BS 8496:2007

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

ICS 03.120.30; 07.100.20

Publishing and copyright information

ISBN 978 0 580 49930 2

The following BSI references relate to the work on this standard:
Committee reference EH/3/4

Publication history

First published January 2007

Amendments issued since publication

Amd. no.	Date	Text affected

Contents

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, pages 1 to 19 and a back cover.

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This British Standard supersedes DD 260:2003, which is withdrawn.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

An estimate of UM, therefore, is meant to demonstrate whether the laboratory uses methods and equipment which ensure a high degree of accuracy, or whether the result is more approximate. The United Kingdom Accreditation Service recommends that any further uncertainty that results from the test sample not being fully representative of the whole should normally be identified separately [4].

4.2 Comparison of estimates of uncertainty of measurement in chemical analyses and microbiological counts

Methods for estimation of UM have been successfully introduced by chemistry laboratories but these do not readily transfer to microbiology because of the assumptions that have to be made regarding homogeneity of the test material. The estimates of UM obtained in chemistry usually involve replicate analyses of sub-samples. In microbiology replicate counts on sub-samples overwhelmingly estimate the natural variation in numbers of organisms present in the different sub-samples, and are also affected by the different characteristics or physiological states of the organisms being counted.

In chemistry laboratories examining water samples the uncertainty of a measured result is considered to be "the interval on the measurement scale within which the true value lies with a high probability, when all sources of error have been taken into account" [5]. The true value applies to the test portion. An estimate of the UM thus reflects the accuracy of the result where accuracy is considered to be the sum of bias plus imprecision.

In practice, an estimate of the UM concentrates on precision and might be based on special studies. It would not be practical to make re-evaluations for each routine sample. Any bias arising from systematic errors is expected to have been minimized by quality assurance checks, which usually include participation in a proficiency testing or external quality assessment scheme.

For estimating precision, and thus for estimating UM in chemistry, attention is paid to each piece of equipment used (some of which will come with a calibration certificate) and each procedure followed. As each stage is considered the contributions to uncertainty may need to be accumulated. Usually the main sources of uncertainty are estimated from repeatability studies which use replicate testing of sub-samples from the relevant sample.

The level of precision estimated from repeatability studies includes the following:

a) variation due to laboratory inaccuracies or inadequacies, together with random errors (see Note); and

b) variation in the test material between the sub-samples used for the replicates.

In chemistry, the latter variation can be expected to be small because the analyte can be expected to be homogeneously distributed in the sample at the detection levels attained in analytical chemistry. Random variation in the distribution of molecules is likely to be below the level of detection [6].

NOTE There needs to be a clear distinction between "random error" encountered at all stages of the procedures and "random variation" in the density of a solution of a chemical or of a suspension of micro-organisms. Random errors are small imprecisions due to minor variations in environmental conditions, and variation in the reading of instruments or the counting of colonies. These are all included in the repeatability estimates.

In microbiology, the first question that needs addressing is what is meant by the true value. The micro-organisms in a well mixed sample are distributed at random, rather than uniformly, and there could be additional variation in distribution due to organism behaviour such as repulsion and attraction between different strains of the species of interest and between these and background organisms. Figure 1 illustrates a hypothetical sample of water containing 30 micro-organisms which has been optimally mixed to achieve a random distribution. When the sample is divided into 10 equal sized sub-samples the numbers of micro-organisms present in the different sub-samples range from zero to seven. This illustrates the fact that a perfect laboratory, detecting every micro-organism in sub-samples from a perfect sample in which all the micro-organisms remain viable, would not achieve repeatable results. Even if the laboratory were not performing perfectly it is likely that replicate results from a series of sub-samples would give an estimate of repeatability which is overwhelmingly influenced by actual variation in the test material. Any impact of laboratory bias would be hidden. This applies especially with the low counts encountered when testing drinking waters [7].

