BRITISH STANDARD

BS ISO
21187:2004

Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results

ICS 07.100.30; 67.100.01

BSI
British Standards

National foreword

This British Standard reproduces verbatim ISO 21187:2004 and implements it as the UK national standard.

The UK participation in its preparation was entrusted to Technical Committee AW/5, Milk and milk products, which has the responsibility to:

— aid enquirers to understand the text;

— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee can be obtained on request to its secretary.

Cross-references

The British Standards which implement international publications referred to in this document may be found in the *BSI Catalogue* under the section entitled "International Standards Correspondence Index", or by using the "Search" facility of the *BSI Electronic Catalogue* or of British Standards Online.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, the ISO title page, pages ii to vi, pages 1 to 13 and a back cover.

The BSI copyright notice displayed in this document indicates when the document was last issued.

Amendments issued since publication

Amd. No.	Date	Comments

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 7 December 2004

ISBN 0 580 45010 4

INTERNATIONAL STANDARD

ISO 21187

IDF 196

First edition
2004-12-01

Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results

Lait — Mesure quantitative de la qualité bactériologique — Lignes directrices pour établir et vérifier une relation de conversion entre les résultats de la méthode de routine et les résultats de la méthode d'ancrage

Reference numbers
ISO 21187:2004(E)
IDF 196:2004(E)

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.

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 21187|IDF 196 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 the National Committees casting a vote.

ISO 21187 | IDF 196 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.

All work was carried out by the Joint ISO/IDF/AOAC Action Team, *Routine analysis in quantitative microbiology*, of the Standing Committee on *Quality assurance, statistics of analytical data and sampling*, under the aegis of its project leader, Mr H.J.C.M. van den Bijgaart (NL).

Introduction

Conversion in quantitative microbiology means expressing the result of a quantitative determination of the bacteriological status of a test sample as obtained with a routine method in units of another method, generally a reference or anchor method. Through this, quantitative results obtained with routine methods can be compared to values or limits that are stated in reference or anchor method units. For establishing and applying a conversion relationship, a number of prerequisites should be met. These are referred to in this International Standard, but are generally described elsewhere.

Although a considerable part of the applied principles for conversion coincides with those applied for the calibration of indirect or routine methods against a reference method, or by means of (certified) reference materials, it is stressed that the background and aims for applying conversion are different from those for calibration. Calibration involves the determination of the adjustment needed for each level of an analyte to closely approximate the true value of its concentration or number. However in quantitative microbiology, a true value in its strict sense cannot be established and is only defined by the method description applied. When applying routine methods in the quantitative determination of bacteriological quality, one is often dealing with different methodological principles and therefore also other units. Conversion is used to transfer results obtained with different methods to a common scale.

Milk — Quantitative determination of bacteriological quality — Guidance for establishing and verifying a conversion relationship between routine method results and anchor method results

1 Scope

This International Standard gives guidelines for the establishment of a conversion relationship between the results of a routine method and an anchor method, and its verification for the quantitative determination of the bacteriological quality of 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 3534-1, *Statistics — Vocabulary and symbols — Part 1: Probability and general statistical terms*

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

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 3534-1, ISO 8196-1|IDF 128-1, ISO 8196-2|IDF 128-2 and the following apply.

3.1
routine method
alternative method
method of analysis allowing quantification of the bacteriological status of a test sample

NOTE 1 The method can be proprietary or non-commercial.

NOTE 2 The term “routine” or “alternative” in this International Standard refers to the entire method. It includes all aspects (such as sample pretreatment, materials and instruments) required for the execution of the method.

NOTE 3 The term “routine method” is used in this International Standard.

3.2
anchor method
reference method
method of analysis internationally recognized by experts or by agreement between parties, and used, for instance, in legislation when expressing official limits for bacteriological quality

NOTE It is stressed that, in quantitative microbiology, any obtained value is only defined by the method description applied. This applies to any routine method as well as, for instance, to the standard plate count for the enumeration of microorganisms. For the purpose of conversion, the term anchor method is preferred over the term reference method and therefore is used throughout this International Standard.

