products.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 8261|IDF 122, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*.

3 Terms and definitions

For the purposes of this International Standard, the following terms and 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*
detection of the presence or absence of these microorganisms, in a particular mass or volume, when tests are carried out in accordance with this International Standard

4 Principle

4.1 General

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

4.2 Pre-enrichment in non-selective liquid medium

Inoculation of the pre-enrichment medium with the test portion, and incubation at 37 °C for 16 h to 20 h.

4.3 Enrichment in selective liquid media

Inoculation of Rappaport-Vassiliadis modified magnesium chloride/malachite green medium and of selenite/cystine medium with the culture obtained in 4.2.

Incubation of the Rappaport-Vassiliadis modified magnesium chloride/malachite green medium in the water bath or incubator (6.4) set at 41,5 °C for 24 h and then a further 24 h.

Incubation of the selenite/cystine medium in the incubator (6.3) set at 37 °C for 24 h and then a further 24 h.

4.4 Streaking out and recognition

From the cultures obtained (4.3), inoculation of two selective solid media (brilliant green/phenol red agar and any other suitable solid selective medium).

NOTE Suitable media allow the recovery of lactose-fermenting *Salmonella* strains.

Incubation of the brilliant green/phenol red agar in the incubator (6.3) set at 37 °C and examination after 20 h to 24 h and, if necessary, again after 40 h to 48 h to check the presence of colonies which, from their characteristics, are considered to be presumptive *Salmonella*.

Incubation of the second selective solid medium at the appropriate temperature and examination after the appropriate time to check the presence of colonies which, from their characteristics, are considered to be presumptive *Salmonella*.

4.5 Confirmation

Subculturing of colonies of presumptive *Salmonella* (4.4) and confirmation by means of appropriate biochemical and serological tests.

5 Culture media, reagents and sera

In order to improve the reproducibility of the results, it is recommended that, for the preparation of culture media, dehydrated basic components or dehydrated complete media are used. In that case, follow the manufacturer's instructions rigorously.

Use only reagents of recognized analytical grade, unless otherwise specified.

The pH values given refer to a temperature of 25 °C. Adjustments, if necessary, are made by adding either hydrochloric acid [$c(\mathrm{HCl}) = 1$ mol/l] or sodium hydroxide solution [$c(\mathrm{NaOH}) = 1$ mol/l].

If not used immediately, store the prepared culture media and reagents under conditions that do not produce any change in their composition, in the dark at a temperature between 0 °C and + 5 °C, for no longer than 1 month, unless otherwise stated.

5.1 Water

Use distilled or demineralized water or water of equivalent purity. The water shall be free from substances that might inhibit the growth of microorganisms under the test conditions specified in this International Standard.

5.2 Culture media

5.2.1 Pre-enrichment medium: Buffered peptone water

5.2.1.1 Composition

Peptone	10,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

5.2.1.2 Preparation

Dissolve the components in the water by heating. Adjust the pH so that after sterilization it is 7,0 ± 0,1.

Transfer the medium in quantities of 225 ml into flasks (6.9) of capacity 500 ml (or multiples of 225 ml into flasks of suitable capacity). Sterilize in the autoclave (6.1) set at 121 °C for 15 min. Cool to room temperature.

5.2.2 First selective enrichment medium: Rappaport-Vassiliadis modified magnesium chloride/malachite green medium (RVS broth)

5.2.2.1 Solution A

5.2.2.1.1 Composition

Enzymatic digest of soya	5,0 g
Sodium chloride (NaCl)	8,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,4 g
Dipotassium hydrogen phosphate (K_2HPO_4)	0,2 g
Water	1 000 ml

5.2.2.1.2 Preparation

Dissolve the components in the water by heating to about 70 °C. Prepare solution A on the day of preparation of the complete RVS medium.

5.2.2.2 Solution B

5.2.2.2.1 Composition

Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	400,0 g
Water	1 000 ml

5.2.2.2.2 Preparation

Dissolve the magnesium chloride in the water. As this salt is very hygroscopic, it is advisable to dissolve the entire contents of $MgCl_2 \cdot 6H_2O$ from a newly opened container. For instance, 250 g of $MgCl_2 \cdot 6H_2O$ is added to 625 ml of water, giving a solution of total volume of 795 ml and a concentration of about 0,3 g/ml of $MgCl_2 \cdot 6H_2O$.

Solution B can be stored in an airtight brown glass bottle at room temperature for at least 2 years.

5.2.2.3 Solution C

5.2.2.3.1 Composition

Malachite green oxalate	0,4 g
Water	100 ml

5.2.2.3.2 Preparation

Dissolve the malachite green oxalate in the water.

Solution C can be stored in a brown glass bottle at room temperature for at least 8 months.