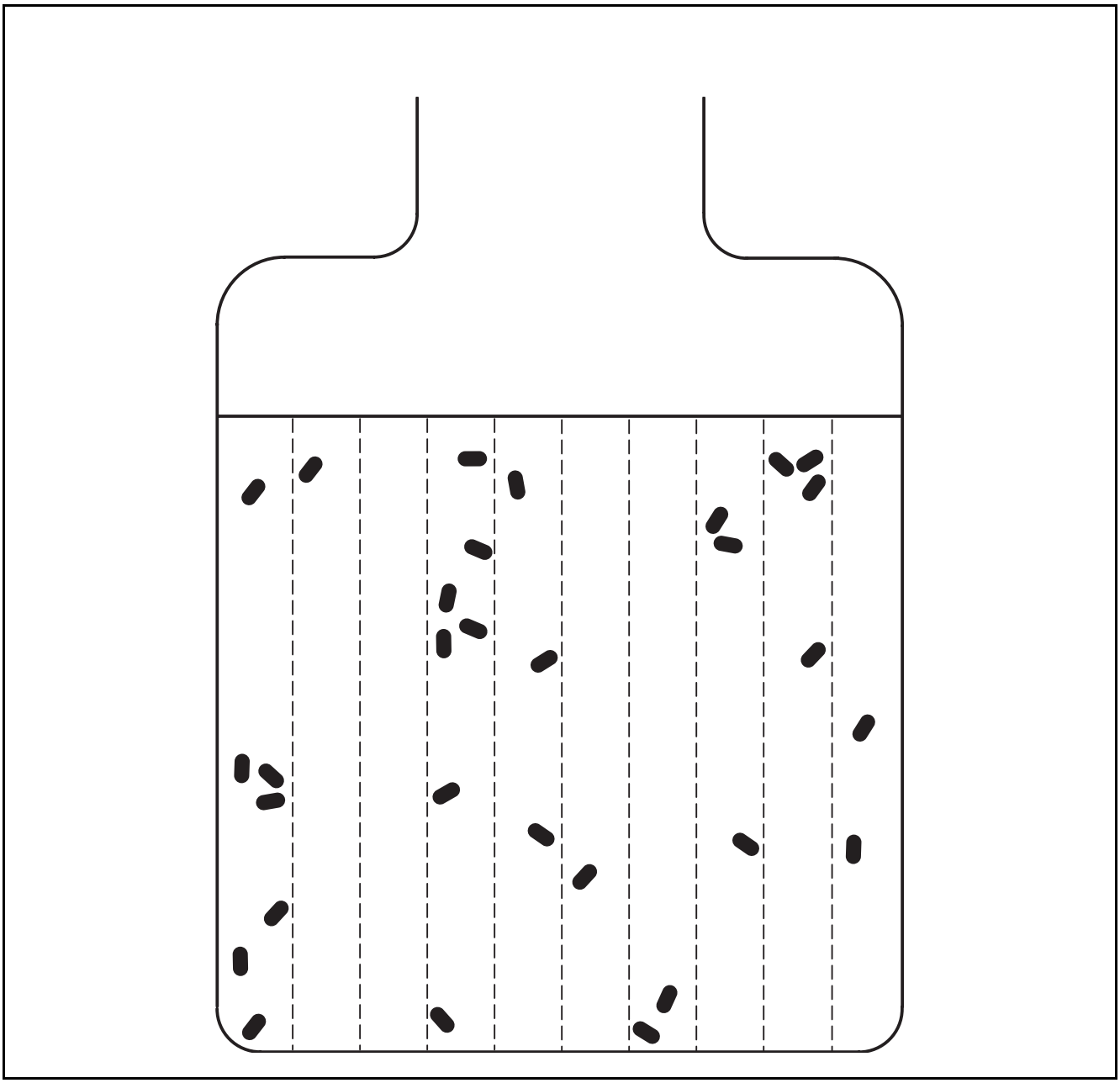
Additionally, not all microbial cells, even at a species level, behave the same within a sample, as they might be at differing stages of growth, stress response and metabolic activity at the time the analysis starts. This will impact on the ability of the cells to grow on, or in, a particular isolation medium. In contrast, it can be assumed that all the molecules of a chemical analyte would behave the same during analysis.

It is also important to note that in microbiological analyses any one test portion can only be tested once, and the micro-organisms in another test portion from the same sample cannot be expected to be in the same condition after a period of storage. Thus it is not possible to return to a sample to perform a repeat analysis and get an equivalent result.

