SLOVENSKI STANDARD
oSIST prEN ISO 15213-1:2021

01-november-2021

Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje prisotnosti in števila Clostridium spp. - 1. del: Preštevanje Clostridium spp., ki reducirajo sulfit, s tehniko štetja kolonij (ISO/DIS 15213-1:2021)

Microbiology of the food chain - Horizontal method for the detection and enumeration of Clostridium spp. - Part 1: Enumeration of sulfite-reducing Clostridium spp. by colony-count technique (ISO/DIS 15213-1:2021)

Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zum Nachweis und zur Zählung von Clostridium spp. - Teil 1: Zählung von Sulfit reduzierenden Clostridium spp. durch Koloniezählverfahren (ISO/DIS 15213 1:2021)

Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche et le dénombrement de Clostridium spp. - Partie 1: Dénombrement de Clostridium spp. sulfito-réducteur par la technique par comptage des colonies (ISO/DIS 15213-1:2021)

Ta slovenski standard je istoveten z: **prEN ISO 15213-1**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology

oSIST prEN ISO 15213-1:2021 **en,fr,de**

DRAFT INTERNATIONAL STANDARD

ISO/DIS 15213-1

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

Secretariat: **AFNOR**

Voting begins on:
2021-09-13

Voting terminates on:
2021-12-06

Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. —

Part 1:
Enumeration of sulfite-reducing *Clostridium* spp. by colony-count technique

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche et le dénombrement de Clostridium *spp. —*

Partie 1: Dénombrement de Clostridium *spp. sulfito-réducteur par la technique par comptage des colonies*

ICS: 07.100.30

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 15213-1:2021(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.*

This first edition of ISO 15213-1, together with ISO 15213-2 and ISO 15213-3, cancels and replaces ISO 15213:2003 and ISO 7937:2004, which have been technically revised. The main changes in this first edition, compared to ISO 15213:2003, are the following:

— the scope is enlarged to samples from the primary production stage;

— the scope of the method is changed from bacteria to *Clostridium* spp.; therefore, typical colonies on the iron sulfite agar plates are confirmed;

— the concentration of sulfite in the Iron Sulfite Agar (ISA) is reduced from 1,0 g/l into 0,5 g/l;

— the heat treatment of 10 min at 80 °C is optional, in case of high background flora, or for the enumeration of only spores of sulfite-reducing *Clostridium* spp. present in the sample;

— the option for using tubes is removed;

— the option for incubating the samples at 50 °C for the enumeration of thermophilic sulfite-reducing bacteria is removed;

— a description is given how the confirmation of typical colonies has to be performed;

— in Annex C the performance characteristics are given;

— Annex D provides a special protocol for the enumeration of sulfite-reducing *Clostridium* spp. in feed.

Introduction

Sulfite-reducing *Clostridium* spp. are anaerobic, Gram-positive, spore-forming, rod-shaped bacteria which belong to the family of the *Bacillaceae*. The most important species which belong to this group are *Clostridium (C.) perfringens, C. bifermentans, C. sporogenes* and *C. botulinum*. Some species can cause foodborne illness. As ubiquitous bacteria they are predominantly found in nature.

Sulfite-reducing *Clostridium* spp., including *C. perfringens,* are widely used as microbial indicators of clostridial contamination in the manufacturing of foods (e.g. meat production). These have the capacity to produce heat resistant spores. Outside the dairy industry, the use of sulfite-reducing *Clostridium* spp. as a microbial indicator is limited to a relatively small number of foods. Its current application in non-dairy foods is either an indication of faecal contamination (especially *C. perfringens*, see also ISO 15213-2 and ISO 15213-3) and/or as an indicator of sanitation/process control related to potential growth and survival of anaerobic spore-forming bacteria.

This part of ISO 15213 describes the horizontal method for the enumeration of sulfite-reducing *Clostridium* spp. in food, feed, environmental samples, and samples from the primary production stage. The method for the enumeration of *C. perfringens* is described in part 2. Part 3 describes the method for the detection of *C. perfringens*. These three parts are published into one series of International Standards because the methods are closely linked to each other. These methods are often conducted in association with each other in a laboratory and the media and their performance characteristics may be similar.

The main technical changes listed in the Foreword, introduced in this document compared to ISO 15213:2003 are considered as major (see ISO 17468).

These changes have a major impact on the performance characteristics of the method.

Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. —

Part 1: Enumeration of sulfite-reducing *Clostridium* spp. by colony-count technique

1 Scope

This document specifies the enumeration of sulfite-reducing anaerobes and sulfite-reducing *Clostridium* spp. by the colony-count technique. This part of ISO 15213 is applicable to

— products intended for human consumption,

— products intended for animal feeding,

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

— samples from the primary production stage.

This horizontal method was originally developed for the examination of all samples belonging to the food chain. Based on the information available at the time of publication of this document, this method is considered to be fully suited to the examination of all samples belonging to the food chain. However, because of the large variety of products in the food chain, it is possible that this horizontal method is not appropriate in every detail for all products. Nevertheless, it is expected that the required modifications are minimized so that they do not result in a significant deviation from this horizontal method.

This technique is suitable for, but not limited to, use for the enumeration of microorganisms in test samples and is based on a minimum of 10 colonies counted in a plate. This corresponds to a level of contamination that is expected to be higher than 10 cfu/ml for liquid samples or higher than 100 cfu/g for solid samples.

NOTE This method has been validated in an interlaboratory study for the following food categories:

1) Ready-to-eat, ready-to-reheat meat products;

2) Eggs and egg products (derivates);

3) Processed fruits and vegetables;

4) Infant formula and infant cereals;

5) Multi-component foods or meal components;

and for the following other categories: Pet food and animal feed, Environmental samples (food or feed production).

