SLOVENSKI STANDARD
oSIST prEN ISO 6579-1:2014

01-september-2014

Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje prisotnosti, števila in serotipov Salmonella - 1. del: Horizontalna metoda za ugotavljanje prisotnosti Salmonella spp. (ISO/DIS 6579-1:2014)

Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Horizontal method for the detection of Salmonella spp. (ISO/DIS 6579-1:2014)

Mikrobiolgie der Lebensmittelkette - Horizontales Verfahren zum Nachweis, zur Zählung und zur Serotypisierung von Salmonellen - Teil 1: Nachweisverfahren (ISO/DIS 6579-1:2014)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et la sérotypie des Salmonella spp. - Partie 1: Méthode horizontale pour la recherche des Salmonella spp. (ISO/DIS 6579-1:2014)

Ta slovenski standard je istoveten z: **prEN ISO 6579-1**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

oSIST prEN ISO 6579-1:2014 **en**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 6579-1

ISO/TC **34**/SC **9**

Secretariat: **AFNOR**

Voting begins on:
2014-06-05

Voting terminates on:
2014-11-05

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

Part 1:
Horizontal method for the detection of Salmonella spp.

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

Partie 1: Méthode horizontale pour la recherche des Salmonella spp.

ICS: 07.100.30

ISO/CEN PARALLEL PROCESSING

This draft has been developed within the European Committee for Standardization (CEN), and processed under the **CEN lead** mode of collaboration as defined in the Vienna Agreement.

This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel five month enquiry.

Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month approval vote in ISO and formal vote in CEN.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Reference number
ISO/DIS 6579-1:2014(E)

Contents

Page

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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 the member bodies casting a vote.

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

ISO 6579-1 was prepared by the European Committee for Standardization (CEN), in collaboration with Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

ISO 6579 consists of the following parts, under the general title *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella*:

— *Part 1: Horizontal method for the detection of Salmonella spp.*

— *Part 2: Enumeration by a miniaturized most probable number technique* [Technical Specification]

— *Part 3: Guidelines for serotyping of Salmonella spp. [Technical Report]*

This fifth edition cancels and replaces the fourth edition (ISO 6579:2002), which has been technically revised. It also cancels and replaces ISO 6785:2007 [4] and Amendment 1 of ISO 6579 (2007) [2].

Annexes A and B and D form a normative part of this International Standard. Annexes C and E are for information only.

Main changes in this fifth edition:

— ISO 6785 has been incorporated in this version of ISO 6579-1;

— samples from the primary production stage have been added to the scope;

— detection of *Salmonella* Typhi and *Salmonella* Paratyphi is described in a normative annex;

— for selective enrichment there is a choice between using the broth or the semi-solid agar of Rappaport Vassiliadis medium (RVS or MSRV);

— 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,* 4 more suspect isolates from different media combinations shall be tested;

— it is allowed 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), one confirmation test has been deleted (Voges-Proskauer reaction).

— in this part of ISO 6579, serological confirmation (to serogroup level) is described. For guidance on serotyping (to serovar level) reference is made to part 3 of ISO 6579;

— Table 1 (Interpretation of biochemical tests) 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.

Introduction

This part of ISO 6579 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 Amendment 1 of ISO 6579:2002).

With this horizontal method, most of the *Salmonella* serovars will be detected. For the detection of some specific serovars additional culture steps may be needed. For *Salmonella* Typhi and *Salmonella* Paratyphi this is described in a separate annex.

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

Guidance for serotyping of *Salmonella* spp. is described in part 3 of ISO/TR 6579.

Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Horizontal method for the 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 International Standard should be familiar with normal laboratory practice. This standard 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 International Standard specifies a horizontal method for the detection of *Salmonella*. Additional culture steps for the detection of *Salmonella* Typhi and *Salmonella* Paratyphi are specified in an Annex of this International Standard.

Subject to the limitations discussed in the Introduction, this International Standard is applicable to:

— 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, swabs.

The selective enrichment medium for detection of *Salmonella* in samples from the primary production stage (MSRV) is intended for the detection of motile Salmonellae and is not appropriate for the detection of non-motile Salmonellae and/or brilliant-green sensitive *Salmonella* strains.

