INTERNATIONAL STANDARD

ISO 17994

First edition
2004-05-15

Water quality — Criteria for establishing equivalence between microbiological methods

Qualité de l'eau — Critères pour établir l'équivalence entre les méthodes microbiologiques

Reference number
ISO 17994:2004(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

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Foreword

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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 17994 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Introduction

This International Standard presents the criteria and procedures for assessing the average quantitative equivalence of the results obtained by two microbiological analytical methods one of which may but need not be a standard or reference method.

The methods considered are based on counts of colonies or of positive and negative liquid enrichment tubes (MPN and presence/absence methods).

NOTE It is possible that a method that is not quantitatively equivalent with a reference method would be accepted, especially if it appears “better” than the reference either quantitatively or otherwise.

Water quality — Criteria for establishing equivalence between microbiological methods

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This International Standard defines an evaluation procedure for comparing two methods intended for the detection or quantification of the same target group or species of microorganisms.

This International Standard provides the mathematical basis for the evaluation of the average relative performance of two methods against chosen criteria of equivalence.

Any two enumeration methods based on counts (of colonies or positive tubes) or any two detection methods [presence/absence (P/A) methods] intended for the same purpose can be compared.

This International Standard provides no solution to directly compare a quantitative method (colony count or MPN) with a detection method (P/A).

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/TR 13843:2000, *Water quality — Guidance on validation of microbiological methods*

3 Terms, definitions and symbols

3.1 Terms and definitions

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

3.1.1 General terms

3.1.1.1
reference method
prescribed analytical method to analyse a given group or species of microorganisms

NOTE As a rule, the reference method is a standard or a commonly used method.

3.1.1.2
trial method
any method which is to be tested for equivalence with a reference method

3.1.1.3
equivalent method
method considered quantitatively equivalent with another method when the average relative difference of their confirmed counts is found “not different” when following the calculations specified in this International Standard

3.1.1.4
standard uncertainty
uncertainty of the result of a measurement expressed as a standard deviation

[GUM]

3.1.1.5
expanded uncertainty
quantity defining an interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand

NOTE The fraction may be viewed as the coverage probability or level of confidence of the interval. To associate a specific level of confidence requires explicit or implicit assumptions regarding the probability distribution. The level of confidence may be attributed to this interval only to the extent to which such assumptions may be justified.

[GUM]

3.1.1.6
coverage factor
numerical factor used as a multiplier of the (combined) standard uncertainty in order to obtain an expanded uncertainty

NOTE The coverage factor, $k = 2$ is chosen for this International Standard because the distribution of the relative difference is unlikely to be normal; the expanded uncertainty corresponds only approximately to the 95 % confidence interval.

3.1.2 Specific terms

3.1.2.1
count
observed number of objects, e.g. colonies or cells of microorganisms, plaques of bacteriophages

NOTE In this International Standard, the result of an MPN estimation is also considered a count.

3.1.2.2
presumptive count
number of objects that according to their outward appearance should presumably be included in the count

3.1.2.3
confirmed count
count corrected for false positive results by further testing of the presumptive objects

3.1.2.4
relative difference
RD
difference between two results, a and b, measured on a relative (natural logarithmic) scale

NOTE The value of RD, x, expressed in percent, is given by

$$x = [\ln(a) - \ln(b)] \times 100\ \%$$

Essentially the same result is given by

$$x = \frac{2(a-b)}{(a+b)} \times 100\ \%$$

until the ratio between a and b is greater than about 3. This accounts for the usage of the term “relative difference” in both cases.

3.2 Symbols and abbreviated terms

A	the (symbol for the idea of) trial method
a	a test result by Method A
a_i	the test result (confirmed count) of Method A in sample i
B	the (symbol for the idea of) reference method
b	a test result by Method B
b_i	the test result (confirmed count) of Method B in sample i
C	coefficient for computing the number of samples, given the value of the experimental variance
D	maximum acceptable deviation (value of confidence limit) in the case Methods A and B are “not different”
i	running index
k	coverage factor used for calculating the expanded uncertainty
L	smallest significant (i.e. maximum acceptable) relative difference between Methods A and B
MPN	most probable number quantitative method
m	number of parallel tubes per dilution in an MPN series
n	number of samples
n_A	number of samples where for the P/A Method, A is positive and B negative
n_B	number of samples where for the P/A Method, A is negative and B positive
P/A	presence/absence detection method
s	experimental standard deviation (standard uncertainty)
s^2	experimental variance
$s_{\bar{x}}$	standard deviation (standard uncertainty) of the mean
U	expanded uncertainty
x	relative difference
x_i	value of the relative difference between a_i and b_i in sample i
$\bar{x}$	arithmetic mean of x_i (i = 1,2,…,n)
x_L	value of the relative difference at the approximate lower 95 % confidence limit, derived by subtracting the value of the expanded uncertainty from the mean
x_H	value of the relative difference at the approximate upper 95 % confidence limit, derived by adding the value of the expanded uncertainty to the mean
X^2	experimental Poisson index of dispersion
y	conditional variable used in computing the number of samples for equivalence testing and/or verification

4 Principle

The basic data are pairs of confirmed counts (a_i, b_i) obtained from the examination of two equal portions taken from the same vessel of a carefully mixed test sample, one determination (count) per method. The complete design consists of a large number of similar determinations.

