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Technical Guide 2

A Guide on Measurement Uncertainty in Chemical & Microbiological Analysis

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Second Edition

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We are grateful to EURACHEM/CITAC measurement uncertainty working group for permission to utilize sections from the EURACHEM/CITAC Guide on Quantifying Uncertainty in Analytical Measurement (available from http://www.vtt.fi/ket/eurachem).

Note: Use of the material does not imply equivalence with the EURACHEM/CITAC guide.

Contents

1.0 Introduction

1.1 The International Standard ISO/IEC 17025:2005 on "General Requirements for the Competence of Testing and Calibration Laboratories" [1] has included a series of clauses on the estimation of measurement uncertainty for calibration and testing laboratories. It requests the assessment of uncertainty of test results during method validation and requires testing laboratories to have and apply procedures for estimating uncertainties of measurement in all test methods except when the test methods preclude such rigorous estimations.

1.2 The SAC-SINGLAS 002 document on "General Requirements for the Competence of Calibration and Testing Laboratories" [2] also states that a laboratory shall use appropriate methods and procedures, including an estimation of uncertainty in all measurements, and indicate the quantitative results accompanying with a statement of the estimated uncertainty.

1.3 The SAC-SINGLAS Technical Guide 1 on "Guidelines on the Evaluation and Expression of the Measurement Uncertainty" [3] was first produced in July 1995 with an aim to harmonize the procedure for expressing measurement uncertainty. The document has been well written and widely accepted. However, it only covers guided examples in the field of calibration and physical measurements. Whilst the corrections are small and experimental error may be negligible in physics (metrology), the estimation of uncertainty of results in chemical analysis is more complicated. This is because chemical testing usually requires several steps in the analytical process, very often with the use of a few analytical equipments, and, each of these actually involves certain element of uncertainty.

1.4 It is the aim of this Guide to give general information of the application of uncertainty to chemical analysis and microbiological analysis and its effects on compliance. This Guide outlines the current thinking of methodology, based on the methods prescribed in the ISO Technical Advisory Group on Metrology (TAG4's) lengthy document ISO guide 98 on "Guide to the Expression of Uncertainty in Measurement", commonly known as GUM [4] in 1995, the EURACHEM document on "Quantifying Uncertainty in Analytical Measurement" [5] and ISO/TS 21748:2004(E) on “Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation”. [10]. Simplified methods adopted elsewhere are also considered. Guidance is also given on the expression and reporting of uncertainty values.

1.5 However, it must be noted that although the concept of uncertainty itself is well accepted, there are different opinions among many learned establishments on how it should be estimated and, to a lesser extent, how it should be referred to. Hence, it is anticipated that this Guide will require constant reviewing and updating, in order to keep up with the most current methodology.

1.6 The appendices accompanying this document are several detailed examples of uncertainty evaluation processes taken from different areas of chemical analysis. These examples are intended to illustrate the application of the procedures described in this Guide.

1.7 A summary of definitions as stated in the ISO 5725-1 (1994) on "Accuracy (Trueness and Precision) of Measurement Methods and Results - Part 1: General Principles and Definitions) [6], ISO TAG4 [4] and EURACHEM [5] is given in Appendix A.

2.0 What is Uncertainty of Measurement?

2.1 The word "uncertainty" means doubt, and thus in its broadest sense "uncertainty of measurement" means doubt about the validity of the result of a measurement.

2.2 Measurement uncertainty is defined as "parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand" [5]. The word "measurand" is further defined in analytical chemistry term as "particular quantity or concentration of a species subject to measurement" (such as copper content in water).

2.3 This definition is also consistent with other concepts of uncertainty of measurement, such as:

- a measure of the possible error in the estimated value of the measurand as provided by the result of a measurement;
- an estimate characterizing the range of values within which the true value of a measurand lies.

2.4 When uncertainty is evaluated and reported in a specified way, it indicates the level of confidence that the value actually lies within the range defined by the uncertainty interval.

3.0 Reasons for Estimating Uncertainty

3.1 There is a growing awareness that analytical data for use in any decision process must be technically sound and defensible. Limits of uncertainty are required which need to be supported by suitable documentary evidence in the form of statistical control as for some kind of ‘quality assurance’. When a measurement process is demonstrated by such statistical control, the accuracy of the process can be implied to characterize the accuracy of all data produced by it.

3.2 It is a recognized fact that any chemical analysis is subject to imperfections. Such imperfection gives rise to an error in the final test result. Some of these are due to random effects, typically due to unpredictable variations of influence quantities, such as fluctuations in temperature, humidity or variability in the performance of the analyst. Other imperfections are due to the practical limits to which correction can be made for systematic effects, such as offset of a measuring instrument, drift in its characteristics between calibrations, personal bias in reading an analogue scale or the uncertainty of the value of a reference standard.

3.3 Every time a measurement is taken under essentially the same conditions. Random effects give rise to random errors from various sources and this affects the measured value. Repeated measurements will show variation and a scatter of test results on both sides of the average value. Statisticians say that random errors affect the precision, or reproducibility. A number of sources may contribute to this variability, and their influence may be changing continually. They cannot be completely eliminated but can be reduced by increasing the number of replicated analysis.

3.4 Systematic errors emanate from systematic effects. They cause all the results to be in error in the same sense, i.e. either producing consistently higher or lower results than the true value. They remain unchanged when a test is repeated under the same conditions. These effects also cannot be eliminated but may be reduced or corrected with a correction factor if a systematic effect is recognized. In fact, systematic errors must be first dealt with before estimating any uncertainty in a chemical analysis.

3.5 Hence, measurement uncertainty is a quantitative indication of the quality of the test result produced. It reflects how well the result represents the value of the quantity being measured. It allows the data users to assess the reliability of the result and have confidence in the comparability of results generated elsewhere on the same sample or same population of the samples. Such confidence is important in the attempt to remove barriers to trade internationally.

3.6 An understanding of the measurement uncertainty helps also in the validation of a new test method or a modified test method. One can suggest additional experiments to fine tune the test method if the uncertainty of the results is found to be large. One can also optimize the critical steps in a chemical analytical procedure in order to reduce uncertainty.

3.7 By quoting measurement uncertainty, the laboratory operator reflects well on the technical competence of his laboratory staff performing the analysis and helps to communicate the limitations of test results to his customer.

4.0 Sources of Uncertainty in Chemical Measurement

4.1 There are many possible sources of uncertainty of measurement in testing, including but not limiting to:

a) Non-representative sampling - the sample analyzed may not be representative of the defined population, particularly when the it is not homogeneous in nature;

b) Non-homogeneity nature of the sample, leading to uncertainty in testing a sub-sample from the sample;

c) Incomplete definition of the measurand (e.g. failing to specify the exact form of the analyte being determined, such as Cr^{3+} and Cr^{6+});

d) Imperfect realization of the definition of the test method. Even when the test conditions are defined clearly, it may not be possible to produce these conditions in a laboratory;

e) Incomplete extraction and pre-concentration of the test solution before analysis;

f) Contamination during sample and sample preparation;

g) Inadequate knowledge of the effects of environmental conditions on the measurement or imperfect measurement of environmental conditions;

h) Matrix effects and interference;

i) Personal bias in reading measurements (e.g. colour readings);

j) Uncertainty of weights and volumetric equipment

k) Uncertainty in the values assigned to measurement standards and reference materials;

l) Instrument resolution, or discrimination threshold, or errors in the graduation of the scale;

m) Approximations and assumptions incorporated in the measurement method and procedure;

n) Values of constants and other parameters obtained from external sources and used in the data reduction algorithm;

o) Random variation in repeated observations of the measurand under apparently identical conditions. Such random effects may be caused by short term environmental fluctuations (e.g. temperature, humidity, etc.) or variability between analysts.

It is to be noted these sources are not necessarily independent and, in addition, unrecognized systematic effects may exist that are not taken into accounts but which contributed to an error. However, such errors may be reduced, for example, from examination of the results of an inter-laboratory proficiency programme.

5.0 Evaluation Methods

5.1 The ISO Guide 98, ISO/TS 21748:2004 and the EURACHEM document have all adopted the approach of grouping uncertainty components into two categories based on their method of evaluation, i.e. *Type A* and *Type B* evaluation methods.

5.2 This categorization, based on the method of evaluation rather than on the components themselves, applies to uncertainty and is not substitutes for the words "random" and "systematic". It avoids certain ambiguities - a random component of uncertainty in one measurement may become a systematic component in another measurement that has, as its input, the result of the first measurement. For example, the overall uncertainty quoted on a certificate of calibration of an instrument will include the component due to random effects, but, when this overall value is subsequently used as the contribution in the evaluation of the uncertainty in a test using that instrument, the contribution would be regarded as systematic.

5.3 *Type A* evaluation of uncertainty is based on any valid statistical method in analysis of a series of repeated observations. The statistical estimated standard uncertainty is called, for convenience, a *Type A standard uncertainty*.

5.4 Component of *Type A* evaluation of standard uncertainty arises from random effect. The Gaussian or Normal Law of Error forms the basis of the analytical study of random effects. (See Appendix B)

5.5 It is a fact that the mean of a sample of measurement provides us with an estimate of the true value, μ of the quantity we are trying to measure. Since, however, the individual measurements are distributed about the true value with a spread which depends on the precision; it is most unlikely that the mean of the sample is exactly equal to the true value of the population.

5.6 For this reason, it is more useful to give a range of values within which we are almost certain the true value lies. The width of the range depends on two factors. The first is the precision of the individual measurements, which in turn depends on the variance of the population. The second is the number of replicates made in the sample. The very fact that we repeat measurements implies that we have more confidence in the mean of several values than in a single one. Most people will feel that the more measurements we make, the more reliable our estimate of μ, the true value.

5.7 In most cases, the best available estimate of the expected value of a measurand quantity x that varies randomly, is the arithmetic mean $\bar{x}$ for n number of replicates:

$$\bar{x} = \sum x_i / n \quad \ldots (1)$$

5.8 The experimental standard deviation s is used to estimate the distribution of x as:

$$s = \sqrt{[\sum (x_i - \bar{x})^2/(n-1)]} \quad \ldots (2)$$

Alternatively, it can be simplified to the following form:

$$s = \sqrt{[(\sum (x_i)^2 / (n-1)) - (\sum (x_i)^2 / n\,(n-1))]} \quad \ldots (3)$$

5.9 The experimental standard deviation of mean, or standard error of the mean (s.e.m.), σ_x, or a distribution of sample means has an exact mathematical relationship between it and the standard deviation, σ, of the distribution of the individual measurements, which is independent of the way in which they are distributed. If N is the sample size, this relationship is:

$$\text{s.e.m. } \sigma_x = \sigma / \sqrt{N} \quad \ldots (4)$$

5.10 From the equation (4) above, it is noted that the larger N is, the smaller the spread of the sample means about μ. This universally used term, the standard error of the mean, might mislead us into thinking that $\sigma/\sqrt{N}$ gives the difference between $\bar{x}$ and μ. This is not so. The $\sigma/\sqrt{N}$ gives a measure of uncertainty or confidence involved in the estimating μ from $\bar{x}$.

5.11 On the other hand, *Type B* evaluation is by means other than used for *Type A* such as:

- from data provided in calibration certificates and other reports;
- from previous measurement data;
- from experience with, or general knowledge of the behaviour of the instruments;
- from manufacturers' specifications;
- from all other relevant information.

Components evaluated using *Type B* methods are also characterized by estimated standard uncertainty.

5.12 When we are considering Type B uncertainty, we have to convert the quoted uncertainty to a standard uncertainty expressed as standard deviation. We can convert a quoted uncertainty that is a stated multiple of an estimate standard deviation to a standard uncertainty by dividing the quoted uncertainty by the multiplier.

Example:

A calibration report for reference weights states that the measurement uncertainty of a 1-gm weight is 0.1 mg at 2 standard deviations. The standard uncertainty is therefore 0.1 mg divided by 2 which gives 0.05 mg.

5.13 The quoted uncertainty can also be converted to a standard uncertainty from the knowledge of the probability distribution of the uncertainty. These probability distributions can be in the standard form of rectangular, triangular, trapezoidal and normal or Gaussian. See Appendix B. Divide the quoted uncertainty by a factor which depends on the probability distribution.

5.14 It may be stressed that those uncertainty components quantified by means other than repeated analysis are also expressed as standard deviations, although they may not always be characterised by the normal distribution. For example, it may be possible only to estimate that the value of a quantity lies within bounds (upper or lower limits) such that there is an equal probability of it lying anywhere within those bounds. This is known as a rectangular distribution. There are simple mathematical expressions to evaluate the standard deviation for this and a number of other distributions encountered in measurement.

5.15 The components, evaluated by either *Type A* or *Type B* methods, are combined to produce an overall value of uncertainty known as the combined standard uncertainty. An *expanded uncertainty* is usually required to meet the needs of industrial, commercial, health and safety, and other applications. It is obtained by multiplying the combined standard uncertainty by a *coverage factor, k*. The *k* value can be 2 for a 95% confidence level and 3 for a 99.7% confidence level. The expanded uncertainty defines 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.

6.0 Structure of Analytical Procedure

6.1 Before the discussion on the methods for estimating uncertainty, it is helpful to first of all break down the analytical method into a set of generic steps in order to identify the possible sources of uncertainty:

a. Sampling
b. Sample preparation
c. Use of certified reference materials to the measuring system
d. Calibration of instrument
e. Analysis for data acquisition
f. Data processing
g. Presentation of results
h. Interpretation of results

6.2 Each of these steps can be further broken down by contributions to the uncertainty for each. The following list, though not exhaustive, demonstrates the various factors that need to be considered when determining the sources of measurement uncertainty.

6.2.1 *Sampling*

- The physical state of the population (bulk) for sampling (gas, liquid or solid);
- Is the population (bulk) static or flowing?
- Does the population consist of discrete units?
- How homogeneous is the population (bulk)?
- Any temperature and pressure effects;
- Physical and chemical stability of the sample.

6.2.2 *Sample preparation for analysis*

- Sub-sampling for analysis
- Extraction
- Dissolution
- Combustion
- Derivatization e.g. esterification (chemical effects)
- Column and thin layer chromatography for separating measurands
- Dilution errors
- Pre-concentration errors
- Contamination

6.2.3 *Use of a certified reference materials (CRM) in the measurement system*

- Uncertainty in true value of CRM
- Residue carry-over in auto-sampler or auto-analyzer
- Does the CRM in a matrix match with the analyte in the sample?

6.2.4 *Calibration of instrument*

- Instrument calibration errors using a biased CRM
- Reference material and its uncertainty
- Sample match to the calibration standards

6.2.5 *Analysis*

- Analyst personal effects for systematic errors (e.g. colour blindness)
- Avoidance of contamination and cross-contamination
- Reagent purity
- Instrument parameter settings, e.g. GC conditions

6.2.6 *Data Processing*

- Statistics and averaging
- Control of rounding and truncating
- Electronic calculations
- Processing algorithms (model fitting, e.g. least squares)

6.2.7 *Presentation of results*

- Final result calculations
- Estimate of uncertainty
- Confidence level

6.2.8 *Interpretation of results*

- Against upper or lower limits
- Regulatory compliance
- Fitness for purpose

7.0 Process for Estimating Uncertainty

7.1 Uncertainty estimation is simple in principle. Appendix C shows the flow diagram of the evaluation process. The following steps summarise the tasks that need to be performed in order to obtain an estimate of the uncertainty associated with a measurement.

7.2 ***Step 1 – Specifications***

7.2.1 Chemical measurements usually are made because a quantitative value for some substance or measurand thereof is needed for some purpose. A suitable system should be available to make the desired measurement, and the system should be maintained in a state of statistical control throughout the measurement process. A further requirement is that the measurement system can be and is calibrated with respect to the substance of interest.

7.2.2 Chemical measurement is basically a comparison of an unknown with a known. In some cases, the comparison is direct, as in the determination of mass using an analytical balance. Direct chemical measurements consist of comparisons on a real time basis or intermittent alternations of standards and unknowns. In indirect measurement, the scale readout of an analytical instrument may be calibrated at intervals of time which should be selected so that no significant changes of the scale factor occur during that period of time.

7.2.3 Hence, a chemical measurement result is obtained at the end of a series of steps in a procedure. This is a numerical value for the measurand that is dependent upon a number of intermediate or input quantities. These may be other measurands or constants (constants also have uncertainties).

7.2.4 In general, the measurand has a relationship to these other quantities which, in principle can be expressed algebraically as:

$$x = f(a,b,c,\ldots) \quad \ldots (5)$$

7.2.5 Such an approach is useful for a theoretical discussion but in practice, except in the simplest cases, it is rarely utilized. It is more useful to break down the measurement procedure into a number of blocks. The results of the uncertainty evaluations on these simple blocks can then be used to obtain the combined uncertainty.

7.2.6 Therefore, for the purpose of uncertainty calculations, it is advisable to break down the relationship between the measurand and the input quantities into simple expressions that conform to one of the standard probability distribution forms as stated in Appendix B.

7.3 ***Step 2 - Identifying Uncertainty Sources***

7.3.1 From the structure of the analytical method used, write down a clear statement of what is being measured, including relationship between the measurand and the other parameters (such as measured quantities, constants used, calibration standards, etc) upon which it depends. Where possible, include corrections for known systematic effects. For each parameter of this relationship, list out all the possible sources of uncertainty, including any chemical assumptions.

7.3.2 Typical sources of uncertainty are:

a) <u>*Sample Uncertainty*</u>

The uncertainties in the data due to the sample always need to be evaluated. The sampling operation from a population can introduce both systematic and random errors. Calibration errors can cause problems of the first type, whilst variability of operation such as sieving or extraction are examples of the latter kind, it may be impossible to quantify the individual components of sampling variance. However, the overall sampling variance can be evaluated by taking a number (at least 7) of samples under conditions where the samples are expected to be essentially identical. The total variance consists of the sum of that due to the samples and to their measurement. Thus:

$$s^2_{total} = s^2_{sample} + s^2_{measurement} \quad \ldots (6)$$

The measurement variance is subtracted from the total variance to obtain sample variance.

In this instance, the variance of the samples measured related to that of the population and sampling is not considered when analysis is done on the sample given.

However, certain conditions of the samples have to be considered. Some samples could be affected by deterioration during collection, transit or storage, e.g. environmental water samples for BOD test. Interactions with other constituents, container walls, and transfer lines are other sources of uncertainties.

Stratification is an insidious source of error in analytical samples. Samples that were initially well-mixed may separate, partially or fully, over a period of time. It may be difficult (perhaps impossible) to reconstitute them. Melting of margarine for certain analysis is a good example. When margarine melts completely for sub-samples to be taken, the original creamy, emulsified form no longer exists as the high water content tends to settle out after melting.

Therefore, whenever stratification is possible, care must be taken to reconstitute the sample, to the extent possible, each time a sub-sample is withdrawn. Otherwise, problems caused by poor mixing can become even more serious as the ratio of sample increment to residual sample increases. Any apparent uncompensated uncertainties resulting from segregation in its various aspects should be considered when evaluating measurement data.

When degradation is possible, samples should be measured before any significant change has occurred. This leads to the concept of "holding time", defined as the maximum period of time that can elapse from sampling to measurement before significant deterioration can be expected to occur.

Statistically, it can shown that if the sampling uncertainty is 3 times that of the measurement variance or uncertainty, the fractional error of ignoring the measurement uncertainty in the total uncertainty estimation is about 5%. It is up to the professional judgement of the laboratory personnel to decide if the measurement uncertainty could be ignored when the total uncertainty of the population measurement is considered.

b) *Instrument Bias*

Systematic errors can occur in an analytical instrument. Many analysts tend to make false assumption about the accuracy of the instrument. For example, the monochromators in spectrometers gradually go out of adjustment, so that errors of several manometers in wavelength settings are not uncommon, yet many photometric analyses are undertaken without appropriate checks being made. Very simple devices such as stop-watches, pH meters and thermometers can all show substantial systematic errors too.

c) *Purity of Reagents and Chemical Standards*

The molarity or normality of a volumetric solution will not be known exactly even if the parent material has been assayed, since some uncertainty related to the assaying procedure remains. Many organic chemicals, for instance, are not 100% pure and can contain isomers or trace inorganic salts. The purity of such substances is usually stated by manufacturers as being *not less than* a certain percentage. Any assumptions about the degree of purity will introduce an element of uncertainty.

Hence, the uncertainty in the composition of chemical standards will depend on the degree of experimental realization of the calculated composition based on the knowledge of the purity of constituents, on the accuracy of the preparative process, and on consideration of stability.

d) *Human Bias*
Systematic errors can also arise from human bias. Some chemists suffer from astigmatism or colour-deficiencies which might introduce errors into their readings of instruments and other observations. Serious errors can be made by them in the titration process using colour indicator.

e) *Computational Effects*
The increasing availability of instruments controlled by micro-processors or microcomputers has reduced to a minimum the number of operations and the level of skill required of their operators. In these circumstances the temptation to regard the instruments' results as beyond reproach is overwhelming. However, the uncritical use of computer software can introduce errors into the reported results as the programmes are subject to conceptual errors such as coding population instead of sample standard deviation. There may be error in selecting an inappropriate calibration model, e.g. using a straight line calibration on a curved response. Early truncation and rounding off can also lead to inaccuracies in the final test result.

f) *Calibration Uncertainties*
Ideally, the calibration process is undertaken to eliminate deviations in the accuracy of measurements or instruments. However, this cannot be glibly assumed. In fact, as the limits of measurement are approached, the uncertainties of calibration may increase in a similar manner and can be the limiting factor in attainable accuracy.

Uncertainty of calibration may be characterised according to the confidence in the standards used and in the uncertainties of their use in the measurement process. Even having the uncertainty in the composition of chemical standards determined, the reliability of the process for transferring the standards to the system calibrated is a further consideration. Both systematic and random sources of error are involved in all the above and will need to be minimized to meet the accuracy requirements of the data. Repetitive calibrations will decrease the random component of uncertainty but not any biases. As calibration uncertainty and measurement uncertainty approach each other, calibration can become a major activity, even in routine measurements.

g) *Cross Contamination*
In any trace analysis, analysts must be fully aware of the possibility of cross contamination between samples and contamination from the laboratory environment as a result of poor working practices. For example, in the analysis of trace volatile organic compounds by headspace or purge and trap technique, any solvent extraction process in the nearby vicinity of a gas chromatograph will certainty affect the final results. Hence, such a risk of uncertainty must be minimized whenever possible.

7.3.3 It is a good practice to write down all possible sources of uncertainty in a chemical analysis and then simplify by re-grouping them under more general headings. For example, instead of writing down temperature, pressure and calibration errors as sources of uncertainty for every determination of weight by difference, it may be more sensible to regard all these three factors as parts of a single heading: ‘weighing uncertainty’ which can be evaluated directly.

7.4 ***Step 3 - Quantifying Uncertainty***

7.4.1 It is to be aware that not all the components of uncertainty are going to make a significant impact to the combined uncertainty to be evaluated. Indeed, in practice, it is likely that only a small number will. Hence, the first step in the quantification of uncertainties identified is to make a preliminary estimate of the contribution of each component to the combined uncertainty and to eliminate those which are not significant. Regroup certain sources of uncertainty with a view of simplification and evaluate them as a single component.

7.4.2 Calibration process for example, will provide a combined uncertainty associated with the blocks of the measurement process and a detailed evaluation of each component within the block is therefore not necessary.

7.4.3 Where uncertainty sources are grouped in this manner, the groups should be identified and the uncertainty sources included should be checked against the list generated by Step 2. This provides an auditable record of which contributions to uncertainty have been included.

7.4.4 Four basic methods can be used to estimate the individual uncertainty component:

- by measuring standard deviation of measured values in repeated experiments
- by making measurements on reference materials
- by utilizing the data and results of previous works carried out elsewhere, such as those collated values from an inter-laboratory study and method performance data
- by personal judgement of the analyst based on past experience

7.4.5 *Experimental Quantification*

7.4.5.1 The standard uncertainty arising from random errors is typically measured from repeated measurements and is quantified in terms of standard deviation of the measured values. In practice, not less than 14 replicates are normally considered as acceptable, unless a high precision is required.

7.4.5.2 However, it must be stressed that before any repeated experiments are to be carried out for this purpose, systematic errors present, in any, which occur in a definite and known sense, must first of all be dealt with.

7.4.5.3 The analyst must consider in the beginning of a measurement in an experiment the likely sources of systematic error such as the instrument functions that need calibrating, and the steps of the analytical procedure where errors are most likely to occur. He should also consider the checks that he can subsequently make for systematic errors. The most formidable protection against systematic errors is the use of standard reference materials and methods. If a non-standard method is to be used, then it is a good practice to compare the results of the method against those obtained by another chemically and physically unrelated method or a standard reference method. If both methods consistently yield results showing only random differences, it is a reasonable presumption that no significant systematic errors are present. On the other hand, if systematic differences do occur, a correction factor for the non-standard method has to be established after repeated analysis.

7.4.5.4 In most analytical procedure, only a few components of the uncertainty dominate. Hence, it is realistic to vary these parameters to the fullest practicable extent in the experiment so that the evaluation of uncertainty is based as much as possible on observed data.

7.4.6 *Use of Reference Materials*

7.4.6.1 In the most general terminology, a reference material is a substance for which one or more properties are established sufficiently well for use to calibrate a chemical analyzer or to validate a measurement process. A Certified Reference Material is a reference material issued and certified by an organisation generally accepted to be technically competent to do so.

7.4.6.2 As the reference material will be used for measurement quality assessment, its property certified must be accurately known. The uncertainty in the certified values takes into account that due to measurement and any variability (such as non-homogeneity) between and/or within sample of the material. Thus, definitive methods are used for establishing the values of the certified properties or they are measured by two or more independent reliable methods. Hence, the uncertainty of the values stated is quite minimal.

7.4.6.3 Therefore, measurements on such reference materials provide very good data for the assessment of uncertainty since they provide information on the combined effect of many of the potential sources of uncertainty. This method of measurement uncertainty is recommended if reference material is available.

7.4.6.4 However, there are other sources of uncertainty in such process that have to be taken into account, such as:

- the uncertainty on the assigned value of the reference material as discussed
- the reproducibility of the measurements made on the reference material
- any significant difference between the measured value of the reference material and its assigned value
- differences in the response of the measurement process to the reference material and the sample due to interference or matrix effects

These sources of uncertainty are relatively easier to be assessed than to work systematically through an assessment of the effect of every potential source of uncertainty.

7.4.6.5 To overcome the matrix effects, the analyst can use the most recent inter-laboratory cross-check samples of similar nature as the reference material.