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 707, *Milk and milk products: guidance on sampling*

ISO/TR 6579-3, *Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 3 Guidelines for serotyping of Salmonella spp.*

ISO 6887-1, *Microbiology of the food chain — 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 6887-2, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products*

ISO 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

ISO 6887-6, *Microbiology of food and animal feed — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 6: Specific rules for the preparation of samples taken at the primary production stage*

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

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

ISO 13307, *Microbiology of food and animal feed — Primary production stage — Sampling techniques*

ISO 17604, *Microbiology of the food chain* — Carcass sampling for microbiological analysis

ISO/TS 17728, *Microbiology of the food chain — Sampling techniques for microbiological analysis of food and feed samples*

ISO 18593, *Microbiology of food and animal feeding stuffs — Horizontal method for sampling techniques from surfaces using contact plates and swabs*

3 Terms and definitions

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

3.1
Salmonella
microorganisms which form typical or less 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 *Salmonella* (3.1), in a particular mass or volume of product or surface area or object (e.g. bootsocks), 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 also Annex A).

NOTE *Salmonella* can be present in small numbers and is often accompanied by considerably larger numbers of other *Enterobacteriaceæ* or other families. Furthermore, 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 certain foodstuffs the use of other pre-enrichment procedures is necessary. See 9.1.2.

For large quantities (e.g. 1 litre or more), it is recommended to pre-heat, the buffered peptone water to 34 °C to 38 °C before inoculating 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 is intended for the detection of motile Salmonellae and is not appropriate for the detection of non-motile Salmonellae and/or brilliant-green sensitive *Salmonella* strains.

4.4 Plating out and identification

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

— Xylose lysine deoxycholate agar (XLD agar);

— any other solid selective medium complementary to XLD agar (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 recommendations.

4.5 Confirmation of identity

Colonies of presumptive *Salmonella* are subcultured and their identity is confirmed 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

Because of the large number of culture media and reagents, it is considered preferable, for clarity, to give their compositions and preparations in Annex B. Alternatively, dehydrated complete media or diluents may be used. Follow, in that respect, the manufacturer’s instructions.

5.2.1 Non-selective pre-enrichment medium: Buffered peptone water

See B.1.

5.2.2 First selective enrichment medium: Rappaport-Vassiliadis medium with soya (RVS broth) or Modified semi-solid Rappaport-Vassiliadis (MSRV) agar

See B.2 or B.3.

5.2.3 Second selective enrichment medium: Muller-Kauffmann tetrathionate novobiocin broth (MKTTn broth)

See B.4.

5.2.4 Solid selective plating-out media

5.2.4.1 First medium: Xylose lysine deoxycholate agar (XLD agar)

See B.5.

5.2.4.2 Second medium

The choice of the second appropriate medium is left to the discretion of the testing laboratory (see Annex E). The manufacturer's instructions should be followed regarding its preparation for use.

5.2.5 Non-selective agar medium

The choice of the non-selective agar medium for purity check is left to the discretion of the testing laboratory. The manufacturer’s instructions should be followed regarding its preparation for use. An example of a non-selective agar medium is Nutrient agar (see B.6).

5.2.6 Triple sugar/iron agar (TSI agar)

See B.7.

NOTE As an alternative, a double sugar/iron agar can be used (Kligler-Hajna).

5.2.7 Urea agar (Christensen)

See B.8.

5.2.8 L-Lysine decarboxylation medium

See B.9.

5.2.9 Reagent for detection of β-galactosidase (or prepared paper discs used in accordance with the manufacturer's instructions) – Optional

See B.10.

Additional toluene is needed.

5.2.10 Medium and reagents for indole reaction - Optional

See B.11.

5.2.11 Saline solution

See B.12.

5.3 Antisera

Several types of agglutinating 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 only anti-sera prepared by a supplier recognized as competent (for example, by an appropriate government agency).

6 Apparatus and glassware

Disposable apparatus 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)**

See ISO 7218.

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

6.3a Incubator, capable of operating in the range 34 °C to 38 °C.

6.3b Incubator, capable of operating 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 - 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 Sterile loops, of approximate diameter 3 mm (10 μl volume), and of 1μl volume, and inoculation needle or wire.

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

6.11 Tubes or **flasks,** of appropriate capacity.

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

6.12 Graduated pipettes or **automatic pipettes**, of nominal capacities 10 ml, 1 ml and 0,1 ml.

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

For sampling of food and animal feed, see ISO/TS 17728. For sampling at the primary production stage, see ISO 13307. For sampling of carcasses, see ISO 17604.

