

**Milk - Bacterial count - Protocol for the evaluation of  
alternative methods (ISO 16297:2013)**

## EESTI STANDARDI EESSÕNA

## NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 16297:2014 sisaldab Euroopa standardi EN ISO 16297:2014 inglisekeelset teksti.	This Estonian standard EVS-EN ISO 16297:2014 consists of the English text of the European standard EN ISO 16297:2014.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 07.05.2014.	Date of Availability of the European standard is 07.05.2014.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

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English Version

**Milk - Bacterial count - Protocol for the evaluation of alternative methods (ISO 16297:2013)**

Lait - Dénombrement bactérien - Protocole pour l'évaluation des méthodes alternatives (ISO 16297:2013)

Milch - Bestimmung der Gesamtkeimzahl - Protokoll für die Bewertung alternativer Verfahren (ISO 16297:2013)

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## Foreword

The text of ISO 16297:2013 has been prepared by Technical Committee ISO/TC 34 "Food products" of the International Organization for Standardization (ISO) and has been taken over as EN ISO 16297:2014 by Technical Committee CEN/TC 302 "Milk and milk products - Methods of sampling and analysis" the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2014, and conflicting national standards shall be withdrawn at the latest by November 2014.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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### Endorsement notice

The text of ISO 16297:2013 has been approved by CEN as EN ISO 16297:2014 without any modification.

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## 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 co-ordinates 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 on to an 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 reference (or official or anchor) method in this International Standard 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 reference 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 International Standard gives guidelines for the evaluation of instrumental alternative methods for total bacterial count in raw milk from animals of different species.

NOTE The document is considered complementary to ISO 16140 and ISO 8196|IDF 128 (see Clause 2 and Reference [1]).

## 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 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 8196-1|IDF 128-1, *Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 1: Analytical attributes of alternative methods*

ISO 8196-2|IDF 128-2, *Milk — Definition and evaluation of the overall accuracy of alternative methods of milk analysis — Part 2: Calibration and quality control in the dairy laboratory*

ISO 16140-1, *Microbiology of food and animal feed — Method validation — Part 1: Vocabulary*

ISO 16140-2, *Microbiology of food and animal feed — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

ISO 21187|IDF 196:2004, *Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results*

## 3 Terms and definitions

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

For the definitions of precision, repeatability and reproducibility, see ISO 5725-1, ISO 5725-2, ISO 8196-1|IDF 128-1, and ISO 16140-1.

## 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 of quantitative microbiological parameters. Thus, in general, transformation of the raw data is necessary for approximation of normality. This is usually a common logarithmic transformation or a square root transformation for low bacteria levels. The most appropriate transformation can be checked by comparing histograms. All statistics are then computed from the