7.4.7 *Estimation Based on Previous Results/Data*

7.4.7.1 Uncertainty Evaluation Using Method Performance Data

The stages in estimating the overall uncertainty using existing data about the method performance are:

- *Reconcile the information requirements with the available data*

  Examine the list of uncertainty sources to see which sources of uncertainty are accounted for by the available data, whether by explicit study of the particular contribution or by implicit variation with the course of whole-method experiments.

- *Obtain further data as required*

  For sources of uncertainty not adequately covered by existing data, one may obtain additional information from the literature or standing data (certificates, equipment specifications, etc.). One may also plan experiments to obtain the required additional data. Additional experiments may take the form of

specific studies of a single contribution to uncertainty, or the usual method performance studies conducted to ensure representative variation of important factors.

7.4.7.2 Uncertainty Estimation Based on Repeatability and Reproducibility Data

One of the EURACHEM's approaches and the ISO/TS 21748 attempt to use the existing laboratory quality assurance/quality control data, repeatability, reproducibility and bias on an analytical method to estimate its uncertainty of measurement.

It may be noted that repeatability data are used as a check on precision, which, in conjunction with other tests, confirms that a particular laboratory may apply reproducibility and trueness data in its estimation of uncertainty.

The ISO/TS 21748:2004 lists out the following procedure for evaluating measurement uncertainty:

a) obtain estimates of the repeatability, reproducibility and trueness of the analytical method from published information about the method;

b) establish whether the laboratory bias for the measurements is within that expected on the basis of the data obtained in (a);

c) establish whether the precision attained by the current measurement is within that expected on the basis of the repeatability and reproducibility estimated obtained in (a);

d) identify any influences on the measurements which were not adequately covered in the studies referenced in (a) and quantify the variance that could arise from these effects, taking into account the sensitivity coefficients and the uncertainties of each influence;

e) where the bias and precision are under control, as demonstrated in (b) and (c), combine the reproducibility estimate (a) with the uncertainty associated with trueness [(a) and (b)] and the effects of additional influences (d) to form a combined uncertainty estimate.

In other words, a laboratory should first of all demonstrate, in its implementation of measurement uncertainty in a method that bias is under control (i.e. the laboratory component of bias is within the range expected from the collaborative or proficiency study). There should also be a continued verification process of such performance, through appropriate quality control including regular checks on bias and precision. Uncertainty of sampling and sub-sampling shall also be taken into account.

The combined standard uncertainty takes the form of:

$$s^2 = s^2_{repeatability} + s^2_{reproducibility} \quad \ldots (7)$$

It should be stressed however, that one must not doubly estimate a measurement uncertainty. If the approach of this clause is adopted, the component-by-component approach taking the uncertainty budgets of individual analytical steps should be carefully evaluated. As the reproducibility standard deviation taken from a collaborative study might have been obtained from samples of different matrices, the matrix effect would have been taken care of but one may have to investigate separately to ensure the laboratory bias is under control, such as via the recovery study.

7.4.8 *Estimation Based on Personal Judgement*

7.4.8.1 There are many instances in chemical analysis that repeated measurements cannot be practically performed or do not provide a meaningful measurement of a particular component of uncertainty. For example:

a) An assessment of spiked recovery and its associated uncertainty cannot be made for every single sample. The analyst may, for example, make such assessment for batches of samples of similar matrix (e.g. soil) carried out on the same day. He then applies the standard uncertainty to all samples. In this instance, the degree of similarity is itself an unknown factor of uncertainty.

b) Although the use of reference material is highly recommended, there remains uncertainty regarding not only its true value, but also regarding the relevance of a particular reference material for the analysis of a specific sample. A judgement is required of the extent to which a proclaimed standard substance reasonably resembles the nature of the sample in a particular situation.

c) Another source of uncertainty arises when the measurand is only defined through a test procedure. Consider the determination of chemical oxygen demand of water that is undoubtedly different whether one analyzes river water or estuaries. The high chloride content and other constituents in estuaries will certainly affect the final test result and its uncertainty.

d) It is a common practice in analytical chemistry to call for spiking with a single or a group of substances, which are close structural analogue or isotopomers, from which either the recovery of the respective native substance or even that of a whole class of compounds is judged. For example, the US EPA 8270C method [8] for the analysis of semi-volatile compounds in water and solid waste suggests the use of various deuteriated surrogate organic compounds such as phenol-d_6, nitrobenzene-d_5 and 4-terphenyl-d_{14} in the spiked recovery analysis.

Clearly, the associated uncertainty is experimentally assessable provided one is ready to study this recovery exercise at all concentration levels and ratios of measurands to the spike, and all ‘relevant’ matrices. But such experimentation is not practical, considering many semi-volatile organic compounds (about 100 of them) are involved. Instead, judgement is made on the concentration dependence on the recoveries of spikes and of measurand made.

7.4.8.2 Judgement of this type is not based on immediate experimental results, but rather on experience with, or general knowledge of the behaviour and property of relevant materials and instruments. It is quite a subjective probability, an expression which can be used synonymously with ‘degree of belief’, ‘intuitive probability’ and ‘credibility’. Such degree of belief is not based on a snap judgement, but on a well considered mature professional judgement of probability through expert knowledge by earlier experiments and observations. It constitutes typical *Type B* evaluation as it does not rely on replicated experiments performed just for a specific evaluation of uncertainty.

7.4.8.3 For the purpose of estimating combined uncertainty, two features of degree of belief estimations are essential:

a) degree of belief is regarded as interval valued which is to say that a lower and an upper limits similar to a classical probability distribution is provided;

b) the same computational rules apply in combining such 'degree of belief' contributions of uncertainty to a combined uncertainty as for standard deviations derived by other methods.

7.4.9 *Estimating Standard Uncertainty*

7.4.9.1 All uncertainty contributions are eventually expressed as standard uncertainties, i.e. in the form of standard deviations. This may involve conversion from some other measure of dispersion.

7.4.9.2 The following guidelines for converting an uncertainty component to a standard deviation are to be noted:

a) For experimental estimation, the uncertainty component can readily be expressed as a standard deviation;

b) Where an uncertainty estimate is derived from previous results and data, it may already be expressed as a standard deviation. However, there are instances where a confidence interval with a confidence level is given in the form of ± *a* at p% confidence. In this case, the value of *a* is to be divided by an appropriate *z* value of the standard normal probability distribution for the *p%* level of confidence given. See Appendix B for the areas under the standard normal probability distribution.

Example

A specification is given that an analytical balance reading is within ± 0.2mg with 95% confidence.

From the standard table giving z values and areas under the Standard Normal Probability Distribution, a figure of z=1.96 is found to give 95.0% area under curve. Using this figure gives a standard uncertainty of (0.2/1.96) = 0.1.

In the case where limits of ± *x* are given without a known confidence level, then it may be appropriate to assume a rectangular distribution, with a standard deviation of $x/\sqrt{3}$. (See Appendix B)

7.4.9.3 Where an estimate is made upon judgement, it may be possible to estimate the component directly as a standard deviation. If this is not possible, an estimate should be made of the maximum value which could reasonably occur in practice.

7.5 **Step 4 – Calculating the Combined Uncertainty and Expanded Uncertainty**

7.5.1 When all the uncertainty contributions are identified and expressed as standard deviations, the uncertainty components are then combined using a spreadsheet method or algebraically.

7.5.2 The standard uncertainty of *y,* where y is the estimate of the measurand *Y* and thus the result of the measurement, is obtained by appropriately combining the standard uncertainties of the input estimates *a,b,c,….* This combined standard uncertainty of the estimate of y is denoted by $u_c(y)$.

7.5.3 The combined standard uncertainty, $u_c(y)$ is the positive square root of the combined variance $u_c^2(y)$ which is given by:

$$u_c^2(y) = \sum\{[\partial f/\partial a]^2 u^2(a) + [\partial f/\partial b]^2 u^2(b) + [\partial f/\partial c]^2 u^2(c) + \ldots \quad \ldots (8)$$

7.5.4 For practical purposes, the following simple rules for combining standard deviations are shown below:

a) For models involving only a sum or difference of quantities,
e.g. $y = k(a + b + c + \ldots)$

where k is a constant, the combined standard uncertainty $u(y)$ is given by:

$$u(y) = k\sqrt{[u_{(a)}^2 + u_{(b)}^2 + u_{(c)}^2 + ...]} \quad \ldots (9)$$

b) For models involving only a product or quotient,
e.g. $y = k(abc\ldots)$

where k is a constant, the combined standard uncertainty $u(y)$ is given by:

$$u(y)/y = k\sqrt{\left[u(a)/a\right]^2 + \left[u(b)/b\right]^2 + \left[u(c)/c\right]^2 + ...} \quad \ldots (10)$$

7.5.5 The final stage is to multiply the combined standard uncertainty by the chosen coverage factor in order to obtain an expanded uncertainty. The coverage factor is chosen after considering a number of issues like the level of confidence required and any knowledge of underlying distributions. For most purposes, a coverage factor k of 2 is normally chosen which gives a confidence level of approximately 95%.

7.5.6 The expanded uncertainty is required to provide an interval which may be expected to encompass a large fraction of the distribution of values which could reasonably be attributed to the measurand.

7.6 *Sensitivity Coefficient*

7.6.1 In chemical testing, analysts frequently encounter factors that have impact on the measurement results. However, very often, the extent of such an impact cannot be clearly expressed by a simple mathematical model. For example, measurement uncertainty arising from oven temperature setting has an impact on the determination of moisture content of a sample.

In such a case, the laboratory should carry out a series of tests to evaluate the impact of such factors on the results. One common example is the temperature effect on water volume expansion. Nowadays, analysts often use water expansion coefficient of 2.1 x 10^{-4} per °C per ml to estimate measurement uncertainty of volume due to temperature change. Such water expansion coefficient is in fact a sensitivity coefficient. It was obtained by changing the temperature and accurately determining the resultant volume. From the volume changes, one can work out the volume change per °C per unit volume. Similarly, oven temperature effect and moisture content can be determined in such a manner.

7.7 Overview on Different Approaches to MU

7.7.1 While there are a number of approaches available to estimate measurement uncertainty, and each approach has its own merits, a correct approach will ensure completeness in such estimation. To help testing laboratories in this aspect, a flow chart and step-by-step procedures are given in Appendix C.

8.0 Reporting Uncertainty

8.1 The information necessary to document a measurement ultimately depends on its intended use but it should contain enough information to allow the result to be re-evaluated if new information or data become available.

8.2 A complete analysis report should include the followings:

a) a description of the methods used to calculate the result and its uncertainty;

b) the values and sources of all corrections and constants used in both the calculation and the uncertainty analysis;

c) a list of all the components of uncertainty with full documentation on how each was evaluated.

The data and analysis should be presented in such a manner that its important steps can be readily followed and if necessary repeated.

8.3 *Reporting Expanded Uncertainty*

8.3.1 Unless it is required otherwise, the result should be reported together with the expanded uncertainty, *U,* calculated using a coverage factor k = 2.

8.3.2 The calculated expanded measurement uncertainty, *U*, represents half of the measurement uncertainty interval. The following standardised format is usually applied to express the entire measurement uncertainty interval, accompanied with a statement:

(Analyte): Result x (units) ± *U* (units)

- *The reported measurement uncertainty is an expanded measurement uncertainty according to this Guide, calculated using 2 as the coverage factor [which gives a confidence level of approximately 95%].*

Note that the texts within square brackets [ ] may be omitted or abbreviated in a suitable manner.

Example
*Total Oil Content : 9.80 ± 0.15% w/w**

** The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a confidence level of approximately 95%.*

8.3.3 Although a coverage factor of 2 is commonly used for 95% confidence reporting, coverage factors of either k =1 or k = 3 may be considered in some cases. These correspond to confidence levels of 68% and more than 99%, respectively.

8.4 *Reporting Standard Uncertainty*

8.4.1 When a coverage factor k = 1 is used, i.e. the measurement uncertainty is estimated to one standard deviation, the uncertainty is called standard measurement uncertainty, designated as u. In such cases, the following report format is recommended:

(Analyte): Result x (units) with a standard measurement uncertainty u (units)

Note that it is not recommended to use the symbol ± when reporting standard measurement uncertainty, because this symbol is usually associated with high confidence intervals such as 95% and above.

8.5 *Reporting Significant Figures*

8.5.1 In chemical analysis, only significant figures of a test result are reported generally. Whether expanded uncertainty U or a standard uncertainty u is given, it is seldom necessary to give more than two significant digits for the uncertainty. Hence, test results should also be rounded to be consistent with the uncertainty given.

9.0 General Remarks

9.1 It is important that one should not doubly count an uncertainty component. When the GUM approach is adopted where the standard uncertainty of each component is considered fully, one should not introduce the repeatability and reproducibility of the test method as the other uncertainty components because repeatability and reproducibility represents the total performance of the test method in terms of its precision and accuracy, respectively.

9.2 However, should sampling uncertainty be an important consideration due to heterogeneous nature of the sample, then repeated sub-sampled analyses could be carried out and the standard uncertainty of the sampling could be added as another uncertainty component to the whole budget.

9.3 The GUM approach does not take the possible result bias into consideration but in chemical analysis, there is always a possibility of systematic error which could then be minimized or eliminated. One has to either estimate a correction factor to adjust the test result back into its true value or estimate the bias uncertainty of reporting such biased result.

10. Measurement Uncertainty for Quantitative Microbiological Testing

10.1 *Introduction*

10.1.1 This Guide adopts the “top-down” or “global” approach to MU, which is based on the standard deviation of reproducibility of the final result of the measurement process. The same approach has been endorsed for a more general use by ISO/TS 21748 elaborated by ISO/TC 69, Application of Statistical Methods, SC 6, Measurement Methods and Results [11], and detailed in the ISO/TS 19036, ”Microbiology of Food and Animal feeding stuffs – Guidelines for the Estimation of Measurement Uncertainty for Quantitative Determinations” [12]. The latter document clarifies that the step-by step approach and the global approach are not mutually exclusive, since all the MU components can be considered to be included in the overall performance of the analytical process, which can be characterized by the observable precision and bias.

10.1.2 The current consensus has been that the “step-by-step” approach does not apply satisfactorily in the case of microbiological analysis, where it is difficult to build a really comprehensive model of measurement process. It appears difficult to quantify accurately the MU contribution of each individual step of the microbiological measurement process, where (1) the analyte is a living organism present in a natural sample that can be in variable physiological state in their natural environment, e.g. in various stage of growth or in injured condition on exposure to the adverse environmental conditions or manufacture processes, (2) the target organism includes different strains, different species or different genera, and (3) there are no truly certified reference preparations of micro-

organisms of standard concentration, and /or representing the micro-organisms in their natural habitats.

10.2 *Scope*

10.2.1 This Guide provides guidance for the estimation and expression of MU associated with quantitative microbiological methods, in which the quantitative estimate is based on counting of particles on the basis of growth (multiplication) into colonies. These methods are commonly known as the heterotrophic plate count, total aerobic microbial count, spiral plate count (instrument method), and colony counts of specific target organisms on selective media, e.g. faecal coliform count, and coagulase-positive *Staphylococcus* count

10.2.2 The approach based on standard deviation of reproducibility of final result is applicable to the quantitative analysis of microorganisms based on colony count of the following products:

- food and animal feedstuff
- drinking water, non-potable water such as recreation water and reservoir water, waste-water
- pharmaceutical products, herbal medicinal products and health supplements
- cosmetic, toiletry and fragrance products

10.2.3 It is not applicable to

- enumeration using a most probable number technique, or
- the analysis of low levels of microorganisms, where the results of plate count are less than 10 colony forming units (cfu). These results may be well below the limit of quantification.

10.3 *Sources of uncertainty in microbiological tests*

10.3.1 Many of the "Sources of Uncertainty in Chemical Measurement" listed under Section 4 of this Guide are also relevant to microbiological measurement.

10.3.2 The following sources of uncertainty have been shown to influence the precision and hence measurement uncertainty of microbiological results:

10.3.2.1 Sampling

a) Nature of sample
   - Homogeneity of the sample
   - Background microflora and their concentration over the target organism to be counted

b) Sample matrix, liquids, powders, solids:
   - The physical state of the sample significantly affects the standard deviation of two sources of uncertainty, the one linked to the matrix (including sub-sampling for the test portion) and the one linked to the preparation of the initial dilution, and hence the reproducibility standard deviation of the final result [12].

c) Method of sampling, e.g. techniques and sampling apparatuses used in sub-surface water sampling, in solid food sampling from bulk or batch packaging

d) Transportation time and temperature of sample

e) Storage time and temperature of sample after receipt until analysis

10.3.2.2 Method of Analysis

a) Source (USP, BP, BAM, APHA, AOAC, ASTM, ISO)

b) Definition of measurand

c) Robustness of test method

- Many standard methods such as USP and APHA specify a range of recommended incubation time and temperature. Colony counts are time and temperature sensitive. The users of these standard methods have to determine and specify the incubation temperature and time of the plate count tests, appropriate for the purpose and performance requirements of the tests including precision and reproducibility.

10.3.2.3 Culture Media and Reagents

- Source, brand
- Formulation specifications
- Water quality
- Quality / performance consistency/batch-to-batch variation, in particular, selective media that contain toxic biological/chemical selective agents
- Storage conditions and shelf-life

10.3.2.4 Analytical Procedure

- Sample homogenization/mixing, e.g. using Stomacher, blender or turbo mixer.
- Sub-sampling
- Preparing and dispensing dilutions
- Inoculation procedure, e.g. Filtration technique, pour-plate, spread-plate and spiral plate techniques
- Incubation conditions
- Reading, interpreting and reporting results
- Microbial concentration in each culture plate

10.3.2.5 Equipment

- Precision and accuracy
- Maintenance
- Calibration
- Repair

10.3.2.6 Analysts

- Training
- Validating & Maintaining Competency

10.4 Approach for Estimation of Measurement Uncertainty of Microbiological Test based on Relative Standard Deviation of Reproducibility (RSD_R) from duplicate pair analysis

10.4.1 Define measurand and standard method or validated in-house method to be used.

10.4.2 Identify individual components of uncertainty and demonstrate that they are under control, for example, regular checking of performance of culture media, incubators, weighing balance, pipettors and other instrument, and within-analyst repeatability.

10.4.3 Analyze the sample using all steps of the test method.

10.4.4 Perform analysis of at least 15 samples that are set up on different days in duplicate pairs, different analysts on different days, using different equipment (e.g. pipettors, incubators, if more than one pipettor or incubator is used for the same test) on the different days and possibly using different batches of reagents and media on different days.

Duplicate pair analysis refers to the analysis of the same sample two times, each time using the same procedure by the same analyst on the same day within a short period of time over which the level of the microorganisms remains stable.

10.4.5 The data should be collected over an extended period of time, e.g. 1 year. The samples should consist of low, medium and high concentrations of microorganisms that normally encountered in the natural samples. The recommended counting range of colonies per plate stipulated in the standard methods can be used as a starting point.

10.4.6 Natural samples should be used as far as possible, since they enable a more realistic estimation of MU. If spiking is required, spikes should be designed to mimic natural contamination as far as possible, e.g. by use of organisms harvested and concentrated from fluid sample by centrifugation. When this is not feasible, reference organisms may be used as spikes.

10.4.7 Calculate Relative Standard Deviations of Reproducibility (RSD_R) using the following formula (modified from ISO/TS 19036) to assess the measurement uncertainties for counts using the following equation:

$$RSD_R = \sqrt{[\sum_{i=1}^{n} ((\log a_i - \log b_i) / x_i)^2 / 2n]} \quad \ldots (11)$$

where

$(\log a_i - \log b_i)/ x_i$ = the relative difference between the duplicate logarithmic results

i =1,2,….n
n = number of duplicate pairs in the analysis

In this case the relative difference in each pair is calculated. This is done by dividing the difference of each pair with the mean value of the pair. This difference is then squared. These are summed. The sum is divided by 2 times the number of duplicate determinations. The square root is taken for that value to give the Relative Standard Deviation of Reproducibility (RSD_R).

10.4.8 The combined uncertainty associated with the procedure is the value of RSD_R :
$u = RSD_R$

10.4.9 Expanded uncertainty $U = k$ (coverage factor for 95% confidence) x RSD_R

where *k* is the appropriate coverage factor, usually 2, unless it is required otherwise, e.g. to determine compliance with certain microbial limit, one may wish to use appropriate t statistic for 95% confidence level.

Example:
If the RSD_R is 0.02 and the count/g, c, is 3.00×10^4, and we assume a coverage factor of $k = 2$

Expanded Uncertainty, $U = 2 \times RSD_R = 2 \times 0.02 = 0.04$

The Expanded Uncertainty, U as an RSD_R %,

$$U = 0.04 \times 100 = 4\%$$

To obtain the MU for the count, c, then use the following equation,

$MU = \log_{10}(c) \pm U = \log_{10}(c) \pm [4\% \times \log_{10}(c)]$

The common logarithm of the count/g, c, 3.00×10^4 is 4.4771.

MU = 4.4771± 0.17908 or 4.2980 to 4.6562, Antilog = 19,860 to 45,310

Alternatively, use the following equation to obtain MU for the count,

$MU = \log_{10}(c) \pm [k \times RSD_R \times \log_{10}(c)]$

MU = 4.4771 ± [2 x 0.02 x 4.4771]
= 4.4771 ± 0.17908 Antilog = 19,860 to 45,310

The range for count/g after round up would be 2.0×10^4 to 4.5×10^4

During any intermediate stages in the calculations, e.g. when transforming counts to $\log_{10}$ values, calculating the mean, etc, try and keep figures as accurate as possible and only round the final results to the desired precision. For colony counts, not more than two significant figures shall be used for reporting the values of the result and the uncertainty interval.

The calculation of the MU from the above equation produces a range of common logarithms of the count. This range is that which could reasonably be expected if similar samples were to be tested by same group of operators within this laboratory, and the counts of these samples fall within the range or concentrations of counts used in the RSD_R estimation.

Format for reporting MU :

The following standardised format is used to express the entire measurement uncertainty interval, accompanied with a statement :

Using the above example : Colony forming units /g : 3.0×10^4 with confidence interval of 2.0×10^4 to 4.5×10^4

- The reported uncertainty is an expanded uncertainty calculated from relative standard deviations of laboratory reproducibility and using a coverage factor of 2 which gives a confidence level of approximately 95%.

10.4.10 This approach assumes RSD_R is constant over the recommended counting range of the method. Where sufficient data is available, this assumption should be verified for subsets of data of duplicate pairs obtained by the method by comparing RSD_R values for low, medium and high concentration ranges.

10.4.11 The relative measurement uncertainty, RSD_R, should be re-determined, when there is a significant change in the operating conditions of the laboratory, such as significant staff changes or changes of equipment.

10.5 The Standard Grubbs Test for Identification of Outliers of Duplicate Pairs

10.5.1 Examine the dataset of duplicate pair analysis, such as the cfu count of duplicate plates and the relative difference of counts/mean for suspected outlier. The Standard Grubbs Test for identification of outlier of duplicate pairs can be used to determine whether suspected outlier can be reasonably removed, at a selected risk of false rejection. The Grubbs test calculates how much a suspected outlier differs from the population mean, measured in units of standard deviation.

10.5.2 The following equation for Grubbs Test is used [13] :

$$T = I\,RD\,I\ /\ (\sqrt{2}\ x\ RSD_R) \qquad \ldots (12)$$

where

RD = Relative difference for each duplicate pair, as a decimal fraction

[$RD = (a_i - b_i) / \bar{x}$]

RSD_R = Relative standard deviation of single measurements from within sets of duplicate pairs calculated using Equation 12.

10.5.3 Compare the calculated T value to the appropriate critical value in the following Table based on the number of data points in the set and the risk that can be tolerated for false rejection. A 5% risk of false rejection is recommended. In the Table, the “Number of Data Points” column refers to the number of duplicate pairs used in the calculation of RSD_R.

Critical T Value of Grubbs Test

Number of Data Points, n	Risk of False Rejection		
	5%	2.5%	1%
3	1.15	1.15	1.15
4	1.46	1.48	1.49
5	1.67	1.71	1.75
6	1.82	1.89	1.94
7	1.94	2.02	2.10
8	2.03	2.13	2.22
9	2.11	2.21	2.32
10	2.18	2.29	2.41
11	2.23	2.36	2.48
12	2.29	2.41	2.55
13	2.33	2.46	2.61
14	2.37	2.51	2.66
15	2.41	2.55	2.71
16	2.44	2.59	2.75
17	2.47	2.62	2.79
18	2.50	2.65	2.82
19	2.53	2.68	2.85
20	2.56	2.71	2.88
21	2.58	2.73	2.91
22	2.60	2.76	2.94
23	2.62	2.78	2.96
24	2.64	2.80	2.99
25	2.66	2.82	3.01
50	2.96	3.20	3.34

Where the calculated T value for a result exceeds the appropriate value in the table (interpolate if necessary), the result is a probable outlier from the data population, and it may be reasonable to remove it. Attempts should be made to find out the cause of the outlier result, whether it was a mistake such as bad pipetting, holes in filter, contaminated medium, computation error etc, before resorting to exclusion based solely on the Grubbs test.

If outliners are excluded, re-calculate the RSD_R with the outliners removed.

10.6 Uncertainty Specified in the Standard Methods

10.6.1 In those cases where well recognized standard test methods (such as AOAC, APHA, ASTM and BP/USP methods) that specify limits to the values of the major sources of uncertainty of measurement and specifies the form of presentation of calculated results, the laboratory should follow the reporting instructions.

Example:
Pour Plate Counting using Standard Method Estimation Dairy Product(SMEDP):

Relative Standard Deviation of Repeatability, RSD_r

$$RSD_r \leq 7.7\% \ (0.077)$$

Relative Standard Deviation of Reproducibility, RSD_R

$$RSD_R \leq 18.2\% \ (0.182)$$

Calculation of combined standard uncertainty, u :

Sum of Squares: $(0.077)^2 + (0.182)^2 = 0.0391$ or 3.9%
Combined uncertainty = $\sqrt{0.0391} = 0.198$ or 19.8%

Expanded uncertainty: U (Use coverage factor k = 2 for 95% confidence)

$$\begin{aligned} U &= k \times u \\ &= 2 \times 19.8\% \\ &= 39.6\% \end{aligned}$$

Note: When using the standard method, the laboratory is required to demonstrate their ability to meet the established performance requirements of the standard method, a pre-requisite for making use of the established expanded uncertainty of the standard method.

10.7 Evaluation of Results Against a Microbial Limit

10.7.1 For a result to be considered as having exceeded a microbial limit, the lower limit for the confidence interval of measurement uncertainty is required to be above this value.