3.3
analyte
component or property which is measured by the method of analysis

NOTE The analyte may be the microorganism, stained particles (e.g. microscopic count), components of microorganisms (e.g. lipopolysaccharides), the result of their ability to multiply (e.g. colony-forming units) or their metabolic activity (e.g. change in conductivity/impedance).

3.4
organizing laboratory
laboratory, possibly appointed by the competent authorities, having the qualified staff and skills to organize, to coordinate and to report on the outcome of the activities for the establishment or the maintenance of a conversion relationship

4 Principles

4.1 Prerequisites

For establishing and verifying a conversion relationship between the results of a routine method and the anchor method, the following prerequisites apply.

a) The routine method should have been validated according to ISO 16140[1)]. Procedures for sampling, test sample preservation, sample transport, sample storage, sample pre-treatment, analysis and calculation of results should be documented, strictly standardized and controlled in agreement with ISO/IEC 17025, EA-4/10, or comparable standards.

b) The anchor method should have been validated, documented, strictly standardized and controlled in agreement with ISO/IEC 17025, EA-4/10 or comparable standards.

c) The protocol for the establishment of the conversion relationship and its verification should be documented. It should follow the guidelines of this International Standard. Approval by competent authorities should be sought where the final aim is that results from a routine method are to be judged against official limits stated in the anchor method units.

4.2 Organizational set-up

The establishment and verification of a conversion relationship is based on the examination of test samples with both methods, covering the field of application and the spectrum of the samples as analysed using the routine method.

A number of situations can be distinguished, as follows.

a) Both the routine method and the anchor method are fully carried out in the same laboratory.

1) Additional guidance on aspects relevant to milk and not covered by ISO 16140 can be found in IDF 161A.

b) The routine method is carried out in a number of laboratories and the anchor method is carried out in one laboratory (not necessarily a routine laboratory). In this situation, sub-samples must be prepared and transported from the location where the routine method is to be carried out to the laboratory where the anchor method is to be carried out, or the other way around.

c) Both the routine method and the anchor method are carried out in a number of laboratories. As in b), the preparation and the transport of sub-samples must be properly considered.

Due to the instability and variability of the bacteriological status of milk samples, the most robust conversion relationships will be obtained where the routine method and the anchor method are undertaken on the same test samples, at the same place, at the same time; i.e. situation a).

In all cases, the organizational set-up should include all the necessary provisions to guarantee that the obtained conversion relationship is representative of the circumstances under which the routine method is carried out and the resulting conversion relationship is later applied.

The organizing laboratory should provide guidance to the collaborating laboratories. Furthermore, it should collect information on critical points in the procedure. All collaborators should be asked to record relevant information, such as details on the method(s) used, quality control data, and possible data about storage and transport conditions.

5 Establishing a conversion relationship

5.1 Consideration of influencing factors and their consequences

5.1.1 General

A number of factors can influence the outcome of routine method or anchor method determinations, or both. The relative magnitude of the effects can differ between test samples and is not necessarily the same for both methods. This implies that certain factors can also influence the conversion relationship. In the evaluation of a routine method, all relevant factors should be identified and should be considered since it is necessary to cover the consequences of their variation in one conversion relationship, or otherwise to establish distinct conversion relationships.

In general, when distinction between samples cannot be made, or is not being made in routine testing circumstances, the variation in the underlying variables should be covered in one conversion relationship. Where a factor is shown to have a significant effect on the conversion relationship, more than one conversion relationship may need to be established and applied.

A number of possible influencing factors are listed below as examples. The explanation provided with each of these factors is at the same time meant as guidance on how to deal with other factors.

5.1.2 Type of bacteria and growth phase

In determinations of the total bacterial or viable count, no differentiation is made between the type of bacteria, their growth phase or metabolic activity. The quantification is the result of different degrees of susceptibility for detection by the method concerned. The normal variation in this should be included in a conversion relationship.

5.1.3 Storage conditions of the product

The storage conditions of the product will affect the number of bacteria and their growth phase. When official limits are given depending on the storage conditions (e.g. time, temperature) and those conditions show a significant effect on the conversion relationship, distinct conversion relationships should be established and should be applied.

