INTERNATIONAL STANDARD

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IDF 169-2

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Milk and milk products — Quality control in microbiological laboratories —

Part 2: Determination of the reliability of colony counts of parallel plates and subsequent dilution steps

Lait et produits laitiers — Contrôle de qualité en laboratoire microbiologique —

Partie 2: Détermination de la fiabilité des comptages de colonies en boîtes parallèles et des dilutions décimales suivantes

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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 14461-2|IDF 169-2 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.

ISO 14461|IDF 169 consists of the following parts, under the general title *Milk and milk products — Quality control in microbiological laboratories*:

— *Part 1: Analyst performance assessment for colony counts*

— *Part 2: Determination of the reliability of colony counts of parallel plates and subsequent dilution steps*

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 the National Committees casting a vote.

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All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Statistics of analytical data*, of the Standing Committee on *Quality assurance, statistics of analytical data and sampling*, under the aegis of its project leaders, Dr. H. Glaeser (EU) and Prof. Dr. H. Weiss (DE).

This edition of ISO 14461-2 | IDF 169-2, together with ISO 14461-1 | IDF 169-1, cancels and replaces IDF 169:1994, which has been technically revised.

ISO 14461 | IDF 169 consists of the following parts, under the general title *Milk and milk products — Quality control in microbiological laboratories*:

— *Part 1: Analyst performance assessment for colony counts*

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Introduction

Every microbiological method consists of several steps that are followed in a specific sequence (sub-sampling, diluting, plating and counting). The final result has a margin of uncertainty that is determined by the variability of all the steps involved.

In order to obtain results with a margin of uncertainty not much larger than what can be expected from the correct application of the method, it is necessary to follow the rules of Good Laboratory Practice (GLP).

The three most important factors in obtaining a correct plate count are

— the homogeneity of the sample material,

— the exactness with which the dilutions are performed, and

— the technique of inoculation and/or counting of the plates.

By homogenizing a sample material very well, making multiple dilution series, and inoculating several plates from the same dilution, it is possible to assess how well a laboratory can perform the colony-count technique, taking into account the expected variability of the method.

Too large a variability indicates that at least one of the steps in the performance of the method is out of control. The identification of those steps is carried out by comparison of the replicate inoculations, the different dilution levels and the dilution series. When the steps with excessive variability have been identified, necessary measures should be taken to bring these steps under control.

Milk and milk products — Quality control in microbiological laboratories —

Part 2:
Determination of the reliability of colony counts of parallel plates and subsequent dilution steps

1 Scope

This part of ISO 14461|IDF 169 describes a routine procedure for the evaluation of results of the enumeration of microorganisms using colony-count methods with subsequent 10-fold dilution steps and one plate or two parallel plates within each dilution step.

This routine procedure is applied regularly in each laboratory performing colony counts. It provides criteria for the acceptability of differences between the results from parallel plates and subsequent dilution steps, as follows.

a) The results (colony counts) obtained from parallel plates are compared with tabulated limits for given colony counts. If these limits are exceeded, a technical problem when performing the parallel determinations may be indicated.

b) The results (sums of colony counts) of two parallel plates of two subsequent 10-fold dilution steps are compared with tabulated limits for given sums of colony counts. If these limits are exceeded, a technical problem when performing the dilutions may be indicated.

c) If the limits mentioned above are exceeded in more cases than expected, this indicates that the test procedure lacks reliability.

NOTE The formulae for calculating the values in Table 1 and 2 are given and explained in Clause 7.

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 14461-1|IDF 169-1, *Milk and milk products — Quality control in microbiological laboratories — Part 1: Analyst performance assessment for colony counts*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply

3.1
colony count
number of microorganisms found, as determined by the method specified in ISO 14461-1|IDF 169-1

NOTE The number of microorganisms is expressed per gram or per millilitre of test sample.