10.7.2 A χ^2 (chi-square test) can be used to find out whether a microbiological result has exceeded the stipulated microbial limit:

$$\chi^2 = (C-L)^2 / L$$

where C = colony count, L = Limit value

10.7.3 The microbial limit will be exceeded if either $\chi^2 > 4$ or if the count, C, is $> L + 2\sqrt{L}$ at 95% confidence limits.

10.7.4 For microbial limit of 100, the number of cfu in the sample would have to be >120 to be statistically in excess of the limit guideline.

10.8 Most Probable Number Methods (MPN)

10.8.1 The APLAC Uncertainty Guideline accepts the data in the McCrady's table as reasonable estimates of uncertainty for MPN results.

10.8.2 For the purposes of SAC-SINGLAS's Policy, the McCrady's table can be used as estimates of uncertainty for a test, provided the laboratory has reviewed the resulting data and identified any unusual combinations of results.

10.8.3 Any unusual combinations of positive and negative tubes in excess of 1% of all MPN results are to be treated as non-conforming to the McCrady's table. Root causes should be identified and corrected.

10.9 Proficiency Testing (PT) Programme

PT organisers rarely specify the methods that the participating laboratories must follow. Pooling of data derived from different methods diminishes the usefulness of the PT information for MU estimation. There may be also matrix differences between the PT samples and the routine samples tested by a laboratory.

10.10 Data Handling

10.10.1 Microbial distributions are not necessarily symmetrical. Bacterial counts often are characterised as having a skewed distribution. Pooling of cfu counts from different samples containing wide range of concentration of micro-organisms may lead to an arithmetic mean that is considerably larger than the median, and result in an unreasonably large variance. Under these circumstances, it would be more appropriate to convert the data to log values to achieve approximately normal distribution of the counts, before doing any statistical analyses.

10.10.2 The expanded uncertainty determined from data over the entire counting range of colonies per filter or plate may overestimate or underestimate uncertainty depending upon whether the data is weighted to high or low counts. Canadian Association for Environmental Analytical Laboratories (CAEAL) Uncertainty Policy [14] recommended that data be separated into ranges (as indicated below) and, the combined uncertainty (U) determined for each range.

The following colony forming unit (cfu) ranges are suggested for membrane filtration techniques for water samples:

- 1-19 cfu/Filter
- 20-80 cfu/Filter
- 81-100 cfu/Filter

The following cfu ranges are suggested for plating (e.g. spread plate) procedures for water samples:

- 1-29 cfu/Plate
- 30-99 cfu/Plate
- 100-300 cfu/Plate

10.11 Qualitative Methods (e.g. Presence-Absence)

10.11.1 Presence/absence tests do not result in an enumeration, therefore uncertainty of measurement cannot be estimated using the above approach. SAC-SINGLAS current policy does not require the estimation of measurement uncertainty for qualitative microbiological tests.

APPENDIX A

GLOSSARY OF STATISTICAL TERMS

The following definitions of statistical terms are quoted from the following documents:

GUM : ISO Guide 98, Guide to the Expression of Uncertainty in Measurement

VIM : International Vocabulary of Basic General Terms in Metrology

ISO/IEC : Guide 2

ISO : ISO 3534 Part 1 and ISO 3534 Part 2

AOAC : Association of Official Analytical Chemists

IUPAC : International Union of Pure and Applied Chemists

A.1 Accuracy

The closeness of agreement between a test result and a true value of the measurand.

Note 1: "Accuracy" is a qualitative concept.
Note 2: The term "precision" should not be used for "accuracy".

A.2 Analyte

The specific component measured in a chemical analysis.

A.3 Arithmetic Mean; Average $\bar{x}$ *or* μ

1) Arithmetic mean value of a *sample* of *n* results:

$\bar{x} = \sum x_i / n$ where $\bar{x}$ = mean of the sample
n = sample size (n results of the sample)

2) Arithmetic mean value of a *population* of *N* results:

$\mu = \sum x_i / N$ where μ = mean of the population
N = Population size (*N* results of the population)

A.4 Bias

The difference between the expectation of the test results and an accepted reference value.

Note 1: Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias. A larger systematic difference from the accepted reference value is reflected by a larger bias value.

A.5 Calibration

Comparison of a measurement standard or instrument with another standard or instrument to report or eliminate, by adjustment, any variation or deviation in the accuracy of the item being compared.

Note 1: Calibration result allows a measurand value to be specified with respect to the indicated value or correction to be determined relative to the indicated value. The calibration results may be recorded in documents called calibration certificates or calibration reports.

A.6 Central Line

A line on a control chart representing the long-term average or a pre-specified value of the statistical measure being plotted.

A.7 Certified Reference Material (CRM)

A reference material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body.

A.8 Coefficient of Variation

The standard deviation *(s)* divided by the mean ($\bar{x}$) value of the parameter measured.

$$CV = \frac{s}{\bar{x}}$$

A.9 Control Chart

A chart, with upper and/or lower control limits, on which values of some statistical measure for a series of samples or sub-groups are plotted, usually in time or sample number order. The chart frequently shows a central line to assist the detection of a trend of plotted values towards either control limits.

A.10 Control Chart (Shewhart)

A control chart to show if a process is in statistical control.

A.11 Control Chart Limits (Upper and/or Lower)

In a control chart, the limit below which (upper limit) or above which (lower limit) or the limits between which the statistic under consideration lies with a very high probability (say, 95% confidence) when the process is under control.

- **action limits; action control limits (upper and/or lower)**

In a control chart, the limits below which (upper limit) or above which (lower limit) or the limits outside which the statistic under consideration lies when action should be taken.

- **warning limits (upper and/or lower)**

The warning limits are always within the action limits and are between the upper and/or lower limits and the central line. When the value of the statistic computed from a sample is outside the warning limits but inside the action limits, increased supervision of the process is generally necessary and rules may be made for action in particular processes. In other words, at the warning limits, attention is called to the possibility of out-of-control conditions, but further action is not necessarily required.

A.12 Coverage Factor k

Numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty.

Note 1: A coverage factor is typically either 2 or 3.

A.13 Correction

Value added algebraically to a specific uncorrected result of measurement to compensate for systematic error.

A.14 Correction Factor

Numerical factor by which a specific uncorrected result is multiplied to compensate for systematic error.

Note 1: It is impossible to determine systematic error precisely. Therefore, compensation cannot be perfect.

A.15 Cumulative Sum Chart (CUSUM Chart)

A control chart on which the plotted value is the cumulative sum of deviations of successive sample statistics from a target value. When a process change is made, the sum is returned to zero. The ordinate (y-axis) of each plotted point represents the algebraic sum of the previous ordinate and the most recent deviation from the target.

A.16 Deviation

Difference between a value and its reference value.

A.17 Drift

Moderate changes in the measurement characteristics of a measuring instrument.

A.18 Duplicate Measurement

A second measurement made on the same or identical sample of material to assist in the evaluation of measurement variance.

A.19 Duplicate Sample

A second sample randomly selected from a population of interest to assist in the evaluation of sample variance.

A.20 Error (of measurement)

The result of a measurement minus a true value of the measurand.

A.21 Error (Random)

Result of a measurement minus the mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions.

Note 1: Random error is equal to error minus systematic error.

Note 2: Because only a finite number of measurements can be made, it is possible to determine only an estimate of random error.

A.22 Error (Systematic)

Mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions minus a true value of the measurand.

Note 1: Systematic error is equal to error minus random error.

Note 2: Like true value, systematic error and its causes cannot be known.

A.23 Fitness for Purpose

Degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose.

A.24 Limit of Detection

The lowest content that can be measured with reasonable statistical certainty.

Note 1: It is expressed as the concentration or quantity which is derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The value of X_L is given by the equation:

$$X_L = X_{blank} + k\, s_{blank}$$

Where X_{blank} is the mean of the blank measures and s_{blank}, the standard deviation of the blank measures, and k, a numerical factor chosen according to the confidence level desired.

A.25 Measurand

Particular quantity subject to measurement.

A.26 Measurement

Set of operations having the object of determining a value of a quantity.

A.27 Measurement Procedure (or Measurement Method)

Set of operations, described specifically, used in the performance of measurements according to a given method.

A.28 Method of Measurement

A logical sequence of operations, described generically, used in the performance of measurement.

A.29 Metrology

Scientific execution of measurement.

Note 1: Metrology includes all theoretical and experimental aspects of measurements, regardless of the magnitude of uncertainty or the applicable scientific or technical field.

A.30 Moving Average Control Chart

A control chart for evaluating process level in terms of an arithmetic average of the latest *n* observations in which the current observation has replaced the oldest of the latest *(n+1)* observations.

A.31 Non-conformity

The non-fulfillment of a specified requirement.

A.32 Outlier

A value which appears to deviate markedly from that for other members of the sample in which it occurs.

A.33 Population

A generic term denoting any finite or infinite collection of individual things, objects or events. It is the totality of items under consideration.

A.34 Precision

The closeness of agreement between independent test results obtained under stipulated conditions.

Note 1: Precision depends only on the distribution of random errors and does not relate to the true value of the specified value. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results.

Note 2: "Independent test results" means results obtained in a manner not influenced by any previous result on the same or similar test object. Quantitative measures of precision depend critically on the stipulated conditions.

A.35 Proficiency Testing

A systematic testing programme in which similar samples are analyzed by a number of laboratories to measure the competence of a group of laboratories to undertake certain analyses.

A.36 Probability

The likelihood of the occurrence of any particular form of an event, estimated as the ratio of the number of ways or times that the event may occur in that form, to the total number of ways that it could occur in any form.

A.37 Quality Assurance

All those planned and systematic actions or characteristics that cover different sets of needs for products or services intended for the same functional use.

A.38 Quality Control

Operational techniques and activities that are used to fulfill requirements for quality.

A.39 Random Sample

A sample selected from a population using a randomization process. It can be a sample of *n* items taken from a population of *N* items in such a way that all possible combinations of *n* items have the *same* probability of being selected.

A.40 Range (Measuring – Working)

Set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits.

A.41 Recovery

The fraction of analyte added to a test sample (fortified or spiked sample) prior to analysis, the unfortified and fortified samples, the percentage recovery (%R) is calculated as follows:

$$\%R = \frac{C_F - C_U}{C_A} \times 100$$

Where C_F is the concentration of analyte measured in the fortified sample; C_U is the concentration of analyte measured in unfortified sample and C_A, the concentration of analyte added (measured value and not determined by method) in fortified sample.

A.42 Reference Material (RM)

A material or substance one or more properties of which are sufficiently well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assignment of values to materials.

A.43 Relative Standard Deviation (RSD)

The coefficient of variation expressed as a percentage.

$$RSD = \frac{s}{\bar{x}} \times 100\%$$

A.44 Repeatability

Precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

A.45 Replicate

A counterpart of another, usually referring to an analytical sample or a measurement. It is the general case for which duplicate, consisting of two samples or measurements, is the special case.

A.46 Reproducibility

Precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.

Note 1: A valid statement of reproducibility requires specification of the conditions changed. Reproducibility may be expressed quantitatively in terms of the dispersion of the results.

A.47 Result of a Measurement

Value attributed to a measurand, obtained by measurement.

Note 1: When the term "result of a measurement" is used, it should be made clear whether it refers to:

- *The indication*
- *The uncorrected result*
- *The corrected result*

and whether several values are averaged.

A.48 Sample

A portion of a population or lot. It may consist of an individual or groups of individuals; it may also refer to objects, materials, or to measurements conceived to be part of a larger group (population) that could have been considered.

A.49 Sampling

The process of drawing or constituting a sample.

A.50 Sampling Size

The number of sampling units in the sample.

A.51 Specification

Document that prescribes the requirements with which the product, process or service has to conform. It is desirable that the requirements be expressed numerically in terms of appropriate units together with their limits.

A.52 Standard

A substance or material, the properties of which are believed to be known with sufficient accuracy to permit its use to evaluate the same property of another. In chemical measurements, it often describes a solution or substance, commonly prepared by the analyst, to establish a calibration curve or to determine the analytical response function of an instrument.

A.53 Standard Deviation (Sample) s

An estimate of the population standard deviation σ from a sample of n results:

$$s = \sqrt{\sum [(x_i - \bar{x})^2/(n-1)]}$$

A.54 Standard Deviation (Population) σ

The standard deviation of a population using *all N* data in that population:

$$\sigma = \sqrt{\sum [(x_i - \mu)^2/N]}$$

The terms “standard error” or “standard deviation of the mean” have also been used to describe the same quantity.

A.55 Standard Error of the Mean (s.e.m.) $\sigma_{\bar{X}}$

For a normally distributed population with mean μ and standard deviation σ, the standard deviation $\sigma_{\bar{X}}$ of the sample mean $\bar{x}$ if *N* samples taken from that population are given by:

$$\sigma_{\bar{X}} = \sigma / \sqrt{N}$$

A.56 Standard Method

A method or procedure of test developed by a standards-writing organisation, based on consensus opinion or other criteria, and often evaluated for its reliability by a collaborative testing procedure.

A.57 Sub-sample

It is a portion taken from a sample. A laboratory sample may be a sub-sample of a gross sample; similarly, a test portion may be a sub-sample of a laboratory sample.

A.58 Systematic Sampling

Sampling by some systematic method. For example, if the sampling units in a population have been arranged in order or on some systematic basis (such as in order of production), and numbered 1 to *N*, a systematic sample of n sampling units is constituted by taking the sampling units numbered:

$$h,\ h+k,\ h+2k,\ \ldots..,\ h+(n-1)k$$

where h and k are integers satisfying the relations

$$nk \leq N < n(k+1) \quad and \quad h \leq k$$

and *h* is generally taken at random from the first *k* integers.

In bulk sampling, the systematic sampling is achieved by taking items at fixed distances or after time intervals of fixed length.

A.59 Tolerance

Difference between the upper and the lower tolerance limits

A.60 Tolerance Interval; Tolerance Zone

Variate values of the characteristic between and including the tolerance limits.

A.61 Tolerance Limits, Limiting Values, Specification Limits

That range of values, calculated from an estimate of the mean and the standard deviation, within which a specified percentage of individual values of a population of measurements or samples, are expected to lie with a stated level of confidence. They are specified values of the characteristics giving upper and/or lower bounds of the permissible value.

A.62 Traceability

The property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually international or national standards, through an unbroken chain of comparisons; all having a stated uncertainty.

It is the characteristic of a measurement result or standard value that allows linking to a standard, international or national, through a chain of seamless traceable comparisons, all of whose uncertainties are denoted.

Note 1: This concept is often expressed by the adjective "traceable".

A.63 True Value

Value consistent with the definition of *a* given particular quantity.

Note 1: This is a value that would be obtained by a perfect measurement but it is by nature, not determinate.

A.64 Type A Evaluation of Uncertainty

Method of evaluation of uncertainty by the statistical analysis of series of observations.

Note 1: A Type A standard uncertainty is obtained by taking the square root of the statistically evaluated variance.

A.65 Type B Evaluation of Uncertainty

Method of evaluation of uncertainty by means other than the statistical analysis of series of observations.

Note 1: When determining a Type B standard uncertainty, it is more convenient to evaluate a non-statistical equivalent standard deviation first and then to obtain the equivalent variance by squaring the standard deviation.

A.66 Uncertainty (of a Measurement)

Parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand.

Note 1: The parameter may be, for example, a standard deviation (or a given multiple of it), or the width of a confidence level.

Note 2: Uncertainty of measurement comprises, in general, many components. Some of them may be evaluated from the statistical distribution of the results of a series of measurements and can be characterised by experimental standard deviations. The other components, which can also be characterised by standard deviations, are evaluated from assumed probability distributions based on experience or other information available.

A.67 Uncertainty (Standard) $u(x_i)$

Uncertainty of the result x_i of a measurement expressed as a standard deviation.

A.68 Uncertainty (Combined Standard) $u_c(y)$

Standard uncertainty of the result of a measurement when the result is obtained from the values of a number of other quantities, equal to the positive square root of a sum of terms, the terms being the variances or covariances of these other quantities weighted according to how the measurement result varies with these quantities.

A.69 Uncertainty (Expanded) U

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 1: The fraction may be considered as the coverage probability or level of confidence of the interval.

Note 2: To associate a specific level of confidence with the interval defined by the expanded uncertainty requires explicit or implicit assumptions regarding the probability distribution characterised by the measurement result and its combined standard uncertainty. The confidence level that may be attributed to this interval can be known only to the extent to which such assumptions can be justified.

Note 3: An expanded uncertainty U is calculated from a combined standard uncertainty u_c and a coverage factor k using the following formula:

$$U = k\,u_c$$

A.70 Variance

A measure of dispersion, which is the sum of squared deviations of observations from the average divided by one less than the number of observations, i.e. variance is the square of standard deviations.

Note 1: The symbols for sample and population variances are s^2 and σ^2, respectively.

Note 2: Variance is an important statistical tool. It is used to estimate and test hypotheses. From the variances of several samples, an analysis of variance procedure can show if the arithmetic means of several populations are likely to be equal.

A.71 α-Risk

The chance of rejecting the null hypothesis when the null hypothesis is true, i.e. concluding that there is a difference between treatments when no difference actually exists, as stated in the null hypothesis.

APPENDIX B

DISTRIBUTION FUNCTIONS

B.1 The followings are some common probability distribution functions that can be used to calculate a standard uncertainty. It may be noted that a probability distribution gives the probability for each of the values of a random variable. The choice of an appropriate distribution function depends on the knowledge of the probability distribution of the uncertainty. The standard uncertainty is then obtained by dividing the quoted uncertainty by a factor, which depends on the probability distribution.

B.2 Rectangular Probability Distribution

It is used when uncertainties are given by maximum bound within which all values are equally probable. The standard uncertainty is computed by dividing the half-interval 'a' by squared root of 3, i.e. $\sqrt{3}$.

B.3 Triangular Probability Distribution

The triangular distribution is a better model to be adopted if it is known that most of the values are likely to be near the centre of the distribution. The standard uncertainty is computed by dividing the half-interval 'a' by squared root of 6, i.e. $\sqrt{6}$.

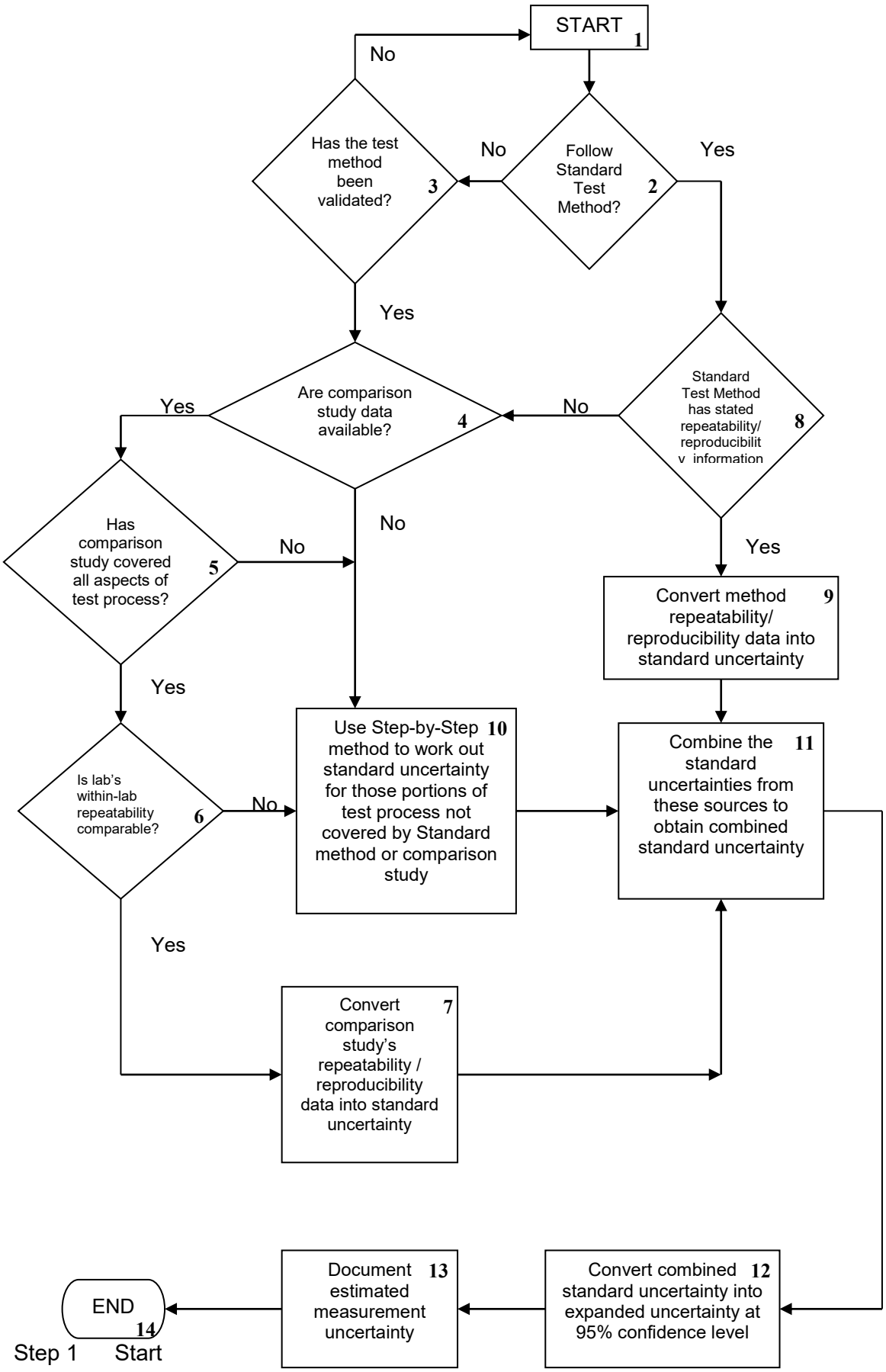
B.4 Normal (Gaussian) Probability Distribution

The normal probability distribution is by far the most common and most important continuous probability distribution. The normal curve is symmetrical and, because of its appearance, it is sometimes called a 'bell-shaped' curve. This distribution form can be assumed for an uncertainty that defines a confidence interval having a given level of confidence of say 95% or 99%. The standard uncertainty is obtained by dividing the quoted uncertainty by an appropriate factor for such a distribution. In general, the following factors are commonly used:

Commonly Used Confidence Coefficients & Confidence Intervals for a Large Sample

Confidence Coefficient	z value	General Form of the Interval Estimate
90	1.645	$\bar{x} - 1.645\sigma_{\bar{x}} < \mu < \bar{x} + 1.645\sigma_{\bar{x}}$
95	1.96	$\bar{x} - 1.96\sigma_{\bar{x}} < \mu < \bar{x} + 1.96\sigma_{\bar{x}}$
99	2.575	$\bar{x} - 2.575\sigma_{\bar{x}} < \mu < \bar{x} + 2.575\sigma_{\bar{x}}$

APPENDIX C
FLOW DIAGRAM OF THE OVERVIEW ON DIFFERENT APPROACHES TO MEASUREMENT UNCERTAINTY ESTIMATION



Step 2 Is Test Laboratory following Standard Test Method? If yes, proceed to Step 8. If no, proceed to Step 3.

Step 3 Has the test method been validated? If yes, proceed to Step 4. If no, Test Laboratory should validate the test method first before it attempts to estimate measurement uncertainty, proceed back to Step 1.

Step 4 Are comparison study data available for the test method? If yes, proceed to Step 5. If no, proceed to Step 10.

Step 5 Has the comparison study covered all aspects of test process? If yes, proceed to Step 6. If no, proceed to Step 10. If a comparison study covers only parts of the whole test process, for those portions covered, Test Laboratory may still proceed to Step 6. For the remaining portions not covered, the Laboratory need to proceed to Step 10. However, the Laboratory may proceed to Step 10 for the whole test process without taking into consideration of comparison study data.

Step 6 Is Test Laboratory's own within-lab repeatability comparable to repeatability reported in the comparison study report? If yes, proceed to Step 7. If no, proceed to Step 10.

Step 7 Test Laboratory converts comparison study's repeatability / reproducibility data into standard uncertainty (see Working Example F5). Proceed to Step 11.

Step 8 Have the Standard Test Method stated repeatability / reproducibility information? If yes, proceed to Step 9. If no, proceed to Step 4. If the Standard Test Method covers only parts of the whole test process, for those portions covered, Test Laboratory may still proceed to Step 9. For the remaining portions not covered, the Laboratory need to proceed to Step 4. However, the Laboratory may proceed to Step 4 for the whole test process without taking into consideration of the data from the Standard Test Method.

Step 9 Test Laboratory converts the Standard Test Method's repeatability / reproducibility data into standard uncertainty (see Working Example F5). Proceed to Step 11.

Step 10 Test Laboratory should use the step-by-step component-by-component estimation method (see for example Working Example F5) to work out standard uncertainty for those portions of test process that are not covered by Standard Test Method or comparison study.

Step 11 Test Laboratory then combines the standard uncertainties from all aspects covered under Steps 7, 9 and 10 to obtain the combined standard uncertainty.

Step 12 Test Laboratory converts the combined standard uncertainty into the expanded uncertainty at appropriately 95% confidence level using appropriate Student t-factor.

Step 13 Test Laboratory shall then document the estimated measurement uncertainty including the above calculations together with supporting data files to ensure data traceability for future reference.

Step 14 End.

APPENDIX D

ISO/TS 21748 APPROACH

The following write-up is extracted from Appendix B of Technical Guide 3, Guidance Document on Measurement Uncertainty for Civil Engineering and Mechanical Testing Laboratories

1. Introduction

1.1 Technical Specification ISO/TS 21748 provides an appropriate methodology for estimating uncertainty associated with results of a wide range of standard test methods subjected to collaborative study in accordance with ISO 5725-2. The methodology complies fully with the relevant principles of the GUM, whilst taking into account the method performance data obtained by collaborative study.

1.2 The general approach used in this Technical Specification requires that

- Estimates of the repeatability, reproducibility and trueness of the method in use, obtained by collaborative study as described in ISO 5725-2, are available from published information about the test method in use. These provide estimates of the intra- and inter-laboratory components of variance, together with an estimate of uncertainty associated with the trueness of the method;

- The laboratory confirms that its implementation of the test method is consistent with the established performance of the test method by checking its own bias and precision. This confirms that the published data are applicable to the results obtained by the laboratory;

- Any influences on the measurement results that were not adequately covered by the collaborative study are identified and the variance associated with the results that could arise from these effects be quantified.