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 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 diagrams in Annex A)

9.1 Test portion and initial suspension

9.1.1 General

For the preparation of test samples and initial suspensions of specific products, see the procedures as described in ISO 6887 (all parts).

For preparation of the initial suspension, in the general case, use as diluent the pre-enrichment medium specified in 5.2.1 (buffered peptone water). Pre-warm the BPW to room temperature before use.

In general, an amount of sample (mass or volume) is added to a quantity of BPW (mass or volume) to yield a 1/10 dilution (generally this concerns 25 g of sample added to 225 ml of BPW). However, for some type of samples it may be necessary to use another ratio (e.g. bootsocks).

NOTE When more than one 25 g test portion from a specified lot of product has to be examined, and when evidence is available that combining test portions does not affect the result for that particular food, the test portions can be pooled. More information on pooling of samples, as well as a procedure to test the influence of pooling on the sensitivity of the method, can be found in ISO 6887-1.

9.1.2 Specific preparations of the initial suspension for certain foodstuffs

The following preparations of the initial suspension are specific for *Salmonella*. Specific preparations applicable for the determination of several microorganisms, including *Salmonella*, are described in ISO 6887-2, ISO 6887-3, ISO 6887-4, ISO 6887-5 and ISO 6887-6.

9.1.2.1 Cocoa and cocoa-containing products

See ISO 6887 part 4.

9.1.2.2 Acidic and acidifying foodstuffs

See ISO 6887 part 1 and part 4.

9.1.2.3 Raw milk, heat-treated milk and liquid milk products

Pipette 25 ml of the test sample into a flask (6.11) containing 225 ml of BPW (5.2.1) and mix.

9.1.2.4 Dried milk products

Prepare a stoppered flask (6.11) with 225 ml of BPW (5.2.1).

Weigh 25 g of the test sample aseptically and pour it over the surface of the liquid in the flask. Stopper the flask, but do not shake. Allow to stand undisturbed at room temperature for 60 min ± 10 min before incubation. Adjustment of pH is not necessary. If after 1 h the dried milk is still not dissolved, mix the contents of the flask by shaking manually or stirring with a sterile spatula.

9.1.2.5 Lactose

Weigh 25 g of the test sample aseptically into a stoppered flask (6.11) containing 225 ml BPW (5.2.1) and shake to dissolve.

9.1.2.6 Casein, caseinates, cheese

Weigh 25 g of the test sample aseptically into the sterile container of a high-speed or peristaltic-type blender. Add 225 ml BPW (5.2.1) preheated to 45 °C (6.7). Blend until the test sample is thoroughly dispersed (1min to 3 min). Ensure that the temperature of the dispersion does not exceed 45 °C.

9.1.2.7 Butter

Weigh 25 g of the test sample aseptically into a sterile flask (6.11) and place in a water bath set at 45 °C (6.7). Keep in the water bath until the whole test portion has just melted. Add 225 ml BPW (5.2.1) preheated to 45 °C (6.7) and mix.

9.1.2.8 Frozen milk products (including edible ices)

Pipette 25 ml of the melted test sample, preheated to no more than 37 °C, into a flask (6.11) containing 225 ml BPW (5.2.1) and mix.

9.1.2.9 Fermented milks, yoghurt, custards, desserts

Weigh 25 g of the test sample aseptically into a stoppered flask (6.11) containing glass beads and 225 ml BPW (5.2.1) and shake to disperse.

9.2 Non-selective pre-enrichment

Incubate the initial suspension (9.1) between 34 °C and 38 °C (see 6.3a) for 18 h ± 2 h.

It is permissible to store the pre-enriched sample after incubation at 5 °C (6.8) for a maximum of 72 h.

9.3 Selective enrichment

9.3.1 General

Allow the selective enrichment media, RVS or MSRV (5.2.2) and MKTTn (5.2.3), to equilibrate at room temperature if they were stored at a lower temperature.

Minimise the transfer of particulate material from the pre-enrichment into the selective enrichment media.

Care should be taken that the maximum permitted incubation temperatures (38 °C and 42,5 °C) are not exceeded.

After incubation it is permissible to store the selective enrichment at 5 °C (6.8) for a maximum of 72 h.