INTERNATIONAL STANDARD

ISO 11866-1

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Milk and milk products — Enumeration of presumptive *Escherichia coli* —

Part 1:
Most probable number technique

*Lait et produits laitiers — Dénombrement d'*Escherichia coli *présumés —*

Partie 1: Technique du nombre le plus probable

Reference number
ISO 11866-1:1997(E)

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.

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.

This part of ISO 11866 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC INTERNATIONAL, and will also be published by these organizations.

ISO 11866 consists of the following parts, under the general title *Milk and milk products — Enumeration of presumptive* Escherichia coli:

— *Part 1: Most probable number technique*

— *Part 2: Most probable number technique using 4-methylumbelliferyl-β-D-glucuronide (MUG)*

— *Part 3: Colony-count technique at 44 °C using membranes*

The method specified in ISO 11866-1 is preferred for samples in which comparatively low numbers of *Escherichia coli* are suspected.

Annex A forms an integral part of this part of ISO 11866. Annex B is for information only.

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Milk and milk products — Enumeration of presumptive *Escherichia coli* —

Part 1:

Most probable number technique

1 Scope

This part of ISO 11866 specifies a method for the enumeration of presumptive *Escherichia coli* by means of the culture technique involving a liquid medium, and calculation of the number of presumptive *Escherichia coli* per gram or per millilitre by the most probable number (MPN) technique after incubation at 37 °C then incubation at 44 °C.

The method is applicable to

— milk, liquid milk products;
— dried milk, dried sweet whey, dried buttermilk, lactose;
— acid casein, lactic casein and rennet casein;
— caseinate and dried acid whey;
— cheese and processed cheese;
— butter;
— frozen milk products (including edible ices);
— custard, desserts and cream.

The method specified in this part of ISO 11866 is preferred for samples in which comparatively low numbers of presumptive *Escherichia coli* (less than 100 per gram or 10 per millilitre) are suspected.

CAUTION — Some *Escherichia coli* pathogenic species do not grow at 44 °C. The applicability of this part of ISO 11866 is limited by the susceptibility of the method to a large degree of variability. The method should, therefore, be used and the results interpreted in the light of the information given in clause 12.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 11866. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 11866 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 7218:1996, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations.*

ISO 8261:1989; *Milk and milk products — Preparation of test samples and dilutions for microbiological examination.*

3 Definition

For the purposes of this part of ISO 11866, the following definition applies.

3.1 presumptive *Escherichia coli:* Bacteria which at 44 °C cause fermentation of 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 part of ISO 11866.

4 Principle

4.1 Inoculation of three tubes of double-strength liquid selective enrichment medium [5.3.1.1 a)] with a specified quantity of test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

4.2 Inoculation of three tubes of single-strength liquid selective enrichment medium [5.3.1.1 b)] with a specified quantity of test sample if the initial product is liquid, or with a specified quantity of the initial suspension in the case of other products.

Then, under the same conditions, inoculation of single-strength medium [5.3.1 b)] with decimal dilutions of the test sample or of the initial suspension.

4.3 Incubation of the tubes of double- and single-strength medium at 37 °C for 24 h to 48h. Examination of the tubes for gas formation.

4.4 Inoculation, from the tubes of double- and single-strength medium which have given rise to gas formation, of a new series of tubes containing the second selective medium (5.3.2).

4.5 Incubation at 44 °C for 24 h to 48 h and examination of the new series of tubes (4.4) for gas formation.

4.6 Inoculation, from the tubes of liquid selective medium (4.5) which have given rise to gas formation, of a new series of tubes containing tryptone water (5.4).

4.7 Incubation at 44 °C for 24 h to 48 h and examination of this new series of tubes (4.6) for indole production.

4.8 Identification of those tubes inoculated originally in 4.1 and/or 4.2, which show in step 4.5 production of gas from the second selective medium at 44 °C and in step 4.7 formation of indole from tryptone water at 44 °C, as being positive for presumptive *Escherichia coli.*

4.9 Determination of the MPN index from the numbers of positive tubes (4.8) of selected dilutions by means of an MPN table (annex A) and calculation of the most probable number (MPN) of presumptive *Escherichia coli* per gram or per millilitre of the original sample.

5 Dilution fluid, culture media and reagent

5.1 General

For current laboratory practice, see ISO 7218 and ISO 8261.

If the prepared culture media and reagents are not used immediately, they shall, unless otherwise stated, be stored in the dark at a temperature between 0 °C and +5 °C for no longer than 1 month, under conditions which do not produce any change in their composition.

5.2 Dilution fluid

See ISO 8261.