Thus in microbiology the true value refers only to the number of micro-organisms in the test portion at the time of examination, in contrast to chemistry where it refers to the concentration of the analyte in the entire sample. This contrast between chemistry and microbiology is described more fully, together with formulae for the sources of variation, in Tillett and Lightfoot [6].

Figure 1 **Illustration of hypothetical water sample containing 30 micro-organisms distributed at random throughout 10 equal sized sub-samples**

The number per sub-sample ranges from 0 to 7



5 Procedure

5.1 Identifying and minimizing components of uncertainty of measurement

The basic test method to be used should be chosen for its ability to select for, and detect, the relevant micro-organisms, with the required level of specificity and sensitivity. A method should be validated when it is first developed and its performance verified when it is introduced into a particular laboratory. These data can be useful for precision estimates.

The technical procedures such as mixing the sample, drawing off a sub-sample, counting colonies or judging positive reactions should be performed by trained staff and should be monitored as part of the laboratory's quality assurance (QA) programme.

It is essential that materials and equipment used should be certified and/or calibrated wherever possible and their performance should be checked as part of the laboratory's QA programme.

Significant inaccuracy (significant in the microbiological or public health sense) due to bias in any of the above should be detected by the QA programme, particularly by participation in external quality assessment (EQA) or proficiency testing schemes or by use of certified reference material. Inaccuracy due to imprecision (such as random errors caused by environmental factors or fluctuations in material quality, or inconsistency, for example inconsistent performance by analysts) will be part of the variation measured by repeatability studies, using counts from replicate sub-samples. However, as stated before, these repeatability measurements will also include the natural variation in numbers of micro-organisms between sub-samples and this is likely to mask any imprecision generated by the laboratory's procedures.

5.2 Approaches to estimation of variation

The best approach to estimation of variation, which will include that due to UM, is to accumulate evidence from replicate counts. These data can be expected to show random variation in counts [or variation in most probable numbers (MPNs) of a magnitude related to the number of tubes as well as to random variation, see **5.6**]. If there is greater variation than this (i.e. over-dispersion) then this might be attributable to organism behaviour or it might indicate excessive imprecision. If a laboratory is finding such over-dispersion then it should record or log this observation, record details of, and review, the nature of the samples involved and check its procedures (see **5.7**). If the data consistently show just random variation (i.e. no over-dispersion) then the laboratory statement on impact of UM can record this and say that significant imprecision is not apparent (see **5.8**).

5.3 Providing suitable data

These will come from preliminary work (development and introduction of a method) and from on-going quality assurance procedures. The development and introduction of a basic method can be divided into four stages as follows.

a) *Stage 1*. When the basic method is developed, validation data should be produced which demonstrate repeatability, in accordance with DD ENV ISO/TR 13843:2001. If these data are published then the findings should be kept on file to provide a baseline against which precision estimates for using this method in the particular laboratory can be compared.

b) *Stage 2*. The basic method should then be compared with a standard or reference method. This work may have been done exclusively by other laboratories. Examples are given in *The microbiology of drinking water Part 3*, 2002 [8] and in BS EN ISO 17994:2004. Repeatability data should be obtained from paired results from samples split between the two methods. It is essential that the study design includes samples from a variety of sources representative of those tested by the laboratory, but it may include replicate sub-samples, in which case data could be extracted on the within-method repeatability. These repeatability data should be collected and recorded for use in a participating laboratory or made available to other participating laboratories wishing to introduce the method.

c) *Stage 3*. When the basic method is introduced into a particular laboratory not involved in Stage 2 then it should be verified that the method works in that laboratory and with their routine samples. This is the stage at which gathering of repeatability data relevant to this laboratory's assessment of any detectable impact of UM should start (see **5.5** and the examples given in Annex A).

d) *Stage 4*. Periodic analyses of variation within replicate sub-samples should be carried out and the results should be recorded with the information from Stages 1 to 3. The data for this should be obtained from the laboratory's on-going routine quality control checks with samples split into at least two sub-samples (for counting methods) or more (for MPN methods; see **5.6**).

