International Standard 5541/2

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION•МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ•ORGANISATION INTERNATIONALE DE NORMALISATION

Milk and milk products — Enumeration of coliforms — Part 2: Most probable number technique at 30 °C

Lait et produits laitiers — Dénombrement des coliformes — Partie 2: Technique du nombre le plus probable après incubation à 30 °C

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 5541/2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*,

NOTE — The method specified in this International Standard has been developed jointly with the International Dairy Federation (IDF) and the Association of Official Analytical Chemists (AOAC) and will also be published by these organizations.

Users should note that all International Standards undergo revision from time to time and that any reference made herein to any other International Standard implies its latest edition, unless otherwise stated.

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Milk and milk products — Enumeration of coliforms — Part 2: Most probable number technique at 30 °C

1 Scope and field of application

This part of ISO 5541 specifies a method for the enumeration of coliforms by means of the culture technique involving a liquid medium, and calculation of the most probable number (MPN) after incubation at 30 °C.

The method is applicable to

— milk, and liquid milk products;

— dried milk, dried sweet whey, dried buttermilk, and 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.

This method is to be preferred for samples in which comparatively low numbers of coliforms (less than 100 per gram or 10 per millilitre) are suspected.

NOTE — For samples with larger numbers of coliforms (more than 100 per gram or 10 per millilitre) see ISO 5541/1.

2 Reference

ISO 707, *Milk and milk products — Methods of sampling.*

3 Definition

For the purpose of this part of ISO 5541, the following definition applies.

coliforms: Bacteria which, at 30 °C, cause fermentation of lactose with the production of gas and form characteristic growth under the operational conditions described.

4 Principle

4.1 Inoculation of a test portion and/or a series of decimal dilutions of the sample in triplicate into the selective liquid medium prescribed in test tubes containing Durham tubes.

4.2 Incubation of the tubes at 30 °C for 48 h.

4.3 From presumed positive tubes, i.e. those tubes showing gas production in the Durham tube in lactose bile brilliant green broth, inoculation on eosin-methylene blue agar.

4.4 Incubation at 30 °C for 24 h.

4.5 From confirmed positive tubes, i.e. those tubes showing gas production in lactose bile brilliant green broth and characteristic growth on eosin-methylene blue agar, calculation of the number of coliforms per millilitre or per gram of sample (i.e. the MPN) using a table.

5 Diluents and media

5.1 Basic materials

In order to improve the reproducibility of the results, it is recommended that, for the preparation of diluents and culture media, dehydrated basic components or complete dehydrated media should be used. The manufacturer's instructions shall be rigorously followed.

The chemical products used shall be of recognized analytical quality.

The water used shall be distilled from glass apparatus or shall be deionized water. It shall be free from substances that might influence the growth of micro-organisms under the test conditions. This shall be periodically checked, particularly in the case of deionized water.

Solutions of sodium hydroxide and hydrochloric acid (approximately 0,1 mol/litre) should be used to adjust the pH of diluents and media.

5.2 Diluents for general use

5.2.1 Peptone/saline solution

NOTE — Peptone/saline solution is the diluent selected by ISO for general use.

Composition

Peptone	1,0 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml