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Milk — Quantitative determination of microbiological quality — Guidance for establishing and verifying a conversion relationship between results of an alternative method and anchor method results

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

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Foreword

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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).

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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*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 302, *Milk and milk products — Methods of sampling and analysis,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement), 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 21187 | IDF 196:2004), which has been technically revised. The main changes compared with the previous edition are as follows:

— the formula describing the conversion relationship has been based on grouped data rather than data from individual samples;

— examples of how to perform outlier tests, and calculation and verification of conversion relationships have been given in a spreadsheet.

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.

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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 (S11) of the *Standing Committee on Statistics and Automation* under the aegis of its project leaders, Ms B. Asmussen (DK), Ms V. Tzeneva (NL), Mr R. Kissling (NZ) and Ms B. Müller (DE).

Introduction

Conversion in quantitative microbiology means expressing the result of a quantitative determination of the microbiological status of a test sample obtained with an alternative method in units of another method, generally an anchor method. Through this, quantitative results obtained with alternative methods can be compared to values or limits that are stated in anchor method units. For establishing and applying a conversion relationship, a number of prerequisites should be met. These are referred to in this document, 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 alternative methods against an anchor 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 alternative methods in the quantitative determination of microbiological 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 microbiological quality — Guidance for establishing and verifying a conversion relationship between results of an alternative method and anchor method results

1 Scope

This document gives guidelines for the establishment of a conversion relationship between the results of an alternative method and an anchor method, and its verification for the quantitative determination of the microbiological quality of milk.

NOTE The conversion relationship can be used a) to convert results from an alternative method to the anchor basis or b) to convert results/limits, expressed on an anchor basis, to results in units of an alternative method.

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 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 the food chain — Method validation — Part 1: Vocabulary*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8196-1 | IDF 128-1, ISO 8196-2 | IDF 128-2, ISO 16140-1 and the following 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/

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

Note 1 to entry: The method can be proprietary or non-commercial.

Note 2 to entry: The term “alternative” in this document refers to the entire method. It includes all aspects (such as test sample pre-treatment, materials and instruments) required for the execution of the method.

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

Note 1 to entry: It is stressed that, in quantitative microbiology, any obtained value is only defined by the method description applied. This applies to any alternative method as well as, for instance, to the standard plate count for the enumeration of microorganisms.

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

Note 1 to entry: The analyte can 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 body
organization, possibly appointed by a competent authority, having the qualified staff and skills to organize, coordinate and report on the outcome of the activities for the establishment and/or the maintenance of a conversion relationship

3.5
measuring range
range wherein data with known precision and accuracy can be obtained

Note 1 to entry: Precision and accuracy data are determined in a validation study (e.g. by the instrument manufacturer or a responsible organization).

3.6
range of interest
numerical values for alternative method results, typical of routine samples when analysing in a laboratory

Note 1 to entry: If applicable, the range of interest shall include official limits and limits related to specific quality schemes.

4 Principles

4.1 General

The establishment and verification of a conversion relationship is based on the examination of test samples with an alternative method and the anchor method.

4.2 Guidance for applied methods and laboratories

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

The alternative method should have been evaluated and validated in accordance with ISO 16140-2 and/or ISO 16297 | IDF 161. Procedures for sampling, test sample preservation, test sample transport, test sample storage, sample pre-treatment, analysis and calculation of results should be documented, strictly standardized and controlled in agreement with ISO/IEC 17025, the Eurachem Guide[6] or comparable standards.

Regular participation in proficiency tests and training according to the relevant standards, e.g. ISO 4833-1, ISO 14461-2 | IDF 169-2, is strongly recommended.

The anchor method should have been validated, documented, strictly standardized and controlled in agreement with ISO/IEC 17025, the Eurachem Guide or comparable standards.

The protocol for the establishment of the conversion relationship and its verification should be documented. It should follow the guidelines of this document.

4.3 Organizational set-up

There are various possible organizational set-ups, e.g. both the alternative and the anchor method are fully carried out in the same laboratory, or several laboratories are involved in the trial.

Due to the instability and variability of the microbiological status of milk samples, the most robust conversion relationships will be obtained where the alternative method and the anchor method are undertaken on the same test samples, at the same place, at the same time. It is recommended to ensure that either the sequence of testing does not impose significant influence on the test results or the method with the lowest influence on the milk sample is applied first.