It is a challenge in water microbiology to select and prepare suitable and typical samples. If a laboratory worked only with a certified reference material then this strain of the relevant organism might give an atypical estimate of repeatability. If another laboratory used natural waters (with a spectrum of target organism strains in varying conditions of viability and all interacting with background organisms) then greater variation might appear in the results. The recommendations for method comparison are for water samples from a representative range of sources and levels of microbial load to be used to illustrate "real life". Microbiology deals with living organisms, with all the inherent variability associated with biological systems. It is essential that a sample of sufficient size to provide all the necessary sub-samples is prepared and used immediately. It is not possible to go back to a stored sample and expect to obtain comparable repeat sub-samples. Microbiological water quality monitoring involves selecting and

identifying target organisms which include a large variety of strains or, as in the case of the coliform bacteria, a range of species and strains, potentially in a range of differing metabolic or stressed states.

5.4 Recommended samples

Data for estimation of variation, which includes UM, should be obtained separately for each type of water to be tested, e.g. potable, bathing, pool, cooling and surface waters. This is because of differences in chemical properties and background organisms associated with each type of water. Guidance on the preparation of suitable samples for testing methods for potable water are given in *The microbiology of drinking water Part 3*, 2002 [8]. Similar procedures can be derived for other types of water samples. If a laboratory does not have suitable natural samples for replicate analyses for use at Stage 4, appropriate samples should be generated by spiking dechlorinated tap water with surface water or wastewater effluent.

5.5 Statistics for counts

For Stage 3 [see **5.3**c)] there should be a minimum of 30 test results, as recommended in *The microbiology of drinking water Part 3*, 2002 [8]. The samples used should be representative of the differing water sources/types typically analysed by the laboratory. If there is a large range of sources/types analysed this should be reflected in the number and type of samples analysed. In practice, 10 replicate sub-samples per sample from a single source gives useful information on variation. A minimum of 3 sources should be used, more for a large laboratory with a wide catchment area examining a wide range of sample types. In the example given in **A.1** five samples, each from a different source, are used and 10 replicate sub-samples studied per sample, giving a total of 50 test results. An index of dispersion should be calculated for each water source/type and tested for excess over a Poisson distribution, as illustrated in **A.1**. A Poisson distribution would be obtained if there were a fully random distribution of particles in a perfectly mixed suspension. If the index of dispersion is not in excess of a Poisson distribution then it can be said that there is no excess variation due to imprecision seen in n samples and therefore no detectable impact of UM. This may then be recorded in connection with the results of the method.

Ongoing checks should be carried out to ensure that the statement of "no detectable impact of UM" still holds. This can be done utilizing the results from the regular analyses of duplicate sub-samples from environmental, or laboratory generated, samples that are typically performed by laboratories undertaking water analyses as part of their QA programme, following the approach recommended in *The microbiology of drinking water Part 3*, 2002 [8]. Data from these duplicate sub-samples can be collated and used to compile an index of dispersion at regular intervals (e.g. every month, or when 20 to 30 pairs of duplicate sub-sample results have accumulated, if fewer than 30 pairs of sub-samples are examined each month). An example of the use of duplicate sub-sample data for the assessment of any detectable impact of UM is given in **A.2**.

5.6 Statistics for MPNs

An estimate of the most likely number of organisms present in the water examined is calculated based on the numbers of positive and negative reactions in the tubes/wells used (see *The microbiology of drinking water Part 3*, 2002 [8]). The confidence interval relating to this estimate from the test portion of water can also be calculated, but if this is to be reported then it should be explained that this relates only to the result and does not reflect variation in counts at the water source. It is however a reflection that the method carries an extra factor of uncertainty not applicable to counting methods.

Repeatability studies are nevertheless relevant to estimates of UM in MPN methods. The observed dispersion between replicate MPNs will have a different pattern from that between replicate counts because there are numerical intervals between the possible MPNs, especially if the number of tubes in the series examined is small. Information from Stages 1 and 2 may indicate an optimum baseline for variation. The particular laboratory's Stage 3 data can be used to give baselines for dispersion estimates for typical samples and ranges of counts dealt with in this laboratory. Continued replicate sample studies as part of QA checks should be used to provide similar on-going monitoring of any detectable impact of UM. Split-pairs may not provide enough information and larger numbers of replicates should be considered or more frequent split-pairs.