5.1.4 Regional influence and production conditions

In the examination of raw milk, this factor comprises those described in 5.1.2 and 5.1.3. It relates to the general characteristics of dairy farming and milk production, such as the method of milking and the collection intervals. Where this can effect the conversion relationship, it should be evaluated whether statistically significant differences can be shown, for instance for different regions. Then separate conversion relationships should be established and be applied. Again, it should be noted that the application of different conversion relationships is limited to situations where the relevant sub-populations of test samples are distinguished under routine testing conditions.

5.1.5 Seasonal influences

The bacterial flora can vary with the time of the year. This variation may be detected to different degrees by different methods, but should be considered as a consequence of measuring different characteristics of the same product by different methods. Where a seasonal influence on the conversion relationship is apparent, the conversion relationship should be based on a data set containing all-year-round data.

5.1.6 Test sample preservation

With certain routine methods, test sample preservation can be applied for stabilization purposes. It should be proven that the detectability of the analyte by the routine method is not influenced. When samples are analysed in parallel with the anchor method, care should be taken that it is the bacteriological status of the samples just before the addition and mixing with preservatives for routine method purposes that is monitored.

5.1.7 Sampling, test sample storage, transport and pretreatment

Sampling, storage, transport and pretreatment of the test sample are not part of the measurement itself but are part of the total analytical procedure and may affect the outcome. Structural changes, even within the limits stated in the description of the procedure, may necessitate an adaptation of a conversion relationship.

5.1.8 Chemicals

Minor changes in the characteristics of chemicals to be used in an analytical method should not influence the outcome of the measurements. However, in particular, chemicals from natural sources can show fluctuating properties. Where a significant effect on the obtained quantitative results is apparent, this should be accommodated for by an adaptation of an established conversion relationship.

5.2 Test samples

5.2.1 Calculation of number of test samples

Assuming a linear regression, the required number of test samples, n, in the final sample set can be calculated from t-test statistics using the following equation (see Annex A):

$$n = \left[t^2 (1 - r^2) \big/ (\delta^2 \cdot r^2) \right] + 1$$

where

n is the required number of test samples;

t is the numerical value of the Student t-distribution at the 95 % confidence level;

δ is the numerical value of the relative error of the estimation for the regression (working with δ = 0,1 is considered appropriate for the purpose concerned);

r is the numerical value of the estimated correlation coefficient between the results of the routine method and those of the anchor method.

NOTE The aim is to determine a required size of a sample set for estimating the regression coefficients from a bivariate normal distribution for a presumed correlation coefficient and a preset relative error of estimation at a chosen confidence level. It is stressed that this is different from the checking of a calibration with subsequent evaluation of the regression coefficients.

In the case where it appears from calculation (see 5.5.2) that the presumed correlation coefficient was an underestimate, the required number of extra data pairs should be included and the calculation should be repeated.

The number of test samples should be large enough to represent (if relevant) the variation

a) in the bacterial composition, level and properties of the sample population,

b) in the geographical region,

c) over the year, and

d) over different laboratories/instruments, when applying the routine method for situations b) and c) in 4.2.

5.2.2 Range of samples

The levels should, within the measuring range, uniformly cover the range of interest for the routine method concerned.

Where data are to be transformed before statistical treatment (see 5.5), the data pairs should uniformly cover the transformed scale.

5.2.3 Representative samples

It is of the highest priority to work with natural test samples. The test samples should be truly representative of the different levels in the population under consideration (see 5.1).

Test samples of raw milk may, in particular, be susceptible to changes between the time of taking the sample and the time of analysis, if sample quality is already poor at sampling. This involves a higher risk of ambient influences during transport, mixing and storage of test samples. Proper precautions should be taken to avoid this.

With some routine methods, results are almost instantly available. When both the routine and the anchor method are carried out in the same laboratory, an efficient selection of samples can be based on the outcome of measurements with the routine method. If this is not the case, it is necessary to select a surplus of samples for analysis with both the routine method and the anchor method to follow the guidance given in 5.2.1 and 5.2.2. Another reason for needing a surplus is that some data pairs are likely to become invalidated (see 5.5.1).