1.3 An uncertainty estimate is made by combining the relevant variance estimates in the manner prescribed by GUM.

1.4 The ISO/TS 21748 assumes that recognised, non-negligible systematic effects are corrected, either by applying a numerical correction as part of the method of measurement, or by investigation and removal of the cause of the effect.

2. General Principles

2.1 Individual Results and Measurement Process Performance

2.1.1 Measurement uncertainty relates to individual results. Repeatability, reproducibility, and bias, by contrast relate to the performance of a measurement or testing process.

2.1.2 The ISO/TS 21748 requires that process performance figures derived from method-performance studies are relevant to all individual measurement results produced by the process. It will be seen that this condition requires supporting evidence in the form of appropriate quality control and assurance data for the measurement process.

2.1.3 It should also be noted that difference between individual test items may additionally need to be taken into account. However, it is unnecessary to undertake individual and detailed uncertainty studies for every test item for a well characterised and stable measurement process.

2.2 Applicability of Reproducibility Data

2.2.1 The application of the principles of the ISO/TS 21748 is based on two principles

- First, the reproducibility standard deviation obtained in a collaborative study is a valid basis for measurement uncertainty evaluation;

- Second, effects not observed within the context of the collaborative study must be demonstrably negligible or explicitly allowed for.

APPENDIX E

GENERIC WORKED EXAMPLES

The following generic worked examples are intended to show how the principles of this Guide can be applied in specific analytical processes. The readers are encouraged to prepare their own generic worked examples to suit their own needs and make their decision on which causes of errors can be ignored due to insignificant contribution to the overall uncertainty to be measured.

As the overall measurement uncertainty in a test method covers all components of uncertainty contributors, the component-by-component approach gives us the flexibility of considering only a specific component when it is changed, whilst keeping the other components intact.

Readers will appreciate the convenience of using computer spreadsheet in this aspect and are strongly recommended to store all information of each contributing component and cross-link all these components in one spreadsheet for each test method.

E.1 WEIGHING

E.1.1 Purpose

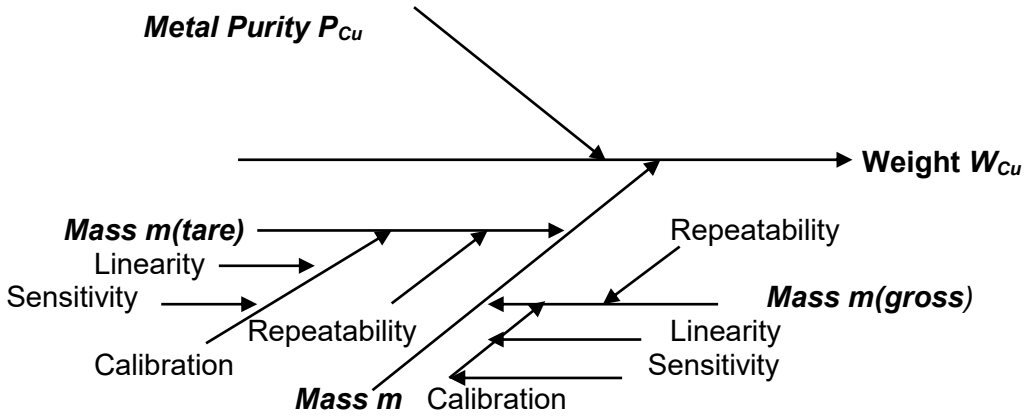
E.1.1.1 To weigh 500 mg copper wire by the weigh-by-difference method.

E.1.1.2 Weighing records:

Wt of container + Cu, g	32.5829
Wt of container, g	32.0822
Wt of Cu metal, g	0.5007

E.1.2 Identification of Uncertainty Sources

E.1.2.1 Cause-and-Effect Diagram



E.1.3 Quantification of Component Uncertainties

E.1.3.1 Purity of Copper Metal

The supplier quotes the purity of the copper wire in its certificate of analysis as 99.99 ± 0.01% without mentioning its degree of confidence.

As there is no idea of the confidence limit of this purity, we take the quoted uncertainty as the rectangular distribution, so the standard uncertainty u(P_{Cu}) is:

$$\frac{0.0001}{\sqrt{3}} = 0.000058$$

E.1.3.2 Weighing Process

E.1.3.2.1 Linearity by Calibration

The external calibration of the balance used states that the difference from the actual weight on the scale pan and the reading of the scale is within ± 0.05 mg with a 95% confidence.

Under the normal distribution, a 95% confidence gives a factor of 1.96.

Therefore, the associated uncertainty expressed as standard deviation is:

$$\frac{0.05}{1.96} \text{ or } 0.026 \text{ mg}$$

NOTE: This component uncertainty has to be taken into account twice because of two weighings involved, one before adding the copper metal and one after.

E.1.3.2.2 *Repeatability*

10 repeated measurements of a tare and gross weight gave a standard deviation of the differences in weighing as 0.06 mg at a range of 20 gm to 100 gm.

NOTE: We account for repeatability only once because it has already accounted for the weight by difference, being a standard deviation of weight differences.

E.1.3.2.3 *Sensitivity*

Sensitivity of the balance can be neglected because the weight by difference is done on the same balance over a very narrow range.

E.1.3.2.4 *Calculating the Combined Standard Uncertainty in Weighing Process*

$$u(m_{Cu}) = \sqrt{2(0.026)^2 + (0.06)^2} = 0.07 \text{ mg}$$

E.1.4 Summary of Values of Uncertainties

Description	Value x	*u(x)*	*u(x) / x*
Purity of Cu metal, P	0.9999	0.000058	0.000058
Wt of Cu metal, mg	500.7	0.07	0.00014

E.1.5 Calculation of Combined and Expanded Uncertainties

Therefore, the combined uncertainty for $u(W_{Cu})/W_{Cu}$

$= \sqrt{0.000058^2 + 0.00014^2} = 0.00015$

The expanded uncertainty using a coverage factor of 2 is:

$U(W_{Cu})/W_{Cu} = 0.00015 \times 2 = 0.00030$

For the copper weight of 500.7 mg, the report of uncertainty is therefore,

500.7 ± 0.15 mg with a coverage factor of 2

Note: that the uncertainty contribution of purity of the copper metal is quite small and can be neglected.

E.2 VOLUME PREPARATION

E.2.1 Purpose

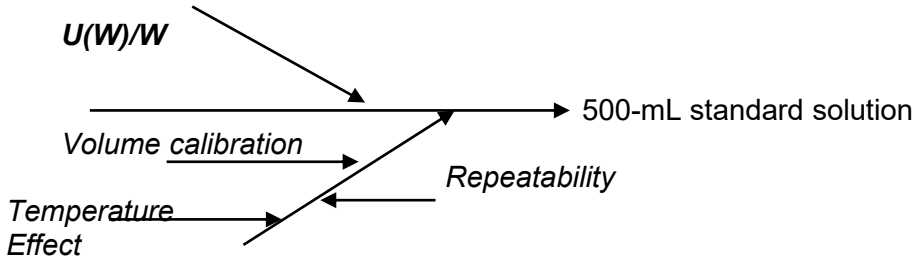
E.2.1.1 To prepare an acid digested copper nitrate standard solution from 500.7 mg copper wire in 500 mL volumetric flask.

E.2.1.2 The steps are:

- weigh 500 mg clean copper wire in a beaker
- acid digest the copper wire with 5 mL concentrated nitric acid
- when the reaction subsides and the copper has completely dissolved in the acid solution, quantitatively transfer the solution to a 500-mL volumetric flask and make up to the mark with more distilled water.

E.2.2 Identification of Sources of Uncertainty

E.2.2.1 Cause and Effect Diagram



E.2.3 Quantification of Component Uncertainties

E.2.3.1 Weighing uncertainty has been previously established at 500.7 mg ± 0.15 mg with a coverage factor of 2.

E.2.3.2 Manufacturer's volume calibration

The manufacturer states that for the flask of 500-mL, the error is ± 0.15 mL at a temperature of 20°C. As it has been given without any confidence level stated, we may assume a triangular distribution because the actual volume is more likely to be at the centre rather than at the extremes of the range.

Hence, uncertainty in calibration is $0.15/\sqrt{6}$ or 0.06 mL.

E.2.3.3 Repeatability of volume measurements

A series of 10 fill-and-weigh exercise on a typical 500-mL volumetric flask gave a standard uncertainty in the form of standard deviation as 0.04 mL. This will be used in the final calculation directly.

E.2.3.4 Temperature effect

According to the manufacturer, the flask has been calibrated at 20°C whereas the laboratory temperature varies between the limits of ± 4°C. The uncertainty from this effect can be calculated from the estimate of temperature range and the

coefficient of volume expansion. As the volume expansion of liquid is larger than that of the flask, only the liquid expansion needs to be considered here. Take the coefficient of expansion of water as 0.00021per ^{o}C.

Hence, volume expansion is:

500 mL x ± 4^{o}C x 0.00021per ^{o}C or ±0.420 mL

Calculate the standard uncertainty for the temperature variation by using a rectangular distribution: $\sqrt{3}$ or 0.24 mL

E.2.3.5 Calculation of Combined Standard Uncertainty for Volume Measurement u(V):

$$u(V) = \sqrt{0.06^2 + 0.04^2 + 0.24^2} = 0.25$$

E.2.4 Summary of Values of Uncertainties in volume preparation VOL

Description	Value x	*u(x)*	*u(x) / x*
Weighing Cu metal, mg	500.7	0.07	0.00014
Volume made up, mL	500	0.25	0.0005

E.2.5 Calculation of Combined Uncertainty and Expanded Uncertainty

Combined uncertainty of preparing 500.7 mg Cu in 500 mL solution, considering uncertainties in weighing and volume preparation is:

$$u(Conc)/Conc = \sqrt{[u(W)/W]^2 + [u(V)/V]^2}$$

$$= \sqrt{[0.07/500.7]^2 + [0.25/500]^2}$$

$$= 0.00052$$

As the concentration of Cu solution is 500.7 mg/500 mL or 1001.4 mg/L,

$U(Conc)$ = 0.00052 x 1001.4 mg/L = 0.52 mg/L

Expanded Uncertainty of preparing 500.7 mg Cu in 500 mL solution or a concentration of 1001.4 mg/L is 0.52 x 2 or 1.04 mg/L with a coverage factor of 2.

Hence, the concentration of Cu solution prepared is 1001.4 ± 1.0 mg/L with a coverage factor of 2.

E.2.6 Remarks:

E.2.6.1 As it can be seen from the above, contribution of uncertainty in weighing is much smaller than that in volume preparation.

E.3 CALCULATING THE MOLECULAR WEIGHT OF A SOLUTE

E.3.1 Purpose

On preparation of a molar or a normal solution, the molecular weight of the solute needs to be known and hence its uncertainty in estimating the molecular weight. For example, we are required to calculate the uncertainty in calculating the molecular weight of potassium permanganate, $KMnO_4$.

E.3.2 IUPAC Commission on Atomic Weights and Isotopic Abundances

IUPAC has published a list of elements with their individual atomic weight and associated uncertainty in its journal *Pure Appl. Chem*., Vol 69, pp. 2471-2473 (1997). A list of common elements is quoted below for easy reference:

Element Name	**Atomic Weight**	**Associated Uncertainty**
Hydrogen H	1.00794	0.00007
Carbon	12.0107	0.0008
Nitrogen	14.00674	0.00007
Oxygen	15.9994	0.0003
Fluorine	18.9984032	0.0000005
Sodium	22.989770	0.000002
Magnesium	24.3050	0.0006
Aluminum	26.981538	0.000002
Phosphorus	30.973761	0.000002
Sulphur	32.066	0.006
Chlorine	35.4527	0.0009
Potassium	39.0983	0.0001
Calcium	40.078	0.004
Chromium	51.9961	0.0006
Manganese	54.938049	0.000009
Iron	55.845	0.002
Cobalt	58.933200	0.000009
Nickel	58.6934	0.0002
Copper	63.546	0.003
Zinc	65.39	0.02
Arsenic	74.92160	0.00002
Bromine	79.904	0.001
Silver	107.8682	0.0002
Cadmium	112.411	0.008
Tin	118.710	0.007
Antimony	121.760	0.001
Iodine	126.90447	0.00003
Barium	137.327	0.007
Mercury	200.59	0.02
Lead	207.2	0.1

A complete list of all elements and their uncertainties can be found on website: http://www.chem.qmw.ac.uk/iupac/AtWt/

E.3.3 Calculation of Molecular Weight of $KMnO_4$ and Its Uncertainties

E.3.3.1 Atomic weights and listed uncertainties (from IUPAC tables) for the constituent elements of $KMnO_4$ are as follows:

Element	Atomic Weight *AW(e)*	Quoted Uncertainty *u(e)*	Standard Uncertainty *u(e)/√3*
K	39.0983	0.0001	0.000058
Mn	54.938049	0.000009	0.0000052
O	15.9994	0.0003	0.00017

Note: $\sqrt{3}$ is being used here by treating the IUPAC quoted uncertainty as forming the bounds of a rectangular distribution.

E.3.3.2 The calculated molecular weight of $KMnO_4$ is:

$$MW_{KMnO4} = 39.0983 + 54.938049 + 4 \times 15.9994$$

$$= 158.0339 \text{ g.mol}^{-1}$$

$$u(MW_{KMnO4}) = \sqrt{0.000058^2 + 0.0000052^2 + (4 \times 0.00017)^2}$$

$$= 0.0007 \text{ g.mol}^{-1}$$

E.3.4 The elemental contribution to $KMnO_4$ is simply the sum of the single atom contributions. Hence, combined uncertainty would be calculated as a square root of the sum of squares of each contributing atom.

E.4 CALIBRATION CURVE

E.4.1 Linear Corelationship

An analytical method or instrument is often calibrated by observing the responses, y, to different levels of the analyte, *x*. In most cases this relationship is taken to be linear, i.e. $y = a + bx$ with *a* being the intercept and *b* being the slope of the calibration curve. In this case, the concentration x_{obs} of the analyte from a sample which produces an observed response y_{obs} is then given by $x_{obs} = (y_{obs} - a)/b$.

In some cases, analytical methods require such linear relationship with forced-zero, i.e. intercept $a = 0$. In these cases, linear relationship is $y = bx$ and $x_{obs} = y_{obs}/b$.

The common method of fitting a linear relationship based on individual calibration data pairs (x_i, y_i) is by using linear least squares calibration method (with or without forced zero).

E.4.2 Sources of Uncertainty

There are four main sources of uncertainty to consider when estimating uncertainty of x_{obs}:

a) Random variations in measurement of y (inclusive of y_i and y_{obs});

b) Random effects in assigned reference value x_i;

c) constant unknown offset on x_i and y_i;

d) the assumption of linearity may not be valid.

Of these four sources, the most significant one is (a). Method for estimating (a) introduced below is through variance of residuals, *S*. *S* can be calculated from

$$S^2 = \Sigma(y_i - y_c)^2/(n-2)$$

whereby

y_i is reading of i^{th} calibration point,
y_c is the calculated reading from the relation $y = a + bx$,
n is the number of calibration points.
and,
$u(x_{obs},y)=\sqrt{var(x)}$ with $var(x) = S^2/b^2$

E.4.3 A Worked Example

For this example, 3 levels of calibration standards are used and their responses are:

Concentration, x_i	**Response, y_i**
5	125
50	1,197
200	4,754

For $y=a+bx$ to be fitted to the above calibration, its a and b can be worked out as:

$$b = \frac{\sum x_i y_i - n\bar{x}\bar{y}}{\sum x_i^2 - n\bar{x}^2} \qquad a = \bar{y} - b\bar{x}$$

In this example,

	x	***y***	***xy***	***x²***
	5	125	625	25
	50	1197	59850	2500
	200	4754	950800	40000
Sum	255	6076	1011275	42525
Average	85	2025.333		

Therefore,

$$b = \frac{\sum x_i y_i - n\bar{x}\bar{y}}{\sum x_i^2 - n\bar{x}^2} = \frac{1011275 - 3 \times 85 \times 2025.333}{42525 - 3 \times 85^2} = 23.732$$

$a = \bar{y} - b\bar{x}$ = 2025.333 – 23.7321343 x 85 = 8.102

Thus, $y = a + bx$ = 8.102 + 23.732x.

With this equation, one can work out calculated responses y_c with known x and their corresponding square of difference $(y-y_c)^2$:

x	***Y***	***Calculated y_c***	***$(y-y_c)^2$***
5	125	126.76259	3.10672
50	1197	1194.7086	5.25036
200	4754	4754.5288	0.27961

Thus,

$S^2 = \Sigma (y_i-y_c)^2/(n-2)$
= (3.10672+5.25036+0.27961)/(3-2)
= 8.63669.

$var(x) = S^2/b^2$
= 8.63669/23.7321343²
= 0.0153346

$u(x_{obs},y) = \sqrt{var(x)}$
= √ 0.0153346
= 0.124

E.5 APPLICATION OF GC-MS

E.5.1 Purpose

The following example shows the measurement of uncertainties in GC-MS (gas chromatographic – mass spectrometric) technique.

E.5.2 The following steps are taken to appraise the measurement uncertainty concerned.

E.5.2.1 Step 1: Specification

The chemist uses the GC-MS technique to analyse biphenyl impurity in benzene. The standard used for calibration is a 50 µg/mL standard solution and a blank solution (i.e. 0 µg/mL).

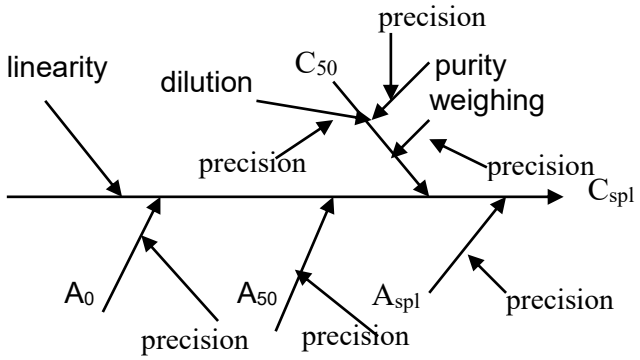
The concentration (µg/mL) of biphenyl in benzene can be calculated using two-point calibration method (bracketing method):

$C_{spl} = A_{spl} \times C_{50}/(A_{50} - A_0)$

where,

A_{spl} Area response of GC-MS for biphenyl in the sample
A_{50} Area response of GC-MS for biphenyl in the standard
A_0 Area response of GC-MS for biphenyl in the blank
C_{50} Concentration of biphenyl in the standard solution which has a nominated concentration of 50 µg/mL biphenyl

E.5.2.2 Step 2: Identify Uncertainty Sources



E.5.2.3 Step 3: Quantifying Uncertainty Components

C_{50}:

The standard solution is prepared from biphenyl solid, first by weighing and then by dissolution and dilution in benzene.

For weighing by difference, a standard uncertainty of 0.000206 g is obtained as shown in the *previous example* for 0.052 g of biphenyl.

The purity of biphenyl is stated to be more than 99.0% by the supplier. Thus, the purity of the raw material is calculated to be 99.5% associated with a standard uncertainty (100%-99%) / $\sqrt{12}$ = 0.289%.

The solid biphenyl is then dissolved and diluted to 1,000 mL using volumetric flask. The specification of the nominal 1L volumetric flask used states the accuracy of 1000.22 ± 0.20 mL. In this case, a triangular distribution is used as past QC checks

has shown that centre distribution is more likely than those near the bounds. Thus, the standard uncertainty from glassware certification is 0.20 / $\sqrt{6}$ = 0.0816 mL.

Repeated filling and weighing has shown a standard uncertainty of 0.15 mL.

For temperature variation effect on the volume of benzene used for dissolution and dilution, as there is no available data on expansion coefficient of benzene, we assume that its expansion is about twice as much as water at ambient temperature (which has an expansion coefficient of 2.1×10^{-4} per °C). From our experience, we know that this estimation should be sufficient.

Thus, the standard uncertainty arising from temperature variation is
$1000.22 \times (4.0/2) \times 4.2 \times 10^{-4} = 0.840$ mL.

The standard uncertainty due to dissolution and dilution is

$\sqrt{(0.0816^2 + 0.15^2 + 0.840^2)} = 0.857$ mL.

Therefore, C_{50} and its standard uncertainty are calculated as follows:

		m	P	V
	Value	0.052	99.5%	1000.22
	Uncertainty	0.000206	0.289%	0.857
m	0.052	0.052206	0.052	0.052
P	99.5%	99.5%	99.789%	99.5%
V	1000.22	1000.22	1000.22	1001.077
C_{50}	**51.72862**	51.9335	51.8789	51.6843
		0.20492	0.15025	-0.04428
	0.066529	0.04199	0.02257	0.00196
$u(C_{50})$	**0.257933**			

A_0, A_{50}, A_{spl}

Replicate measurements have given the following results:

	GC-MS Area Response		
	A_0	A_{50}	A_{spl}
1	2	390	265
2	0	397	260
3	0	395	269
4	1	394	266
5	0	398	263
6	2	396	268
7	2	391	265
8	1	392	262
9	0	396	267
10	1	395	265
Mean	**0.9**	**394.4**	**265**
SD	0.876	2.633	2.749
SD of the Mean	**0.277**	**0.833**	**0.869**

The standard deviations of means in the above table are used directly as the standard uncertainty associated with the mean values, which will be used in final calculation.

Linearity:

Two-point calibration (bracketing method) assumes linearity within the concentration range to be determined. However, studies have shown that, by analysing biphenyl solution at various known concentration levels, the maximum deviation from the true results are 1.0 µg/mL. A rectangular distribution is assumed and thus, the standard uncertainty due to linearity is

$1.0/\sqrt{3}$ = 0.577.

E.5.2.4 Step 4: Calculate Total Uncertainty

As the linearity is on the final result, it will be combined later.

First, the standard uncertainties due to C_{50}, A_0, A_{50} and A_{spl} are combined by spreadsheet method as shown in the next page to give a concentration in the sample as 34.836 µg/mL with a standard uncertainty of 0.266 µg/mL.

Thus, the total combined standard uncertainty is $\sqrt{(0.222^2 + 0.577^2)}$ = 0.618 µg/mL.

		C_{50}	**A_0**	**A_{50}**	**A_{spl}**
	Value	51.72862	0.9	394.4	265
	Uncertainty	0.257933	0.277	0.833	0.869
C_{50}	51.72862	51.98655	51.72862	51.72862	51.72862
A_0	0.9	0.9	1.177	0.9	0.9
A_{50}	394.4	394.4	394.4	395.233	394.4
A_{spl}	265	265	265	265	265.869
C_{spl}	**34.8363**	**35.01**	**34.861**	**34.7627**	**34.9505**
		0.173703	0.0245	-0.07359	0.11424
	0.04924	0.030173	0.0006	0.00542	0.01305
$u(C_{spl})$	**0.2219**				

To calculated expanded uncertainty at 95% confidence, k = 2.26 has to be used as only 10 determinations are available (degrees of freedom = 9). Expanded uncertainty is thus $U(C_{spl})$ = 0.618 x2.26 = 1.397 µg/mL.

Therefore, the result is:

34.8 ± 1.4 (µg/mL)*

*The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2.26 for 9 degrees of freedom which gives a level of confidence of approximately 95%

E.5.3 Alternative Way of Combining Standard Uncertainties:

As C_{50}=*1000000 x mP/V,*

thus,

$C_{spl} = A_{spl} \times C_{50}/(A_{50}–A_0) = 1000000 \times mPA_{spl}/[V(A_{50}–A_0)]$:

		m	***P***	***V***	A_0	A_{50}	A_{spl}
	Value	0.052	99.5%	1000.22	0.9	394.4	265
	Uncertainty	0.000206	0.289%	0.857	0.277	0.833	0.869
m	0.052	0.052206	0.052	0.052	0.052	0.052	0.052
P	99.5%	99.5%	99.789%	99.5%	99.5%	99.5%	99.5%
V	1000.22	1000.22	1000.22	1001.077	1000.22	1000.22	1000.22
A_0	0.9	0.9	0.9	0.9	1.177	0.9	0.9
A_{50}	394.4	394.4	394.4	394.4	394.4	395.233	394.4
A_{spl}	265	265	265	265	265	265	265.869
C_{spl}	**34.8363**	34.9743	34.9375	34.8065	34.8608	34.7627	34.9505
		0.13801	0.10118	-0.0298	0.02454	-0.0736	0.11424
	0.04924	0.01905	0.01024	0.00089	0.0006	0.00542	0.01305
$u(C_{spl})$	**0.221902**						

This gives the same result as in the previous spreadsheet.

E.6 ESTIMATION OF BIAS BASED ON THE RECOVERY DATA

E.6.1 In general, test recovery is defined as: "Proportion of the amount of analyte, present in or added to the analytical portion of the test material, which is then extracted and presented for measurement." Such recovery studies form an essential component of the validation and use of all analytical methods to check their accuracies.

E.6.2 Recovery data *R* is obtained as the ratio of the concentration of analyte found by the method to that stated to be present (known or true value) and can be used to determine the bias present, if any, in that particular test method. If the bias does exist (i.e. the test results obtained are consistently higher or lower than the true value), an investigation must be made to find out the cause of such systematic error and minimize it, if possible. If not, the test result must be adjusted by a correction factor.

E.6.3 To get a recovery data, one has to add a known amount of analyte to a matrix and the whole matrix is then subject to a normal analysis. The amount recovered minus the original amount present should indicate the recovery factor.

E.6.4 In a perfect situation, *R* would be exactly unity (1) but in reality, circumstances such as imperfect extraction often give observations that differ from the ideal.

E.6.5 Hence, we must take note of the sources of uncertainty in recovery estimation. Some of them are:

a. repeatability of the recovery experiment
b. uncertainties in reference material values
c. uncertainties in added spike quantity (in terms of weight or volume)
d. poor representation of native (originally present) analyte by the added spike
e. poor or restricted match between experimental matrix and the full range of sample matrices encountered
f. effect of analyte / spike level on recovery and imperfect match of spike or reference material analyte level and analyte level in samples.

E.6.6 We can test the recovery for any significant departure from unity by the *t*-test. Such significance testing considers the question:

" Is $|R - 1|$ greater than u_R, the uncertainty in the determination of *R*?"

The significance testing can be done as follows:

H_0 : $|R - 1| / u_R \leq t$ ⟶ *R does not differ significantly from 1*

H_1 : $|R - 1| / u_R > t$ ⟶ *R differs significantly from 1*

where *t* is the critical value based either on:

- a ' coverage factor' allowing for practical significance, or
- where the test is entirely statistical, $t(n-1, \alpha/2)$ being the relevant value of Student's *t*-distribution table for a level of confidence $1-\alpha$.

