SLOVENSKI STANDARD
oSIST prEN ISO 6579-4:2024

01-marec-2024

Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje prisotnosti, števila in serotipov Salmonella - 4. del: Identifikacija monofazne Salmonelle Typhimurium (1,4,[5],12:i:-) z verižno reakcijo s polimerazo (PCR) (ISO/DIS 6579-4:2023)

Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 4: Identification of monophasic Salmonella Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR) (ISO/DIS 6579-4:2023)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zum Nachweis von Salmonella spp. - Teil 4: Identifizierung von monophasischen Salmonella Typhimurium (1,4,[5],12:i:-) durch Polymerase□Kettenreaktion (PCR) (ISO/DIS 6579-4:2023)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche, le dénombrement et le sérotypage des Salmonella - Partie 4: Identification du variant monophasique de Salmonella Typhimurium (1,4,[5],12:i:-) par réaction de polymérisation en chaîne (PCR) (ISO/DIS 6579-4:2023)

Ta slovenski standard je istoveten z: **prEN ISO 6579-4**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

oSIST prEN ISO 6579-4:2024 **en,fr,de**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 6579-4

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

Secretariat: **AFNOR**

Voting begins on:
2023-12-22

Voting terminates on:
2024-03-15

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

Part 4:
Identification of monophasic Salmonella Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR)

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

Partie 4: Identification de Salmonella Typhimurium (1,4,[5],12:i:-) monophasique par réaction de polymérisation en chaîne (PCR)

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 6579-4:2023(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

A list of all parts in the ISO 6579 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

In several (international, regional, national) laws, regulatory limits are set to ensure the 'absence' of *Salmonella* spp. in samples of the food chain. Moreover, several (EC) Regulations also demand the absence of particular *Salmonella* serovars which have shown to cause a relatively high percentage of human salmonellosis. One of these *Salmonella* serovars for which legal criteria are set is *Salmonella* Typhimurium, including its monophasic variant 1,4[5],12:i:- (for example Regulation (EC) No. 1086/2011[8]). Hence, it is important to know that a serovar found with antigenic formula 1,4[5],12:i:- is indeed the monophasic variant of *Salmonella* Typhimurium (1,4[5],12:i:1,2) and not the monophasic variant of another *Salmonella* serovar for which no criteria are set, like *S*. Lagos (1,4[5],12:i:1,5), *S*. Agama (4,12:i:1,6), *S.* Farsta (4[5],12:i:e,n,x), *S*. Tsevie (1,4,12:i:e,n,z_{15}), *S.* Gloucester (1,4,12,27:i:l,w), or *S*. Tumodi (1,4,12:i:z_6). Confirmational distinction between *Salmonella* Typhimurium and *Salmonella* non-Typhimurium serovars can be determined using molecular analysis, like the PCR technique(s) described in this document.

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

Part 4: Identification of monophasic Salmonella Typhimurium (1,4,[5],12:i:-) by polymerase chain reaction (PCR)

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting, enumerating and (sero)typing *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 document should be familiar with normal laboratory practice. This document 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.

1 Scope

This document specifies a horizontal *in vitro* method for the molecular identification and differentiation of the monophasic variant of *Salmonella enterica* subsp. *enterica* serovar Typhimurium (1,4[5],12:i:-) lacking, or not expressing, the second H phase H:1,2, starting from isolates. The method detects specific DNA sequences of an intergenic region of the first H phase flagellin cluster for identification of *Salmonella enterica* subsp. *enterica* serovar Typhimurium (further called *Salmonella* Typhimurium) and specific DNA sequences of genes associated with second H phase flagellar antigen expression.

The method is applicable for:

— differentiation of the isolate under analysis between monophasic *Salmonella* Typhimurium and the monophasic variant of another *Salmonella* non-Typhimurium serovar that has the same antigenic formula;

— identification of the isolate under analysis being either monophasic *Salmonella* Typhimurium or (biphasic) *Salmonella* Typhimurium.

This document is applicable for the analysis of a pure culture belonging to the genus *Salmonella*, isolated from:

— products intended for human consumption;

— products intended for animal feeding;

— environmental samples in the area of food and feed production and handling;

— samples from the primary production stage.

This document can also be applied in other domains for identification of monophasic *Salmonella* Typhimurium (e.g. environmental, human health, animal health).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 6579-1, *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/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 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 20836, *Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of microorganisms — Thermal performance testing of thermal cyclers*

ISO 20837, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for sample preparation for qualitative detection*

ISO 20838, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for amplification and detection for qualitative methods*

ISO 22119:2011, *Microbiology of food and animal feeding stuffs — Real-time polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

ISO 22174, *Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection and quantification of microorganisms — General requirements and definitions*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22174 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online Browsing Platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
monophasic *Salmonella* Typhimurium
variant of *Salmonella enterica* subsp. *enterica* serovar Typhimurium lacking the second H phase, having the antigenic formula 1,4[5],12:i:-

