SLOVENSKI STANDARD
SIST-TS ENV ISO 13843:2004

01-junij-2004

Kakovost vode – Navodilo za validacijo mikrobioloških metod (ISO/TR 13843:2000)

Water quality - Guidance on validation of microbiological methods (ISO/TR 13843:2000)

Wasserbeschaffenheit - Richtlinie zur Validierung mikrobiologischer Verfahren (ISO/TR 13843:2000)

Qualité de l'eau - Lignes directrices pour la validation des méthodes microbiologiques (ISO/TR 13843:2000)

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English version

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This European Prestandard (ENV) was approved by CEN on 7 April 2001 as a prospective standard for provisional application.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Foreword

The text of the Technical Report from Technical Committee ISO/TC 147 "Water quality" of the International Organization for Standardization (ISO) has been taken over as a European Prestandard by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

This European Prestandard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2001, and conflicting national standards shall be withdrawn at the latest by November 2001.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this European Prestandard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of the Technical Report ISO/TR 13843:2000 has been approved by CEN as a European Prestandard without any modifications.

TECHNICAL REPORT

ISO/TR 13843

First edition
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Water quality — Guidance on validation of microbiological methods

Qualité de l'eau — Lignes directrices pour la validation des méthodes microbiologiques

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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.

In exceptional circumstances, when a technical committee has collected data of a different kind from that which is normally published as an International Standard ("state of the art", for example), it may decide by a simple majority vote of its participating members to publish a Technical Report. A Technical Report is entirely informative in nature and does not have to be reviewed until the data it provides are considered to be no longer valid or useful.

Attention is drawn to the possibility that some of the elements of this Technical Report may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO/TR 13843 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Water quality — Guidance on validation of microbiological methods

1 Scope

This Technical Report deals with validation of microbiological methods, with particular emphasis on selective quantitative methods in which the quantitative estimate is based on counting of particles either directly, with the aid of a microscope, or indirectly, on the basis of growth (multiplication) into colonies or turbidity.

The principles and procedures within this scope are commonly known as the presence/absence (P/A), most probable number (MPN), colony count and direct (microscopic) count.

This Technical Report does not apply to the validation of the so-called rapid or modern methods which mostly depend on measuring products or changes due to microbial activity but do not address the detection of individual particles.

2 Terms and definitions

For the purposes of this Technical Report, the following terms and definitions apply.

2.1
accuracy of measurement
closeness of the agreement between a test result and the accepted reference value

NOTE The term “accuracy”, when applied to a set of test results, involves a combination of random components and a common systematic error or bias component.

[ISO 3534-1:1993, 3.11]

2.2
analyte
measurand
particular quantity subjected to measurement

NOTE 1 See reference [5].

NOTE 2 In microbiology the analyte is ideally defined as a list of taxonomically defined species. In many cases, in practice the analyte can only be defined by group designations less accurate than taxonomic definitions.

2.3
analytical portion
test portion
volume of particle suspension inoculated into a detector unit

NOTE Examples of a detector unit are agar plate, membrane filter, test tube, microscopic grid square.

2.4
application range
range of particle concentrations routinely subjected to measurement by a method

2.5
categorical characteristic
method performance characteristic numerically expressed as a relative frequency based on P/A or +/– classification

2.6
CFU, deprecated
colony-forming unit, deprecated
CFP, deprecated
colony-forming particle, deprecated

NOTE The term was originally introduced to convey the idea that a colony may originate not only from a single cell but from a solid chain or aggregate of cells, a cluster of spores, a piece of mycelium, etc. It mistakenly equates the number of colonies observed to the number of living entities seeded on the medium. Growth unit, viable particle, **propagule** (2.27) and **germ** (2.13) are terms with similar meanings but convey the original idea better and apply not only to colony-count methods but also to MPN and P/A.

2.7
coefficient of variation
CV
relative standard deviation
for a non-negative characteristic, the ratio of the standard deviation to the average

NOTE 1 The ratio may be expressed as a percentage.

NOTE 2 The term "relative standard deviation" is sometimes used as an alternative to "coefficient of variation", but this use is not recommended.

[ISO 3534-1:1993, 2.35]

NOTE 3 In this Technical Report the term coefficient of variation (CV) is used when the relative standard deviation is expressed in percent (CV % = 100 RSD).