5.2.2.4 Complete medium

5.2.2.4.1 Composition

Solution A (5.2.2.1)	1 000 ml
Solution B (5.2.2.2)	100 ml
Solution C (5.2.2.3)	10 ml

5.2.2.4.2 Preparation

Add to 1 000 ml of solution A, 100 ml of solution B and 10 ml of solution C. Adjust the pH, if necessary, so that after sterilization it is 5,2 ± 0,1. Dispense 10 ml quantities of the thus-obtained solution into test tubes (6.9) or into sterile flasks (6.8) of suitable capacity to obtain the portions necessary for the test. Sterilize in the autoclave (6.1) set at 115 °C for 15 min.

Store the prepared medium in the refrigerator at 3 °C ± 2 °C.

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

5.2.3.1.1 Composition

Tryptone	5,0 g
Lactose	4,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	10,0 g
Sodium hydrogen selenite	4,0 g
Water	1 000 ml

5.2.3.1.2 Preparation

Dissolve the first three basic components in the water by boiling for 5 min. After cooling, add the sodium hydrogen selenite. Adjust the pH, if necessary, to 7,0 ± 0,1. Do not sterilize.

5.2.3.2 L-Cystine solution

5.2.3.2.1 Composition

L-Cystine	0,1 g
Sodium hydroxide solution, $c\,(\text{NaOH}) = 1$ mol/l	15 ml
Sterile water	approx. 85 ml

5.2.3.2.2 Preparation

Add the components to a sterile 100 ml one-mark volumetric flask. Dilute to the mark with sterile water. Do not sterilize.

5.2.3.3 Complete medium

5.2.3.3.1 Composition

Base (5.2.3.1)	1 000 ml
L-Cystine solution (5.2.3.2)	10 ml

5.2.3.3.2 Preparation

Add the L-cystine solution aseptically to the base. Adjust the pH, if necessary, to $7{,}0 \pm 0{,}1$. Dispense the medium aseptically into sterile flasks of suitable capacity to obtain the portions necessary for the test.

The medium may be used until a red precipitate occurs.

5.2.4 First selective solid medium: Brilliant green/phenol red agar (Edel and Kampelmacher)

5.2.4.1 Base

5.2.4.1.1 Composition

Meat extract powder	5,0 g
Peptone	10,0 g
Yeast extract powder	3,0 g
Disodium hydrogen phosphate (Na_2HPO_4)	1,0 g
Sodium dihydrogen phosphate (NaH_2PO_4)	0,6 g
Agar	12 g to 18 g [a]
Water	900 ml

[a] Depending on the gel strength of the agar.

5.2.4.1.2 Preparation

Dissolve the components or the dehydrated complete base in the water by heating, if necessary. Adjust the pH, if necessary, so that after sterilization it is $7{,}0 \pm 0{,}1$. Transfer the base to tubes (6.9) or flasks (6.8) of appropriate capacity. Sterilize in the autoclave (6.1) set at 121 °C for 15 min.

5.2.4.2 Sugar/phenol red solution

5.2.4.2.1 Composition

Lactose	10,0 g
Sucrose	10,0 g
Phenol red	0,09 g
Water	approx. 80 ml

5.2.4.2.2 Preparation

Dissolve the components in approximately 50 ml of water in a 100 ml one-mark volumetric flask. Dilute to the mark with the water. Heat the solution in a water bath (6.5) set at 70 °C for 20 min. Cool in another water bath (6.5) set at 55 °C. Use the solution immediately after cooling.

5.2.4.3 Brilliant green solution

5.2.4.3.1 Composition

Brilliant green (see specification in annex B)	about 0,5 g
Water	100 ml

5.2.4.3.2 Preparation

Dissolve the brilliant green in the water. Store the solution for at least one day in the dark to allow auto-sterilization to occur.

5.2.4.4 Complete medium

5.2.4.4.1 Composition

Base (5.2.4.1)	900 ml
Sugar/phenol red solution (5.2.4.2)	100 ml
Brilliant green solution (5.2.4.3)	1 ml

5.2.4.4.2 Preparation

Add the brilliant green solution (5.2.4.3) aseptically to the sugar/phenol red solution (5.2.4.2) cooled in a water bath (6.5) to 55 °C. Add this to the base, preheated in the water bath to 55 °C, and mix. The temperature of the water bath should be kept between 50 °C and 55 °C while mixing.

5.2.4.4.3 Preparation of the agar plates

Place in each of an appropriate number of large Petri dishes (6.12) about 40 ml of the freshly prepared complete medium (5.2.4.4). If large dishes are not available, place about 15 ml of the medium in small Petri dishes (6.12). Allow to solidify.

If prepared in advance, store the agar plates for no longer than 4 h at room temperature or no longer than 1 week between 0 °C and + 5 °C.

Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven (6.2) set at 50 °C or in the laminar airflow cabinet (6.2) until the surface of the agar is dry.