3.2
presumptive monophasic *Salmonella* Typhimurium strain (isolate)
a pure culture characterized as belonging to the genus *Salmonella*, giving a positive reaction for O-antigen O:4 and H-antigen H:i and with a negative reaction for the second H phase H:1,2

3.3
threshold cycle crossing point
point of the amplification curve at which the fluorescence signal rises above the baseline or crosses a predefined threshold setting

[SOURCE: ISO 22119:2011, 3.17]

3.4
quantification cycle
C_q, C_t, C_p, TOP
in real-time PCR, the cycle at which the fluorescence signal can be distinguished from the background fluorescence level entering into the exponential phase of target amplification

Note 1 to entry: Quantification cycle (C_q) is a generic term which includes cycle threshold (C_t), crossing point (C_p), take off point (TOP) and all other instrument specific terms referring to the fractional cycle used to detect or quantify the target in the real-time PCR assay.

Note 2 to entry: The quantification cycle is based either on a threshold applied to all samples or on a regression analysis of the signal, for each sample.

[SOURCE: ISO 22174, 3.58]

4 Principle

4.1 General

The identification of the monophasic variant of *Salmonella* Typhimurium comprises of the following three successive steps (see 4.2 to 4.4).

4.2 Preparation of well-isolated colonies

The culture is streaked onto the surface of a (pre-dried) non-selective solid nutrient medium and incubated between 34 °C and 38 °C for 24 h, to obtain well-isolated colonies.

4.3 Suspension of a colony

A well-isolated colony is selected and suspended in 100 µl saline solution (0,85 % w/v) or in 100 µl PCR grade water.

4.4 Amplification and detection

The suspended bacterial cells are analysed by PCR for detection of the genetic sequences unique to *Salmonella* Typhimurium (1,4[5],12:i:1,2) and its monophasic variant lacking the second H phase (1,4[5],12:i:-), as well as for detection of specific genetic sequences of genes associated with the second H phase flagellar antigen expression. Specific PCR assays including primers and probes are described in Annexes B to D.

5 Culture media and reagents

Follow current laboratory practices in accordance with ISO 7218. For the steps in 4.3 and 4.4, molecular grade reagents and consumables suitable for molecular biology shall be used as indicated in ISO 20837 and ISO 20838. The composition of culture media and reagents and their preparation are specified in Annex A. For performance testing of culture media, follow the procedures in accordance with ISO 11133 and Clause A.4. The primers and probes for identification of the monophasic variant of *Salmonella* Typhimurium (1,4[5],12:i:-) are listed in Annexes B to D.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications. The usual microbiological laboratory equipment (see ISO 7218) and, in particular, the following shall be used.

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

NOTE The range 34 °C to 38 °C for incubation of culture media includes the use of incubators set at 35 °C ± 1 °C, 36 °C ± 2 °C or 37 °C ± 1 °C.

6.2 Sterile loops, of approximate diameter 3 mm (10 µl) and 0,3 mm (1 µl), or an inoculation needle/wire.

6.3 Water bath, capable of operating at 47 °C to 50 °C.

6.4 Refrigerator, capable of operating at 5 °C ± 3 °C.

6.5 Drying cabinet or **oven**, capable of operating between 25 °C and 50 °C.

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

6.7 Equipment for suspension of a colony, e.g. (micro)centrifuge tubes.

6.8 Graduated pipettes and **pipette filter tips**, for handling volumes between 0,2 µl and 13,55 µl, depending on the PCR assay used. For more reactions per mix, larger volumes are needed.

6.9 Mixer.

6.10 Sterile Petri dishes, with a diameter of approximately 90 mm.

6.11 Equipment for PCR and **real-time PCR**, e.g. microcentrifuge or plate spinner**.**

6.12 Thermal cycler or **real-time PCR thermal cycler**, calibrated in accordance with ISO 20836.

6.13 Associated consumables for conventional or real-time PCR, e.g. PCR tubes, optical plates and seals, optical plate holder, suitable for use with the selected PCR machine.

6.14 Apparatus for dry sterilization (oven) or **wet sterilization (autoclave)**.
As specified in ISO 7218.

7 Presumptive monophasic *Salmonella* Typhimurium strain (isolate)

The isolate to be used for further identification shall be a pure culture characterized as belonging to the genus *Salmonella* (see ISO 6579-1). A presumptive monophasic *Salmonella* Typhimurium isolate will show a positive reaction for O-antigen O:4 and H-antigen H:i and a negative reaction for the second H phase H:1,2 (see ISO/TR 6579-3).

8 Culturing the isolate

Streak the culture of Clause 7 (e.g. with a 10 µl loop; 6.2) on the surface of a non-selective agar medium (e.g. Nutrient agar; A.2) to obtain well-isolated colonies. Incubate the plates, inverted, between 34 °C and 38 °C for 24 h ± 3 h.

9 Procedure

9.1 Preparation of cell suspension or DNA

By means of an inoculating wire or a sterile loop (6.2), pick and suspend (a portion of) one colony in 100 µl saline solution (0,85 % w/v; A.3) or in 100 µl PCR grade water.