2.8
collaborative test
method or laboratory performance test where several laboratories join in an experiment planned and co-ordinated by a leader laboratory

NOTE Collaborative tests are mainly of two types. Intercalibration exercises are made to allow laboratories to compare their analytical results with those of other participating laboratories.

Method performance tests produce precision estimates (repeatability, reproducibility) out of data accumulated when several participating laboratories study identical samples with a strictly standardized method.

2.9
confirmed [verified] colony count
x
presumptive colony count corrected for false positives

$$x = pc = \frac{k}{n}c$$

where

c is the presumptive count;

p is the true positive rate;

n is the number of presumptive positives isolated for confirmation;

k is the number confirmed.

2.10
control chart
two-dimensional scattergram for monitoring method performance with control values obtained by a Type A study

NOTE In control charts the horizontal axis is usually in the time scale or ordinate scale and the control variable is the mean or some precision measure (s, CV, RSD).

2.11
detector
particle detector
plate of solid matrix or a tube of liquid containing a nutrient medium for counting or detecting living microbial particles

2.12
detection set
detector set
combination of plates or tubes on which quantitative estimation of microbial concentration in a sample is based

NOTE The detection set is the set of plates or tubes utilized for numerical estimation of a single value.

EXAMPLES Parallel plates of a suspension, plates from consecutive dilutions, 3 × 5 tube MPN system, microtitre plate.

2.13
germ
living entity capable of producing growth in a nutrient medium

cf. **propagule** (2.27)

2.14
guidance chart
two-dimensional scattergram for presenting method performance data (quantity or precision) with arbitrary guide values or guide values obtained by Type B reasoning

NOTE In guidance charts, the horizontal axis is usually the colony count per detector.

2.15
heterogeneous Poisson distribution
distribution arising when the mean of a Poisson distribution varies randomly from occasion to occasion

NOTE 1 See reference [11].

NOTE 2 See also negative binomial distribution (2.19).

2.16
limit of detection
particle number x (per analytical portion) where the probability p_0 of a negative result equals 5 %

NOTE 1 Probability of a positive result $p(+) = 1 - p_0$.

NOTE 2 a) Calculation of x via Poisson distribution:

$$x = \ln\left(\frac{1}{p_0}\right) = \ln\left(\frac{1}{0,05}\right) = \ln(20) = 3,00$$

b) Calculation of x via negative binomial distribution:

$$x = \frac{\left(p_0{}^{-u^2} - 1\right)}{u^2} = \frac{0,05^{-u^2} - 1}{u^2} = \frac{20^{-u^2} - 1}{u^2}$$

2.17
limit of determination
lowest average particle concentration x per analytical portion where the expected relative standard uncertainty, equals a specified value (RSD)

NOTE a) Calculation of x via Poisson distribution:

$$x = \frac{1}{(\mathrm{RSD})^2}$$

b) Calculation of x via negative binomial distribution:

$$x = \frac{1}{(\mathrm{RSD})^2 - u^2}, \text{ given overdispersion factor} = u$$

2.18
linearity
linear dependence of the signal on concentration of the analyte

cf. **proportionality** (2.28)

2.19
negative binomial distribution
a particular "overdispersed" statistical distribution of counts

NOTE 1 Its variance can be expressed as

$$\sigma^2 = \mu + u^2 \mu^2$$

where μ is the mean.

NOTE 2 In this Technical Report the square of the overdispersion factor (u) is substituted for the inverse of the exponent ($1/k$) of the standard formula for the negative binomial distribution.

2.20
overdispersion
variation in excess of Poisson randomness

NOTE It is detected qualitatively by the Poisson index of dispersion, and measured quantitatively by estimating the parameter u (overdispersion factor) of the negative binomial distribution.

2.21
overdispersion factor
u
additional random uncertainty of determination in excess of the Poisson distribution, measured in terms of relative standard deviation

2.22
overlap error
crowding error
systematic depression of colony counts due to confluence of colonies

NOTE Quantitatively, overlap error depends primarily on the fraction of available growth space occupied by colonial growth.

2.23
parallel counts
particle or colony numbers in equal analytical portions drawn from the same suspension

2.24
Poisson distribution
fully random distribution of particle numbers when sampling a perfectly mixed suspension

NOTE The probability $P(k)$ of observing exactly k units in a test portion when the mean equals μ is calculated from

$$P(k) = \frac{\mu^k}{k!} e^{-\mu}$$

2.25
precision
closeness of agreement between independent test results obtained under stipulated conditions

NOTE Precision does not relate to the true value or the specified value. It is usually expressed in terms of imprecision and computed as a standard deviation of the test results.

