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Microbiology of the food chain — Detection and enumeration of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables and berry fruits

Microbiologie de la chaîne alimentaire — Recherche et dénombrement de Cryptosporidium *et* Giardia *dans les légumes verts frais à feuilles et les fruits à baies*

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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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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Introduction

Cryptosporidium spp. and *Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis*) are protozoan parasites that can cause enteric illness in humans. Both organisms are characterized by a robust transmission stage, the *Cryptosporidium* oocyst and the *Giardia* cyst, which can survive in moist environments for prolonged periods. These transmission stages are hereafter referred to collectively as (oo)cysts. *Cryptosporidium* oocysts in particular are highly resistant to chlorine at the concentrations used in the treatment of drinking water, and chemical disinfection of leafy green vegetables and berry fruits, where performed during processing, may also be ineffective. Consequently, the absence of vegetative bacteria on fresh produce as indicators of faecal contamination does not necessarily indicate the absence of (oo)cysts. No practical method exists to culture *Cryptosporidium* spp. and *Giardia duodenalis* for the purpose of detection, and therefore, in order to detect contamination with these parasites, direct removal of the (oo)cysts from the food sample must be performed, followed by visualization of the (oo)cysts by microscopy. The methods described in this International Standard are for determining whether *Cryptosporidium* and/or *Giardia* (oo)cysts are present on the surfaces of fresh produce and for their enumeration. This International Standard is based on published methods that have been tested in a multicentre collaborative trial. Alternative methods can be used following a demonstration of their equivalence with this International Standard following the protocol described in ISO 16140.[1]

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WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, 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 method that is applicable for the detection and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts on or in food products that are described herein as fresh leafy green vegetables and berry fruits. With suitable controls, it may also be applicable for the examination of other fresh produce.

The microscopy descriptions are for *Cryptosporidium* spp. oocysts and *Giardia duodenalis* cysts of size ranges which include those species (*Cryptosporidium*) or assemblages (*Giardia*) known to be pathogenic to humans.

This method does not include any molecular analysis and therefore is not suitable for the determination of the species or genotypes/assemblages of *Cryptosporidium* oocysts and *Giardia* cysts. The method will detect all species and genotypes/assemblages that are known to be pathogenic for humans and also others that are not. For further identification, molecular typing assays are required. However, these cannot be reliably performed if process positive controls have been spiked into the samples, as the result of molecular typing assays will be obfuscated.

This method does not allow the determination of viability or infectivity of any *Cryptosporidium* oocysts and *Giardia* cysts which may be present.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 7218:2007, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

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

3.1
***Cryptosporidium* oocyst**
transmission stage of *Cryptosporidium* spp.

Note 1 to entry: Its detection is based on reaction with specific anti-*Cryptosporidium* antibodies and morphological characteristics as described in Clause 8.

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3.2
***Giardia* cysts**
transmission stage of *Giardia* spp.

Note 1 to entry: Its detection is based on reaction with specific anti-*Giardia* antibodies and typical morphological characteristics as described in Clause 8.

3.3
fresh leafy green vegetable
plant leaves eaten as a vegetable, which have not been subjected to any process, except perhaps cutting and washing

3.4
fresh berry fruit
small, round or oblong, fleshy and juicy fruit, which has not been subjected to any process except perhaps cutting and washing

3.5
internal extraction control
(oo)cysts labelled with specific fluorogenic reporters that may be added in defined numbers to the sample prior to processing to assure that the method is operating properly

3.6
positive control
sample to which (oo)cysts have been added in defined numbers prior to extraction to verify that the method after the elution step is operating efficiently

3.7
negative control
sample having an equivalent quantity of material to the tested sample, which is considered to be free of (oo)cysts and processed in the same manner as the tested sample

4 Principle

The principle of the method is based on removal of the (oo)cysts from the sample by elution procedures, followed by concentration in the eluate by centrifugation and isolation by immunomagnetic separation (IMS). Detection of the (oo)cysts is performed by microscopy after labelling with specific monoclonal antibodies (mAbs) conjugated to a fluorochrome.

5 Reagents

5.1 Reagents required for eluting (oo)cysts from leafy green vegetables and berry fruits

5.1.1 Glycine buffer, pH 5,5 for leafy green vegetables (A.2.1).

5.1.2 Glycine buffer, pH 3,5 for berry fruits (A.2.2).

5.2 Reagents required for concentrating, fixing, staining, detection, and quality control

5.2.1 Methanol, analytical grade.

5.2.2 Paramagnetic beads, coupled with antibodies specific to the walls of *Cryptosporidium* oocysts and/or *Giardia* cysts.

5.2.3 Hydrochloric acid (HCl), 0,1 mol/l (A.3.1).

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