The statistics used will need careful planning. There is no general solution that can be recommended because the statistics depend on the numbers of tubes/wells used. A laboratory working with an MPN method may need to consult a statistician.

An approach covering currently used MPN methods is outlined here.

Samples should not be used which would result in the majority of tubes/wells being positive, so as to avoid MPNs in ranges where the intervals between MPN values will be large.

a) If the number of tubes/wells is large (greater than 50) and the replicates give, on average, half or fewer of the tubes with a positive reaction, then the numerical intervals between possible MPNs will be small. In this case the variation can be expected to be approximately Poisson and the indices of dispersion recommended for counts can be applied.

b) If the number of tubes is moderate (21 to 50) then the observed variance , s^2, from replicates should be recorded and compared. Variation can increase as the mean MPN increases and it might be easier to record and compare coefficients of variation (also known as relative standard deviation). The coefficient of variation, v, is given by the formula: $v = s/\bar{x}$ where s is the standard deviation and $\bar{x}$ is the mean MPN.

Formal statistical comparisons of observed values of s^2 pose problems. The traditional methods, such as the F test to compare the ratio of two variations, are not suitable because they are sensitive to non-normally-distributed data. MPN data are discrete numbers with potentially large intervals which often makes these statistical tests unsuitable, even if the MPNs are transformed onto a logarithmic or square root scale to make them symmetrical. Sequential plots of v or s^2 should be monitored for stability and non-parametric tests should be used to test the null hypothesis of no increase in variation.

c) If the number of tubes is small (11 to 20) then there will be large numerical intervals between possible MPNs and therefore a great deal of inherent imprecision in the method rendering it semi-quantitative. The uncertainty due to variations in numbers of organisms present in sub-samples of the test material, together with imprecision inherent in the MPN method is likely to exceed any uncertainty of measurement due to laboratory performance. The laboratory statement on impact of UM should state this.

5.7 Action if significant impact of UM is detected

If a significant impact of UM is detected at Stage 3 this means that the application of the method in the laboratory concerned is unsatisfactory. Investigations of the causes, remedial action and reassessment is necessary before the method is adopted for routine use.

If a significant impact of UM is detected at Stage 4 this means that a problem has developed with the application of the method. Immediate investigation and remedial action is essential. For any results that have already been reported, the recipient needs to be informed of the potential impact on the reliability of the reported results.

5.8 Laboratory statement on impact of UM

Each laboratory should have a documented statement on their assessment of any detectable impact of UM for each method, which can be used to demonstrate that the methods are under analytical control within the constraints inherent in water microbiology (see **4.2**). This statement should be kept on file and made available if requested. It should include the following information:

- full description of the relevant method and context (e.g. *E. coli* counts from drinking water samples) including recovery, sensitivity, specificity and limit of detection;
- literature or records of typical repeatability recorded with this method;
- cross-reference to QA procedures in this laboratory and data relating to assessment of any laboratory bias;
- list of critical components such as measuring volume and making dilutions, and reasons why their contribution is minimal (or has been studied and shown to be negligible) and how it is controlled routinely in the laboratory QA programme;

- performance in external quality assurance for the method confirming lack of significant laboratory bias;
- reports of replicate studies to demonstrate repeatability, and also, in a larger laboratory, reproducibility between analysts and equipment.

The following is an example of a suggested format.

*NOTE The data used in the example come from the worked examples in **A.1** and **A.2**.*

	Dates and samples	**Summary and comments**
Stage 3	June 2002 50 test results comprising 10 replicate sub-samples from 5 samples representing 5 individual sources. Data collected to verify the performance of the method to be introduced	5 index of dispersion tests D^2 = 6.0, 6.6, 15.9, 8.9, 13.9 (Degrees of freedom = 9) None of these is significant at the 5% level. Conclusion: no variation demonstrated above natural random variation.
Stage 4	October 2002 31 pairs of results from duplicate sub-samples from routine samples used in QA	Index of dispersion D^2 = 20.1 (Degrees of freedom = 31) $p > 0.5$ Conclusion: no variation demonstrated above natural random variation.
	November 2002 30 pairs of results from duplicate sub-samples from routine samples used in QA	Index of dispersion D^2 = 11.7 (Degrees of freedom = 30) $p > 0.5$ Conclusion: no variation demonstrated above natural random variation.