The normal procedure for testing with the routine method should be followed. This implies that the conditions for sampling, test sample storage, and its transport during the whole procedure should also closely mimic the conditions under which the conversion equation is to be applied.

5.3 Pretreatment of test samples

5.3.1 Storage conditions of test samples

After sampling, the test samples should be stored under identical conditions for both the routine method and the anchor method, and within the stated requirements for both methods (e.g. storage at between 0 °C and + 4 °C, and analysis within 36 h after sampling).

5.3.2 Preparation and distribution of sub-samples

Preferably, analysis by both methods should be carried out using the same test sample, within a short interval of time. However, this is not always feasible. A number of situations can be distinguished.

With reference to the situations given in 4.2, the following applies when routine laboratories select samples.

a) If possible, sub-samples should be avoided. Analysis by both methods should preferably be carried out on the same sample within a short interval of time.

b) From each sample, two sub-samples should be prepared shortly before dispatch. One sub-sample should be stored at the laboratory for analysis by the routine method, and the other should be transported to the laboratory appointed for testing by the anchor method. Analysis by both the routine method and the anchor method should be carried out at the same time.

c) If testing by the anchor method is carried out locally, the analysis by both methods should preferably be carried out on the same test sample within a short interval of time. If testing by the anchor method is carried out in another laboratory, two sub-samples should be prepared from each sample shortly before dispatch. One sub-sample should be stored at the laboratory for analysis with the routine method, and the other should be transported to the laboratory appointed for testing with the anchor method. Analysis by both the routine method and the anchor method should be carried out at the same time.

If test samples are selected centrally, sub-samples should be prepared and distributed among the participating laboratories, accompanied by detailed information on the handling of samples and the time of analysis.

Preparation of sub-samples consists of

— effective mixing of the cold sample by gently inverting the sample container at least 25 times, and

— pouring a sub-sample into a clean, dry and sterile sample container.

During the preparation of sub-samples, both the original test sample and the sub-samples should be kept at a temperature within the specified temperature range, for example at between 0 °C and + 4 °C.

During transport, the individual test samples should be sealed to ensure that any leakage from one sample does not affect the integrity of the other samples.

It should be checked that the transport packaging is suitable for its purpose. Ideally, a suitable means of temperature monitoring during transport is desirable.

During storage and transport of the sub-samples, it should be ensured that storage conditions for the different sub-samples are the same and still representative of the conditions under which the conversion equation will be applied.

5.4 Analysis

Each test sample should be analysed in duplicate, both with the routine method and the anchor method, thereby closely adhering to the standardized procedures.

When using decimal dilutions with the anchor method, these should be chosen in such a way that valid results in the range of interest are obtained.

5.5 Calculation

5.5.1 General

Before any calculation is made, a scatter diagram (i.e. plotted distribution of two-dimensional arrays) of observed values should be checked visually to obtain a first impression of the character of the relationship. The scatter diagram will show whether the relationship between the results of both methods tends to be linear over the whole range. If not, an appropriate data transformation should be used to achieve a linear relationship (see ISO 16140:2003, 6.2.1.3.2).

If the repeatability error on one or both variables is dependent on the level, it is better to apply a weighted least-squares method (see ISO 11095). It is recommended that a statistician's expertise be used for an analysis in such a case.

For the purpose of this International Standard, a linear relationship is assumed.

In the general case of regression, the vertical y-axis (dependent variable) is used for the routine method and the horizontal x-axis (independent variable) is used for the anchor method. If the repeatability error on the anchor method results is much larger (ratio > 2) than the repeatability error on the routine method results, x- and y-axes should be permuted before performing regression (see ISO 16140:2003, 6.2.1.3.2).

5.5.2 Validity of results

The collected results should be evaluated for validity.

Results should be excluded when there is a sound microbiological reason to do so. Examples are damage to the samples during transportation, abuse of specified temperature conditions, and reported deviations from the test protocol.

Data pairs for which either a routine method result or an anchor method result is below the lower quantification limit, or above the upper quantification limit, for the respective method should be excluded.

Duplicate results exceeding the established limit for repeatability should be excluded. If relevant [situation c) in 4.2], results exceeding the established limit for reproducibility should also be excluded.