Subsampling should be avoided. However, in case of two or more participating laboratories subsamples may be necessary.

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 alternative method is carried out and the resulting conversion relationship is later applied. Factors to consider are listed in Clause 5.

The organizing body 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, details on the testing of samples, quality control data, and possibly data about storage and transport conditions.

5 Consideration of factors influencing the conversion relationship

5.1 General

A number of factors can influence the outcome of alternative 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 an alternative 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 test 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, e.g. with collection of milk from farms twice daily and every three days.

Influencing factors are grouped into environmental factors affecting the milk sample, e.g. content of type of bacterial flora or background noise from the sample matrix, and analytical factors which relate to the analysis itself, e.g. reagents. Some factors that can influence the conversion relationship in raw milk analysis are given in 5.2 and 5.3. Some of these factors can also be applied to other situations.

5.2 Environmental factors

5.2.1 General

The microbiological flora of a milk sample, i.e. the type of microorganisms, their growth phase or metabolic activity, influences the outcome of the measurement depending on the principle of the

method. For example, with the plate count method according to ISO 4833-1 only microorganisms viable under the respective growth conditions are determined, whereas with a flow-cytometric method all stainable microorganisms with a signal above the discriminator level are counted. This can have a significant impact on the conversion relationship. The normal variation of microbiological flora should be included in the test sample set that is used to determine a conversion relationship. Microorganisms in milk originate from the udder, the teat skin, from the air and from contamination from feedstuff, milking equipment and containers. The number and type of bacteria in milk can depend on the general characteristics of milk production such as the method of milking, storage conditions and collection intervals. The growth phase is dependent on the sample handling. Thus, there are numerous environmental factors influencing the microbiological flora of a milk sample. Some of these factors, which should be considered in the organizational set-up of the trial, are listed in 5.2.2 to 5.2.7.

5.2.2 Animal species

Animal species can have an impact on the bacterial flora in their milk, either directly through differences in the type of flora or in the production environment, or indirectly through composition and properties of the milk influencing the growth conditions for microorganisms. This can affect analytical results with different methods in different ways and therefore the conversion relationship.

5.2.3 Bulk milk storage conditions

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

5.2.4 Seasonal variations

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 or be adapted to the seasonal influence, e.g. by using a rolling system, including data from all seasons (see 9.1).

5.2.5 Sampling and pre-treatment of the test samples

Sampling, test sample storage, transport and pre-treatment of the test sample can affect bacterial growth, even within the stated limits for allowed storage time and temperature. Structural changes can necessitate an adaption of a conversion relationship.

5.2.6 Test sample preservation

Preservation of test samples should be avoided. However, with certain alternative methods, test sample preservation can be applied for stabilization purposes. It should be proven that the detectability of the analyte by the alternative method as well as the anchor method is not influenced.

5.2.7 Milk production conditions

These factors relate to the general characteristics of milk production such as the method of milking and collection intervals. Where this can affect 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.

5.3 Analytical factors

5.3.1 Instrument make and model

Even within the same methodology, different instrument models or brands can apply slightly different pre-treatment (e.g. in arranging for sample homogeneity) or counting procedures, which can influence the conversion relationship.

5.3.2 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, organic materials 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.

5.3.3 High somatic cell counts

Elevated somatic cell counts (e.g. 1 000 000 cells/ml) can cause increased background noise and higher count values.

6 Test samples

6.1 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 Formula (1) (see Annex A):

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

where

n is the required number of test samples;

t is the numerical value of the Student's t-distribution at the 95 % confidence level and n–1→∞;

δ is the numerical value of the relative error of the estimation for the regression (for payment and regulatory purposes, it is recommended to work with δ = 0,05; however, under certain circumstances, e.g. if the workload gets too big, δ = 0,10 can be applied);

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

The aim is to determine a required size of a test 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.

As conversion is basically different from calibrations known from chemical methods, its validation is also different. It should follow the fundamentals of conversion, i.e. using more data, to address the large natural variations and errors of microbiological testing including the anchor method. Data may be pooled from several years to reach sufficient numbers.

In cases where it appears from calculation (see 8.1) that the presumed correlation coefficient was an overestimate, the required number of extra data pairs should be included and the calculation should be repeated.

