INTERNATIONAL STANDARD

ISO 11866-1

IDF 170-1

Second edition
2005-12-01

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

Part 1: Most probable number technique using 4-methylumbelliferyl-β-D-glucuronide (MUG)

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

Partie 1: Technique du nombre le plus probable avec utilisation de 4-méthylumbelliferyl-β-D-glucuronide (MUG)

Reference numbers
ISO 11866-1:2005(E)
IDF 170-1:2005(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

International Dairy Federation
Diamant Building • Boulevard Auguste Reyers 80 • B-1030 Brussels
Tel. + 32 2 733 98 88
Fax + 32 2 733 04 13
E-mail info@fil-idf.org
Web www.fil-idf.org

Published in Switzerland

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 11866-1|IDF 170-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

This edition of ISO 11866-1|IDF 170-1 cancels and replaces ISO 11866-2:1997, 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 11866-1 | IDF 170-1 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 11866-1 | IDF 170-1 cancels and replaces the former part 2 of IDF 170A:1999, while the former part 1 has been replaced by ISO 7251:2005.

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

Part 1:
Most probable number technique using 4-methylumbelliferyl-β-D-glucuronide (MUG)

1 Scope

This part of ISO 11866 | IDF 170 specifies a combined method for the enumeration of presumptive *Escherichia coli* and of presumptive coliforms by means of a culture technique involving a liquid medium with MUG, and calculation of the number of presumptive *Escherichia coli* and/or coliforms per gram or per millilitre by the most probable number (MPN) technique after incubation at 30 °C.

It is a more rapid method than that described in ISO 7251 as the incubation time is reduced (omission of several enrichment steps).

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 preferred for samples in which comparatively low numbers of presumptive *Escherichia coli* and/or other presumptive coliforms (less than 100 per gram or 10 per millilitre) are suspected.

CAUTION — The applicability of this part of ISO 11866 | IDF 170 is limited by the susceptibility of the method to a large degree of variability. The method should, therefore, be applied and the results interpreted in the light of the information given in Clause 12.

NOTE The methods described in ISO 4831 apply for the enumeration of coliforms for reference purposes.

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 30 °C cleave 4-methylumbelliferyl-β-D-glucuronide (MUG), with the production of fluorescence, and which produce indole from tryptophan, under the conditions specified in this part of ISO 11866|IDF 170

3.2
coliforms
bacteria which at 30 °C cause fermentation of lactose with the production of gas under the conditions specified in this part of ISO 11866|IDF 170

4 Principle

4.1 Three tubes of double-strength liquid selective enrichment medium are inoculated 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 Three tubes of single-strength liquid selective enrichment medium are inoculated 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, the single-strength medium is inoculated with specified quantities of decimal dilutions of the test sample or of the initial suspension.

4.3 The tubes of double- and single-strength medium are incubated at 30 °C for 24 h to 48 h.

4.4 Those tubes showing fluorescence and formation of indole are identified as being positive for presumptive *Escherichia coli*.

4.5 Those tubes showing gas formation are identified as being positive for presumptive coliforms.

4.6 The MPN index is determined from the numbers of positive tubes (4.4) of selected dilutions by means of an MPN table (Annex A) and the most probable number (MPN) of presumptive *Escherichia coli* per gram or per millilitre of the original sample is calculated.

4.7 The MPN index is determined from the numbers of positive tubes (4.5) of selected dilutions by means of an MPN table (Annex A) and the most probable number (MPN) of coliforms per gram or per millilitre of the original sample is calculated.

5 Dilution fluid, culture media and reagents

5.1 General

For current laboratory practice, see ISO 7218 and ISO 8261 | IDF 122.

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.

5.3 Culture media

5.3.1 Modified lauryl sulfate tryptose broth (selective enrichment medium)

5.3.1.1 Composition

	a) Double-strength medium	b) Single-strength medium
Tryptose	40,0 g	20,0 g
Lactose	10,0 g	5,0 g
Dipotassium hydrogen phosphate (K_2HPO_4)	5,5 g	2,75 g
Potassium dihydrogen phosphate (KH_2PO_4)	5,5 g	2,75 g
Sodium chloride	10,0 g	5,0 g
Sodium lauryl sulfate [$CH_3(CH_2)_{11}OSO_3Na$]	0,2 g	0,1 g
4-Methylumbelliferyl-β-D-glucuronide (MUG)	0,2 g	0,1 g
Tryptophan	2,0 g	1,0 g
Water	1 000 ml	1 000 ml

5.3.1.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization it is 6,8 at 25 °C.