If the difference in duplicate results is below the mentioned limits, they should be averaged before regression.

5.5.3 Principle of the regression method

The conversion relationship should be calculated according to ISO 16140:2003, 6.1.2.4, 6.2.1.3 and Annex S.

An outlier is defined as an extreme data pair, which normally appears randomly for less than 1 % of the data pairs. For such data pairs, the absolute deviation differs more than $(2{,}58 s_{y,x})$, where $s_{y,x}$ is the residual standard deviation from the estimated points by regression.

Outliers should be discarded, after which a recalculation should be performed.

5.6 Expression of results

The conversion relationship may be expressed as

a) a mathematical equation for the range of validity,

b) a table, listing equivalent values in routine method units and in anchor method units for the range of validity, or

c) equivalence points (i.e. specific values of results in routine method units that meet, for instance, with stated legislative limits in anchor method units).

The availability of a properly established conversion relationship provides the possibility to express the result of a quantitative determination of the bacteriological quality of a test sample in either routine method units or in anchor method units.

6 Verification of a conversion relationship

6.1 Frequency of verification

The exactness of the conversion relationship should be regularly checked and, if necessary, updated. The check and any necessary update should be carried out

a) at regular intervals,

b) after changes in milk production factors and/or sampling routines, which can be presumed to affect the composition and the properties of the bacterial flora,

c) after relevant changes in the procedure for the routine method and/or the anchor method, or

d) by rolling (i.e. continuously refreshing the data set with new data-pairs, thereby deleting the oldest ones and recalculating and evaluating the conversion relationship frequently).

In all cases, the representativity of the data set should be ensured.

6.2 Test samples

See 5.2.

6.3 Pretreatment of test samples

See 5.3.

6.4 Analysis

See 5.4.

6.5 Calculation and verification of a conversion relationship

A conversion relationship should be checked according to 5.5 and based on the new data set. It should also be checked whether the newly obtained conversion relationship significantly differs from the one applied so far. If yes, it should be adapted; if no, it should be left as it is.

For example, when the conversion relationship is the result of a linear regression procedure, it should be checked whether the newly calculated regression coefficients a and b are not significantly different from the one applied so far (see ISO 8196-1|IDF 128-1 and ISO 8196-2|IDF 128-2.

7 Test report

The test report of the organizing laboratory shall specify:

a) the set-up of the study for establishing or verifying the conversion relationship;

b) full details on sampling, sample storage procedures, sample transport and sample pre-treatment;

c) any assumptions made;

d) the methods used, with reference to this International Standard;

e) the results obtained;

f) where more than one laboratory was involved, details on interlaboratory quality assurance to minimize variabilities;

g) the resulting conversion relationship or the changes therein and its validity.

Annex A
(informative)

Number of samples for linear regression

A.1 For comparison of an estimated and a hypothetical regression coefficient, the test equation for a two-tailed test with probability $(1 - \alpha)$ is

$$t = \left| b_{yx} - \beta_{yx} \right| \Big/ s_{byx} \tag{A.1}$$

where

t is $t_{(n-1;1-\alpha/2)}$ which is the numerical value of the Student t-distribution at its $(1 - \alpha)$ probability level;

b_{yx} is the estimated regression coefficient;

β_{yx} is the hypothetical regression coefficient;

s_{byx} is $s_{yx} \Big/ s_x (n-1)^{0,5}$;

n is the number of data pairs.

Replacing $\left| b_{yx} - \beta_{yx} \right|$ by d results in

$$t = d \Big/ \left[s_{yx} \Big/ s_x (n-1)^{0,5} \right] \tag{A.2}$$

This can be rewritten as

$$n = t^2 (s_{yx}{}^2 \Big/ s_x{}^2) \Big/ d^2 + 1 \tag{A.3}$$

Since $s_{yx}{}^2$ may be approximated by $s_y{}^2 - b_{yx}{}^2 \cdot s_x{}^2$ with higher values of n, Equation (A.3) may also be written as

$$n \approx t^2 (s_y{}^2 \Big/ s_x{}^2 - b_{yx}{}^2) \Big/ d^2 + 1 \tag{A.4}$$

A.2 As the value of the Student t-distribution is dependent on the degrees of freedom, Equation (A.4) has to be solved iteratively. Since the values for $s_x{}^2$, $s_y{}^2$ and b_{yx} are estimates, the value of n will be an estimate and the demand for a predetermined probability $(1 - \alpha)$ cannot be maintained correctly.