5.9 Statement on laboratory reports

Laboratory reports should include a statement to indicate that data on the performance of the method used, which includes information about the variability associated with the reported result, is available upon request.

Annex A (informative) Worked examples

A.1 Analysis of replicate counts from different water sources from secondary validation studies

NOTE In this example real data are used from one laboratory which introduced a new method for testing potable water and compared its performance against a reference method using the procedures given in The microbiology of drinking water Part 3, *2002 [8]. The samples were derived from material from four river sites and one sewerage works. The counts are the actual numbers of colonies on the plates, not corrected for any dilutions.*

A.1.1 The data from ten replicate sub-samples from a sample from each of 5 sources were used to study variation between replicates using the new method to determine whether the impact of UM was negligible in that it was obscured by natural random variation.

The results of the replicate counts are shown in Table A.1.

A.1.2 The index of dispersion, D^2, tests the null hypothesis that the observed variation within each set of 10 replicate counts is distributed at random. In this case D^2 is distributed approximately as a chi-squared variable with $(n - 1)$ degrees of freedom. The value of D^2 is given by the following equation:

$$D^2 = \sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{\bar{x}}$$

for n replicate counts $x_1, x_2 \ldots\ldots x_n$ with a mean of $\bar{x}$.

Degrees of freedom = $n - 1$.

Table A.1 **Counts on 10 replicate sub-samples from a sample from each of 5 water sources**

	Sample from source 1	Sample from source 2	Sample from source 3	Sample from source 4	Sample from source 5
	46	13	38	48	61
	41	14	40	41	62
	47	6	35	51	72
	47	14	38	50	84
	48	10	44	53	85
	42	9	51	56	82
	37	10	32	65	69
	45	14	36	56	63
	32	10	30	64	74
	38	8	57	59	91
Column sum	423	108	401	543	743
Mean, $\bar{x}$	42.3	10.8	40.1	54.3	74.3
Index of dispersion, D^2	6.0	6.6	15.9	8.9	13.9

A.1.3 As an example, using the equation given in **A.1.2**, the value of D^2 for the first sample in Table A.1 was calculated as follows:

$$D^2 = \frac{(46-42.3)^2}{42.3} + \frac{(41-42.3)^2}{42.3} + \frac{(47-42.3)^2}{42.3} + \frac{(47-42.3)^2}{42.3} + \frac{(48-42.3)^2}{42.3} + \frac{(42-42.3)^2}{42.3} + \frac{(37-42.3)^2}{42.3} + \frac{(45-42.3)^2}{42.3} + \frac{(32-42.3)^2}{42.3} + \frac{(38-42.3)^2}{42.3}$$

$$= 0.32 + 0.04 + 0.52 + 0.52 + 0.77 + 0.09 + 0.66 + 0.17 + 2.51 + 0.44$$

$$= 6.04$$

which, rounded to one decimal place, gives $D^2 = 6.0$

A.1.4 The value of D^2 for each of the five sources is given in Table A.1. From standard chi-squared tables (see Note), for 9 degrees of freedom, a value of D^2 of 16.9 or above is significant at the 5% level. Thus, a D^2 value of 16.9 or above would indicate significant excess variation at the 5% probability level, which would mean that the null hypothesis would be rejected. As the value of D^2 for each of the five sources is below 16.9 it can be concluded that the uncertainty of measurement (UM) was not large enough to be detected in any of the five sets of ten replicates.

NOTE Standard statistical tables are available on the internet.

A.2 Analysis of counts from routine quality assurance duplicate sub-samples

A.2.1 When the new method was introduced for routine work, as part of the QA programme the laboratory examined duplicate sub-samples each day the analytical method was used (see *The microbiology of drinking water Part 3*, 2002 [8]). These data were accumulated monthly and analysed to determine whether variation between replicates exceeded that expected from random variation in organism numbers in sub-samples. Pairs of replicate counts from the first two months are shown in Table A.2.

