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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*



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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

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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).

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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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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

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.