Another reason for needing a surplus of samples for analysis is that some data pairs are likely to become invalidated (see 8.1). A surplus of samples can also be necessary to ensure that the guidance given in 6.2 and 6.3 is followed.

6.2 Range of test samples

The levels should, within the measuring range, uniformly cover the range of interest for the alternative method concerned. Where data are to be transformed before statistical treatment (see 8.1), the data pairs should uniformly cover the transformed scale.

With some alternative methods, results are almost instantly available, so an efficient selection of samples can be based on the outcome of these measurements. This screening method should not have an impact on the results of the following analyses.

6.3 Representativeness of samples

It is of the highest priority to work with natural test samples. It is not possible to use samples spiked with pure cultures and incubated samples as this will lead to non-representative measurement results.

The test samples used to establish (and verify) a conversion relationship should truly represent the relevant variation of the routine sample population. It should be checked what factors have a significant influence on the variation (see Clause 5).

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

6.4 Pre-treatment of test samples

6.4.1 General

Preferably, test samples should be taken by a routine laboratory (e.g. a participating laboratory) to make sure that circumstances in the trial (e.g. sampling, test sample storage and test sample transport) are as close as possible to routine circumstances. If this is not feasible, test samples can also be collected centrally from another organization.

Test samples shall be analysed with both the alternative and anchor method at the same time or close to it. The time between alternative and anchor method analysis should be as short as at all possible. It may vary by up to 2 h, but it should preferably be less than 30 min (see ISO 16297 | IDF 161), whereby samples are kept at 0 °C to 4 °C. The time of analysis for both methods shall be noted.

If testing is done in different laboratories, sub-samples should be prepared (see 6.4.2) and should be transported to the laboratories appointed for testing (see 4.2). The sub-samples should be continuously kept at 0 °C to 4 °C. Test results on sub-samples should be accompanied by detailed information on the handling of test samples and the time of analysis.

6.4.2 Preparation of sub-samples

It is of utmost importance for establishing and verifying a conversion relationship that analyses are done with samples as equal as possible. To reach this, several factors are considered during sub-sample preparation, as follows.

— To obtain homogeneity of sub-samples, before dividing the original sample into sub-samples an effective mixing of the cold sample by rapidly inverting the sample container at least 25 times should be done. There should be no foaming or any foam should be allowed to disperse. The interval between mixing and removing the test portion should not exceed 3 min.

— The sub-sample should be poured into a clean, dry and sterile sample container. The sample containers should be sealed tightly to prevent any leakage from one sample affecting the integrity of the other samples during transport.

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

6.4.3 Storage and transport of sub-samples

It should be checked that the transport packaging is suitable for its purpose. A suitable 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 are as representative as possible of the conditions under which the conversion relationship will be applied (see 5.2).

7 Analysis

It is of utmost importance that analysis with the alternative method and the anchor method is carried out at the time stated in the description accompanying the samples (see 6.4.1).

Each test sample should be analysed as a minimum in duplicate, both with the alternative method and the anchor method, thereby closely adhering to the standardized procedures (see 4.2).

NOTE An anchor method duplicate is considered to be two dilution series and at least one plate from each relevant dilution step.

8 Establishing a conversion relationship

8.1 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, a base-10 logarithmic transformation can be applied (see ISO 16297 | IDF 161).

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

In the general case of regression, the vertical *y*-axis (dependent variable) is used for the alternative method and the horizontal *x*-axis (independent variable) is used for the anchor method.

An example for the calculation of the conversion relationship is given in Annex B.

NOTE Plotting scatter diagrams during the trial helps to find out if the experimental design or protocols need adjustment.

8.2 Validity of results

The results in the data set 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 and abuse of specified temperature conditions.

Data pairs for which either an alternative 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, results exceeding the established limit for reproducibility should also be excluded.

Results should also be excluded in cases of reported deviation from the test protocol.

8.3 Conversion relationship

The conversion relationship can be expressed as:

— a mathematical formula for the range of validity;

— a table, listing equivalent values in alternative method units and in anchor method units for the range of validity; or

— equivalence points (i.e. specific values of results in alternative 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 microbiological quality of a test sample in either alternative method units or in anchor method units.

8.4 Calculations

8.4.1 General

Before starting any calculation, the results obtained with the anchor and alternative method should be transformed in a base-10 logarithmic scale.

Subsequently for each data pair the duplicate results from each method should be averaged.