Transfer the media in quantities of 10 ml to tubes of dimensions 16 mm × 160 mm (6.2) containing inverted Durham tubes (6.3) in the case of single-strength medium, and to test tubes of dimensions 20 mm × 200 mm (6.2) containing inverted Durham tubes (6.3) in the case of the double-strength medium.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

The inverted Durham tubes shall not contain air bubbles after sterilization.

5.4 Indole reagent (Kovacs reagent)

5.4.1 Composition

4-Dimethylaminobenzaldehyde	5,0 g
2-Methylbutan-1-ol or pentan-1-ol	75,0 ml
Hydrochloric acid (ρ_{20} = 1,18 g/ml to 1,19 g/ml	25,0 ml

5.4.2 Preparation

Dissolve the 4-dimethylaminobenzaldehyde in the alcohol by heating gently to between 50 °C and 55 °C by means of the water bath (6.5).

Cool and add the hydrochloric acid.

Protect from light and store at approximately 4 °C. The colour of the reagent shall be light yellow to light brown.

5.5 Sodium hydroxide solution, c(NaOH) ≈ 0,5 mol/l.

5.5.1 Composition

Sodium hydroxide	2 g
Water	100 ml

5.5.2 Preparation

Dissolve the sodium hydroxide in the water.

6 Apparatus and glassware

For general requirements, see ISO 7218 and ISO 8261 | IDF 122. Glassware shall be resistant to repeated sterilization.

Usual microbiological laboratory apparatus and, in particular, the following.

6.1 Autoclave, capable of operating at 121 °C ± 1 °C.

For details, see ISO 7218.

6.2 Test tubes, of dimensions approximately 16 mm × 160 mm and 20 mm × 200 mm, or **flasks** or **bottles** of suitable capacity.

Test tubes should be checked for absence of autofluorescence before being used.

6.3 Durham tubes, of a size suitable for use in the test tubes (6.2).

6.4 Incubator, capable of maintaining a temperature of 30 °C ± 1 °C at all points within it.

6.5 Water bath, capable of operating at between 50 °C and 55 °C.

6.6 Long-wave ultraviolet (UV) lamp, of wavelength between 360 nm and 366 nm, preferably in a UV cabinet or in a dark room, or covered by a box or a carton which provides dark conditions.

NOTE Short-wave UV (germicidal) lamps are unsatisfactory.

6.7 pH-meter, accurate to within ± 0,1 pH units at 25 °C.

6.8 Total-delivery pipettes, with nominal capacities of 1 ml and 10 ml.

6.9 Vortex mixer

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 11866 | IDF 170. A recommended sampling method is given in ISO 707 | IDF 50.

8 Preparation of test sample

Prepare the test sample according to the method given in ISO 8261 | IDF 122.

9 Procedure

9.1 Test portion, initial suspension and further dilutions

Prepare the test portion, initial suspension (primary dilution) and further decimal dilutions according to the method given in ISO 8261 | IDF 122.

Prepare a sufficient number of dilutions to ensure that all tubes for the final dilution will yield a negative result.

9.2 Inoculation of selective enrichment medium

9.2.1 Take three tubes of double-strength enrichment medium [5.3.1.1 a)]. Using a sterile pipette (6.8), transfer to each of these tubes 10 ml of the test sample if liquid, or 10 ml of the initial suspension (primary dilution) in the case of other products.

9.2.2 Then take three tubes of single-strength enrichment medium [5.3.1.1 b)]. Using a fresh sterile pipette (6.8), transfer to each of these tubes 1 ml of the test sample if liquid, or 1 ml of the initial suspension (primary dilution) in the case of other products.

9.2.3 For each of the further dilutions, proceed as specified in 9.2.2. Use a fresh sterile pipette for each dilution.

9.2.4 Carefully mix the inoculum with the medium by means of a mixer (6.9). Avoid the introduction of air into the Durham tubes (6.3).

9.3 Incubation

Incubate all inoculated tubes (from 9.2.1 to 9.2.3) in the incubator (6.4) set at 30 °C for 24 h ± 2 h. If, at this stage, neither gas formation nor opacity preventing the observation of gas formation is observed, incubate for up to 48 h ± 2 h.