INTERNATIONAL STANDARD

ISO 16297

IDF 161

Second edition
2020-01

Milk — Bacterial count — Protocol for the evaluation of alternative methods

Lait — Dénombrement bactériologique — Protocole pour l'évaluation de méthodes alternatives

Reference numbers
ISO 16297:2020(E)
IDF 161:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B
B-1030 Brussels
Phone: +32 2 325 67 40
Fax: +32 2 325 67 41
Email: info@fil-idf.org
Website: www.fil-idf.org

Published in Switzerland

Contents

Page

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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document 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 second edition cancels and replaces the first edition (ISO 16297 | IDF 161:2013), which has been technically revised with the following main changes:

— the number of samples and the calculation of the lower limit of quantification has been changed and aligned with ISO 16140-2;

— an example of carry-over effect given in Figure 1 has been omitted;

— the requirements for the evaluation of the accuracy of the estimate and the accuracy profile have been clarified and aligned with ISO 16140-2;

— Annex A (informative) has been omitted.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

This document was prepared by the IDF *Standing Committee on Statistics and Automation* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

The work was carried out by the IDF/ISO Action Team (S18) of the *Standing Committee on Statistics and Automation* under the aegis of its project leaders, Mrs V. Tzeneva (NL) and Mrs I. Andersson (SE).

Introduction

Any quantitative measurement in microbiology should consider that there is a requirement for the microbiological state in a sample to be regarded as one point within the coordinates of a multidimensional system, which is to be projected on to the one-dimensional scale of the method applied, i.e. plate count, flow cytometry. Aspects such as flora (types and numbers of microorganisms and their distribution), growth phase, sub-lethal damage, metabolic activity, and history, influence to a greater or lesser extent any parameter that is measured. It is evident that any projection of an *n*-dimensional situation onto a one-dimensional scale is bound to provide a picture of the real situation that is rather restricted. In this respect, one has to bow to the inevitable, regardless of which method of measurement is preferred.

The term “anchor method” in this document means a method internationally recognized by experts, used in legislation or by agreement between the parties. There are requirements for evaluation of an alternative method to refer to the anchor method and to be based on the examination of suitable samples for its intended use.

Milk — Bacterial count — Protocol for the evaluation of alternative methods

1 Scope

This document specifies a protocol for the evaluation of instrumental alternative methods for total bacterial count in raw milk from animals of different species.

NOTE The document is complementary to ISO 16140-2 and ISO 8196 | IDF 128 (all parts).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

ISO 8196 | IDF 128 (all parts), *Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis*

ISO 16140-1, *Microbiology of the food chain — Method validation — Part 1: Vocabulary*

ISO 21187 | IDF 196, *Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between results of an alternative method and anchor method results*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 5725-1, ISO 5725-2, ISO 8196-1 | IDF 128-1, ISO 8196-2 | IDF 128-2, ISO 8196-3 | IDF 128-3 and ISO 16140-1 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Transformation of results

A prerequisite for statistics most common in the evaluation of measuring methods is the approximation of a normal distribution of the data. The exponential multiplication of microorganisms usually leads to a right-tailed distribution with quantitative microbiological parameters. Thus, in general, transformation of the raw data is necessary for approximation of normality. This is usually a common logarithmic transformation. The most appropriate transformation can be checked by comparing histograms. All statistics are then computed from the transformed data, unless specified otherwise, and only the final results are re-transformed to give a more expressive idea of the situation to the user (see Reference [2]).

5 Attributes of the alternative method

5.1 General

For each alternative method, only the relevant parameters outlined in this clause shall be evaluated. For example, the measuring range (see 5.3) of the plate loop method is determined by the loop(s) used and the determination of the parameter is not relevant.

5.2 Description of the method to be evaluated

5.2.1 Description

The description of the method under study shall be in line with the checklist in 5.2.2.

Most of the information is found in the specification of the method given by the responsible supplier or any other source (author) of the technique specified.

5.2.2 Checklist

a) Principle of the method.

b) Parameter or unit.

c) Technical design of the measurement procedure.

d) Field of application:

   1) purpose, e.g. research, screening, milk grading;

   2) matrix, e.g. raw milk from cows.

e) Supplier(s) of instrument, reagents and standards.

f) Specification of the method given by the producer or the author:

   1) prerequisites for sampling (often compared to the situation of fat analysis);

   2) possibilities for sample preservation [reagent(s), storage condition(s)];

   3) quantitative (units: method under study or anchor method) and qualitative (the kind of microorganisms covered) spectrum;

   4) precision (in units of the method under study or in anchor method units);

   5) accuracy of the estimate (in anchor method units);

   6) samples per hour;

   7) list of references.

5.3 Measuring range

5.3.1 Lower limit of quantification

The lower limit of quantification is defined on milk samples without or with a very low concentration of bacteria. The standard deviation of n-fold measurements is estimated; generally, n = 20. The standard

deviation s_0 is calculated in units of the alternative method without any transformation of the data using Formula (1):

$$s_0 = \sqrt{\frac{1}{n-1}\sum_{j=1}^{n}\left(y_j - \bar{y}\right)^2} \quad (1)$$

where

n is the total number of test portions used;

y_j is the not transformed result of the test portion j;

$\bar{y}$ is the average not transformed result of all test portions.

The lower limit of quantification is calculated as $L_q = 10 s_0$ (see also ISO 16140-2).

5.3.2 Upper limit of quantification

The upper limit of quantification is determined by the highest possible reading of the method or by methodological limitations, e.g. coincidence effects, inaccuracy in the upper measuring range, clogging of filters. Coincidence is when two or more elements of the measurand are detected simultaneously and identified as only one unit. For example, with flow cytometry, if two bacterial cells pass the detector simultaneously, they are detected as one. The coincidence effect is higher with higher concentrations of a measurand.

The upper limit of quantification is determined as the highest concentration where the instrument is still linear according to 5.3.3.

5.3.3 Linearity of the instrument signal

The relationship between the instrument readings and the expected values shall be linear within the concerned range of bacterial counts. Deviations from linearity may stem from non-specific signals and coincidence effects.

To evaluate linearity, use the raw data expressed in units of the alternative method without logarithmic or any other transformation.

A linearity check is at first performed visually using appropriate graphs to obtain an impression of the shape of the relationship. Whenever deviation from linearity appears evident, a quantitative parameter is calculated to indicate whether the observed trend is acceptable or not.

To achieve this, use a high bacterial count milk diluted serially with low bacterial count milk, resulting in a set of at least 10 samples covering the concentration range of interest.

Measure all samples at least four times and calculate the average result for each sample. This gives the measured value per sample. Use the measured values for the high-count milk and the low count milk to calculate values for the intermediate samples from the applied mixing ratios. This results in an expected value for each sample. Then, apply linear regression with the expected values per sample, C_e, on the x-axis and the measured values per sample, C_{meas}, on the y-axis.

Calculate, for each sample, the residuals $\Delta C_{1i} = C_{meas,i} - (a \times C_{e,i} + b)$ from the regression.