

BRITISH STANDARD

Water quality – Enumeration of micro-organisms in water samples – Guidance on the estimation of variation of results with particular reference to the contribution of uncertainty of measurement

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Foreword

Publishing information

This British Standard was published by BSI and came into effect on 31 January 2007. It was prepared by Subcommittee EH/3/4, *Microbiological methods*, under the authority of Technical Committee EH/3, *Water quality*. A list of organizations represented on this committee can be obtained on request to its secretary.

Supersession

This British Standard supersedes DD 260:2003, which is withdrawn.

Relationship with other publications

This British Standard gives guidance on the interpretation of the requirements in BS EN ISO/IEC 17025:2005 for estimating uncertainty of measurement, in the context of microbial counts in water samples. It is intended to form an adjunct to BS ISO 8199 which gives guidance on the enumeration of micro-organisms in water samples.

Use of this document

As a guide, this British Standard takes the form of guidance and recommendations. It should not be quoted as if it were a specification and particular care should be taken to ensure that claims of compliance are not misleading.

Any user claiming compliance with this British Standard is expected to be able to justify any course of action that deviates from its recommendations.

Presentational conventions

The provisions in this standard are presented in roman (i.e. upright) type. Its recommendations are expressed in sentences in which the principal auxiliary verb is “should”.

Commentary, explanation and general informative material is presented in smaller italic type, and does not constitute a normative element.

Contractual and legal considerations

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

Introduction

BS EN ISO/IEC 17025:2005, 5.4.6.2 specifies that “Testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement”. This is difficult to apply to water microbiology because the distribution and behaviour of microbial cells in water is not uniform. The purpose of the present document is to provide practical guidance on how to interpret and implement these requirements within the context of a water microbiology laboratory. It is intended to form an adjunct to BS ISO 8199:2005.

1 Scope

This British Standard gives guidance on interpretation of the requirements specified in BS EN ISO/IEC 17025:2005 with regard to the uncertainty of measurement in microbial counts in water samples. It gives guidance on the estimation of variation in results between replicate sub-samples, which will include that due to uncertainty of measurement, in order to assess whether the variation due to uncertainty of measurement has been controlled in accordance with the requirements specified in BS EN ISO/IEC 17025:2005. It is applicable to examination of sub-samples as part of a quality assurance system. It is applicable to samples of all types of water.

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.

BS EN ISO/IEC 17025:2005, *General requirements for the competence of testing and calibration laboratories*

DD ENV ISO/TR 13843:2001, *Water quality – Guidance on validation of microbiological methods*

3 Terms and definitions

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

3.1 accuracy

sum of trueness plus precision

NOTE This equates to the degree of conformity between the result of a measurement and the true value of the measurand.

3.2 bias

systematic errors associated with the methods used

NOTE These errors include those due to dilution, population selection, and calibration.

3.3 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.

[DD ENV ISO/TR 13843:2001, 2.25]

3.4 imprecision

random errors incurred in applying the methods together with random variation in the test material

3.5 limit of detection

lowest number of micro-organisms that need to be present in the test portion of water examined for their presence to be detected by a particular microbiological test

NOTE Limit of detection should not be confused with limit of determination which is the lowest average number of micro-organisms in a body of water that would result in a 95% probability of giving a positive result when a representative test portion is examined by a particular microbiological test.

3.6 measurand

specific quantity that is subjected to measurement

3.7 repeatability

closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions of measurement within a short period of time

NOTE 1 Modified from International vocabulary of basic and general terms in metrology, 1993 [1].

NOTE 2 Conditions of measurement include, the method of analysis; the analyst; the measurement instrument and the conditions under which it is used; and the location.

NOTE 3 Within a short period of time is normally considered to be within one hour.

3.8 reproducibility

closeness of the agreement between the results of measurements of the same measurand carried out under different conditions of measurement

NOTE 1 Modified from International vocabulary of basic and general terms in metrology, 1993 [1].

NOTE 2 Conditions of measurement include, the method of analysis; the analyst; the measurement instrument and the conditions under which it is used; the location; and the time.

