
Water quality — Guidance on validation of microbiological methods

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



PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

This document is a preview generated by EVS

© ISO 2000

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing 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 22 734 10 79
E-mail copyright@iso.ch
Web www.iso.ch

Printed in Switzerland

Contents

Page

Foreword.....	iv
1 Scope	1
2 Terms and definitions	1
3 Arrangement of the document	8
4 Basic concepts.....	8
4.1 General.....	8
4.2 Validation	8
4.3 Detectors	11
4.4 Performance characteristics	11
4.5 Specifications.....	11
5 Limitations and characteristic features of microbiological methods	12
5.1 Recovery of the analyte	12
5.2 Sample variance.....	12
5.3 Particle distribution and overdispersion.....	12
5.4 Interactions in the detector.....	12
5.5 Robustness	13
5.6 Spurious errors	13
5.7 Control and guidance charts	13
6 Mathematical models of variation	14
6.1 Unavoidable basic variation — The Poisson distribution	14
6.2 Overdispersion — The negative binomial model	17
6.3 Statistical and practical limits	20
6.4 General tests for randomness — Detection of overdispersion	21
7 Specifications — Current practice	21
8 Specifications — Recommended approach.....	22
9 Determination and expression of performance characteristics	23
9.1 General.....	23
9.2 Categorical characteristics related to specificity and selectivity.....	23
9.3 Working limits	24
9.4 Working range of MPN procedures.....	25
9.5 Precision	25
10 Procedures and steps of validation	26
10.1 General.....	26
10.2 Primary validation.....	26
10.3 Secondary validation.....	28
11 Designs for determining specifications	28
11.1 A general model for basic quantitative specifications	28
11.2 Precision of the entire analytical procedure.....	29
11.3 Categorical characteristics.....	29
11.4 Unplanned data	29
Annex A Statistical procedures and computer programs	30
Annex B Numerical examples	34
Annex C Example of a validation experiment.....	45
Bibliography	46

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