INTERNATIONAL STANDARD

ISO 17994

Second edition
2014-02-15

Water quality — Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods

Qualité de l'eau — Exigences pour la comparaison du rendement relatif des microorganismes par deux méthodes quantitatives

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbcee0d4-dee0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

Reference number
ISO 17994:2014(E)

© ISO 2014

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbcee0d4-dee0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

COPYRIGHT PROTECTED DOCUMENT

© ISO 2014

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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

© ISO 2014 – All rights reserved

Contents

Page

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbccc0d4-dcc0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

© ISO 2014 – All rights reserved

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.

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, 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, www.iso.org/patents.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

iTeh STANDARD PREVIEW
(standards.iteh.ai)

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/...
6ad69cd5236a/iso-17994-2014

This second edition cancels and replaces the first edition (ISO 17994:2004), which has been technically revised.

 © ISO 2014 – All rights reserved

Introduction

This International Standard specifies criteria and procedures for comparing the average quantitative 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).

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbcee0d4-dee0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

© ISO 2014 – All rights reserved

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbcee0d4-dee0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

Water quality — Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This International Standard specifies an evaluation procedure for comparing two methods with established performance characteristics according to ISO/TR 13843 and intended for the 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 quantitative methods against chosen criteria for the comparison. It does not provide data for assessment of the precision of the methods being compared. It is appropriate that the precision of methods is assessed as part of their performance characterization.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

This International Standard does not provide methods for the verification of method performance characterization in a single laboratory.

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbcee0d4-dee0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

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

3 Terms, definitions and symbols

3.1 Terms and definitions

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

3.1.1 General terms

3.1.1.1
comparison trial
interlaboratory method comparison that involves laboratories which perform paired measurements on several of their own samples with two different methods

© ISO 2014 – All rights reserved

3.1.1.2
not-different method
method considered quantitatively not different to another method when the mean difference between their confirmed counts and stipulated difference lie between predetermined stipulated limits, taking into account all sources of variation

Note 1 to entry: This difference can be assessed by the average relative difference of their confirmed counts.

3.1.1.3
predetermined stipulated limit
permitted average difference (based on a "confidence interval" designated $-2L$ to $+2L$) between results obtained by each method, based on professional practices or regulatory requirements

Note 1 to entry: Reference [1] suggests that, in international and interlaboratory method performance tests, a limit of $2L$ = 10 % for setting the "confidence interval" be the predetermined stipulated limit for drinking water, and this has been widely used. However, for environmental waters, such as bathing waters, Reference [2] proposes a predetermined stipulated limit of $2L$ = 20 %.

3.1.1.4
reference method
method of analysis internationally recognized by experts or by agreement between the parties

Note 1 to entry: As a rule, the reference method is a standard or a commonly used method.

3.1.1.5
standard measurement uncertainty
measurement uncertainty expressed as a standard deviation

[SOURCE: ISO/IEC Guide 99:2007 [3], 2.30]

iTeh STANDARD PREVIEW
(standards.iteh.ai)

3.1.1.6
trial method
any method which is to be tested for comparison with a reference method

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbcee0d4-dee0-4c53-a2cb-6adb9cd5236a/iso-17994-2014

3.1.2 Specific terms

3.1.2.1
count
observed number of objects

EXAMPLE Colonies or cells of microorganisms, plaques of bacteriophages

Note 1 to entry: In this International Standard, the result of an MPN estimation is also considered to be a count.

3.1.2.2
presumptive count
colony count or most probable number (MPN) estimate based on the number of colonies or fermentation tubes that have an outward appearance that is interpreted as typical of a target organism

3.1.2.3
confirmed count
presumptive count multiplied by the confirmation coefficient

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

© ISO 2014 – All rights reserved

3.2 Symbols and abbreviated terms

A	(symbol for the idea of) trial method
a	test result by method A
a_i	test result (confirmed count) of method A in sample i
B	(symbol for the idea of) reference method
b	test result by method B
b_i	test result (confirmed count) of method B in sample i
i	subscript indicating a series
k	coverage factor used for calculating the confidence interval
L	smallest microbiologically significant mean relative difference between the results by methods A and B
MPN	most probable number quantitative method
n	number of samples
s	experimental standard deviation of the relative difference (standard uncertainty)
s^2	experimental variance
$s_{\bar{x}}$	standard deviation of the relative difference (standard uncertainty) of the mean
W	half-width of the confidence interval
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 lower confidence limit, derived by subtracting the value of the half-width of the confidence interval from the mean
x_U	value of the relative difference at the upper confidence limit, derived by adding the value of the half-width of the confidence interval to the mean
X^2	experimental Poisson index of dispersion
y	conditional variable used in computing the number of samples for comparison testing

iTeh STANDARD PREVIEW
(standards.iteh.ai)
ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbcee0d4-dee0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

4 Principle

This International Standard is based on the principle of the paired t-test (see Annex C).

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 “not different” if the mean relative difference of the paired confirmed counts does not differ significantly from zero and the

© ISO 2014 – All rights reserved

confidence interval does not extend beyond the level of the predetermined stipulated limit. The decision rules based on the above principle are given in 7.2 and 7.3 and a flow chart is given in Annex A.

5 Basic requirements for a comparison study

5.1 General

Both methods shall have data on detailed performance characteristics, derived in accordance with the guidance outlined in ISO/TR 13843.