3.9 reference culture

culture of a micro-organism obtained from a recognized national culture collection

3.10 reference material

material containing a defined population of micro-organisms from one or more reference cultures in numbers expected to fall within a defined range for use in the assessment of a measurement method

3.11 certified reference material

reference material accompanied by a certificate stating the expected microbial count with an associated confidence interval

3.12 uncertainty of measurement**UM**

parameter associated with the result of a measurement, that characterizes the dispersion of the values that can reasonably be attributed to the measurand [*Guide to the expression of uncertainty in measurement*, 1995 [2]]

3.13 validation

process providing evidence that a method is capable of serving its intended purpose in detecting or quantifying a specific microbe or microbial group with the required level of precision and accuracy

NOTE Modified from DD ENV ISO/TR 13843:2001, 4.2.1.

3.14 secondary validation

process providing evidence that a method validated elsewhere performs within the user's laboratory according to the specifications determined in the original validation

NOTE 1 Also known as verification.

NOTE 2 Modified from DD ENV ISO/TR 13843:2001, 2.38 and 4.2.3.

3.15 sample

volume of water collected from the body of water under investigation

3.16 sub-sample

volume of water taken from a sample

3.17 test portion

volume of water examined in a particular test

NOTE The test portion may comprise the whole sample or a sub-sample.

4 General

4.1 Significance of requirements specified in BS EN ISO/IEC 17025

BS EN ISO/IEC 17025:2005, 5.4.6.2 specifies:

“Testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement. In certain cases the nature of the test method may preclude rigorous, metrologically and statistically valid, calculation of uncertainty of measurement. In these cases the laboratory shall at least attempt to identify all the components of uncertainty and make a reasonable estimation, and shall ensure that the form of reporting of the result does not give a wrong impression of the uncertainty. Reasonable estimation shall be based on knowledge of the performance of the method and on the measurement scope and shall make use of, for example, previous experience and validation data.”

The document EA-04/10 *Accreditation for microbiological laboratories* [3], produced by the joint EA/EURACHEM Working Group, supplements BS EN ISO/IEC 17025 and states the following in paragraphs 5.2 to 5.4.

NOTE Points of particular importance to the present British Standard are indicated in bold type.

“5.2 Microbiological tests generally come into the category of those precluding rigorous, metrologically and statistically valid calculation of uncertainty of measurement. It is generally appropriate to base the estimation of uncertainty on repeatability and reproducibility data alone, but ideally including bias (e.g. from proficiency testing results). The individual components of uncertainty should be identified and demonstrated to be under control and their contribution to the variability of results evaluated. Some components (e.g. pipetting, weighing and dilution effects) may be readily measured and easily evaluated to demonstrate a negligible contribution to overall uncertainty. Other components (e.g. sample stability and sample preparation) cannot be measured directly and their contribution cannot be evaluated in a statistical manner but their importance to the variability of results should be considered also.

5.3 It is expected that accredited microbiological testing laboratories will have an understanding of the distributions of organisms within the matrices they test and take this into account when sub-sampling. However, **it is not recommended that this component of uncertainty is included in estimates** unless the client's needs dictate otherwise. The principal reasons for this are that uncertainty due to distribution of organisms within the product matrix is not a function of the laboratory's performance and may be unique to individual samples tested and because test methods should specify the sample size to be used taking into account poor homogeneity.

5.4 The concept of uncertainty cannot be applied directly to qualitative test results such as those from detection tests or the determination of attributes for identification. Nevertheless, individual sources of variability, e.g. consistency of reagent performance and analyst interpretation, should be identified and demonstrated to be under control. Additionally, for tests where the limit of detection is an important indication of suitability, the uncertainty associated with the inocula used to determine the limit should be estimated and its significance evaluated. Laboratories should also be aware of the incidence of false positive and false negative results associated with the qualitative tests they use.”

Theoretically, the uncertainty of measurement (UM) associated with a test result relates to the measurement made on the test portion examined and is distinct from any uncertainty associated with the sampling process and/or the selection of the test portion. This is an important distinction where the material being sampled is water and the measurement is of numbers of relevant micro-organisms in the test portion.