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Microbiology of the food chain — Horizontal method for the enumeration of psychrotrophic microorganisms

Microbiologie de la chaîne alimentaire — Méthode horizontale pour le dénombrement des micro-organismes psychrotrophes

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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*.

This second edition cancels and replaces the first edition (ISO 17410:2001), which has been technically revised. It also replaces ISO 6730:2005 | IDF 101:2005[2] and ISO 8552:2004 | IDF 132:2004[4]. The main changes compared with the previous edition are as follows:

— the surface-plating technique is used, as opposed to the pour-plate technique used in ISO 6730:2005 | IDF 101:2005 and ISO 8552:2004 | IDF 132:2004, as psychrotrophic microorganisms are sensitive to heat;

— one horizontal method is used for the enumeration of psychrotrophic microorganisms in a) products intended for human consumption, b) products intended for animal feeding, c) environmental samples in the area of food and feed production, handling, and d) samples from the primary production stage;

— the rapid method has been included as an annex for the estimation of psychrotrophic plate count in raw and pasteurized milk (originating from ISO 8552:2004 | IDF 132:2004);

— performance testing of the culture medium, plate count agar (PCA), has been introduced;

— the expression of results has been changed to be in accordance with ISO 7218.

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

Psychrotrophic microorganisms are able to grow at low temperatures. These microorganisms may cause decay of refrigerated foods (except gas-packaged foods) due to odour and taste deviations. Some psychrotrophic microorganisms present in raw milk are also capable of producing heat stable enzymes. When heated (pasteurization or sterilization) these enzymes are insufficiently inactivated, causing quality defects in the heated product (fat or protein degradation).

For the revision of this document, no performance characteristics were included due to the lack of data and the fact that an interlaboratory study was not organized for the described method, since psychrotrophic microorganisms are a group of microorganisms that are mainly used for process monitoring and considered to be non-pathogenic.

The main technical changes listed in the Foreword, introduced in this document compared to ISO 17410:2001, are considered as minor (see ISO 17468[6]).

Microbiology of the food chain — Horizontal method for the enumeration of psychrotrophic microorganisms

1 Scope

This document specifies a horizontal method for the enumeration of psychrotrophic microorganisms that are able to grow and form colonies on a solid agar culture medium after aerobic incubation at 6,5 °C.

This document 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.

NOTE Annex B specifies a rapid method for the estimated enumeration of psychrotrophic microorganisms in raw and pasteurized milk.

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 835, *Laboratory glassware — Graduated pipettes*

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 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

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 http://www.electropedia.org/

3.1
psychrotrophic microorganism
entity of microscopic size, encompassing bacteria, yeasts and moulds

Note 1 to entry: Psychrotrophic microorganisms are bacteria, yeasts and moulds that are able to produce colonies under the conditions specified in this document.

4 Principle

A specified quantity of a test sample (liquid products), or a specified quantity of an initial suspension in the case of other products (non-liquid products), is surface-plated on a solid agar culture medium contained in Petri dishes.

Other plates are prepared under the same conditions using decimal dilutions of the test sample or of the initial suspension.

The plates are incubated under aerobic conditions at 6,5 °C for 10 days.

Annex B specifies a method for the rapid estimation of psychrotrophic plate count in raw and pasteurized milk by incubating plates at 21 °C for 25 h. However, it should be noted that not all microorganisms able to produce colonies at 6,5 °C will produce colonies if incubated at 21 °C.

The number of psychrotrophic microorganisms per gram of sample or the number of psychrotrophic microorganisms per millilitre of sample is calculated from the number of colonies obtained on the plates containing fewer than 150 colonies.

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 A. For performance testing of culture media, follow the procedures in accordance with ISO 11133 and Annex A.

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 Colony-counting device (optional), consisting, for example, of an illuminated base with a dark background, fitted with a magnifying screen to aid colony detection, and (optionally) a mechanical or electronic digital counter.

6.2 Sterile spreader or **spatula**, made of glass, plastic or steel, for spreading inoculated material on the surface of the culture medium.

NOTE A spreader made of glass can be made from a glass rod of about 3,5 mm in diameter, shaped like a hockey stick about 20 cm long, bent at right angles about 3 cm from one end and flattened at the ends by heating.

6.3 Incubator, capable of operating at a temperature of 6,5 °C ± 1 °C.

6.4 Oven for dry sterilization or **autoclave** for wet sterilization, used in accordance with ISO 7218.

6.5 Petri dishes, made of glass or plastic, of approximately 90 mm or 140 mm diameter.

6.6 pH meter, accuracy to within ±0,1 pH unit at 25 °C.

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

6.8 Total delivery graduated pipettes, sterile, of nominal capacities 0,1 ml and 1 ml, ISO 835 class A, or automated pipettes, ISO 8655-2 with use of sterile tips.

6.9 Tubes, bottles or **flasks**, of appropriate capacity, for preparation, sterilization and, if necessary, storage of culture media.

6.10 Water bath, or **similar apparatus**, capable of operating at a temperature of 47 °C to 50 °C and of boiling water.

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 the following documents:

— ISO/TS 17728 for food and animal feed[8];

— ISO 707 for milk and milk products[1];

— ISO 6887-3 for fish and fishery products[3];

— ISO 13307 for the primary production stage[5];

— ISO 17604 for carcasses[7];

— ISO 18593 for surfaces[9].

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 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 Test portion, initial suspension and dilutions

Use the method described in ISO 6887 (all parts) or the specific International Standard appropriate to the product concerned.