E.6.7 IUPAC (*Pure Appl. Chem. Vol 71, pp 337-348, 1999*) has suggested the following cases depending on the recovery *R* considered:

a. *if R* is not significantly different from 1, *no* correction is applied.

b. *if R* is significantly different from 1 and a correction for R is applied.

c. *if R* is significantly different from1 but, for operational reasons, no correction for R is applied.

E.6.8 For case (b) when a correction of *R* has to be explicitly included in the calculation of the corrected result, i.e.

$$c_{corr} = \frac{c}{R} \quad \text{… Eq [1]}$$

where *c* is the raw results with an uncertainty u_c, it is obvious that we must include uR in the uncertainty budget. This led us to a combined uncertainty u_{corr} on the corrected result given by:

$$\frac{u_{corr}}{c_{corr}} = \sqrt{\left[\frac{uc}{c}\right]^2 + \left[\frac{uR}{R}\right]^2} \quad \text{…Eq [2]}$$

u_{corr} would be multiplied by a coverage factor *k* (usually 2) to obtain the expanded uncertainty *U*.

But, how are we going to calculate the uncertainty of recovery, u_R and uncertainty of bias, u_B?

E.6.9 Example 1:

In a measurement of trace metal analysis in water, the laboratory has shown that a recovery mean for copper contents in 10 samples of distilled water (with added 3.50 mg/L Copper) is 3.65 mg/L with a standard deviation of 0.18 mg/L. The 'native' copper content in the distilled water has been found to be below its detection limit.

Hence, the % recovery of copper in this analysis is = 3.65/3.50 or 104%.

Use the *t*-distribution formula:

$$\mu = x \pm t\,(s/\sqrt{n}) \quad \text{… Eq [3]}$$

In fact, the uncertainty of recovery, u_R as shown by the above equation is the factor, $s/\sqrt{n}$.

Hence,

$$|t| = \mu - \bar{x} \,/\, (s/\sqrt{n})$$

and so,

$$|t| = 3.50 - 3.65 \,/\, (0.18/\sqrt{10})$$

= 2.64, which is ***larger*** *than the critical t- value of 2.26 for 9 degree of freedom at 95% confidence.*

This indicates that the figure 3.65% found by the experiment is significantly larger than the 3.50% spiked amount and therefore a bias exists. The experimental value

can be corrected by applying a correction factor which is to be the reverse of the recovery mean of 104%, i.e. a factor of 0.96.

However, if for some practical reason, the bias cannot be corrected, then the standard uncertainty due to bias, $u_{(B)}$, is to be calculated by incorporating:

(i) the uncertainty of bias, *(1-R)/t* if we do not correct it, and
(ii) the uncertainty of such recovery, u_R, as below:

$$u_{(B)} = \sqrt{\{[(1-R)/k]^2 + u^2 R\}} \quad \text{… Eq [4]}$$

where: R = mean ratio of the recovered data divided by the known value
k = the Student *t*-value at a given degree of freedom
u_R = standard uncertainty of the mean ratio R

By the above example, we have

$R = 1.04$
$t = 2.64$
$u_R = 0.18 / \sqrt{10} = 0.057$

Hence,

$$u_{(B)} = \sqrt{\{\left[(1-1.04) / 2.64\right]^2 + (0.057)^2\}} = 0.059.$$

E.6.10 Example 2:

An estimate of the method bias can be obtained from QC data by comparing testing results with the target value. For example, the target value for a Cu check solution is 10.03 ppb. The last 10 days' testing results are 9.98, 10.33, 10.21, 10.15, 10.23, 10.29, 10.31, 10.27, 10.20, 10.28 ppb. The ratios of laboratory-result over target value are calculated as 0.9950, 1.0299, 1.0179, 1.0120, 1.0199, 1.0259, 1.0279, 1.0239, 1.0169, and 1.0249. The mean (R) of these ratios is R = 1.01942 with a standard deviation (S_R) of S_R = 0.01019. The standard uncertainty is calculated as the standard deviation of the mean: $u(R) = S_R/\sqrt{n} = 0.01019/\sqrt{10} = 0.00322$.

To determine whether there is a significant bias, we need to determine whether R is significantly different from 1. The appropriate test is:

$$t = \frac{|1-R|}{u(R)} = \frac{|1-1.01942|}{0.00322} = 6.03$$

The two-tailed critical t value at 95% for d.f.= n-1 = 9 is 2.26. Therefore, R is significantly different from 1, i.e. bias exists.

It is a general requirement of ISO GUM that corrections should be made for all significant bias. Thus, a correction factor equal to 1/R shall be applied in the mathematical model when calculating the testing results for samples. With this factor applied, u(R) will be included in the calculation of combined standard uncertainty.

If the bias in the above case has not been corrected due to some practical reason, then, standard uncertainty due to bias u(B) is calculated as u(B)=

$\sqrt{\{[(1-R)/k]^2 + u^2 R\}}$, where R is the mean ratio obtained above, u(r) is the standard uncertainty of the mean ratio obtained above, and k is the student t-value at a given degrees of freedom. For the case above, standard uncertainty due to bias without bias correction is u(B) = $\sqrt{\{[(1-R)/k]^2 + u^2 R\}}$ = $\sqrt{\{[(1-1.01942)/2.26]^2+0.00322^2\}}$ = 0.00918.
Assuming there is no bias detected in the above case and the relative standard uncertainty of the check solution is u(std) = 0.0022, then u(B) = $\sqrt{[u^2(std)+u^2(R)]}$ = $\sqrt{[0.0022^2+0.00322^2]}$ = 0.0039.

Recovery data can also be used to determine bias present in a method. The target value for recovery is 100%. For example, for measurement of pesticide in butter, a study has shown a recovery mean for 33 samples is 109% with a standard deviation of 12%. The standard uncertainty of the mean is u(Rec) = 12%/$\sqrt{33}$ = 0.020889. The t test result is t= $|$109%-100%$|$/0.020889 = 4.31. This t value is larger than the critical t value for 32 d.f. at 95% confidence. Therefore, bias exists and has to be corrected by applying a correction factor which is the reverse of the recovery mean.

APPENDIX F

WORKED EXAMPLES

F.1 ACID/BASE TITRATION: DETERMINATION OF CONCENTRATION OF HCl SOLUTION

F.1.1 METHOD

F.1.1.1 Ascertain the 0.1M sodium hydroxide (NaOH) solution by titrating against 0.1M potassium hydrogen phthalate (KHP) solution;

F.1.1.2 Then determine the concentration of approximately 0.1M HCl solution by titrating against the 0.1M NaOH solution.

F.1.2 PROCEDURE

Step 1 : Weigh 5 gm KHP powder accurately

Step 2 : Dissolve the KHP in water and make up to 250 ml volume

Step 3 : Calculate the molarity of KHP

Step 4 : Dissolve 2 gm NaOH pellets in water and make up to 500 ml volume

Step 5 : Pipette 25 ml NaOH solution in a conical flask

Step 6 : Titrate the NaOH solution against the KHP solution from a 50-ml burette

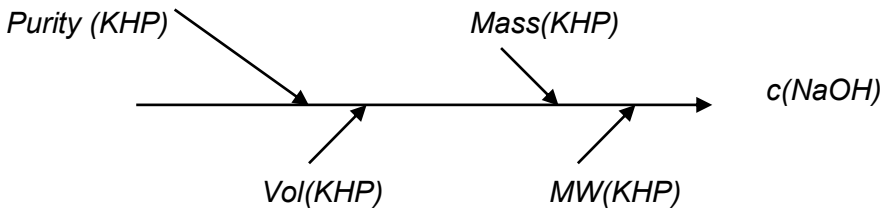
Step 7 : Calculate the concentration of NaOH solution

Step 8 : Pipette an aliquot of 25 ml NaOH solution into a conical flask

Step 9 : Titrate the standardized NaOH solution against the HCl solution from a 50-ml burette

Step 10: Calculate the strength of HCl solution

F.1.3 CAUSE AND EFFECT DIAGRAM (for determining the concentration of NaOH solution)

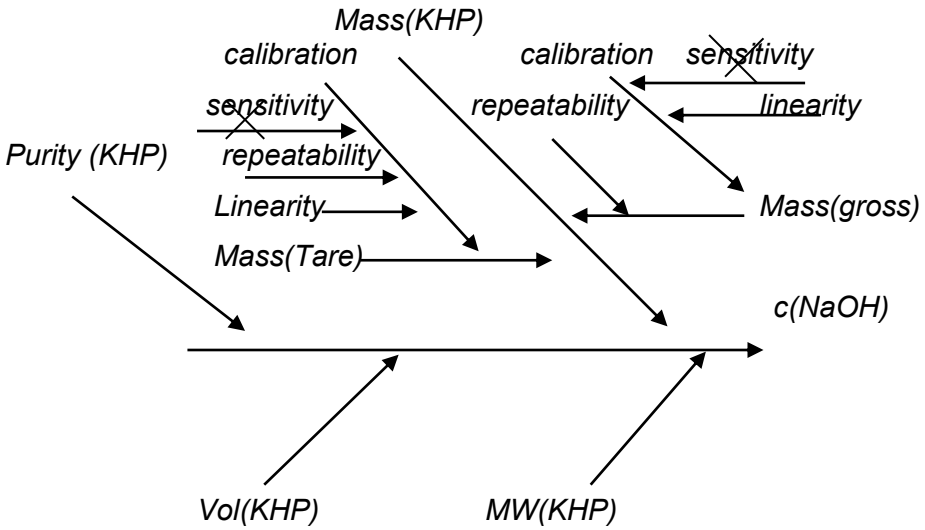


F.1.4 EVALUATION OF UNCERTAINTY COMPONENTS

F.1.4.1 Step 1: Weighing the KHP

F.1.4.1.1 Workings*:*

Container + KHP	33.5895 g
Empty Container	28.5130 g
Weight of KHP	5.0765 g



F.1.4.1.2 Sources of Uncertainties:

a. *Associated with the calibration of the balance used*

   The calibration certificate indicates that at a 95% confidence level, a weight obtained by difference within the same range is within ± 0.1mg of the displayed value. This uncertainty component can be expressed as a standard deviation by dividing 0.1 by 1.96, giving 0.052 mg.

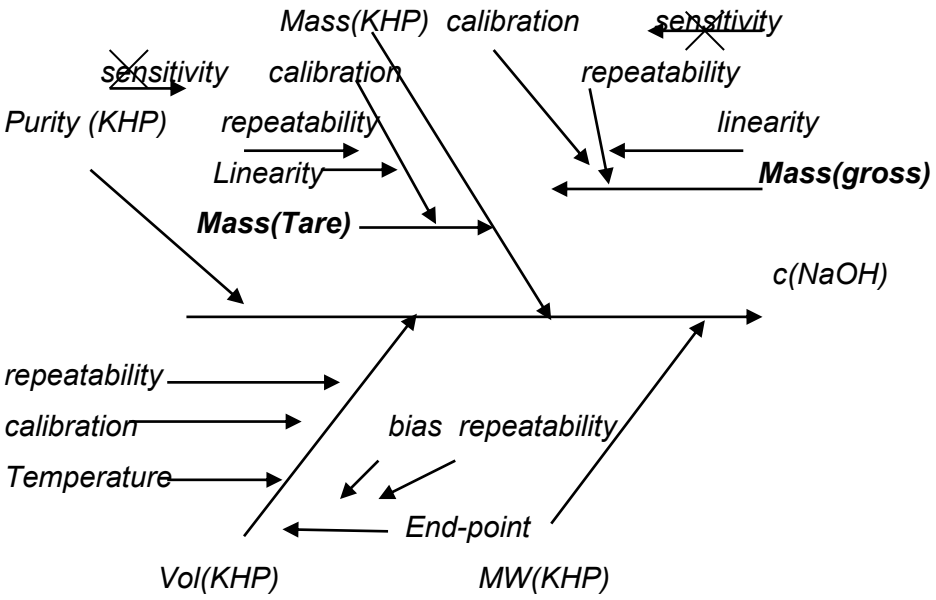
b. *Uncertainty associated with the standard deviation of replicated weighings up to 50 g.*

   The laboratory QA report shows that a standard deviation of 0.09 mg was found in this balance used after 7 replicated weighings.

   Combined uncertainties of weighing $u(W_k)$:

   $u(W_k) = \sqrt{(0.052^2 + 0.09^2)} = \pm 0.104$ *mg*

F.1.4.2 Step 2: Preparation of KHP standard solution



F.1.4.2.1 Workings*:*

Dissolve 5 gm KHP and make up to 250 ml in a volumetric flask.

F.1.4.2.2 Sources of Uncertainties

a. *Uncertainty of the volume of the volumetric flask used*

The manufacturer's catalog states that the 250-ml flask comes with an uncertainty of ± 0.15 ml without mentioning the degree of confidence. Hence, a rectangular distribution of errors is assumed with a factor of $\sqrt{3}$. The standard deviation of the volume is therefore 0.15 / $\sqrt{3}$ = 0.087 ml

b. *Uncertainty in filling up to the volume designated*

The standard deviation for the variation of the total volume of the volumetric flask calculated after a series of replicated (10 times) filling and weighing of water to the mark is found to be 0.014 ml. This value will be used in the later calculation of the uncertainty in volume measurement.

c. *Differences between solution temperature and the calibration temperature of the volumetric flask*

Consider only the coefficient of volume expansion of the solution because it is considerably greater than that of the volume of expansion of flask made of glass, for practical purposes.

Take the coefficient of volume expansion for water as 2.1 x 10^{-4} per °C and the temperature variation between that of the solution and the calibration temperature as 5 degrees. For the volume of 250 ml used, this will give a 95% confidence interval of:

250 ml x 5 °C x 2.1 x 10^{-4} per °C per ml or 0.263 ml

The standard deviation of the temperature difference therefore is:

0.263 / 1.96 *or* 0.13 ml

Combined uncertainties of KHP standard volume, $u(V_k)$ is:

$u(V_k) = \sqrt{(0.087^2 + 0.014^2 + 0.13^2)} = 0.16$ *ml*

F.1.4.3 Step 3: Calculating the concentration of KHP solution

F.1.4.3.1 Workings:

The concentration of this KHP solution, M_k is calculated from the formula:

$M_k = (W_k \times P \times 1000)/(V_k \times MW)$ … [1]

Where,

W_k = *Weight of KHP used (5.0765 g)*
P = Purity of KHP (99.8 ± 0.2%)
V_k = Volume of solution made (250 ml)
MW = Molecular weight of KHP of formula $C_8H_5O_4K$

F.1.4.3.2 Sources of Uncertainty

In addition to the uncertainties of W_k and V_k where have been examined earlier, there are two more uncertainties to be determined, via:

a) Uncertainty for Purity of KHP

Purity of KHP has been provided by the supplier as 99.8% ± 0.2%, meaning P is 0.998 ± 0.002. As there is no confidence level stated for the uncertainty, we have to take a rectangular distribution of error with a factor of $\sqrt{3}$, giving $u(P)$ as:

$u(P) = 0.002/\sqrt{3}$ *or* 0.0012

b) Uncertainty of Molecular Weight (MW) of KHP

The molecular formula of potassium hydrogen phthalate is $C_8H_5O_4K$. Consider the table of atomic weights of elements, C, H, O, and K, including uncertainty estimates published by the *IUPAC Journal of Pure and Applied Chemistry, vol. 66, No. 12 (1994), pages 2423-2444*, as follows:

Element	**Atomic Weight**	**Uncertainty Quoted**	**Standard Uncertainty Calculated**
C	12.011	± 0.001	0.00058
H	1.00794	± 0.00007	0.00004
O	15.9994	± 0.0003	0.00017
K	39.0983	± 0.0001	0.000058

Note: *the standard uncertainties are calculated by dividing the quoted uncertainties by* $\sqrt{3}$.

The contribution of uncertainty of each element to the molecular weight of KHP is then calculated by multiplying each standard uncertainty by the number of atoms of each element in the molecular formula, and the results are tabulated as below:

No. Of Atoms In The formula	**Calculated Weight**	**Calculated Results**	**Uncertainty Contributed**
C_8	8 x 12.011	96.088	0.0046
H_5	5 x 1.00794	5.0397	0.00020
O_4	4 x 15.9994	63.9976	0.00068
K	1 x 39.0983	39.0983	0.000058
	Molecular weight	204.2236	

The molecular weight of KHP is therefore 204.2236 and the combined uncertainty *u(MW)* is the square root of the sum of squares of the individual uncertainties, i.e.

$$u(MW) = \sqrt{(0.0046^2 + 0.0002^2 + 0.00068^2 + 0.000058^2)}$$
$$= 0.0047$$

Having considered all the contributions of uncertainties, we can summarize them as below:

Uncertainty Factor	**Values To Be Used, *V***	**Uncertainty, *u***	**Relative Standard Deviation (RSD) = *u/V***
W_k	5.0765 g	0.000104	2.05×10^{-5}
P	0.998	0.0012	1.202×10^{-3}
V_k	250 ml	0.16	6.4×10^{-4}
MW	204.2236	0.0047	2.3×10^{-5}

The standard uncertainty in the concentration (M_k) of this KHP standard solution therefore is expressed as:

$$\frac{u(M_k)}{M_k} = \sqrt{(0.0000205^2 + 0.001202^2 + 0.00064^2 + 0.000023^2} = 0.0014 \text{ mol } L^{-1}$$

Now, the concentration of KHP solution, M_k, is calculated from equation [1] as follows:

M_k = (5.0765 x 0.998 x 1000) / (250 x 204.2236) = 0.0992 mol L^{-1}

Hence, the standard uncertainty *u(Mk)* in the concentration of KHP solution is:

$u(M_k)$ = 0.0014 x 0.0992 = 0.00014

The concentration of KHP solution is therefore 0.0992 mol L^{-1} with a standard uncertainty of 0.00014 mol L^{-1}.

F.1.4.4 Step 4 : Preparation of NaOH solution

As the sodium hydroxide solution prepared is to be standardized by the KHP standard solution by direct chemical analysis, the uncertainties associated in the

preparation of NaOH solution are not considered although the purity of sodium hydroxide and the volume of total solution prepared have certain uncertainty.

F.1.4.5 Step 5: Pipette an aliquot (25ml) solution of NaOH solution into a conical flask

As in Step 2, the following components are to be considered when transferring an aliquot of 25 ml NaOH solution for titration:

a. *Uncertainty in the stated internal volume of the pipette used*

The pipette manufacturer states that the pipette used has an uncertainty of ±0.03ml. By approximating to a rectangular distribution because of unknown confidence level, the standard deviation of the volume of this pipette to be measured is 0.03 / $\sqrt{3}$ or 0.017 ml.

b. *Uncertainty in the filling of pipette to 25 ml*

Replicate weighing measurements of the volume of 25 ml with this pipette give a standard deviation of 0.0010 ml, which will be used directly in the final calculation of standard uncertainty.

c. *Uncertainty in the variation of volume by the effect of temperature (temperature of measurement Vs calibration temperature of the pipette)*

Taking the possible temperature variation of 5 °C and the coefficient of volume expansion of glass as 2.1 x 10^{-4} per °C, the 95% confidence level of volume measurement due to temperature factor is:

25 x 5 x 2.1 x 10^{-4} ml *or* 0.0263 ml

Therefore, the standard deviation for temperature variation is 0.0263 / 1.96 or ± 0.013 ml.

Combining all these 3 sources of uncertainty, we have the uncertainty $u(V_S)$ in the volume transfer of NaOH solution as the square root of the sum of squares of these 3 standard deviations, giving the result of:

$$u(V_S) = \sqrt{(0.017^2 + 0.0010^2 + 0.013^2)} \text{ or } 0.021$$

F.1.4.6 Step 6: Titration of the NaOH solution against the standard KHP solution (V_a)

The 25 ml NaOH solution is titrated against the standard KHP solution from a 50-ml burette. Again, we need to consider the sources of uncertainty from the point of view of the similar 3 factors discussed earlier, via:

a. *Uncertainty in the stated volume of the 50-ml burette*

The manufacturer states that the burette used has an uncertainty of ± 0.05ml. By approximating to a rectangular distribution because of unknown confidence level, the standard deviation of the volume of this pipette to be measured is 0.05 / $\sqrt{3}$ or 0.029 ml.

b. *Uncertainty in the volume of KHP standard solution used for titration*

As it is expected to use about 25-ml KHP standard solution in the titration exercise, repeated deliveries and weighing of 25-ml volumes from the burette were checked and gave a standard deviation of 0.012 ml.

We shall use this figure as the standard uncertainty of the volume used.

c. *Uncertainty of the temperature effect between the titration temperature at room temperature and the calibration temperature of the burette*

Taking the possible temperature variation of 5 °C as before and the coefficient of volume expansion of glass as 2.1 x 10^{-4} per °C, the 95% confidence level of volume measurement, due to temperature factor is:

25 x 5 x 2.1 x 10^{-4} ml *or* 0.0263 ml

Therefore, the standard deviation for temperature variation is 0.0263 / 1.96 or ± 0.013 ml.

In this titration exercise, 25.20 ml of the KHP solution was found to be used in achieving the end point with the NaOH solution. Hence, using the figures obtained above, the combined standard uncertainty $u(V_a)$ is calculated as :

$$u(V_a) = \sqrt{(0.029^2 + 0.012^2 + 0.013^2)} \text{ or } 0.034 \text{ ml}$$

F.1.4.7 Step 7: Calculation of the concentration of NaOH solution

The formula used in the calculation of the NaOH solution is:

$$M_s = (M_k \times V_a) / V_s \quad \ldots [2]$$

Where,
M_s = concentration of the NaOH solution
M_k = concentration of the KHP standard solution
V_a = Volume of the KHP standard solution used
V_s = Volume of the NaOH solution pipetted for titration

Having considered all the contributions of uncertainties in step 3 to step 6, we can summarize them as below:

Uncertainty Factor	**Values To Be Used, *V***	**Uncertainty, *u***	**Relative Standard Deviation (RSD) = *u/V***
M_k	0.0992 mol l^{-1}	0.00014	1.41 x 10^{-3}
V_k	25.2 ml	0.034	1.35 x 10^{-3}
V_a	25.0 ml	0.021	8.40 x 10^{-4}

The standard uncertainty in the concentration (M_s) of this NaOH solution therefore is expressed as :

$$\frac{u(M_s)}{M_s} = \sqrt{(0.00141^2 + 0.00135^2 + 0.00084^2)} = 0.0021 \text{ mol } L^{-1}$$

Now, the concentration of NaOH solution, M_s, is calculated from equation [2] as follows:

M_s = (25.20 x 0.0992) / 25.0 = 0.100 mol L^{-1}

Hence, the standard uncertainty $u(M_s)$ in the concentration of NaOH solution is:

$u(M_s)$ = 0.0021 x 0.1000 = 0.00021 mol L^{-1}

F.1.4.8 Step 8: Pipette 25-ml volume of NaOH solution for HCl titration (V_b)

As 25-ml volume of the standardized NaOH solution is used for titration, similar considerations can be applied as in Step 5, giving 25 ml volume with a standard uncertainty $u(V_b)$ of ± 0.021 ml.

F.1.4.9 Step 9: Titration of NaOH against the HCl solution (V_c)

As in step 6, the HCl solution is titrating the 25-ml NaOH solution from a 50-ml burette. The combined standard uncertainty, $u(V_c)$ is therefore the same as 0.034 ml under similar assumptions.

F.1.4.10 Step 10: Calculation of the concentration of HCl solution (M_h)

The formula used in the calculation of the HCl solution is:

$M_h = (M_s \times V_b) / V_c$ … [3]

where,
M_h = concentration of the HCl solution
M_s = concentration of the NaOH standard solution
V_b = Volume of the NaOH standard solution used
V_c = Volume of the HCl solution from burette for titration

Having considered all the contributions of uncertainties in step 2 to step 9, we can now summarize them as below:

Uncertainty Factor	**Values To Be Used, *V***	**Uncertainty, *u***	**Relative Standard Deviation (RSD) = *u/V***
M_s	0.100mol l^{-1}	0.00021	2.13 x 10^{-3}
V_b	25.00 ml	0.021	8.4 x 10^{-4}
V_a	25.30 ml	0.034	1.35 x 10^{-3}

The relative standard uncertainty in the concentration (M_h) of this HCl solution therefore is expressed as:

$$\frac{u(M_h)}{M_h} = \sqrt{(0.00213^2 + 0.00135^2 + 0.00084^2} = 0.0027 \text{ mol } L^{-1}$$

Now, the concentration of HCl solution, M_h, is calculated from equation [3] as follows:

M_s = (25.00 x 0.100) / 25.30 = 0.0988 mol L^{-1}

Hence, the standard combined uncertainty $u(M_h)$ in the concentration of HCl solution is:

$u(M_h)$ = 0.0027 x 0.0988 = 0.00027 mol L^{-1}

F.1.4.11 Step 11: Calculation of the Expanded Uncertainty

The expanded uncertainty $U(M_h)$ is calculated by multiplying the standard combined uncertainty by a coverage factor, k, of 2 :

$U(M_h) = 0.00027 \times 2 = 0.00054$ mol L^{-1}

Hence, the concentration of the HCl solution analyzed is found to be :

0.0988 mol L^{-1} ± 0.00054 mol L^{-1}

F.1.5 Remarks:

In this acid/base titrimetry, the followings are its possible sources of error and some of them have been taken into account in this example. If additional sources of error were to be considered significant, they would be considered too:

a. Weighing - balance calibration and repeatability
b. Weighing - buoyancy effect of air in the laboratory, particularly when a micro-balance is used
c. Temperature effect- room temperature versus calibration temperature
d. Purity of chemicals used in standardization
e. Uncertainty of molecular weights of the chemicals used
f. Possible impurities, e.g. other alkaline matter in NaOH pellets
g. Systematic errors in volumetric glassware
h. Variation in end point detection, e.g. personal judgement
i. Competing reactions, such as adsorption of carbon dioxide from the air.

F.2 DETERMINATION OF LINOLEIC ACID OF MILK FAT EXTRACTED FROM MILK POWDER BY GC-FID

F.2.1 Specification

a) Accurately weigh about 10 g of milk powder sample.

b) The sample is extracted in the presence of NH_4OH with alcohol, ethyl ether, and petroleum ether.

c) Fatty acid methyl esters of extracted fat are prepared by using $NaOCH_3$ and $BF_3 \bullet CH_3OH$ esterification.

d) The mixture solution obtained is then evaporated to near-dryness under a stream of nitrogen.

e) The residue is then dissolved in heptane and the solution obtained is transferred to 25 mL volumetric flask and topped up with heptane to the mark.

f) Methyl linoleate is separated and quantified by gas chromatograph – flame ionisation detector. Linear calibration curve used for quantification is constructed based on 4 concentration levels of methyl linoleate with forced zero (using blank).

