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Milk — Estimation of psychrotrophic microorganisms — Colony-count technique at 21 °C (Rapid method)

Lait — Estimation des micro-organismes psychrotrophes — Technique par comptage des colonies à 21 °C (Méthode rapide)

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Foreword

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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.

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ISO 8552|IDF 132 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF), in collaboration with AOAC International. It is being published jointly by ISO and IDF and separately by AOAC International.

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 and AOAC International 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 IDF National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team on *Microbiological harmonization*, of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leader, Dr J. Floor (ZA).

This first edition of ISO 8552|IDF 132 cancels and replaces the first edition of IDF 132A:1991, which has been editorially and technically revised.

Introduction

The rapid method described in this International Standard is essentially an approximate method because the incubation conditions are such as to permit organisms other than psychrotrophs to be counted if they grow sufficiently quickly at 21 °C.

Nevertheless, comparative trials in a number of laboratories have shown that the results obtained by the method described here (based on that of Reference [1]) correlate well with those obtained by the method described in ISO 6730|IDF 101 (with incubation at 6,5 °C for 10 days). The latter method (which will be replaced by the horizontal method ISO 17410) should be used if a more accurate enumeration of psychrotrophic microorganisms is required.

Milk — Estimation of psychrotrophic microorganisms — Colony-count technique at 21 °C (Rapid method)

1 Scope

This international Standard specifies a rapid method for estimating the number of psychrotrophic microorganisms by means of the colony-count technique at 21 °C.

The method is applicable to raw and pasteurized milk.

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 6730, *Milk — Enumeration of colony-forming units of psychrotrophic micro-organisms — Colony count technique at 6,5 degrees C*[1)]

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

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
psychrotrophic microorganisms
bacteria, yeasts and moulds forming countable colonies when incubated aerobically at 6,5 °C for 10 days under the conditions specified in ISO 6730

1) Equivalent to IDF 101A:1991.

4 Principle

4.1 Poured plates are prepared using a specified culture medium and a specified quantity of an appropriate dilution of the test sample.

4.2 The plates are incubated aerobically at 21 °C for 25 h.

4.3 The number of microorganisms per millilitre of sample is calculated from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

5 Diluents and culture medium

5.1 General

See ISO 7218 and ISO/TS 11133-1.

5.2 Diluents

See ISO 8261|IDF 122.

5.3 Culture medium — Plate count milk agar

5.3.1 Composition

Yeast extract	2,5 g
Enzymatic digestion of casein	5,0 g
Skimmed milk powder[a]	1,0 g
Glucose, anhydrous ($C_6H_{12}O_6$)	1,0 g
Agar	9 g to 18 g [b]
Water	1 000 ml

[a] The skimmed milk powder shall be free from inhibitory substances.

[b] Depending on the gel strength of the agar.

5.3.2 Preparation

5.3.2.1 Preparation from commercial dehydrated medium

Follow the manufacturer's instructions but, in all cases, add the skimmed milk powder even if the manufacturer considers such an addition unnecessary. Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

5.3.2.2 Preparation from dehydrated basic components

Dissolve and disperse in the water, in the following order, the yeast extract, the enzymatic digestion of casein, the glucose and, finally, the skimmed milk powder. Heating the water will assist this procedure. Add the agar and heat to boiling while stirring frequently until the agar is completely dissolved.

Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

5.3.2.3 Distribution, sterilization and storage

Dispense the prepared medium into test tubes (6.6), in quantities of 12 ml to 15 ml per tube, or into flasks or bottles (6.7) of capacity not greater than 500 ml. Sterilize for 15 min in an autoclave (6.10) set at 121 °C.

If the medium is to be used immediately, cool it in a water bath (6.4) to between 44 °C and 47 °C before use.

If not, store the medium in the dark at 3 °C ± 2 °C for no longer than three months (see ISO 7218). Before commencing the microbiological examination and in order to avoid any delay when pouring the medium, completely melt the stored medium, then cool it in a water bath (6.4) at between 44 °C and 47 °C before use (see 8.2.4).

With regard to the temperature check of the medium and other requirements, see ISO 7218.

5.3.3 Performance testing for the quality assurance of the culture medium

Test the performance of the medium in accordance with ISO/TS 11133-2.