A.3 An alternative way has been suggested (see [7]), introducing the relative error, δ, of the estimate for the slope of the regression, as follows:

$$\delta = \left| b_{yx} - \beta_{yx} \right| \Big/ \beta_{yx} \tag{A.5}$$

Equation (A.1) is transformed to

$$t \approx \left| b_{yx} - \beta_{yx} \right| \Big/ \left[s_y{}^2 \left(1 - r^2\right) \Big/ (n-1) s_x{}^2 \right]^{0,5} \tag{A.6}$$

where r is equal to $b_{yx}\left(s_x / s_y\right)$.

Extending Equation (A.6) results in

$$t \approx \left(\left|b_{yx} - \beta_{yx}\right| \Big/ \beta_{yx}\right) \Bigg/ \left\{\left[s_y{}^2\left(1-r^2\right) \Big/ (n-1)s_x{}^2\right]^{0,5} \Big/ \beta_{yx}\right\} \tag{A.7}$$

On introducing Equation (A.5), Equation (A.7) becomes

$$t \approx \delta \Bigg/ \left\langle \left\{\left[s_y{}^2\left(1-r^2\right) \Big/ (n-1)s_x{}^2\right]^{0,5} \cdot s_x \right\} \Big/ r \cdot s_y \right\rangle \tag{A.8}$$

or

$$t \approx \delta \Big/ \left[\left(1-r^2\right) \Big/ (n-1)r^2\right]^{0,5} \tag{A.9}$$

Resolving n, results in

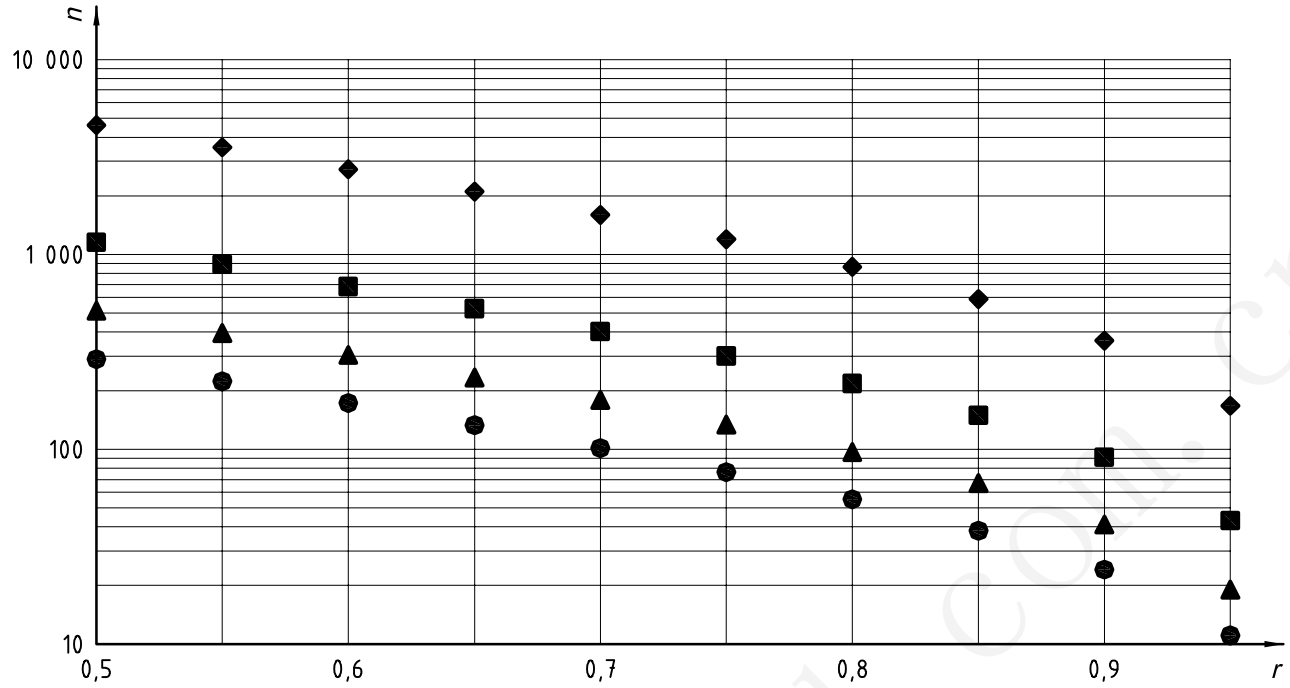
$$n \approx \left[t^2(1-r^2) \Big/ (\delta^2 \cdot r^2)\right] + 1 \tag{A.10}$$