As the test method involves extraction and derivatisation processes, accurate determination of the analyte is therefore very much dependent on the effectiveness of these processes. In order to assess the effectiveness, recovery study has been carried out in parallel with normal analysis.

With recovery rate R available, concentration of linoleic acid in the milk powder sample (C_{spl}) can be calculated by:

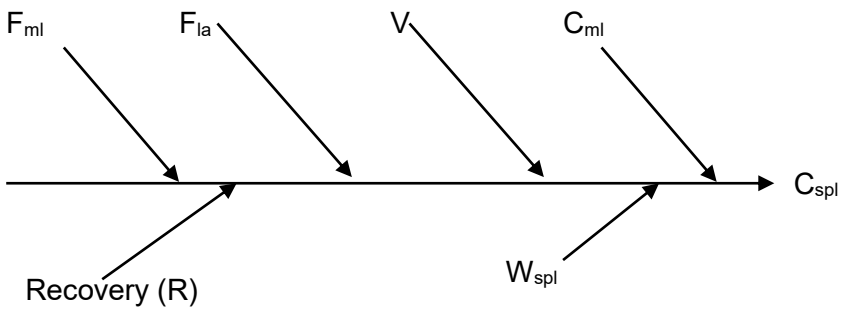
$$C_{spl} = (C_{ml} \times V \times F_{la})/(R \times F_{ml} \times W_{spl})$$

whereby,

C_{spl}: Concentration of linoleic acid in the milk powder sample (in mg/g).
C_{ml}: Concentration obtained from calibration curve for methyl linoleate (in mg/mL).
V: Final volume of the solution before injection (25 mL).
F_{la}: Formula weight of linoleic acid (in g/mol).
F_{ml}: Formula weight of methyl linoleate (in g/mol).
W_{spl}: Milk Powder sample weight (in g).

F.2.2 Identifying Uncertainty Sources

The cause and effect of uncertainty can be constructed as follows:



F.2.3 Quantifying Uncertainty Components

C_{ml}: The C_{ml} result obtained from calibration curve for the sample was 7.15 mg/mL.

For this analysis, 4 level of calibration standards, with a concentration of 1 mg/mL, 2 mg/mL, 4 mg/mL and 10 mg/mL, respectively, were prepared from a 1000 ± 2 mg/mL methyl linoleate in heptane reference standard. The four calibration standards and their blank were measured and the following results were obtained:

Concentration (mg/mL), x_i	**Response (Area)**	**Net Response (Area), y_i**
0	2	0
1	135	133
2	280	278
4	560	558
10	1194	1192

The relationship for forced zero least square fitting is y = bx with b being the slope of the calibration curve:

$$b = \frac{\sum x_i y_i}{\sum x^2}$$

The concentration x_{obs} of the analyte from a sample which produces an observed response y_{obs} is then given by $x_{obs}=y_{obs}/b$. The uncertainty $u(x_{obs},y)$ in a predicted value x_{obs} due to variability in y can be estimated from the variance of residuals S as for Generic Example E.4 above:

	x	**y**	**xy**	**x^2**
	0	0	0	0
	1	133	133	1
	2	278	556	4
	4	558	2232	16
	10	1192	11920	100
Sum	17	2161	14841	121

Thus, $b = \frac{\sum x_i y_i}{\sum x^2}$ = 14841/121 = 122.6528926 ➔ y = 122.6528926*x*. Therefore:

x	**y**	**Calculated y_c**	**$(y-y_c)^2$**
0	0	0	0
1	133	122.652893	107.06263
2	278	245.305785	1068.9117
4	558	490.61157	4541.2005
10	1192	1226.52893	1192.2467

Thus,

$S^2 = \Sigma(y_i-y_c)^2/(n-2)$ = (107.06263+1068.9117+4541.2005+1192.2467)/(5-2)

= 2303.14.

$var(x) = S^2/b^2$ = 2303.14/122.6528926^2 = 0.1531

$u(x_{obs},y) = \sqrt{var(x)} = \sqrt{0.1531} = 0.391$.

The result is $u(C_{ml}) = 0.391$ mg/mL.

V: The volumetric glassware used for topping the final solution to 25 mL has a certified value of 25.040 ± 0.015 mL at 20°C, obtained from supplier's specification. For this study, a rectangular distribution has been chosen. Therefore the uncertainty due to calibration is $0.015/\sqrt{3} = 0.00866$ mL.

The glassware is used in an environment with a temperature variation of ± 4.0 °C (at 95% confidence level; if confidence level is not given, then assume rectangular distribution).

As heptane's expansion coefficiency due to temperature variation is not known, let's assume it is about twice as bad as water which has an expansion coefficiency of $2.1x10^{-4}$ per °C per ml. In this case, the expansion coefficiency of heptane is assumed to be $4.2x10^{-4}$ per °C per ml. From our experience we know such assumption is on the higher side for the temperature range of the lab where analysis is done. The uncertainty due to temperature variation for a volume of 25.04 mL is thus at a maximum of $25.040 \times (4.0/2) \times 4.2x10^{-4} = 0.0210$ mL.

Therefore, $u(V) = \sqrt{(0.00866^2 + 0.0210^2)} = 0.0227$.

F_{la}: As for **Working Example F1**, molecular weight (MW) of linoleic acid ($C_{18}H_{32}O_2$) and its uncertainty are calculated as:

		C	**H**	**O**
	Value	12.0107	1.00794	15.9994
	Uncertainty	0.00046	0.00004	0.00017
C	12.0107	12.01116	12.0107	12.0107
H	1.00794	1.00794	1.00798	1.00794
O	15.9994	15.9994	15.9994	15.99957
F_{la}	**280.44548**	280.454	280.447	280.446
		0.00832	0.00128	0.00035
	7.091E-05	6.9E-05	1.6E-06	1.2E-07
$u(F_{la})$	**0.008421**			

F_{ml}: Similarly, MW of methyl linoleate ($C_{19}H_{34}O_2$) and its uncertainty are:

		C	**H**	**O**
	Value	12.0107	1.00794	15.9994
	Uncertainty	0.00046	0.00004	0.00017
C	12.0107	12.01117	12.0107	12.0107
H	1.00794	1.00794	1.00798	1.00794
O	15.9994	15.9994	15.9994	15.99957
F_{ml}	**294.47206**	294.481	294.473	294.472
		0.00878	0.00136	0.00035
	7.902E-05	7.7E-05	1.8E-06	1.2E-07
$u(F_{ml})$	**0.0088895**			

W_{spl}: The sample weight was 10.0232 g with weighing by difference. Calibration report shows a maximum deviation of 0.4 mg from stated values of standard weight, giving $0.4/\sqrt{3}$ =0.231 mg of standard uncertainty for each weighing. As the weighing of sample involves both tare and the sample weighing, uncertainty due to this deviation has to be counted twice:

$u(W_{spl}) = \sqrt{(0.231^2 + 0.231^2)}$ = 0.327 mg ➔ 0.000327 g.

Recovery (R): During another previous study on a similar sample, repeated recovery tests were done on a single sample and found to have an average of 91.3% recovery with a standard deviation of 5.4%. Thus, the relative standard deviation is 5.4%/91.3%=0.0591. For current sample, recovery was 0.950 (i.e. 95.0%). Thus, the standard uncertainty due to recovery is 95%x0.0591=5.61%.

F.2.4 Calculating Total Uncertainty

Standard uncertainties due to C_{ml}, V, F_{la}, R, F_{ml} and W_{spl} are combined first by the spreadsheet method:

		C_{ml}	**V**	**F_{la}**	**R**	**F_{ml}**	**W_{spl}**
	Value	7.15	25.04	280.44548	95.0%	294.47206	10.0232
	Uncertainty	0.391	0.0227	0.008421	5.61%	0.00889	0.00033
C_{ml}	7.15	7.541	7.15	7.15	7.15	7.15	7.15
V	25.04	25.04	25.0627	25.04	25.04	25.04	25.04
F_{la}	280.44548	280.445	280.445	280.4539	280.445	280.44548	280.445
R	95.0%	95.0%	95.0%	95.0%	100.61%	95.0%	95.0%
F_{ml}	294.47206	294.472	294.472	294.47206	294.472	294.48095	294.472
W_{spl}	10.0232	10.0232	10.0232	10.0232	10.0232	10.0232	10.0235
C_{spl}	**17.906665**	18.8859	17.9229	17.907203	16.9082	17.906125	17.9061
		0.97923	0.01623	0.0005376	-0.9985	-0.000541	-0.00058
	1.9561078	0.95889	0.00026	2.89E-07	0.99695	2.922E-07	3.4E-07
$u(C_{spl})$	**1.3986092**						

The above standard uncertainty obtained is then combined with that due to precision to give total combined standard uncertainty. As precision obtained above is relative standard deviation, thus,

Thus, the total combined standard uncertainty is

$u(C_{spl})$ = 1.398 mg/g

The expanded uncertainty $U(C_{spl})$ at 95% confidence level is obtained by multiplying the combined standard uncertainty with a coverage factor of 2 giving:

$U(C_{spl})$ = 2x1.398 = 2.8 mg/g

The concentration of linoleic acid in the tested milk powder has been found to be:

17.9 ± 2.8 mg/g *

*The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%

F.2.5 Comments:

Significant Components Evaluation

According to the law of propagation of errors, for addition and subtraction relationship, e.g. $x = x_1+x_2$, the combined standard uncertainty is the square root of the sum of square of uncertainty of individual components, i.e. $u(x) = \sqrt{[u^2(x_1)+u^2(x_2)]}$. Thus, contribution of each component to the combined standard uncertainty can be compared directly between $u(x_1)$ and $u(x_2)$.

However, in the case where relationship is of multiplication and/or of division, e.g. $x = x_1/x_2$ The combined standard uncertainty is calculated as:

$$u(x)/x= \sqrt{\{[u(x_1)/x_1]^2 + [u(x_2)/x_2]^2\}}$$

Therefore, in order to compare contribution of individual components to the combined standard uncertainty, one has to compare the relative standard uncertainty of individual components, i.e. $u(x_1)/x_1$ vs. $u(x_2)/x_2$.

For the working example discussed above, the relationship is of multiplication and of division. Thus, to compare each component's contribution, each component uncertainty shall be converted to relative standard uncertainty.

Description	**Value**	**Standard Uncertainty**	**Relative Standard Uncertainty**	**Diagrammatic Contribution**
C_{ml}	7.15 mg/g	0.391 mg/g	0.0547	
V	25.04 mL	0.0227 mL	0.0009	
F_{la}	280.44548 g/mol	0.00842 g/mol	0.00003	
F_{ml}	294.47206 g/mol	0.00889 g/mol	0.00003	
W_{spl}	10.0232 g	0.000327 g	0.00003	
R	95.0%	5.61%	0.0591	
r			0.170	
C_{spl}	17.90667 mg/g	3.35 mg/g	0.192	

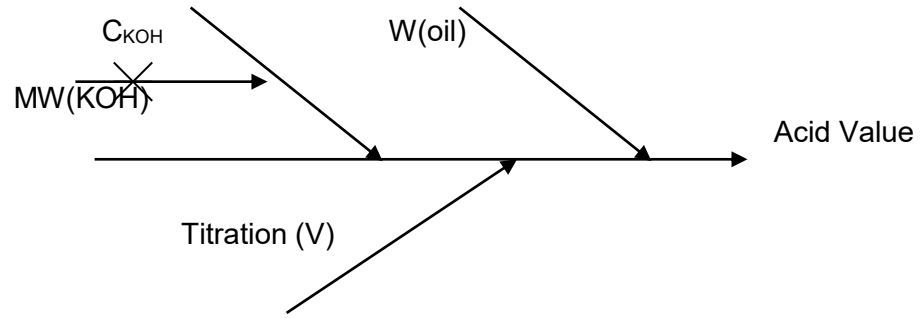
From the table above, it is obvious that the main contributions are from C_{ml}, R and r. In fact, one can work out the combined standard uncertainty from these three sources only by ignoring other minor sources. In this case, $u(C_{ml})/C_{ml}= \sqrt{(0.0547^2 + 0.0591^2 + 0.170^2)} = 0.188$. Thus, $u(C_{ml})=0.188 \times C_{ml} = 0.188 \times 17.90667 = 3.37$ mg/g. Thus, it is obvious that the uncertainty calculated from only major sources is not significantly different from that obtained after considering all sources.

F.3 DETERMINATION OF ACID VALUE IN PALM OIL

F.3.1 Method

An aliquot of oil sample is dissolved in neutralized IPA and titrated against standardized 0.1mol/L KOH solution with phenolphthalein as indicator.

F.3.2 Cause and Effect Diagram



F.3.3 Quantifying Uncertainties

F.3.3.1 Standardizing 0.1M KOH Solution with 0.5M HCl

F.3.3.1.1 Preparation of 0.5M HCl

A commercially prepared HCl solution containing 18.230 g HCl (m_{HCl}) is used to prepare C_{st} 0.5M HCl of volume V = 1000 mL.

- The volumetric flask used for the solution preparation has the volume 1000 mL ± 0.4 mL at 20°C. The appropriate standard deviation of the calibrated volume using a rectangular distribution is 0.4/ $\sqrt{3}$ or 0.23 mL.
- Since the actual temperature and the flask calibration temperature is -3 °C with 95% confidence, at volume coefficient of water expansion 2.1 x 10^{-4} per °C per mL, the possible volume variation is 1000 x 3 x 2.1 x 10^{-4} or 0.63 mL. The corresponding standard deviation is 0.63 /1.96 or 0.32 mL.

The standard deviation of the flask filling is less than 1/3 of the standard deviations for calibration and temperature variation, and is thus neglected.

Combining these two contributions of the uncertainty *u(V)*, we have

$$u(V)/V = \sqrt{(0.23^2 + 0.32^2)} / 1000$$
$$= 0.00039.$$

The concentration of HCl is $m_{HCl} / M_{HCl}.V$ *where* M_{HCl} *is the molecular weight of HCl.*

- The manufacturer of the HCl solution indicates a possible deviation of its titer of 0.02% per °C. Taking a possible temperature difference in the manufacturer's laboratory of -2°C (with 95% confidence), the standard uncertainty of mHCl is:

$u(m_{HCl})$ = 18.230 x 0.02 x 2 / (100 x 1.96)
= 0.004 g

$u(m_{HCl}) / m_{HCl}$ = 0.00022.

- The standard uncertainty of the molecular weight of HCl, according to IUPAC atomic masses and rectangular distribution, is
  $u(M_{HCl})$ = 0.000043.

  It is noted that $u(M_{HCl})/M_{HCl}$ is negligible in comparison with $u(V)/V$ and $u(m_{HCl})/m_{HCl}$, the relative standard uncertainty is

$u(C_{st})/C_{st} = \sqrt{(0.00039^2 + 0.00022^2}$ = 0.00045.

F. 3.3.1.2 Determination of C_{KOH}

The exact concentration of the KOH solution is established before its use by titration against the standardized HCl solution.

Therefore, $C_{KOH} = C_{st}.V_{st} / V_{KOH}$

Where,

V_{st} is the volume (mL) of the standard HCl solution used for titration of the volume V_{KOH} (mL) of the KOH solution.

- As shown above, $u(C_{st})/C_{st} = 0.00045$

- For transfer of an aliquot of the KOH solution to the conical flask, a glass pipette of volume 5 ± 0.01 mL is used. Taking a possible temperature variation of ± 3°C with 95% confidence, and repeatability of filling the pipette (standard deviation) 0.0033 mL, one can calculate $u(V_{KOH})/V_{KOH} = 0.0015$

- The titration is accomplished using a 5-mL microburette graduated in 0.01mL division (supplier's calibration accuracy of ± 0.01mL).

- The possible temperature variation is the same as that mentioned above, the standard deviation of filling is 0.0033 mL, and the standard deviation of end point detection arising due to the drop size of the burette (0.017 mL) is 0.0098 mL.

Thus, the maximum value of $u(V_{st})/V_{st}$ = 0.013 if C_{KOH} = 0.1 mol/L , and the corresponding V_{st} = 1mL.

The uncertainties $u(C_{st})/C_{st}$ and $u(V_{KOH})/V_{KOH}$ are negligible in comparison to $u(V_{st})/V_{st}$;

therefore, $u(C_{KOH})/C_{KOH} = u(V_{st})/V_{st} = 0.013$

F.3.3.2 Acid Value Determination

The acid value is:

$AV = M_{KOH}\ V_{KOH}\ C_{KOH} / m$

- The test method recommends the use of KOH molecular weight of 56.1 Instead of the complete value M_{KOH} = 56.10564; hence, in this case,

  $u(M_{KOH})/M_{KOH}$ = 0.00564/(56.1 x $\sqrt{3}$) = 0.00006

- For free fatty acid titration against KOH solution, we used the 5-mL burette described before; therefore,

  $u(V_{KOH})/V_{KOH} = u(C_{KOH})/C_{KOH} = u(V_{st})/V_{st} = 0.013$

- The uncertainty of oil sample weighing of 2.5 g is say, $u(m)/m$ = 0.0023.

  It is clear that the uncertainties of the molecular weight of KOH and weighing of oil sample are negligible. Hence,

  $$u(A_V)/A_V = \sqrt{[u(V_{KOH})/V_{KOH}]^2 + [u(C_{KOH})/C_{KOH}]^2} = 0.018$$

  The expanded uncertainty with a coverage factor of 2 is:

  $U(A_V)/A_V = 2 \times 0.018$ *or* 0.04.

NOTE: The detection of the end point of the titration is a dominant source of uncertainty. If a commercial burette, for example, has a drop size of 0.043 mL, the expanded uncertainty will increase to 0.07.

Moreover, the colour of the oils and the possible change in the indicator behaviour near the end point in the oil-solvent mixture are not taken into consideration. The same relates also to the influence of atmospheric CO_2 on C_{KOH}.

F.4 KINEMATIC VISCOSITY OF FUEL OIL (ASTM D 445-97)

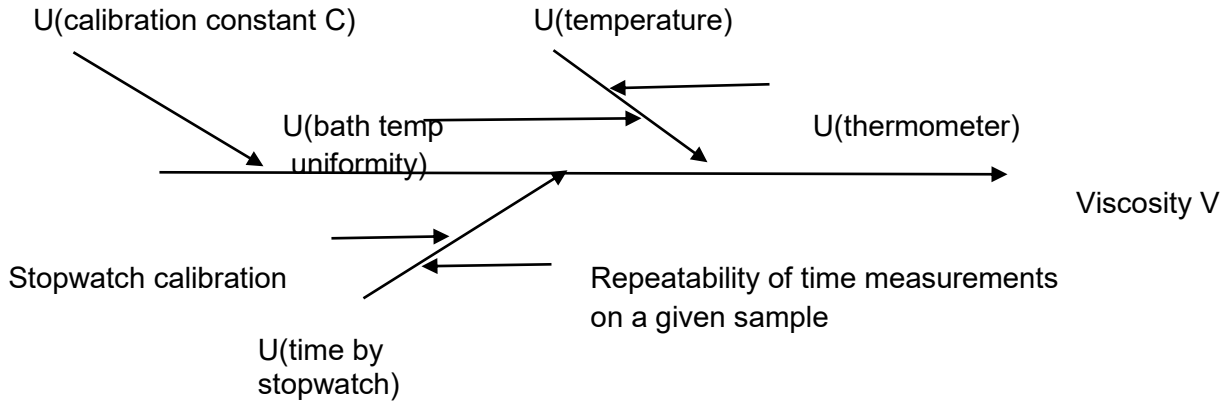
F.4.1 Principle of Test Method

The time is measured for a fixed volume of liquid to flow under gravity through the capillary of calibrated viscometer under a reproducible driving head and at a closely controlled and known temperature. The kinematic viscosity is calculated as the product of the measured flow time and the calibration constant of the viscometer.

i.e. Viscosity, centistokes (mm^2/sec), V = C x t

where, C = calibration constant of the viscometer
and t = measured flow time in second

F.4.2 Uncertainty Components Identified



F. 4.3 Estimation of Standard Uncertainty of Components

a) Calibration Constant of Viscometer
Viscometer R359 calibration constants

Upper bulb = 0.5084 ± 0.00042 (95% confidence)
Lower bulb = 0.3737 ± 0.00056 (95% confidence)

Standard uncertainty of calibration constants
Upper bulb = 0.00021
Lower bulb = 0.00028

b) Measurement of flow time in seconds
   i. Measurement uncertainty of stop watch used = 0.10 (with 95% confidence)
   Standard uncertainty of stop watch used = 0.05

   ii. Repeatability test of time measurement by stop watch on a given sample

351.98	352.65	352.12	351.68	352.69

Mean = 352.22, Std Dev = 0.437

Combined standard uncertainty for time measurement = SQRT (0.11^2+0.086^2) or 0.440148

c) Oil Bath Temperature Control

i. Reference thermometer = ± 0.06 (with 95% confidence)
Standard uncertainty of reference thermometer = 0.030

ii. Temperature distribution in viscosity bath at various locations

50.04	50.08	49.96	50.02	50.04

Mean = 50.028, Std Dev = 0.0438

Combined standard uncertainty for temperature = SQRT (0.030^2+0.0438^2)
= 0.05310

F.4.4 Example

Upon analysis of a fuel oil sample, the following results were obtained:

C = 0.5048 (upper bulb)
t = 352.22
Viscosity (V) = 177.80

F.4.5 Estimation of Uncertainty of Measurement

	Value	Std Uncertainty	Std Unc/Value	(Std Unc/Value)^2
C	0.5048	0.00021	0.000416006	1.73061×10^{-07}
t	352.224	0.440148	0.001249624	1.56156×10^{-06}
Temp	50.0	0.05310	0.001062073	1.128×10^{-06}
			Sum =	2.86262×10^{-06}
			Combined (std u/V) =	0.001691929

Therefore,
Kinematic viscosity of sample found = 177.8 mm^2/sec
Combined standard uncertainty = 0.301 mm^2/sec
Expanded uncertainty with a coverage factor of 2 = 0.60 mm^2/sec

F.5 DETERMINATION OF CRUDE FIBRE IN ANIMAL FEEDING STUFFS (SOURCE: EURACHEM/CITAC GUIDE, 2000)

F.5.1 Step I: Specify the Measurand

Crude fibre is defined in the method scope as the amount of fat-free organic substances which are insoluble in acid and alkaline media. There is no suitable reference material available for this method. However, both collaborative inter-laboratory/proficiency studies (repeatability and reproducibility) and in-house repeatability studies have been carried out to evaluate method performance.

During the analysis, the sample is treated to digest most components, leaving behind all the undigested material. The test method requires blank correction to be done. The percentage of weight loss after blank correction is defined as the **"fibre** content" by the method. Thus, the fiber content as a percentage of the sample by weight, C_{fiber}, is given by:

$C_{fiber} = [(b-c)/a] \times 100$

whereby,
a: original sample weight
b: weight loss for the sample
c: weight loss for the blank (crucible)

F.5.2 Step2: Identify Uncertainty Sources

To make use of data from the collaborative inter-laboratory/proficiency testing studies and from the in-house repeatability studies, the fishbone diagram should be drawn in such a way that all sources contributing to precision are grouped under one bone.

F.5.3 Step 3: Quantify the Uncertainty Components

F.5.3.1 Data from collaborative studies and from in-house repeatability studies

Five different feeding stuffs representing typical fibre and fat concentrations were analysed during the studies. Participants in the studies carried out all stages of the method, including grinding of the samples. The repeatability (s_r) and reproducibility (s_R) estimates obtained from the studies are presented in the table below.

During the in-house repeatability studies, experiments were also done to evaluate the repeatability (within batch precision) for the feeding stuffs at the similar concentration as those for collaborative studies. The results are presented in the last of the column of the table below.

	Fiber Content (%, w/w)			
Sample	**Collaborative Interlaboratory/Proficiency Studies**			**In-House Repeatability Standard Deviation**
	Mean	**Reproducibility Standard Deviation (s_R)**	**Repeatability Standard Deviation (s_r)**	
A	2.3	0.293	0.198	0.193
B	12.1	0.563	0.358	0.312
C	5.4	0.390	0.264	0.259
D	3.4	0.347	0.232	0.213
E	10.1	0.575	0.391	0.327

From the table above, it is obvious that the estimates of repeatability obtained in-house were comparable to those obtained from the collaborative studies. This indicates that the method precision in this particular laboratory is similar to that of the laboratories which took part in the collaborative trial. On this basis (alone), it is acceptable to use the reproducibility standard deviation from the collaborative trial in the uncertainty budget for the method.

F.5.3.2 Extra factors

To complete the uncertainty calculation we need to consider whether there are any other effects not covered by the collaborative studies which need to be addressed. The collaborative studies covered different sample matrices and the pre-treatment of samples, as the participants were supplied with samples which required grinding prior to analysis. The uncertainties associated with matrix effects and sample pre-treatment do not therefore require any additional consideration.

However, repeatability data from collaborative studies and from in-house studies does not reveal individual participating laboratory's bias. This bias should be evaluated separately to determine if it is significant compared to the reproducibility standard deviation.

For this particular laboratory, the "constant weight" was achieved within 2mg only. The uncertainty from this bias is thus $0.002/\sqrt{3}$ = 0.00115g. As the method specified a 1g sample to be used, the standard uncertainty due to weighing bias is thus 0.115%. From the table above, it is obvious that for all fibre concentrations, this uncertainty is smaller than the reproducibility standard deviation, and for all but the lowest fibre concentrations is less than 1/3 of the s_R value. Again, this source of uncertainty can usually be neglected. However for low fibre concentrations (e.g. 2.3% w/w in the table above), this uncertainty is more than 1/3 of the s_R value so an additional term should be included in the uncertainty calculation for such a low level sample.

F.5.4 Step 4: Calculate Total Uncertainty

For 3% w/w or above level, s_R can be used as the standard uncertainty. E.g, for 3.4% w/w level, the standard uncertainty is 0.347% and the expanded uncertainty with k=2 (95% confidence) is 0.69%. For those below 3% w/w level, e.g. 2.3%, the combined standard uncertainty is $\sqrt{(0.115\%^2+0.293\%^2)}$ = 0.31%. The expanded uncertainty is thus 0.62% (k=2 at 95% confidence level).

Remark: F test is assumed to be conducted.