In this International Standard, two methods are considered quantitatively equivalent ("not different") if the mean relative difference of the paired confirmed counts does not differ significantly from zero and the expanded uncertainty does not extend beyond the level of the stipulated maximum acceptable deviation. The decision rules based on the above principle are detailed in 7.2 and 7.3 and a flow chart is given in Annex A.

NOTE 1 Fixing a value for the maximum acceptable deviation (D) implies indirectly that the smallest average difference (L) to be considered significant is one half of that value.

NOTE 2 It has been suggested that in international and inter-laboratory method performance tests a limit of D = 10 % for the "confidence interval" be the maximum acceptable deviation for drinking water[2].

NOTE 3 For chemical methods, mean and precision are used as criteria for equivalence. In microbiology, equal precision (equal variance) is not an equivalence criterion.

5 Basic requirements for an equivalence experiment

5.1 General

Both methods shall fulfil at least the minimum requirements of validity specified in ISO/TR 13843.

The most important basic requirement of equivalence trials is a wide range of samples. Participation by a number of laboratories is usually necessary to expand the sample range over large geographical areas. Also the credibility of a general conclusion is commonly believed to require the participation of several laboratories. The result of the comparison is generally valid only within the range of sample types studied. Collaborative trials are detailed in Annex B.

It is essential that all laboratories taking part in a collaborative study have recognized quality assurance systems in use and apply approved basic techniques of cultivation.

5.2 Types of samples

The requirements for method comparisons differ somewhat from the daily routine situation. It is useful and often necessary to pre-select or prepare special samples. Samples for method comparisons should contain enough bacteria that the likelihood of scoring a zero count is small.

Samples for method comparisons should represent types that are included in the scope of both methods. Natural samples are ideal. Appropriate samples may also be prepared by dilution, spiking, or mixing of different kinds of water to achieve the desired population in a suitable density. Spiking with pure cultures should be considered the last resort.

It may be appropriate to stress the microbial population of some samples by controlled application of disinfectants[2] or by refrigerated storage in order to simulate situations encountered in routine laboratory practice.

5.3 Number of samples and participating laboratories

5.3.1 General

It is not possible to determine beforehand the exact number of samples required for a valid comparison. The number depends on the actual difference observed, on the experimental standard deviation and on the difference considered significant. This International Standard includes an adequacy clause based on a

stipulated "maximum acceptable deviation" and the expanded uncertainty. If the data are found inadequate for deciding that the methods are "not different", more samples are to be collected and examined.

If the methods happen to differ markedly, a small number of samples might suffice to determine this fact. It is therefore advisable to proceed in stages. The first stage should be planned to detect large differences between the methods. If large differences are not found (result inconclusive), more samples are taken until the system is able to detect the average difference that corresponds with the maximum acceptable deviation chosen at the beginning of the trial. Tables are given in 5.3.3 and 5.3.6 to provide help for planning.

5.3.2 The number of laboratories

There are no previous standards or rules about the number of laboratories in inter-laboratory equivalence trials. Six is tentatively suggested as minimum number.

5.3.3 Number of samples, two colony methods

The total number of samples, n, sufficient for the detection of a given average relative difference at about 95 % confidence depends on the experimental variance according to the equation:

$$n = Cs^2$$

where

s^2 is the variance;

C is a coefficient that depends on the chosen least significant difference.

The value of C is derived from the relationship: $C = 4/L^2$. The relationship between D (the maximum acceptable deviation) and L (the least significant difference) is: $L = D/2$ (see Table 1).

Table 1 — Coefficients for determining the number of samples required for the detection of a given relative difference (L)

D[a] %	L %	C
60	30	0,004 4
40	20	0,010 0
30	15	0,017 8
20	10	0,040 0
10	5	0,160 0

[a] The corresponding maximum acceptable deviation (D) is shown for comparison.

EXAMPLE A rather frequently observed value for the experimental standard deviation of the relative difference is approximately $s = 80$. Inserting this value in the equation gives $n = 6\,400C$. In order to detect an average relative difference of 10 % units ($L = 10$ %), $n = 6\,400 \times 0{,}040\,0 = 256$ samples should be sufficient.

5.3.4 Number of samples, two MPN methods

With MPN methods the number (n) of samples depends on the number (m) of parallel tubes according to the equation:

$$n = 1\,700/m$$

With five parallel tubes per dilution, 1 700/5 = 340 samples should suffice for the detection of a 10 % relative difference.