A.4 Using Equation (A.10), the required sample size can be calculated for a demanded relative error of estimation δ and a given correlation r. It is clear that in the case of a bad correlation, the sample size will increase strongly.

As an example, in Table A.1 and Figure A.1, sample sizes are given for α = 0,05 for various values of δ and r.

Table A.1 — Number of samples n for α = 0,05

δ	r	n	δ	r	n
0,05	0,50	4 611	0,15	0,50	514
0,05	0,55	3 544	0,15	0,55	395
0,05	0,60	2 733	0,15	0,60	305
0,05	0,65	2 101	0,15	0,65	234
0,05	0,70	1 600	0,15	0,70	179
0,05	0,75	1 196	0,15	0,75	134
0,05	0,80	865	0,15	0,80	97
0,05	0,85	591	0,15	0,85	67
0,05	0,90	361	0,15	0,90	41
0,05	0,95	167	0,15	0,95	19
0,10	0,50	1 153	0,20	0,50	289
0,10	0,55	887	0,20	0,55	222
0,10	0,60	684	0,20	0,60	172
0,10	0,65	526	0,20	0,65	132
0,10	0,70	401	0,20	0,70	101
0,10	0,75	300	0,20	0,75	76
0,10	0,80	217	0,20	0,80	55
0,10	0,85	149	0,20	0,85	38
0,10	0,90	91	0,20	0,90	24
0,10	0,95	43	0,20	0,95	11



Key

◆ $\delta = 0{,}05$

■ $\delta = 0{,}10$

▲ $\delta = 0{,}15$

● $\delta = 0{,}20$

where δ is the relative error of estimation

Figure A.1 — Plot of number of samples n against correlation r for $\alpha = 0{,}05$

Bibliography

[1] ISO 11095, *Linear calibration using reference materials*

[2] ISO 16140:2003, *Microbiology of food and animal feeding stuffs — Protocol for the validation of alternative methods*

[3] ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

[4] IDF 161A, *Milk — Determination of bacteriological quality — Guidance on the evaluation of routine methods*

[5] EA-4/10, *Accreditation for microbiological laboratories*[2)]

[6] SUHREN, G. and REICHMUTH, J. Interpretation of quantitative microbiological results. *Milchwissenschaft*, **55**, 2000, pp. 18-22

[7] THIGPEN, C.C. A sample-size problem in simple linear regression. *The American Statistician*, **41**, 1987, pp. 214-215

2) Revised version of EAL-G18 (from European co-operation for Accreditation).

BSI — British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover. Tel: +44 (0)20 8996 9000. Fax: +44 (0)20 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: +44 (0)20 8996 9001. Fax: +44 (0)20 8996 7001. Email: orders@bsi-global.com. Standards are also available from the BSI website at http://www.bsi-global.com.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre. Tel: +44 (0)20 8996 7111. Fax: +44 (0)20 8996 7048. Email: info@bsi-global.com.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: +44 (0)20 8996 7002. Fax: +44 (0)20 8996 7001. Email: membership@bsi-global.com.

Information regarding online access to British Standards via British Standards Online can be found at http://www.bsi-global.com/bsonline.

Further information about BSI is available on the BSI website at http://www.bsi-global.com.

Copyright

BSI
389 Chiswick High Road
London
W4 4AL