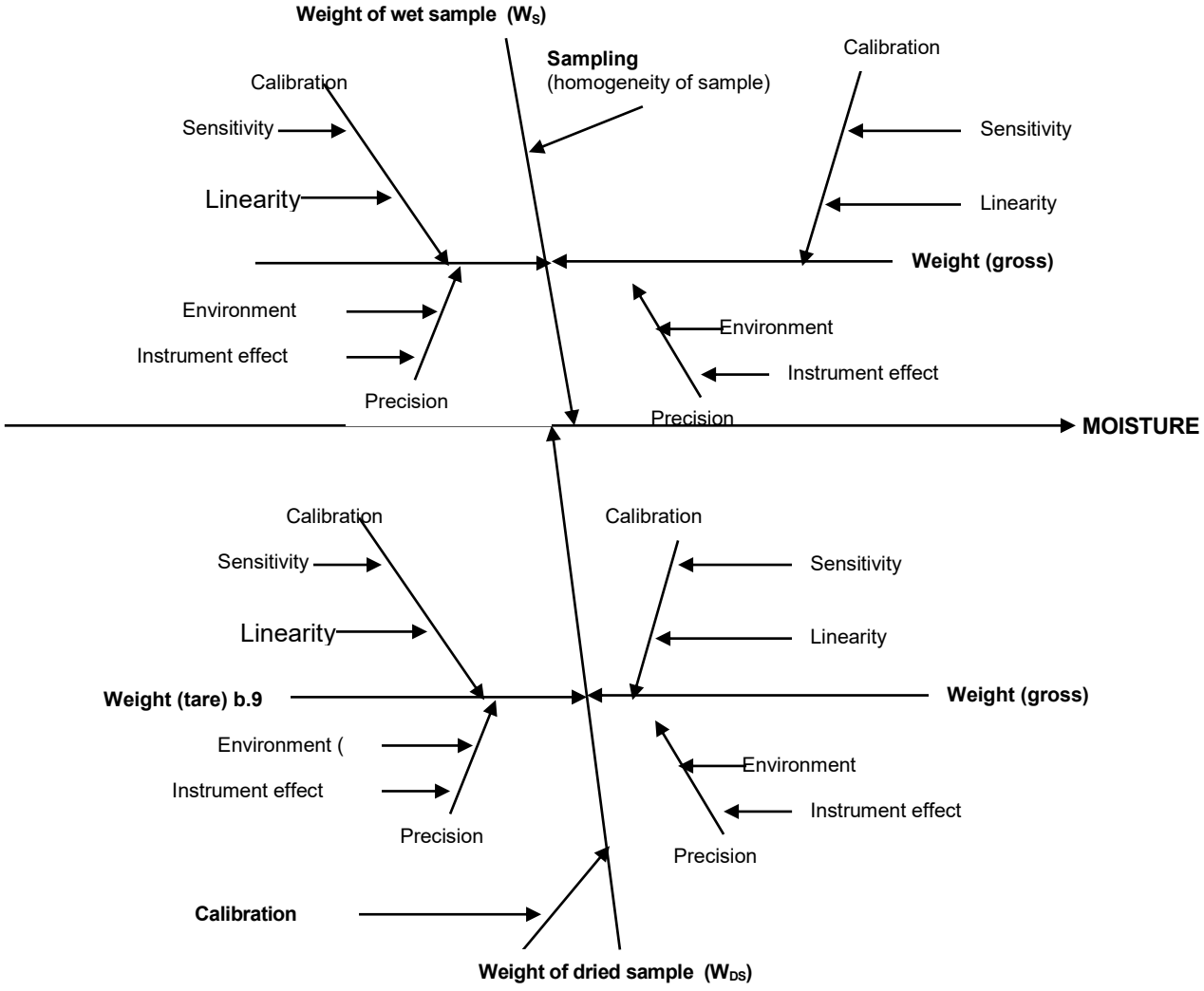
F.6. MOISTURE DETERMINATION IN SCALLOP

F.6.1. Specification of measurand

$$\text{Moisture content (\%)} = \frac{\text{Weight of wet sample, } W_S \text{ (g)} - \text{Weight of dried sample, } W_{DS} \text{ (g)}}{\text{Weight of wet sample, } W_S \text{ (g)}} \times 100$$

F.6.2. Identification of Uncertainty Sources

F.6.2.1. Cause-and-Effect Diagram



F.6.3 Purpose

a. To determine the moisture of 4.8540 g of scallop by the weigh-by-difference method and drying in the oven.

b. Weighing records:

Before drying:

Wt. of container + lid + scallop, g	17.1868
Wt. of container + lid, g	12.3328

Wt. of scallop, g	4.8540

After drying at 102°C:

Wt. of container + lid + dried scallop, g	15.7538
Wt. of container + lid, g	12.3328
Wt. of dried scallop, g	3.4210

$$\% \text{ moisture content} = \frac{(4.8540 - 3.4210)}{4.8540} \times 100 = 29.52$$

F.6.4 Weighing Process

F.6.4.1. Linearity by calibration

The external calibration of the balance used states that the difference from the actual weight on the scale pan and the reading on the scale is within ± 0.5 mg with a 95% confidence.

Under the normal distribution, a 95% confidence gives a factor of 1.96.

Therefore, the associated uncertainty expressed as standard deviation is:

$\frac{0.5}{1.96}$ or 0.255 mg

NOTE: This component uncertainty has to be taken into account twice because of two weighings involved each time. Two sets of weighings were made, one of sample before drying and one of sample after drying.

F.6.4.2. Repeatability (Precision)

10 repeated measurements of a tare and gross weight gave a standard deviation of 0.210 mg at a range of 20000.8 mg to 20001.4 mg.

NOTE: We account for repeatability only once because it has already been accounted for in the weight by difference, being a standard deviation of weight differences.

F.6.4.3. Sensitivity

Sensitivity of the balances can be neglected because the weight by difference is done on the same balance over a very narrow range.

F.6.4.4. Calculating the combined Standard Uncertainty in each Weighing Process

$$U(W_{scallop}) = \sqrt{[2(U_{linearity})^2 + U_{precision}^2]}$$
$$= \sqrt{[2(0.255)^2 + 0.210^2]} = 0.417 \text{ mg}$$

F.6.5. Summary of values of Uncertainties

Description	**Value x**	**U(x)**	**U(x)/x**
Wt of scallop (mg) before drying	4854.0	0.417	0.0001
Wt of scallop (mg) after drying	3421.0	0.417	0.0001

F.6.7. Calculation of combined and expanded Uncertainties

Therefore the combined uncertainty:

u_c(moisture) = $\sqrt{(0.0001^2 + 0.0001^2)}$ x moisture content

= 0.00014 x 29.52 % = 0.0041 %

The expanded uncertainty using a coverage factor of 2 (to get 95% confidence limit) is:

U(moisture) = 0.0041 % x 2

= 0.0082 %

Therefore, the result is 29.52 % ± 0.0082 % with approximately 95% confidence level.

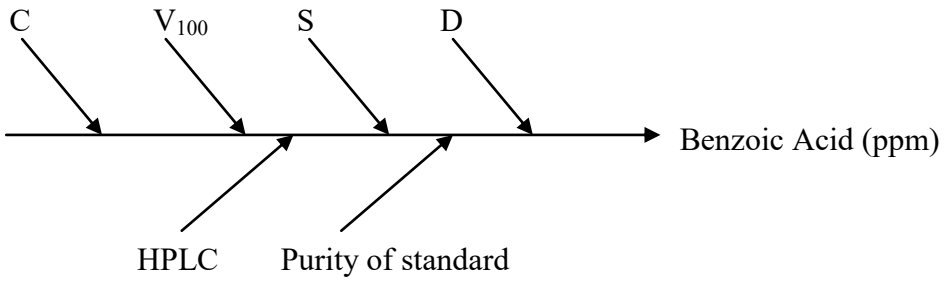
F.7. BENZOIC ACID IN FOOD PRODUCTS

F.7.1 Benzoic Acid in food sample can be calculated by:

Benzoic Acid (ppm) (w/w): $\frac{CxDxV_{100}}{S}$

where C: Concentration of benzoic acid (mg/l) taken from calibration curve
D: Dilution factor of sample if required
S: Sample weight (g)
V_{100}: Extraction volume nominal 100 (ml)

F.7.2 Identification of uncertainty sources



F.7.3 Quantifying Uncertainty Components

C
For this analysis, 4 levels of calibration standards were prepared:
0, 10, 20, 40 and 80 ml/g

The 4 calibration standards and their blank were measured and the following results were obtained:

X Conc'n (mg/l)	y Area (mAU's)	xy	x^2	Calculated y_c	$(y-y_c)^2$
0	0	0	0	0	0.000
10	231.0	2310	100	235.054	16.436
20	462.5	9250	400	470.108	57.885
40	945.7	37828	1600	940.216	30.069
80	1880.1	150408	6400	1880.433	0.111
Sum	3519.3	199796	8500		104.501
				Slope (b) = Sum xy / Sum X^2	23.505
				S2 = sum $(y-y_c)^2$ / (n-2)	34.834
				Var (x) = S^2 / b^2	0.063047
				u(C) = sqrt(S^2 / b^2)	0.251091

u(C) = 0.251091 mg/l

S
Use 2-decimal place balance for weighing of sample

Obtain combined standard uncertainty from calibration record**
u(S) = 0.0139 g

V_{100}

Use a 100ml volumetric flask for dissolving sample extract.
Obtain combined standard uncertainty from calibration record for two glassware.**

	Test 1	Test 2
Glassware ID:	159	158
u(100), µl =	80.11	96.89
u(100), ml =	0.08011	0.09689

D

If no dilution is required:
Pipette and volumetric volume are set as 1, standard uncertainty are set as zero.

If dilution is required:
After extraction, pipette a suitable amount of sample extract into a volumetric flask and make up volume with 70% ethanol. Obtain combined standard uncertainty from calibration record for glassware.**

	Pipette ID	Pipette Vol, ml	u(P), µl	u(P), ml
Test 1	21	10	16.77	0.01677
Test 2	37	10	7.79	0.00779

	V. Flask ID	Vol Flask, ml	u(V), µl	u(V), ml
Test 1	94	50	21.40	0.02140
Test 2	151	50	34.79	0.03479

	Test 1		Test 2	
	Pipette Vol, ml	Vol Flask, ml	Pipette Vol, ml	Vol Flask, ml
Value	10	50	10	50
Std Uncertainty	0.01677	0.02140	0.00779	0.03479
C.V.	0.001677	0.000428	0.000779	0.000696
C.V. sq	0.000003	0.000000	0.000001	0.000000
Sum of C.V. sq	0.000003		0.000001	
Sqrt (Sum of C.V. sq)	0.001732	=u(D)/(D) =A	0.001000	=u(D)/(D) =A
Dilution factor (D)	5	= B	5	= B
u(D)	0.008660	= A*B	0.005000	= A*B

Calculation: Benzoic Acid, mg/kg = $(C*D*V_{100}) / S$

Concentration of benzoic acid (C) in final extraction solution is obtained:

Test 1:	21.010	mg/l
Test 2:	21.055	mg/l

		C	D	V_{100}	S
Test 1	Value	21.010	5	100	10.00
	Std Uncertainty	0.251091	0.008660	0.080110	0.013900
	C.V.	0.011951	0.001732	0.000801	0.001390
	C.V. sq	0.000143	0.000003	0.000001	0.000002
	Sum of C.V. sq	0.000149			
	Sqrt (Sum of C.V. sq)	0.012207	= u(Benzoic Acid)/(Benzoic Acid) = A		
	Benzoic Acid, mg/kg	1050.50	= ($C*D*V_{100}$) / S = B		
	u(Benzoic Acid), mg/kg	12.822987	= A*B		
Test 2	Value	21.055	5.0	100	10.00
	Std Uncertainty	0.251091	0.005000	0.096890	0.013900
	C.V.	0.011926	0.001000	0.000969	0.001390
	C.V. sq	0.000142	0.000001	0.000001	0.000002
	Sum of C.V. sq	0.000146			
	Sqrt (Sum of C.V. sq)	0.012083	= u(Benzoic Acid)/(Benzoic Acid) = A		
	Benzoic Acid, mg/kg	1052.75	= ($C*D*V_{100}$) / S = B		
	u(Benzoic Acid), mg/kg	12.720427	= A*B		
	Ave Benzoic Acid,mg/kg	1051.625000			
	Ave u(Benzoic Acid)	12.77170666	ppm (w/w)		

Purity of Standard

Purity of standard given by the certificate is of below:

Lot: 14141
Purity: 100 ± 0.5%

As there is no confidence limit of the purity, we take the quoted uncertainty as the rectangular distribution.

Standard uncertainty = $0.5 / \sqrt{3}$ = 0.288675 %

u(Purity of standard) = 0.288675 %

F.7.4 Summary of Uncertainty Obtained

Description	**X**	**u(x)**	**u(x) / x**	**$[u(x) / x]^2$**
Benzoic Acid, mg/kg	1051.625	12.771707	0.012145	0.000147
Purity of standard, %	100.00	0.288675	0.002887	0.000008
Sum of $[u(x)/x]^2$	0.000156			
Sqrt {Sum of $[u(x)/x]^2$}	0.012483	=u(x)/x		
Combined uncertainty	13.127562	=u(x)		

The expanded uncertainty u(Benzoic Acid) at 95% confidence level is obtained by multiplying the combined standard uncertainty with a coverage factor of 2 giving

u(Benzoic Acid) = 2 * u(x) = 26.26 ppm (w/w)

The test result takes the form of: 1051.63 ± 26 ppm (w/w)

*** calibration records not shown in details herein. However, working examples of such calibration have been shown in this Guide elsewhere.*

F.8. FLUORIDE CONTENT IN WATER BY SPADNS METHOD (APHA Method 4500-F⁻, D)

F.8.1. Procedure

a. Standard calibration curve

i Prepare fluoride standards in the range 0 to 1.00 mg/l fluoride in 50 ml volumetric flask..

ii Develop the colour by adding 10.00 ml mixed acid zirconyl-SPADNS reagent

iii Obtain the absorbance readings of standards at 570 nm and plot the calibration curve.

b. Sample preparation

i If the sample contains residual chloride, add 1 drop of $NaAsO_2$ solution per 0.1 mg residual chlorine.

ii For colour development, add 10.00 ml of acid zirconyl-SPADNS reagent to a known volume of sample and make up to 50 ml.

iii Adjust temperature to be similar to those used for obtaining the standard curve.

iv Obtain reading of the sample.

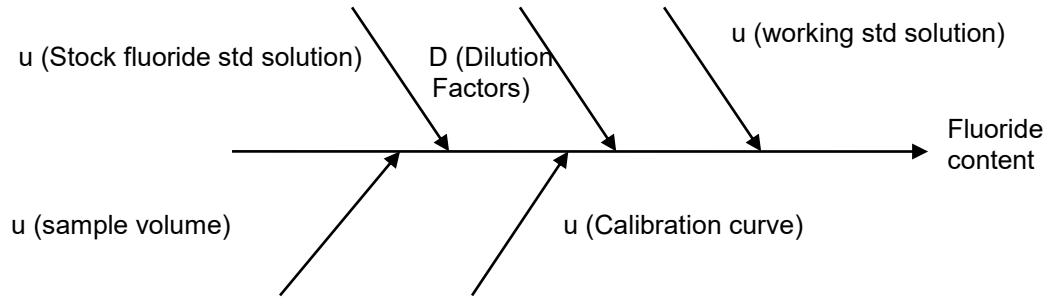
F.8.2. Calculation

mg F^- /L = $\frac{A}{ml\ sample}$ x 50 x dilution factor of sample, if any

where

A = mg/L fluoride determined from plotted curve

F.8.3. Uncertainty components identified



a. Stock fluoride solution: 221 mg (±0.40 mg) NaF (equivalent to 100 mg F) in 100 ml (±0.23 ml) distilled water (i.e. 1000 mg F^-/L)
Std uncertainty u(w) = 0.40/$\sqrt{3}$ = 0.2309
Std uncertainty u(v) = 0.23/2 = 0.115 ml

u(stock std) = 1000 $\sqrt{(0.231/221)^{2} + (0.115/100)^{2}}$ *= 1.554 mg F^-/L.*

b. Two-step Dilutions (100x followed by 10x to obtain1.00 mg F^-/L))

b.1 Intermediate fluoride standard solution: Dilute 1.00 ml (±0.03 mL) stock standard to 100.0 ml (±0.23 mL) solution to give 10 mg F^-/L

u(1.00 mL stock) = 0.03/2 = 0.015
u(100 mL volumetric flask) = 0.23/2 = 0.115

$$\frac{u(Dilution\ factor)}{100} = \sqrt{(0.015/1.00)^2 + (0.115/100)^2}$$

Therefore, u(100x Dilution factor) = 1.504

b.2 Working fluoride standard solution: Dilute 10.0 ml (±0.08 mL) intermediate F standard to 100.0 ml (±0.23 mL) solution to give 1.0 mg F^-/L

u(10.0 mL stock) = 0.08/2 = 0.04
u(100.0 mL volumetric flask) = 0.23/2 = 0.115

$$\frac{u(Dilution\ factor)}{10} = \sqrt{(0.04/10.0)^2 + (0.115/100)^2}$$

Therefore, u(10x Dilution factor) = 0.012

c. Sample volume taken for analysis: 50 mL ± 0.09 mL

Standard uncertainty u(sample) = 0.09/2 = 0.045 mL

d. Fluoride concentration read from the calibration curve

Upon analysis, the sample absorbance = 0.2531, which gave 0.424 mg/L with standard uncertainty of 0.0405mg/L as obtained from the calibration curve.

F.8.4. Calculation of combined uncertainties

Uncertainty Components, x_i	**Uncertainty u**	**RSD u/x_i**
1000 mg/L stock F^- standard	1.554	0.001554
100x Dilution Factor for int. std	1.504	0.01504
10x Dilution Factor for working std	0.012	0.0012
50.0 mL sample size	0.045	0.0009
0.424 mg/ F^- from calibration curve	0.0405	0.0955

The calculated Fluoride F^- content in sample $= \frac{0.424 \times 50}{50} = 0.424$*mg/L*

$$\frac{Combined\ uncertainty,\ u}{0.424} =$$

$$\sqrt{(0.001554)^2 + (0.01504)^2 + (0.0012)^2 + (0.0009)^2 + (0.0955)^2}$$

= 0.0967

Therefore, combined uncertainty, u = 0.424 x 0.0967 = 0.041 and expanded uncertainty, U = 2 x u = 0.082

F.8.5. Results

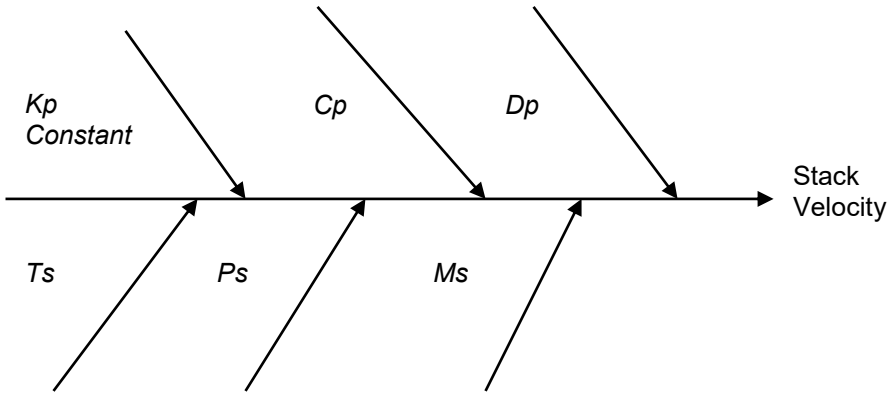
The fluoride content of the sample = 0.42 mg/l ± 0.08 mg/l with a coverage factor of 2 (95% confidence)

F.9. STACK VELOCITY

F.9.1. Formula for Calculation

$$Vs = Kp * Cp * (\sqrt{Dp}) \sqrt{\frac{Ts}{(Ps * Ms)}}$$

F.9.2. Uncertainty Contributors



F.9.3. Uncertainty Components Identified

a. *Dp* – Differential pressure

Reading from the manometer scale in the interval of 1 mm water.
Uncertainty of reading is ½

Standard uncertainty for *Dp* is then: $\frac{1}{2\sqrt{3}}$ = 0.29

As the pressure readings were read twice,
Therefore, the combined standard uncertainty for *Dp* =

$\sqrt{(0.29^2 + 0.29^2)}$ = 0.41 mm Water

b. *Ts* – Temperature of stack
Standard uncertainty of temperature probe of the instrument at 39.9°C = 0.37°C, based on the calibration of the probe against the working thermocouple.

Absolute temperature = (273.0 + 39.9) K

c. *Ps* – Absolute pressure of stack

Atmospheric pressure = 760 mm Hg
Stack static pressure measured = - 7 mm Water
Absolute stack pressure calculated = 759.4 mm Hg

Calibrated barometer reading at 1001 mbar showed an error of +1 mbar. 1001 mbar is equivalent to 750.8 mm Hg and the corresponding error is 0.75 with 95% confidence

Therefore, standard uncertainty of barometer = 0.75 / 1.96 = 0.38 mm Hg

d. *Ms* – Molecular weight of stack air

Molecular weight of stack air is calculated = 29.9 g/g-mole
Uncertainty of dried molecular weight = 1.4 g/g-mole with 95% confidence
Standard uncertainty of molecular weight found = 0.7 g/g-mole

e. *Cp* – Pitot tube coefficient

Cp was given as 0.84 with a reported uncertainty of 0.004

Standard uncertainty therefore = 0.004 / $\sqrt{3}$ = 0.0023

f. *Kp* – Velocity equation constant = 34.97

No uncertainty data given

F.9.4. Calculation of Velocity (Vs)

Given data:
Kp = 34.97
Cp = 0.84
Ts = (273 + 39.9) K = 312.9 K
Ps = 759.4 mm Hg
Ms = 29.9 g/g-mole
Dp = 26.0 mm Water

Therefore, *Vs* = 17.52 m / sec

F.9.5. Estimation of Measurement Uncertainty of Stack Velocity

The various standard uncertainties estimated were tabulated below:

Parameter	**Value X**	**Standard Uncertainty u**	**RSD Squared** $(\frac{u}{X})^2$
Kp	34.97	-	-
Cp	0.84	0.0023	0.0000075
Ts	312.9	0.37	0.0000014
Ps	759.4	0.38	0.0000003
Ms	29.9	0.7	0.0005480
Dp	26.0	0.41	0.0002487
Vs =	17.52	Total sum =	0.0008059

Therefore, the combined standard uncertainty of
Vs = 17.52 * $\sqrt{0.0008059}$ = 0.497

Hence, the expanded uncertainty of *Vs* = 2 * 0.511 = 0.99

F.9.6. Reporting

Velocity of Stack Gas = 17.52 ± 0.99 with a coverage factor of 2 (95% confidence)

F.10 VANADIUM IN FUEL OIL
INDUCTIVELY COUPLED PLASMA EMISSION SPECTROSCOPY (IP501)

F.10.1 Purpose

To evaluate the measurement uncertainty of 60 ppm of V

F.10.2 Procedure

1.1 20-50 gm (W) of sample was pretreated in a clean platinum dish and the residue was made up to volume with water in a 100-mL volumetric flask.

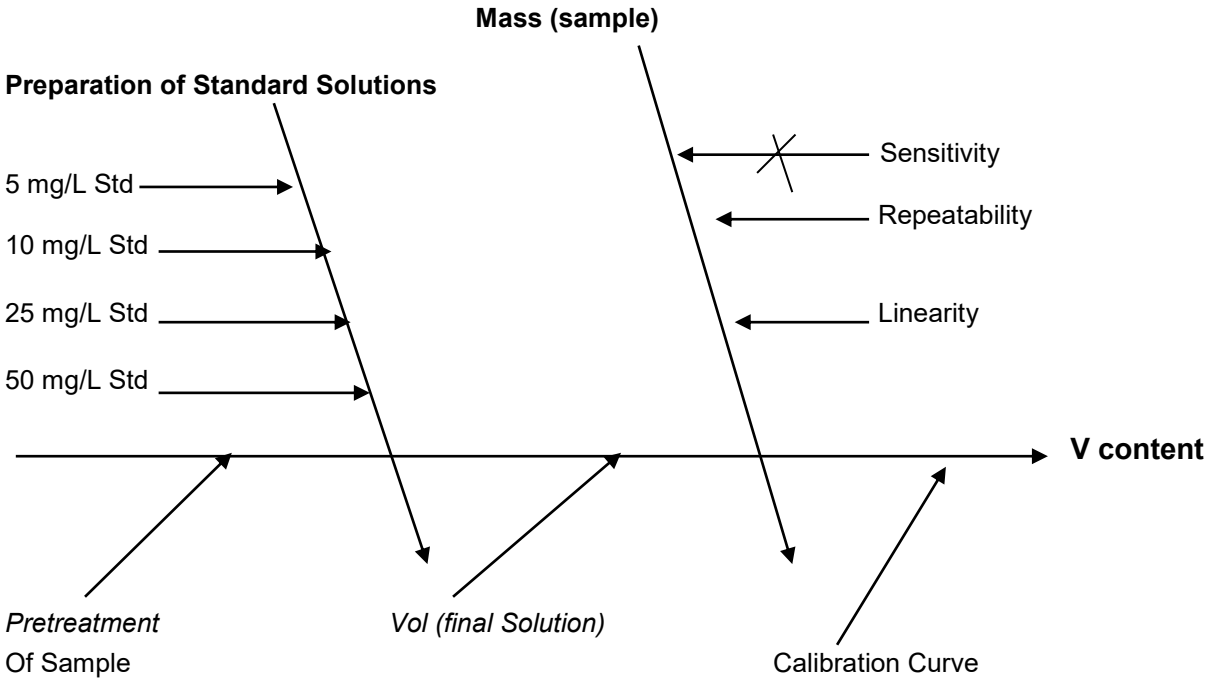
1.2 The stock solution was prepared from a 1000 mg/L commercial standard. 5, 10, 25 and 50 mg/L V standards were prepared from the 250 mg/L stock solution

1.3 The concentration of V in the sample (Cv, mg/L) was obtained from the calibration curve.

1.4 The concentration (C, mg/L) in the original sample was calculated with the following formula.

$$C = \frac{V \times C_V}{W} \quad \ldots [1]$$

F.10.3 Cause and effect diagram



F.10.4 Evaluation of uncertainty components

Step 1: Weighing the sample

The weight of the sample was obtained by subtracting the weight of empty Pt dish from weight of Pt dish and sample.

Sources of Uncertainties:

a. Associated with the calibration of the balance used

The calibration certificate states that at 95% confidence level, a weight obtained by difference within the same range was within ± 0.2 g. This uncertainty component can be expressed as a standard deviation by dividing 0.2 by 1.96, giving 0.102 g.

b. Uncertainty associated with the standard deviation of replicated weighing up to 100 g.