A.2.2 The index of dispersion, D^2, tests the null hypothesis that the variation within pairs is random and that no excess that might be due to UM is significant. In this case D^2 (within pairs) is distributed approximately as a chi-squared variable with n degrees of freedom. The value of $D^2_{(\text{pairs})}$ is given by the following equation:

$$D^2_{(\text{pairs})} = \sum_{i=1}^{n} \frac{(x_{i1} - x_{i2})^2}{(x_{i1} + x_{i2})}$$

for n pairs of results x_{11}, x_{12}; x_{21}, x_{22}…….x_{n1}, x_{n2}.

Degrees of freedom = n.

Table A.2 **Replicate counts on daily duplicate sub-samples**

Month 1			
Replicate 1	**Replicate 2**	$x_1 - x_2$	$x_1 + x_2$
17	15	2	32
14	12	2	26
12	8	4	20
16	14	2	30
68	58	10	126
73	62	11	135
10	8	2	18
26	22	4	48
26	24	2	50
26	19	7	45
17	21	–4	38
32	63	–31	95
22	20	2	42
11	15	–4	26
19	18	1	37
19	16	3	35
11	12	–1	23
12	14	–2	26
15	14	1	29
36	42	–6	78
106	98	8	204
32	29	3	61
31	29	2	60
27	25	2	52
18	15	3	33
68	63	5	131
74	77	–3	151
21	20	1	41
39	28	11	67
23	24	–1	47
48	44	4	92

Month 2			
Replicate 1	**Replicate 2**	$x_1 - x_2$	$x_1 + x_2$
27	21	6	48
30	31	–1	61
51	43	8	94
63	60	3	123
25	30	–5	55
28	26	2	54
54	60	–6	114
26	23	3	49
24	18	6	42
13	12	1	25
25	27	–2	52
40	38	2	78
40	45	–5	85
55	63	–8	128
49	51	–2	100
86	91	–5	177
34	32	2	66
23	21	2	44
12	20	–8	32
17	13	4	30
18	11	7	29
43	36	7	79
33	30	3	63
14	10	4	24
8	6	2	14
18	16	2	34
23	20	3	43
53	51	2	104
73	82	–9	155
82	78	4	160

A.2.3 As an example, using the equation given in **A.2.2**, the value of $D^2_{(\text{pairs})}$ for Month 1 in Table A.2 was calculated as follows:

$$D^2_{(\text{pairs})} = \frac{2^2}{32} + \frac{2^2}{26} + \frac{4^2}{20} + \frac{2^2}{30} + \frac{10^2}{126} + \frac{11^2}{135} + \frac{2^2}{18} + \frac{4^2}{48} + \frac{2^2}{50} + \frac{7^2}{45} + \frac{-4^2}{38} + \frac{-31^2}{95} + \frac{2^2}{42} + \frac{-4^2}{26} + \frac{1^2}{37} + \frac{3^2}{35} + \frac{-1^2}{23} + \frac{-2^2}{26} + \frac{1^2}{29} + \frac{-6^2}{78} + \frac{8^2}{204} + \frac{3^2}{61} + \frac{2^2}{60} + \frac{2^2}{52} + \frac{3^2}{33} + \frac{5^2}{131} + \frac{-3^2}{151} + \frac{1^2}{41} + \frac{11^2}{67} + \frac{-1^2}{47} + \frac{4^2}{92}$$

$$= 0.13 + 0.15 + 0.8 + 0.13 + 0.79 + 0.9 + 0.22 + 0.33 + 0.08 + 1.09 + 0.42 + 10.12 + 0.1 + 0.62 + 0.03 + 0.26 + 0.04 + 0.15 + 0.03 + 0.46 + 0.31 + 0.15 + 0.07 + 0.08 + 0.27 + 0.19 + 0.06 + 0.02 + 1.8 + 0.02 + 0.17$$

$$= 20.1$$

Thus for Month 1, $D^2_{(\text{pairs})} = 20.1$. Degrees of freedom = 31.

For 31 degrees of freedom, a value of D^2 of 45.0 or above is significant at the 5% level. Therefore, there is no statistically significant excess variation.

For Month 2, $D^2_{(\text{pairs})} = 11.7$. Degrees of freedom = 30.

For 30 degrees of freedom, a value of D^2 of 43.8 or above is significant at the 5% level. Therefore, there is no statistically significant excess variation.