8.4.2 Removal of outliers

An outlier is an observation that lies an abnormal distance from other values in a random sample from a population of data. This normally applies with around 1 % of the data pairs.

To identify the outliers in the data set, the following procedure should be applied.

a) Apply ordinary least square (OLS) regression with the results obtained with the anchor and alternative method expressed in their respective units.

b) Calculate the residual standard deviation ($s_{y,x}$).

c) Calculate ($2{,}58 \times s_{y,x}$).

d) With the regression line obtained under a), calculate the estimated value of the alternative method results in units of the anchor method.

e) Calculate for each data pair the difference between the anchor method result and the estimated value from the alternative method result as obtained with d). If the difference is more that ($2{,}58 \times s_{y,x}$) the data pair can be considered an outlier and should be discarded.

8.4.3 Conversion relationship

After outlier removal, the conversion relationship should be calculated as follows.

a) For each data pair, calculate the mean value of the results obtained with the anchor method and with the alternative method in their respective units.

b) Sort the data set according to the means calculated for the anchor method results per test sample given in a).

c) Calculate the average for each 10 anchor method results in increasing order with the units as they are obtained, e.g. average of the first 1 through 10 results, then the average of the 11 through 20 results, etc. These are the consolidated data of the anchor method.

d) Calculate for the same samples the average for each 10 alternative method results with the units as they are obtained, e.g. average of the first 1 through 10 results, then the average of the 11 through 20 results, etc. These are the consolidated data of the alternative method.

e) Make a scatter diagram by placing the consolidated anchor methods results calculated in c) on the x-axis and the consolidated alternative methods results calculated in d) on the y-axis.

f) Compute the conversion relationship by applying OLS regression and compute $s_{y,x;\mathrm{con}}$ (with $s_{y,x;\mathrm{con}}$ expressing $s_{y,x}$ of the consolidated data).

9 Verification of a conversion relationship

9.1 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:

— at regular intervals;

— after changes in environmental factors (see 5.2), e.g. milk production factors and/or sampling routines, which can be presumed to affect the composition and the properties of the microbiological flora;

— after relevant changes in the procedure for the alternative method and/or the anchor method; or

— 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.

9.2 Calculation

A conversion relationship should be checked according to 8.1 and calculated 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 so, the conversion applied so far should be adapted. If not, it should be left as it is.

For example, when the conversion relationship is the result of an OLS 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).

Examples for the verification of the conversion relationship are given in Annexes B and C.

10 Test report

The test report of the organizing body should specify:

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

— full details on the test sample set, sampling, test sample storage procedures, test sample transport and test sample pre-treatment;

— any assumptions made;

— the methods used, with reference to this document, i.e. ISO 21187 | IDF 196:2021;

— the results obtained;

— any deviations from the procedure;

— any unusual feature observed;

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

— the resulting conversion relationship or the changes therein and its validity;

— the date of the test.

Annex A
(informative)

Number of test samples for linear regression

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

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

where

t is $t_{(n-1;1-\alpha/2)}$, which is the numerical value of the Student's 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 $s_{yx} / s_x (n-1)^{0,5}$;

n is the number of data pairs.

Replacing $\left| b_{yx} - \beta_{yx} \right|$ by d results in Formula (A.2):

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

This can be rewritten as Formula (A.3):

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

Since s_{yx}^2 can be approximated by $s_y^2 - b_{yx}^2 \cdot s_x^2$ with higher values of n, Formula (A.3) may also be written as Formula (A.4):

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

where

s_y is the standard deviation of the y-values;

s_x is the standard deviation of the x-values.

A.2 As the value of the Student's t-distribution is dependent on the degrees of freedom, Formula (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 Reference [7]), introducing the relative error, δ, of the estimate for the slope of the regression, as shown by Formula (A.5):

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

Formula (A.1) is transformed to Formula (A.6):

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

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

Extending Formula (A.6) results in Formula (A.7):

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

On introducing Formula (A.5), Formula (A.7) becomes Formula (A.8):

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

or Formula (A.9):

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

Resolving n results in Formula (A.10):

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

A.4 Using Formula (A.10), the required sample size can be calculated for a demanded relative error of estimate 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 $\alpha = 0,05$ for various values of δ and r.

