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Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *Escherichia coli* — Most probable number technique

*Microbiologie des aliments — Méthode horizontale pour la recherche et le dénombrement d'*Escherichia coli *présumés — Technique du nombre le plus probable*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 7251 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This third edition cancels and replaces the second edition (ISO 7251:1993), and also ISO 11866-1:1997 and IDF 170-1:1999.

Clauses 4, 9 and 10 of ISO 7251:1993 have been technically revised. The main changes are as follows:

- the temperature of the second incubation is now 44 °C ± 1 °C (see 4.2.5);
- detection (9.1) and enumeration (9.2) of presumptive *E. coli* are covered;
- the use of five tubes per dilution is allowed for some specific products (see 9.2.2.1);
- some products (dairy products) may hinder the collection of gas (see 9.1.2 and 9.2.2.5);
- the MPN calculation refers to ISO 7218 (see Clause 10).

Introduction

Because of the large variety of products within this field of application, these guidelines may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases every attempt will be made to apply the provided guidelines as far as possible and that deviations from them will only be made if absolutely necessary for technical reasons.

When this International Standard is next reviewed, account will be taken of all information then available regarding the extent to which the guidelines have been followed and the reasons for deviations from them in the case of particular products.

The harmonization of test methods cannot be immediate, and for certain groups of products International Standards and/or national standards may already exist that do not comply with these guidelines. In cases where International Standards already exist for the product to be tested, they should be followed, but it is hoped that when such standards are reviewed they will be changed to comply with this International Standard so that eventually the only remaining departures from these guidelines will be those necessary for well-established technical reasons.

Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *Escherichia coli* — Most probable number technique

1 Scope

This International Standard gives general guidelines for the detection and enumeration of presumptive *Escherichia coli* by means of the liquid-medium culture technique and calculation of the most probable number (MPN) after incubation at 37 °C, then at 44 °C. This International Standard is applicable to

- products intended for human consumption and the feeding of animals, and
- environmental samples in the area of food production and food handling.

WARNING — Some pathogenic strains of *Escherichia coli* do not grow at 44 °C.

A limitation of the applicability of this International Standard is imposed by the susceptibility of the method to a large degree of variability (see Clause 10).

2 Normative references

The following referenced documents are indispensable for the application 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 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

ISO/TS 11133-2, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 2: Practical guidelines on performance testing of culture media*

3 Terms and definitions

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

3.1

presumptive *Escherichia coli*

bacteria which at 44 °C ferment lactose with the production of gas, and which at 44 °C produce indole from tryptophan, when the test is carried out in accordance with the method specified in this International Standard

3.2

enumeration of presumptive *Escherichia coli*

most probable number of *E. coli* per millilitre or per gram of the test sample when the test is carried out according to the method specified in this International Standard

4 Principle

4.1 Detection method

4.1.1 A liquid selective enrichment medium is inoculated with a specified quantity of the initial suspension of the test sample.

4.1.2 The tube is incubated at 37 °C for up to 48 h. The tube is examined for gas production after 24 h and 48 h.

4.1.3 If the tube has given rise to opacity, cloudiness or gaseous emission, it is subcultured to a tube containing a liquid selective medium (EC broth).

4.1.4 The tube obtained in 4.1.3 is incubated at 44 °C for up to 48 h. The tube is examined for gas production after 24 h and 48 h.

4.1.5 If the tube of medium obtained in 4.1.4 has given rise to gaseous emission, it is subcultured to a tube containing indole-free peptone water.

4.1.6 The tube obtained in 4.1.5 is incubated at 44 °C for 48 h. The tube is examined for indole production resulting from the degradation of tryptophan in the peptone constituent.

4.1.7 Tubes showing opacity, cloudiness or gas production in the liquid selective enrichment medium (4.1.1) and whose subcultures have produced gas in the EC broth and indole in the peptone water at 44 °C are considered to contain presumptive *Escherichia coli*. The results are given as the "presence" or "absence" of presumptive *Escherichia coli* in x g or x ml of product.

4.2 Enumeration method

4.2.1 Three tubes of double-strength liquid selective enrichment medium are inoculated with a specified quantity of the initial suspension.

4.2.2 Three tubes of single-strength liquid enrichment medium are inoculated with a specified quantity of the initial suspension.