As this method has been validated for at least five food categories, this method is applicable for a broad range of foods. Since the method is not commonly used for samples in the primary production stage, this category was not included in the validation study. Therefore, no performance characteristics were obtained for this category. For detailed information on the validation see Clause 11 and Annex C.

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 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

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*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
sulfite-reducing *Clostridium* spp
genus of microorganisms of the family of *Clostridiaceae*, usually capable of growth in/on Iron Sulfite Agar (ISA) under anaerobic conditions, forming typical or less typical colonies, and displaying certain characteristics with biochemical confirmation tests

Note 1 to entry: The biochemical confirmation tests are described in 9.6.

3.2
enumeration of sulfite-reducing *Clostridium* spp
determination of the number of colony-forming units (cfu) of sulfite-reducing *Clostridium* spp. (3.1) bacteria per ml or per g or sample when the specified test is conducted

Note 1 to entry: The specified test is described in Clause 9.

4 Principle

4.1 General

A specified quantity of the liquid test sample, or of an initial suspension in the case of other products, is dispensed into an empty Petri dish and mixed well with a specified molten agar culture medium to form a poured plate. Other plates are prepared under the same conditions using decimal dilutions of the test sample. If it is the intention to count only spores, heat treatment of 10 min at 80 °C needs to be performed before plating.

When the number of cfu is expected to be at or near the limit of determination of the method, the use of duplicate plates is preferable. If duplicate plates are used the minimum for the sum of colonies should be 10. In this case the level of contamination is expected to be higher than 5 cfu/ml for liquid samples or higher than 50 cfu/g for solid samples.

A pour-plate technique is especially suited for the enumeration of products expected to contain spreading colonies that can obscure colonies of the target microorganisms.

The enumeration of sulfite-reducing *Clostridium* spp. requires four successive stages as specified in Annex A.

4.2 Preparation of dilutions

For the preparation of decimal dilutions from the test portion, follow the procedure as specified in ISO 6887 (all parts).

4.3 Enumeration

The plates are incubated under anaerobic conditions at 37 °C for 48 h. After incubation, the number of typical colonies, which show black or grey to yellow-brown staining, are counted. The colour of the colonies and the surrounding zone commences due to the formation of iron(II)sulphide as a result of the reaction between sulphide ions and trivalent iron [Fe(III)] present in the medium.

4.4 Confirmation

Typical colonies are picked for confirmation.

NOTE When no confirmation is performed, the results can be reported as 'anaerobic sulfite-reducing bacteria'.

5 Culture media and reagents

Follow current laboratory practices in accordance with ISO 7218. The composition of culture media and reagents and their preparation are specified in Annex B. For performance testing of culture media, follow the procedures in accordance with ISO 11133 and/or Annex B.

6 Equipment and consumables

Disposable equipment 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 Appropriate apparatus for achieving an anaerobic atmosphere.

6.2 Apparatus for dry sterilization (oven) or **wet sterilization (autoclave).**

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

6.4 Incubator, capable of operating at 37 °C ± 1 °C.

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

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

6.7 Sterile flasks, bottles or tubes, of appropriate capacity. Bottles, flasks or tubes with non-toxic metallic of plastic screw-caps may be used.

6.8 Sterile graduated pipettes or **automatic pipettes**, of nominal 10 ml and 1 ml.

6.9 Sterile loops, of approximately 1 µl volume, or inoculation needle or wire.

6.10 Sterile Petri dishes, with a diameter of approximately 90 mm and (optional) large size (diameter approximately 140 mm).

6.11 Water bath, capable of operating at 44 °C to 47 °C and 80 °C ± 2 °C.

7 Sampling

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

Recommended sampling techniques are given in:

— ISO/TS 17728 for food and animal feed;

— ISO 707 for milk and milk products;

— ISO 6887-3 for fish and fishery products;

— ISO 13307 for primary production stage;

— ISO 17604 for carcasses;

— ISO 18593 for surfaces.

It is important that the laboratory receives a sample that is representative. The sample should not have been damaged or changed during transport or storage.

8 Preparation of test sample

Prepare the test sample from the laboratory sample in accordance with the specific International Standard dealing with the product concerned; follow the procedures as specified in ISO 6887 (all parts). If there is no specific International Standard available, it is recommended that the parties concerned come to an agreement on this subject.

9 Procedure

9.1 General

A diagram of the procedure is given in Annex A.

9.2 Test portion, initial suspension and dilutions

Refer to ISO 6887 (all parts) and the specific International Standard dealing with the product concerned. A proposal for preparing the initial suspension of feed samples is given in Annex D.

9.3 Heat pre-treatment to select spores

If it is the intention to count only spores, heat the decimal dilution series to 80 °C in a water bath for 10 min ± 1 min. Heat treatment shall be given within 15 min after preparation of the initial suspension to avoid germination of spores. The temperature should be monitored by placing an appropriate thermometer in a reference bottle of the same size as the sample bottle and containing the same volume of water at the same initial temperature as the sample being treated. The time taken to reach 80 °C shall not exceed 15 min and can be minimised by ensuring the water level to be at least 4 cm above the level of the sample and that water in the water bath is circulated to maximize heat exchange.

Start the time of heating (10 min) when the temperature of the reference sample has reached 80 °C. After heat treatment, the samples should be cooled immediately till approximately 20 °C.

Heat treatment should also reduce the competitive flora in some matrices containing a high level of background flora (e.g. liquid whey, feed silage).