2.26
primary validation
full validation
establishment of the specifications for the performance of a new method and/or experimental verification that a method meets theoretically derived quality criteria

2.27
propagule
a viable entity, vegetative cell, group of cells, spore, spore cluster, or a piece of fungal mycelium capable of growth in a nutrient medium

cf. **germ** (2.13)

2.28
proportionality
agreement of observed particle counts with the volume (or dilution) of a series of analytical portions from a common root suspension

NOTE Proportionality is computed for statistical evaluation as the log-likelihood ratio statistic G^2 with n–1 degrees of freedom.

2.29
qualitative method
method of analysis whose response is either the presence or absence of the analyte in a certain amount of sample

NOTE See reference [10].

2.30
recovery
general term for the number of particles estimated in a test portion or sample, with the understanding that there is a true (although unknown) number of particles of which 100 % or less are "recovered" by the detector

2.31
relative accuracy
degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on identical samples

NOTE See reference [10].

2.32
relative difference
d
difference between two measured values divided by their mean

$$d = \frac{x_A - x_B}{\bar{x}} = \frac{2(x_A - x_B)}{x_A + x_B}$$

$$d\ \% = 100\ d$$

NOTE For all practical purposes, the same value results from the calculation $d = \ln(x_A) - \ln(x_B)$.

2.33
relative recovery
ratio (A/B) of colony counts obtained by two methods tested on equal test portions of the same suspension, where B is the reference (when applicable)

2.34
relative standard deviation
RSD
estimate of the standard deviation of a population from a sample of n results divided by the mean of that sample

$$\text{RSD} = \frac{s}{\bar{x}}$$

cf. **coefficient of variation** (2.7)

2.35
repeatability
closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement

NOTE 1 See *Guide to the expression of uncertainty in measurement* [6].

NOTE 2 Repeatability is computed as $r = 2{,}8 s_r$, where s_r is the repeatability standard deviation.

2.36
reproducibility
closeness of the agreement between the results of measurements on the same measurand carried out under changed conditions of measurement

NOTE 1 See *Guide to the expression of uncertainty in measurement* [6].

NOTE 2 Reproducibility is computed as $R = 2{,}8\ s_R$,

where

s_R is the reproducibility standard deviation usually compounded from the between-laboratories standard deviation s_L and repeatability standard deviation s_r:

$$s_R = \sqrt{s_L^2 + s_r^2}$$

2.37
robustness
insensitivity of an analytical method to small changes in procedure

NOTE 1 See reference [23].

NOTE 2 To examine the robustness it is advisable to “abuse” the method in a controlled way.

2.38
secondary validation
demonstration by experiment that an established method functions according to its specifications in the user's hands

2.39
apparent selectivity
F
ratio of the number of target colonies to the total number of colonies in the same sample volume

$F = \lg(t/n)$

where

t is the apparent concentration of presumptive target types estimated by counting colonies;

n is the concentration of total colonies.

2.40
sensitivity
fraction of the total number of positive cultures or colonies correctly assigned in the presumptive inspection

2.41
specificity
fraction of the total number of negative cultures or colonies correctly assigned in the presumptive inspection

2.42
standard uncertainty
uncertainty of the result of a measurement expressed as a standard deviation

NOTE See reference [5].

2.43
type A evaluation
⟨of uncertainty⟩ method of evaluation of uncertainty by the statistical analysis of a series of observations

EXAMPLE Observations may be e.g. standard deviation, relative standard deviation.

NOTE 1 See references [5] and [6].

NOTE 2 Repeatability and reproducibility are often estimated by carrying out collaborative method performance tests where several laboratories study "identical" samples provided by a central organizer [15].

2.44
type B evaluation
⟨of uncertainty⟩ method of evaluation of uncertainty by means other than the statistical analysis of series of observations e.g. from assumed probability distributions based on experience or other information

NOTE See references [5] and [6].

2.45
uncertainty
⟨of measurement⟩ parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand

NOTE See reference [6].

2.46
uncertainty
⟨of counting⟩ relative standard deviation of results of repeated counting of the colonies or particles of the same plate(s) or field(s) under stipulated conditions