Bibliography

Standards publications

For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

BS ISO 8199:2005, *Water quality – General guidance on the enumeration of micro-organisms by culture*

BS EN ISO 17994:2004, *Water quality – Criteria for establishing equivalence between microbiological methods*

Other publications

[1] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. *International vocabulary of basic and general terms in metrology.* Second edition. Geneva: ISO, 1993.

[2] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. *Guide to the expression of uncertainty in measurement.* Geneva: ISO, 1995.

[3] JOINT EA/EUROCHEM WORKING GROUP. *Accreditation for microbiological laboratories.* Publication reference EA-04/10. European co-operation for Accreditation, 2002[1)].

[4] UNITED KINGDOM ACCREDITATION SERVICE. *The expression of uncertainty in testing*. UKAS publication reference LAB 12, 2000[2)].

[5] LAWN, R., THOMPSON, M., and R. WALKER. *Proficiency testing in analytical chemistry*. London: Published for the LGC by the Royal Society of Chemistry, 1997.

[6] TILLETT, H.E., and N.F. LIGHTFOOT. Quality control in environmental microbiology compared with chemistry: what is homogeneous and what is random? *Water Science and Technology*. 1995, **31**, 471-477.

[7] TILLETT, H.E., and D.P. SARTORY. Measurement uncertainty in water microbiology may not estimate the laboratory's precision. *Accreditation and Quality Assurance*, 2004, **9**, 629-632.

[8] STANDING COMMITTEE OF ANALYSTS. *The microbiology of drinking water (2002) Part 3 – Practices and procedures for laboratories – Methods for the examination of waters and associated materials.* Nottingham: Environment Agency, 2002[3)].

---

1) Available from www.european-accreditation.org.
2) Available from the United Kingdom Accreditation Service web site at www.ukas.com.
3) Available from the UK Drinking Water Inspectorate web site at www.dwi.gov.uk/regs/pdf/micro.htm or the Environment Agency web site at www.environment-agency.gov.uk/nls.

Further reading

BS ISO 3534-1:2006, *Statistics – Vocabulary and symbols – Part 1: General statistical terms and terms used in probability*

BS ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions*

BS ISO 6107-8:1993, *Water quality – Vocabulary – Part 8*

DD ENV ISO 11133-1:2000, *Microbiology of food and animal feeding stuffs – Guidelines on quality assurance and performance testing of culture media – Part 1: General guidelines on quality assurance of culture media in the laboratory*

PD 6532-1:1993, *Reference materials – Part 1: Guide to the contents of certificates of reference materials – Guide to terms and definitions used in connection with reference materials*

NIEMELA, S.I. *Uncertainty of quantitative determinations derived by cultivation of microorganisms*. Helsinki, Finland: Centre for Metrology and Accreditation[4)].

VOYSEY, P.A., and K. JEWELL *Uncertainty associated with microbiological measurement*. Review No.15, Campden and Chorleywood Food Research Association, 1999[5)].

[4)] Available from www.mikes.fi.

[5)] Available from Campden and Chorleywood Food Research Association, Chipping Campden, Gloucestershire, UK.

BSI – British Standards Institution

BSI is the independent national body responsible for preparing British Standards. It presents the UK view on standards in Europe and at the international level. It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover.
Tel: +44 (0)20 8996 9000. Fax: +44 (0)20 8996 7400.

BSI offers members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Buying standards

Orders for all BSI, international and foreign standards publications should be addressed to Customer Services. Tel: +44 (0)20 8996 9001.
Fax: +44 (0)20 8996 7001. Email: orders@bsi-global.com. Standards are also available from the BSI website at http://www.bsi-global.com.

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Library and its Technical Help to Exporters Service. Various BSI electronic information services are also available which give details on all its products and services. Contact the Information Centre. Tel: +44 (0)20 8996 7111.
Fax: +44 (0)20 8996 7048. Email: info@bsi-global.com.

Subscribing members of BSI are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration. Tel: +44 (0)20 8996 7002.
Fax: +44 (0)20 8996 7001. Email: membership@bsi-global.com.

Information regarding online access to British Standards via British Standards Online can be found at http://www.bsi-global.com/bsonline.

Further information about BSI is available on the BSI website at http://www.bsi-global.com.

Copyright

389 Chiswick High Road
London
W4 4AL