6 Apparatus and glassware

Usual microbiological equipment (see ISO 7218) and, in particular, the following.

6.1 Glassware

Disposable glassware is an acceptable alternative to re-usable glassware if it has suitable specifications. Re-usable glassware shall be capable of undergoing repeated sterilization and shall be chemically inert.

6.2 **Incubator**, capable of operating at 21 °C ± 1 °C.

6.3 **pH meter**, having an accuracy of calibration of ± 0,1 pH unit at 25 °C.

6.4 **Water bath**, capable of operating at between 44 °C and 47 °C.

6.5 **Colony-counting equipment**, consisting, for example, of an illuminated base with a dark background, fitted with a magnifying lens to be used at a magnification of at least 2×, and a mechanical or electronic digital counter.

6.6 **Test tubes**, of approximate capacity 20 ml, with suitable stoppers.

6.7 **Flasks** or **bottles**, of appropriate capacity but not greater than 500 ml, with suitable stoppers.

6.8 **Graduated pipettes**, of nominal capacity 1 ml.

6.9 **Petri dishes**, made of glass or plastic, of diameter 90 mm to 100 mm.

6.10 **Apparatus for dry sterilization (oven)** or **wet sterilization (autoclave).**

See ISO 7218.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 707.

8 Procedure

8.1 Preparation of primary dilution and further decimal dilutions

Prepare the primary dilution (10^{-1}) and further decimal dilutions of the test sample in accordance with ISO 8261|IDF 122.

Bacterial colonies obtained with this rapid method tend to be very small and are not readily detectable in a zero dilution plate because of opacity or cloudiness of the media. Therefore, dilute the test samples at least 10-fold (preferably 100-fold or more) in order to be able to also observe small pinpoint colonies.

8.2 Inoculation and incubation

8.2.1 Take two sterile Petri dishes (6.9). Transfer using a sterile pipette (6.8) 1 ml of the primary dilution (8.1) to each dish.

8.2.2 If necessary, repeat the procedure with further decimal dilutions, using a new sterile pipette for each dilution.

8.2.3 If appropriate and possible, select only the critical dilution steps (at least two consecutive decimal dilutions) for the inoculation of the Petri dishes that will give colony counts of between 15 and 300 colonies per plate.

8.2.4 Pour about 12 ml to 15 ml of plate count medium (5.3.2.3) at 44 °C to 47 °C into each Petri dish. The time elapsing between the end of the preparation of the primary dilution and the moment when the medium is poured into the dishes shall not exceed 15 min.

8.2.5 Carefully mix the inoculum with the medium by rotating the Petri dishes. Allow the mixture to solidify by leaving the Petri dishes to stand on a cool horizontal surface.

8.2.6 Invert the prepared dishes and place them for 25 h ± 1 h in the incubator (6.2) set at 21 °C. Do not stack the dishes more than six high. Separate stacks of dishes from one another and from the walls and top of the incubator.

8.3 Counting of colonies

8.3.1 After the specified period of incubation (8.2.6), count the colonies on the plates using the colony-counting equipment (6.5).

Examine the dishes under subdued light. Include pinpoint colonies in the count, but it is essential that the operator avoid mistaking particles of undissolved or precipitated matter in dishes for pinpoint colonies. Examine doubtful objects carefully, using higher magnification where required, to distinguish colonies from foreign matter.

8.3.2 Consider spreading colonies as single colonies. If less than one-quarter of the dish is overgrown by spreading colonies, count the colonies on the unaffected part of the dish and calculate the corresponding number for the entire dish. If more than one-quarter of the dish is overgrown by spreading colonies, discard the count.

9 Calculation and expression of results

For calculation and expression of results, see ISO 7218.

10 Precision

10.1 General

For information about the confidence limits for the estimation of small numbers of microorganisms, see ISO 7218.

NOTE No detailed precision data obtained from a collaborative study are available. The figures given are experimental.

10.2 Repeatability

Experience indicates that if the higher of two individual single test results on a sample with more than 1 000 CFU/ml, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, frequently exceeds the lower result by 50 %, the analyst should examine the procedure to determine sources of error.

11 Test report

The test report shall specify:

a) all the information required for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incident which may have influenced the result(s);

e) the test result(s) obtained.