The most important basic requirement of comparison studies is a wide range of samples. Participation by a number of laboratories is preferable, allowing the expansion of the sample range over large geographical areas. Also the credibility of a general conclusion is commonly believed to require the participation of several laboratories. However, the inclusion of a wide range of sample types by a single laboratory will also be valid. The result of the comparison is generally valid only within the range of sample types studied. Advice on the conduct of comparison studies is given 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 Description of methods

It is important to note that the principles of operation of the two methods being compared should be well understood and that the significance of any differences in the methods on the outcome of the comparative assessment should be recognized. This is particularly important if the confirmed results from each method are based on different principles. Any differences should be detailed in the test report (see Clause 8).

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Performance characteristics data shall be derived in accordance with ISO/TR 13843. Such data for the methods shall be compared in order to assess potential differences in performance.

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbccd5b1-dea0-4e53-a2de-6ad69cd5236a/iso-17994-2014

EXAMPLE Methods for the enumeration of coliform bacteria based on possession of the enzyme β-galactosidase have been shown to produce higher counts than those based on the fermentation of lactose due to the detection of a greater range of coliform bacteria.

5.3 Types of samples

The requirements for method comparisons differ somewhat from the daily routine situation. It is useful and often necessary to preselect or prepare special samples. Samples for method comparisons shall contain enough target organisms that the likelihood of scoring a zero count is small.

Samples for method comparisons shall represent types that are included in the scope of both methods. Natural samples are ideal. Samples to be tested shall represent those water source types relevant to the geographical and environmental area where the method is applied. The water types to be tested shall be included to the scope of the methods under evaluation. 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 shall be considered the last resort.

To avoid the inhibition of the target organisms by other organisms, ensure that the concentration of total bacteria in a sample is not too high. Consult ISO 8199 to ascertain the ranges of the colony counts for different cultivation methods.

It can be appropriate to influence the microbial population of existing samples to simulate situations encountered in routine laboratory practice. Such modifications could be the applications of disinfectants (e.g. chlorine, ozone or UV, Reference [1]), different ranges of temperature or the influence of daylight, in order to simulate different environmental situations from where the samples for laboratories can originate.

 © ISO 2014 – All rights reserved

5.4 Number of samples and participating laboratories

5.4.1 Number of laboratories

The number of laboratories participating in comparison trials shall be sufficient to obtain a representative result for the relative recovery of the two methods being tested. Factors that shall be considered when deciding on the number of participating laboratories in a comparison trial include:

a) whether the alternative method is being assessed as a replacement for the reference method;

b) whether the comparison trials are for statutory or verification purposes;

c) the need to cover the range of geographical areas and water types for which the alternative method may be used;

d) the need to consider seasonal variability in occurrence of the target organisms;

e) the number of test results needed for the assessment of relative recovery;

f) the number of laboratories with sufficient capacity and technical expertise available to participate.

It can be acceptable to have a limited number of participating laboratories analysing a wide range of water types rather than a higher number of laboratories analysing a narrower range of water types appropriate for the methods being compared.

NOTE Several successful comparisons have been achieved with three to six laboratories. In theory, it is possible that one laboratory is able to conduct a suitable comparison study provided they have access to a wide enough range of sample types for which the methods have been characterized.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

5.4.2 Number of samples

ISO 17994:2014

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 "predetermined stipulated limit" and the half-width of the confidence interval. If the data are found inadequate for deciding that the methods are either "different" or "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 relative difference that corresponds to the predetermined stipulated limit chosen at the beginning of the trial.

© ISO 2014 – All rights reserved

The total number of samples, n, for a two-sided evaluation (7.2) that would be sufficient for the detection of a given average relative difference at about 95 % confidence depends on the experimental variance according to Formula (1):

$$n = \frac{4s^2}{L^2} \tag{1}$$

where

n is the number of samples required for the detection of a difference L;

L is the smallest microbiologically significant mean relative difference;

s is the experimental standard deviation.

EXAMPLE A rather frequently observed value for the experimental standard deviation of the relative difference is approximately s = 80. In order to detect an average relative difference of 10 % (L = 10 %), n = 25 600/100 = 256 samples is expected to be sufficient for a two-sided evaluation.

For a one-sided evaluation (7.3) the corresponding number of samples can be calculated according to Formula (2):

$$n = \frac{3s^2}{L^2} \tag{2}$$

iTeh STANDARD PREVIEW
(standards.iteh.ai)

The rationale for the derivation of Formulae (1) and (2) is presented in Annex C.

High variability of counts can be experienced due to irregular behaviour of either laboratories or the range of sample types analysed. Whether it is warranted to continue a comparison of methods study can be ascertained by an examination of the standard deviation of the mean relative difference. Valid comparisons are indicated by standard deviations of less than 100.

ISO 17994:2014
https://standards.iteh.ai/catalog/standards/sist/fbceeb1d4-dee0-4c53-a2cb-6ad69cd5236a/iso-17994-2014

NOTE With some recent methods based on chromogenic substrates, it is possible to estimate two bacterial groups simultaneously. One of the groups can be 10 or more times as numerous as the other. It is possible that the number of samples sufficient for making a final decision of equivalence with the more numerous type is not sufficient for an organism present in low numbers.

 © ISO 2014 – All rights reserved