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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 des bactéries Clostridium *spp. sulfito-réductrices par la technique de comptage des colonies*

Reference number
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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.

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*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition of ISO 15213-1 cancels and replaces ISO 15213:2003, which has been technically revised.

The main changes are as follows:

— the Scope has been expanded to include samples from the primary production stage;

— the scope of the method has been changed from "sulfite-reducing bacteria" to "sulfite-reducing *Clostridium* spp.": therefore, typical colonies on the iron sulfite agar plates are confirmed;

— the concentration of sulfite in the iron sulfite agar has been reduced from 1,0 g/l to 0,5 g/l;

— the heat treatment of 10 min at 80 °C has been made optional, in the 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 for inoculation has been removed;

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

— a description of how the confirmation of typical colonies has to be performed has been added;

— the flow diagram in Annex A giving a short description of the procedure has been revised;

— in Annex C, the performance characteristics have been added;

— Annex D has been added to provide a special protocol for the enumeration of sulfite-reducing *Clostridium* spp. in feed.

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A list of all parts in the ISO 15213 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.

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Introduction

Sulfite-reducing *Clostridium* spp. are obligate anaerobic, Gram-positive, spore-forming, rod-shaped bacteria. 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. The *Clostridium* species inhabit soils and the intestinal tract of animals and humans.

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/TS 15213-3) and/or as an indicator of sanitation/process control related to potential growth and survival of anaerobic spore-forming bacteria.

This document 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 ISO 15213-2. The method for the detection of *C. perfringens* is described in ISO/TS 15213-3. These three parts are published as a 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 can be similar.

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

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

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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

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for the enumeration of sulfite-reducing *Clostridium* spp. 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 the enumeration of sulfite-reducing *Clostridium* spp. by the colony-count technique.

This document is applicable to:

— products intended for human consumption;

— products for feeding animals;

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

— samples from the primary production stage.

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

— ready-to-eat, ready-to-reheat meat products;

— eggs and egg products (derivates);

— processed fruits and vegetables;

— infant formula and infant cereals;

— multi-component foods or meal components.

It has also been validated 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 food. For detailed information on the validation, see Clause 11 and Annex C. Since the method is not commonly used for samples in the primary production stage, this category was not included in the interlaboratory study. Therefore, no performance characteristics were obtained for this category.

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

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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, the enumeration of microorganisms in test samples with a minimum of 10 colonies counted on 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.

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 the food chain — 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 19036:2019, *Microbiology of the food chain — Estimation of measurement uncertainty for quantitative determinations*

3 Terms and definitions

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

ISO and IEC maintain terminology 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 gram, per millilitre, per square centimetre or per sampling device when a specified test is conducted

Note 1 to entry: Specified tests are given 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. After solidification of the agar culture medium, an overlay is used to prevent the

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