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

Part 2:

**Colony-count technique at 44 °C using
membranes**

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

*Partie 2: Technique par comptage des colonies obtenues sur
membranes à 44 °C*



Reference numbers
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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 11866-2|IDF 170-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This edition of ISO 11866-2|IDF 170-2 cancels and replaces ISO 11866-3:1997, of which it constitutes a minor revision.

ISO 11866-1:1997 has been cancelled and replaced by ISO 7251:2005, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*.

ISO 11866|IDF 170 consists of the following parts, under the general title *Milk and milk products — Enumeration of presumptive Escherichia coli*:

- *Part 1: Most probable number technique using 4-methylumbelliferyl- β -D-glucuronide (MUG)*
- *Part 2: Colony-count technique at 44 °C using membranes*

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

Draft International Standards, adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO 11866-2|IDF 170-2 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO/IDF/AOAC Group of Experts on *Pathogenic contaminants* (E102), under the aegis of its chairman, Mrs R. Lodi (IT).

This edition of ISO 11866-2|IDF 170-2 cancels and replaces the former part 3 of IDF 170A:1999, while the former part 1 has been replaced by ISO 7251:2005.

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

Part 2: Colony-count technique at 44 °C using membranes

1 Scope

This part of ISO 11866|IDF 170 specifies a method for the enumeration of presumptive *Escherichia coli* by means of a colony-count technique 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), and
- custard, desserts and cream.

The method specified in this part of ISO 11866|IDF 170 is the preferred method for samples in which comparatively large numbers of presumptive *Escherichia coli* (more than 100 per gram or 10 per millilitre) are suspected.

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

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

ISO 8261|IDF 122, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

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 form indole-positive (pink) colonies on cellulose acetate membranes overlaid on tryptone-bile agar, under the conditions specified in this part of ISO 11866|IDF 170

4 Principle

4.1 Resuscitation

A specified quantity of the test sample or initial suspension is inoculated onto cellulose acetate membranes overlaid on mineral-modified glutamate agar, then they are incubated at 37 °C for 4 h.

NOTE This procedure enables the presumptive *Escherichia coli* damaged by storage under frozen, dried or chill conditions, or damaged by heat or chemical processes, to be resuscitated. It also permits the diffusion of high concentrations of any fermentable carbohydrate present in the test sample which would otherwise interfere with indole production during the subsequent isolation stage.

4.2 Isolation

The membranes from the resuscitation stage on the mineral-modified glutamate agar are transferred to tryptone-bile agar. They are incubated at 44 °C for 18 h to 24 h.

4.3 Detection

The presence of presumptive *Escherichia coli* on the membrane is demonstrated by the production of indole by each colony.

4.4 Calculation

The number of colony-forming units (CFU) of presumptive *Escherichia coli* per gram or per millilitre of sample is calculated from the number of indole-positive colonies obtained on membranes at dilution levels chosen so as to give a significant result.

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|IDF 122.