Example for calculation using Formula (A.10) and $\delta = 0,10$, $r = 0,90$ and t for $\alpha = 0,05$ and n–1→∞:

$n = [1,96^2 \ (1 - 0,90^2) / [0,10^2 \times 0,90^2)] + 1 = 91$

Table A.1 — Number of samples n for $\alpha = 0,05$ (level of significance) and n–1→∞

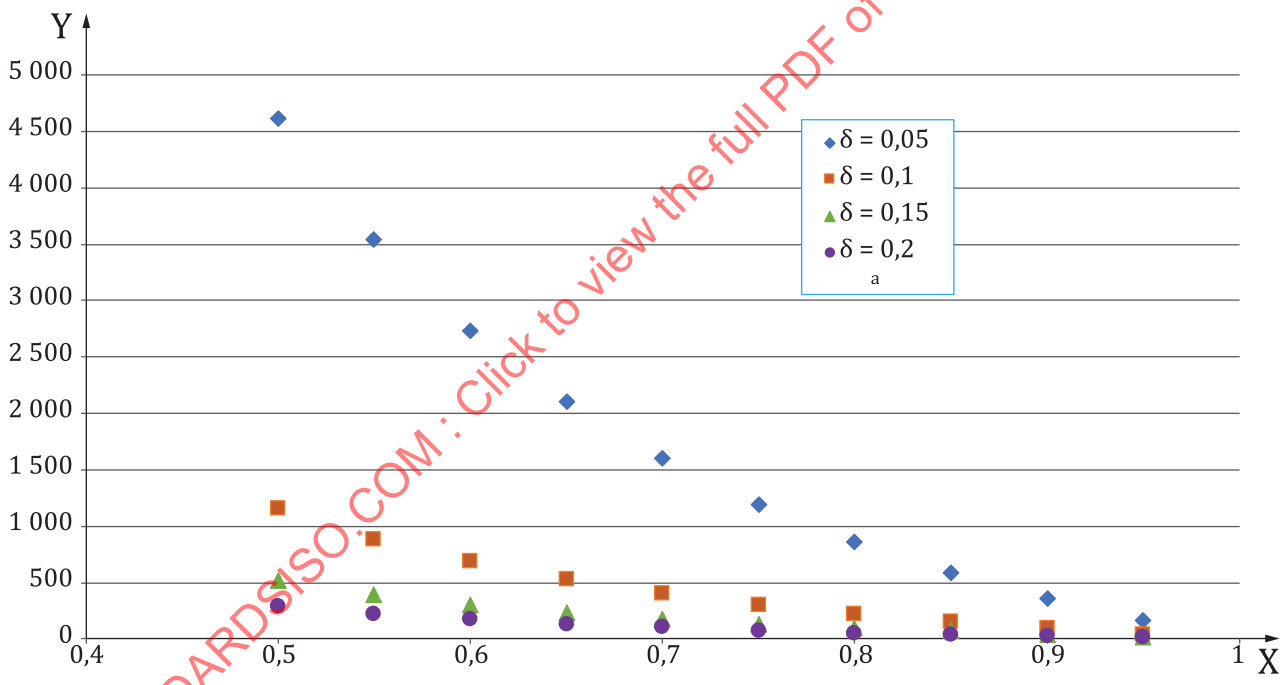
δ[a]	r	n	δ	r	n
0,05	0,50	4 611	0,15	0,50	513
0,05	0,55	3 544	0,15	0,55	395
0,05	0,60	2 733	0,15	0,60	305
0,05	0,65	2 101	0,15	0,65	234
0,05	0,70	1 600	0,15	0,70	179
0,05	0,75	1 196	0,15	0,75	134
0,05	0,80	865	0,15	0,80	97
0,05	0,85	591	0,15	0,85	67
0,05	0,90	361	0,15	0,90	41

[a] The demanded relative error of estimate.

Table A.1 *(continued)*

δ[a]	r	n	δ	r	n
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

[a] The demanded relative error of estimate.



Key

X correlation coefficient, r

Y required number of test samples, n

[a] Where δ is the relative error of estimation.

Figure A.1 — Plot of number of samples n against correlation r for α = 0,05

Annex B
(informative)

Example identification of outliers and calculation of conversion relationship

A full example based on real data can be found in a spreadsheet file at https://standards.iso.org/iso/21187/ed-2/en/.

The data in this file have been supplied by the German National Reference Laboratory for Milk and Milk Products, Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Kiel.