The standard deviation after 10 replicated weighing was 0. We take resolution/3 as the minimum standard deviation. The resolution of this balance was 0.1 g and therefore the minimum standard deviation was 0.03 g.

Combined uncertainties of weighing u(W):

$u(W) = \sqrt{(0.102^2 + 0.03^2)} = \pm 0.107$ g.

Step 2: Pretreating the sample in a clean Pt dish at high temperature

The uncertainty from pretreatment was insignificant compared to other sources.

Step 3: Transferring the residue into a clean 100-mL volumetric flask

Sources of uncertainties:

a. Uncertainty in manufacturer's volume calibration

The manufacturer states that for the 100 ml volumetric flasks, the error was ± 0.1 mL at 20°C without stating the confidence level.

Hence, uncertainty in calibration was $0.1/\sqrt{6}$ or 0.041 mL.

b. Repeatability of volume measurements

10 fill and weigh exercises on the 100-mL volumetric flask gave the standard deviation as 0.03332 mL.

c. Temperature effect

Taking the temperature variation of 5 °C and the coefficient of volume expansion of glass as 2.1×10^{-4} per °C, the 95% confidence level of volume measurement was

$100 \times 5 \times 2.1 \times 10^{-4}$ mL or 0.105 mL

Using a rectangular distribution, the standard deviation for temperature variation was $0.105/\sqrt{3}$ or ± 0.0606 mL.

The combined standard uncertainty u(V) was calculated as:

$u(V) = \sqrt{(0.041^2 + 0.03332^2 + 0.0606^2)}$ or 0.0803 mL

Step 4: Prepare V intermediate standard stock solution (250 mg/l) by diluting 25 mL of 1000 mg/L standard solution to 100 ml with water

$$C_1 \text{ (mg/L)} = \frac{V_1 \text{ x Concentration of solution (P)}}{V_2}$$

Uncertainty of V_1, $u(V_1)$:

a. Uncertainty in manufacturer's volume calibration

   The manufacturer states that for the 25 mL pipette, the error was ± 0.03 mL at a temperature of 20°C. No confidence level was stated.

   Hence, uncertainty in calibration was $0.03/\sqrt{6}$ or 0.01225 mL.

b. Repeatability of volume measurements

   10 fill and weigh exercises on the 25 mL volumetric flask gave the standard deviation as 0.01253 mL.

c. Temperature effect

   Taking the temperature variation of 5 °C and the coefficient of volume expansion of glass as 2.1×10^{-4} per °C, the 95% confidence level of volume measurement was

   $25 \times 5 \times 2.1 \times 10^{-4}$ mL or 0.026 mL

   Using a rectangular distribution, the standard deviation for temperature variation was $0.026 / \sqrt{3}$ or ± 0.01516 mL.

Hence, using the figures obtained above, the combined standard uncertainty $u(V_1)$ was:

$u(V_1) = \sqrt{(0.01225^2 + 0.01253^2 + 0.01516^2)}$ or 0.0232 mL

Uncertainty of V_2, $u(V_2)$:

Similar considerations can be applied as in Step 3, giving a 100 mL volume a standard uncertainty $u(V_2)$ of $\pm$ 0.0803 mL.

Uncertainty of stock concentration, u(P):

Concentration of stock solution (1000 mg/L) has been provided by the supplier as 1012 mg/L. As there is no confidence level stated for the uncertainty, we ignore its uncertainty.

The uncertainties were summarized in the table below:

Uncertainty Factor	Values to be used, V	Uncertainty, u	Relative Standard Deviation (RSD) = u/V
V_1	25 mL	0.0232 mL	9.3×10^{-4}
V_2	100 mL	0.0803 mL	8.0×10^{-4}

Standard uncertainty in the concentration (C_1) of this standard solution (25 mg/L) was,

$$\frac{u(C_1)}{C_1} = \sqrt{(0.000913^2 + 0.00080^2)} = 0.0012$$

The concentration of this solution, C_1, was 253 mg/L. Hence, the standard uncertainty $u(C_1)$ was $u(C_1) = 0.0012 \times 253 = 0.310$ (mg/L)

Step 5: Prepare V standard solution C_5 (5 mg/L), by pipetting 2 mL of C_1(253 mg/L) to 100 mL with water

$$C_5 \text{ (mg/L)} = \frac{V_5 \times C_1}{V}$$

Uncertainty of V_5, $u(V_5)$:

a. Uncertainty in manufacturer's volume calibration

The manufacturer states that for the 2 mL pipette the error was $\pm$ 0.006 mL at a temperature of 20°C without stating the confidence level.

Hence, uncertainty in calibration was $0.006/\sqrt{6}$ or 0.00245 mL.

b. Repeatability of volume measurements

8 fill and weigh exercises on the 2-mL pipette gave the standard deviation as 0.00055 ml.

c. Temperature effect

Taking the temperature variation of 5 °C and the coefficient of volume expansion of glass as 2.1×10^{-4} per °C, the 95% confidence level of volume measurement was,

$2 \times 5 \times 2.1 \times 10^{-4}$ mL or 0.0021 mL

Using a rectangular distribution, the standard deviation for temperature variation was $0.0021/\sqrt{3}$ or ± 0.00121 mL.

Hence, using the figures obtained above, the combined standard uncertainty $u(V_5)$ was,

$u(V_5) = \sqrt{(0.00245^2 + 0.00055^2 + 0.00121^2)}$ or 0.00279 mL

			Deviation (RSD) = u/V
V_5	2 mL	0.00279 mL	1.4×10^{-3}
V	100 mL	0.0803 mL	8.0×10^{-4}
C_1	253 mg/L	0.310 mg/L	1.2×10^{-3}

$$\frac{u(C_5)}{C_5} = \sqrt{(0.0014^2 + 0.00080^2 + 0.0012^2)} = 0.0016$$

The concentration of this solution, C_5, was 5.06 mg/L. Hence, the standard uncertainty $u(C_5)$ was $u(C_5) = 0.0016 \times 5.06 = 0.008$ mg/L.

Step 6 Prepare V standard solution C_{10} (10 mg/L), by diluting 4 mL of C_1(250 mg/L) to 100 mL with water

The procedure was the same as Step 5 and the uncertainties were summarized below:

Uncertainty Factor	Values to Be Used, V	Uncertainty, u	Relative Standard Deviation (RSD) = u/V
V_{10}	4 mL	0.0221 mL	5.5×10^{-3}
V	100 mL	0.0803mL	8.0×10^{-4}
C_1	253 mg/L	0.310 mg/L	1.2×10^{-3}

$$\frac{u(C_{10})}{C_{10}} = \sqrt{(0.0055^2 + 0.00080^2 + 0.0012^2)} = 0.0056$$

The concentration of this solution, C_{10} was 10.12 mg/L. Hence, the standard uncertainty $u(C_{10})$ was $u(C_{10}) = 0.0056 \times 10.12 = 0.057$ mg/L.

Step 7: Prepare V standard solution C_{25} (25 mg/l), by pipetting 10 mL of C_1(250 mg/L) to 100 mL with water

The procedure was the same as Step 5 and the uncertainties were summarized below:

Uncertainty Factor	Values to Be Used, V	Uncertainty, u	Relative Standard Deviation (RSD) = u/V
V_{25}	10 mL	0.016 mL	1.6×10^{-3}
V	100 mL	0.0803 mL	8.0×10^{-4}
C_1	253 mg/L	0.310 mg/L	1.2×10^{-3}

$$\frac{u(C_{25})}{C_{25}} = \sqrt{(0.0016^2 + 0.00080^2 + 0.0012^2)} = 0.0018$$

The concentration of this solution, C_{25}, was 25 mg/L. Hence, the standard uncertainty $u(C_{25})$ was $u(C_{25}) = 0.0018 \times 25.3 = 0.046$ mg/L.

Step 8: Prepare V standard solution C_{50} (50 mg/L), by pipetting 20 mL of C_1(250 mg/L) to 100 mL with water

The procedure was the same as Step 5 and the uncertainties were summarized below:

Uncertainty Factor	Values to Be Used, V	Uncertainty, u	Relative Standard Deviation (RSD) = u/V
V_{50}	20 mL	0.01926 mL	9.6×10^{-4}
V	100 mL	0.0803mL	8.0×10^{-4}
C_1	253 mg/L	0.310 mg/L	1.2×10^{-3}

$$\frac{u(C_{50})}{C_{50}} = \sqrt{(0.00096^2 + 0.00080^2 + 0.0012^2)} = 0.0013$$

Now, the concentration of this solution, C_{50} , is 50 mg/L. Hence, the standard uncertainty $u(C_{50})$ is $u(C_{50}) = 0.0013 \times 50.6 = 0.063$ mg/L.

Step 9: Calibration Curve

The least squares method was used to obtain the relationship between calibration data pairs (x_i, y_i).

There were four main sources of uncertainty to consider when estimating uncertainty of $C_{Al.}$

A. Random variations in measurement of y (inclusive of y_i and y_{obs})
B. Random effects in assigned reference value x_i
C. Constant known offset on x_i and y_i
D. The assumption of linearity may not be valid

Of these four sources, the most significant is A. Method for estimating A introduced below was through variance of residuals, S.

$S^2 = \sum(y_i - y_c)^2/(n-2)$

where

y_i is reading of i^{th} calibration point and y_c is the calculated reading from the relation $y = a + bx$ while n is the number of calibration points. In IP501, n = 5.

Then $u(x_{obs}, y) = \sqrt{(var(x))}$ with $var(x) = S^2/b^2$.

5 levels of calibration standards are used and their responses are

Concentration, x_i	Response, y_i
0	0.4088
5.06	36.58
10.12	71.96
25.30	180.7
50.60	354.7

For y = a+bx to be fitted to the above calibration, a and b can be determined as:

$$b = \frac{\sum x_i y_i - n\bar{x}\bar{y}}{\sum x_i^2 - n\bar{x}^2} \qquad a = \bar{y} - b\bar{x}$$

In this analysis,

	x	Y	xy	x^2
	0	0.4088	0.0	0.0
	5.06	36.58	185.1	25.6
	10.12	71.96	728.2	102.4
	25.30	180.7	4571.7	640.1
	50.60	354.7	17947.8	2560.4
Sum	**91.08**	**644.349**	**23432.9**	**3328**
Average	**18.216**	**128.870**	**4686.6**	**666**

Therefore,

$$b = \frac{\sum x_i y_i - n\bar{x}\bar{y}}{\sum x_i^2 - n\bar{x}^2} = \frac{4686.6 - 5 \times 18.216 \times 128.87}{3228 - 5 \times 18.216^2} = 7.006$$

$a = \bar{y} - b\bar{x}$ = 128.87-7.006 x 18.216 = 1.250

Thus, y = a + bx = 1.250 + 7.006 x
With this equation, calculated response y_c can be determined with known x and their corresponding square of difference $(y-y_c)^2$.

X	Y	Calculated y_c	$(y-y_c)^2$
0	0.4088	1.24952	0.7068126
5.06	36.58	36.27923	0.0143012
10.12	71.96	71.30894	0.0359686
25.30	180.7	176.39807	4.8406479
50.60	354.7	351.54662	1.1028865
Sum			6.701

Thus, $S^2 = \sum(y_i - y_c)^2/(n-2) = 6.701/(5-2) = 2.234$

Var (x) = $S^2/b^2 = 2.234 / 7.090^2 = 0.0455$

$u(C_V) = u(x_{obs},y) = \sqrt{}$ (var(x)) = $\sqrt{}$ 0.0455 = 0.213 (mg/L)

Step 10: Calculation of V concentration (C) in the original sample

Uncertainty Factor	Values to Be Used, V	Uncertainty, u	Relative Standard Deviation (RSD) = u/V
W	21.5 g	0.1073 g	4.99×10^{-3}
V	100 mL	0.117 mL	1.17×10^{-3}
C_V	60.3 mg/L	0.213 mg/L	3.53×10^{-3}
C_{50}	50.6 mg/L	0.063 mg/L	$1.25\ 10^{-3}$
C_{25}	25.3 mg/L	0.046 mg/L	$1.82\ 10^{-3}$
C_{10}	10.12 mg/L	0.057 mg/L	5.63×10^{-3}
C_5	5.06 mg/L	0.008 mg/L	1.58×10^{-3}

$$\frac{u(C)}{C} = \sqrt{(0.00499^2 + 0.00117^2 + 0.00353^2 + 0.00125^2 + 0.00182^2 + 0.00563^2 + 0.00158^2)}$$

$$= 0.0088$$

Now, V concentration of this solution, C, was 60.3 ppm. Hence, the standard uncertainty u(C) is u(C) = 60.3 x 0.0088 = 0.53 (ppm)

Step 11: Calculation of the Expanded Uncertainty

The expanded uncertainty U(C) is calculated by multiplying the standard combined uncertainty by a coverage factor, k, of 2:

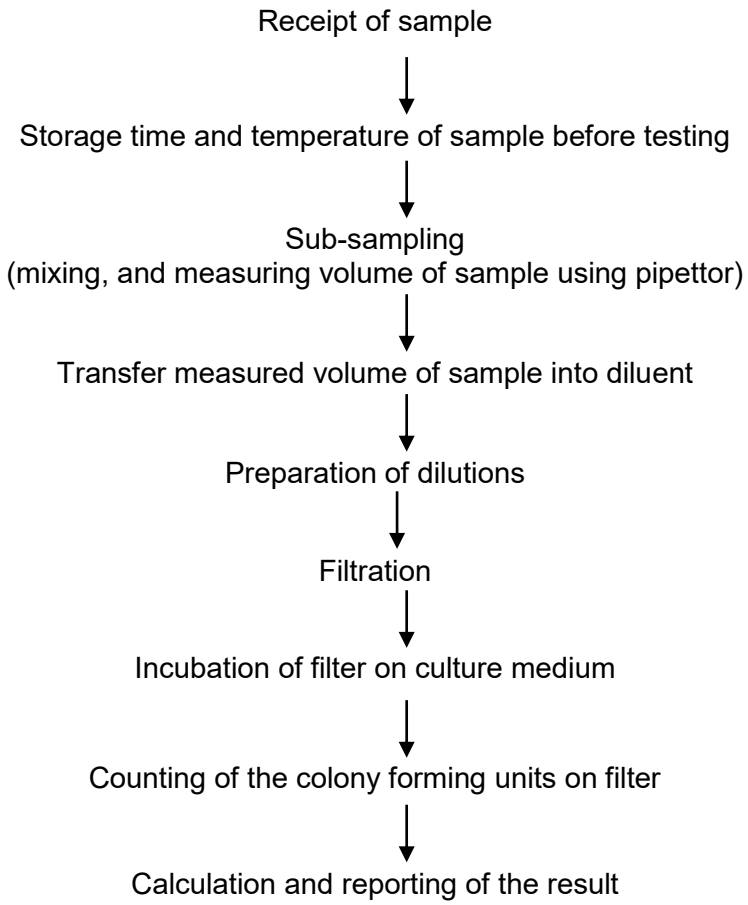
$$U(C) = 0.53 \times 2 = 1.1 \text{ (ppm)}$$

Hence, V concentration in the sample analysed was found to be 60.3 ± 1.1 (ppm)

F.11 TOTAL COLIFORM COUNT OF RESERVOIR WATER

1. APHA standard method for total coliform count of reservoir water samples by membrane filtration was used. The coliform group is defined as those facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that develop red colonies with a metallic sheen within 24 hours at 35 ^{0}C on an Endo-type medium containing lactose. The standard method specifies incubation time of 22 to 24 hours. As coliform counts are time-sensitive, an incubation time of 22 hours, and M-Endo Medium were used in this MU determination.

2. **The flow chart of the analytical procedure is presented as follows:**



3. Individual components of uncertainty were identified as shown in the cause-effect diagram (Diagram 1). Our quality performance checks showed that many of these components were under control, for example,

    - Growth promotion performance of the new batch of M-Endo Medium against the old batch was determined using Student's t statistic as shown in Table 3. The Student's t-test showed that the growth promotion performance of the two batches of medium was acceptable and not statistically different.

    - Comparison of plate counts read by analysts --- the analysts were able to duplicate their own plate count reading within 5%, and the counts of other analysts within 10%.

    - Calibrated apparatuses such as autoclave, balance, incubator, pipettors and thermometer were used, and the certificates of sensitivity and repeatability of apparatuses were within acceptable tolerance limits.

4. All the samples were analysed using all steps of the standard method.

5. 21 samples were set up and analysed on different days in duplicate pairs, analysts on different days, using different equipment (incubators, pipettors) on different days and using different batches of reagents and media on different test days.

6. Relative Standard Deviations of Reproducibility (RSD_R) was calculated for the assessment of the measurement uncertainties for counts using the following equation (modified from ISO/TS 19036).

$$RSD_R = \sqrt{[\sum_{i=1}^{n} [(\log a_i - \log b_i)/x_i]^2] / 2n}$$

where

$(\log a_i - \log b_i)/x_i$ = the relative difference between the duplicate logarithmic results

i =1,2,….n

n = number of duplicate pairs in the determination.

Table 1 : Total Coliform (TC) Tests (APHA Method) on a Series of Different Water Samples from Reservoir

Sample No.	TC / Filter Duplicate 1 (a_i)	TC / Filter Duplicate 2 (b_i)	Log a_i	Log b_i	Mean x_i	Difference log a_i - log b_i	Diff / Mean, D (log a_i -log b_i)/x_i	(Diff / Mean)^2 D^2
1	10	13	1.0000	1.1139	1.0570	-0.1139	-0.1078	0.0116
2	22	23	1.3424	1.3617	1.3521	-0.0193	-0.0143	0.0002
3	28	25	1.4472	1.3979	1.4225	0.0492	0.0346	0.0012
4	41	55	1.6128	1.7404	1.6766	-0.1276	-0.0761	0.0058
5	52	49	1.7160	1.6902	1.7031	0.0258	0.0152	0.0002
6	46	35	1.6628	1.5441	1.6034	0.1187	0.0740	0.0055
7	44	31	1.6435	1.4914	1.5674	0.1521	0.0970	0.0094
8	29	41	1.4624	1.6128	1.5376	-0.1504	-0.0978	0.0096
9	64	50	1.8062	1.6990	1.7526	0.1072	0.0612	0.0037
10	23	21	1.3617	1.3222	1.3420	0.0395	0.0294	0.0009
11	22	19	1.3424	1.2788	1.3106	0.0637	0.0486	0.0024
12	30	42	1.4771	1.6232	1.5502	-0.1461	-0.0943	0.0089
13	37	33	1.5682	1.5185	1.5434	0.0497	0.0322	0.0010
14	18	22	1.2553	1.3424	1.2988	-0.0872	-0.0671	0.0045
15	23	18	1.3617	1.2553	1.3085	0.1065	0.0814	0.0066
16	45	31	1.6532	1.4914	1.5723	0.1619	0.1029	0.0106
17	25	36	1.3979	1.5563	1.4771	-0.1584	-0.1072	0.0115
18	13	11	1.1139	1.0414	1.0777	0.0726	0.0673	0.0045
19	15	19	1.1761	1.2788	1.2274	-0.1027	-0.0836	0.0070
20	66	69	1.8195	1.8388	1.8292	-0.0193	-0.0106	0.0001
21	7	30	0.8451	1.4771	1.1611	-0.6320	-0.5443	0.2963
Grand Mean					1.4462			
Summation								0.4015
Number of duplicate analysis, *n*								21
2 x number of duplicate (2 x *n*) =						42		
Sum of (Diff / Mean)^2/ 2 *n* =						0.009560412		
Relative Standard Deviation, RSD =						0.0978		
Coefficient of Variation, CV% = 100 x RSD						9.7777		

Step 1. Transform the raw data by taking the $\log_{10}$ of the data (Column 4, 5).

Step 2. Calculate the mean of the transformed replicates (Column 6).

Step 3. Calculate the difference between the transformed replicates (Column 7).

Step 4. Divide the difference between the transformed replicates by the Mean (Column 8).

Step 5. Square each of the difference / Mean (Column 9)

Step 6. Add the differences together (Column 9) and divide by 2*n*, where *n* = the total number of pairs of duplicates (for this example *n* = 21) to get 0.009560

Step 7. Take the square root of the results in Step 6; this equals the Relative Standard Deviation of Reproducibility, which is 0.0978.

7. On examination of the data set, the relative error, difference / mean of sample No. 21 was 0.5443 which is much larger than the values obtained from other duplicate pairs. The result of this sample appeared to be outlier. The standard Grubbs test for identification of outliers of duplicate pairs was used,

$T = I\ RDI / (\sqrt{2} . RSD_R)$

Where:

RD = Relative Difference for each duplicate pair, as a decimal fraction [$RD = (a_i - b_i) / X_{mean}$]

RSD_R = Relative Standard Deviation of single measurements from within sets of duplicate pairs.

RD of sample no. 21 = 0.6320 / 1.1611=0.5443

$T = 0.5443 / (\sqrt{2} \times 0.0978) = 0.5443 / 0.1383 = 3.935$

The calculated T value, 3.94, of the result of sample no. 21 exceeded the critical value of 2.58 for 21 data points of the Grubbs T value table at 5% risk of false rejection. Hence, the result of this sample was found to be an outlier. On investigation, the pipettors used for this test were defective. The result was excluded from the calculation of the measurement uncertainty of the determinations. The measurement uncertainty was re-calculated based on 20 sample determinations as follows:

Table 2 : Total Coliform (TC) Tests (APHA Method) on a Series of Different Water Samples from Reservoir

Sample No.	TC / Filter Duplicate 1 (a_i)	TC / Filter Duplicate 2 (b_i)	Log a_i	Log b_i	Mean x_i	Difference log a_i - log b_i	Diff / Mean, D (log a_i -log b_i)/x_i	(Diff / Mean)^2 D^2	Technician
1	10	13	1.0000	1.1139	1.0570	-0.1139	-0.1078	0.0116	A
2	22	23	1.3424	1.3617	1.3521	-0.0193	-0.0143	0.0002	B
3	28	25	1.4472	1.3979	1.4225	0.0492	0.0346	0.0012	A
4	41	55	1.6128	1.7404	1.6766	-0.1276	-0.0761	0.0058	B
5	52	49	1.7160	1.6902	1.7031	0.0258	0.0152	0.0002	A
6	46	35	1.6628	1.5441	1.6034	0.1187	0.0740	0.0055	B
7	44	31	1.6435	1.4914	1.5674	0.1521	0.0970	0.0094	A
8	29	41	1.4624	1.6128	1.5376	-0.1504	-0.0978	0.0096	B
9	64	50	1.8062	1.6990	1.7526	0.1072	0.0612	0.0037	A
10	23	21	1.3617	1.3222	1.3420	0.0395	0.0294	0.0009	B
11	22	19	1.3424	1.2788	1.3106	0.0637	0.0486	0.0024	A
12	30	42	1.4771	1.6232	1.5502	-0.1461	-0.0943	0.0089	B
13	37	33	1.5682	1.5185	1.5434	0.0497	0.0322	0.0010	A
14	18	22	1.2553	1.3424	1.2988	-0.0872	-0.0671	0.0045	B
15	23	18	1.3617	1.2553	1.3085	0.1065	0.0814	0.0066	A
16	45	31	1.6532	1.4914	1.5723	0.1619	0.1029	0.0106	B
17	25	36	1.3979	1.5563	1.4771	-0.1584	-0.1072	0.0115	A
18	13	11	1.1139	1.0414	1.0777	0.0726	0.0673	0.0045	B
19	15	19	1.1761	1.2788	1.2274	-0.1027	-0.0836	0.0070	A
20	66	69	1.8195	1.8388	1.8292	-0.0193	-0.0106	0.0001	B
Summation								0.1052	
Number of duplicate analysis, n								20	
2 x number of duplicate (2 x n) =						40			
Sum of (Diff / Mean)^2/ 2n =						0.0026311			
Relative Standard Deviation, RSD =						0.0513			
Coefficient of Variation, CV% = 100 x RSD						5.1295			

Further Grubbs test on Sample 1, the sample with the highest relative error in the new sets, showed that it was not an outlier. The RSD_R obtained from these sets of results was used in MU estimation of the tests.

A sample from the same matrix/source was found to have a total coliform count of 60 cfu / filter. The cfu was converted to a log value of 1.7782. Using the RSD_R value obtained from the results listed in the above Table 2, the following expanded uncertainty was obtained using the following equation:

$MU = \log_{10}(c) \pm k \times RSD_R \times \log_{10}(c)$

At 95% confidence level, coverage factor, k, 2,

The interval of expanded uncertainty of 60 cfu / filter = Antilog [1.7782 + (2 X 0.0513 X 1.7782)] = Antilog (1.5958 to 1.9606) = 39 to 91

In this example, 10 ml of sample was filtered. Therefore, the total coliform count per 100 ml sample is 60 x 10 cfu/ 100 ml = 600 cfu/ 100 ml. The measurement uncertainty of the total coliform count of the sample is reported as:

Total coliform count, cfu/ 100 ml: 6.0×10^2 with confidence interval of 3.9×10^2 to 9.1×10^2.

The reported uncertainty is an expanded uncertainty calculated from relative standard deviations of laboratory reproducibility and using a coverage factor of 2 which gives a confidence level of approximately 95%.

8. The same approach can be used for the estimation of personal repeatability RSD in the laboratory. Using the above example, the personal repeatability RSD_r of Technician A can be calculated as follows:

$$\text{Technician A Repeatibility } RSD_r = \sqrt{\frac{\sum_{i=1}^{i=n}[(\log a_i - \log b_i)/x_i]^2}{2n}}$$

$$= \sqrt{\frac{0.0116 + 0.0012 + ... + 0.0070}{2x10}} = 0.0523$$

Coefficient of Variation, CV % = 100 X RSD_r = 5.23%

$$\text{Technician B Repeatibility } RSD_r = \sqrt{\frac{\sum_{i=1}^{i=n}[(\log a_i - \log b_i)/x_i]^2}{2n}}$$

$$= \sqrt{\frac{0.0002 + 0.0058 + ... + 0.0001}{2x10}} = 0.0503$$

Coefficient of Variation, CV % = 100 X RSD_r = 5.03%

Table 3. Comparison of Growth Promotion Performance of Medium A (New Batch) with Medium B (Old Batch)

Medium A	Log A	A - A_{mean}	(A - A_{mean})SQD	Medium B	Log B	B - B_{mean}	(B - B_{mean}) SQD
101	2.004321	0.016382	0.000268	85	1.929419	-0.050707	0.002571
98	1.991226	0.003287	0.000011	98	1.991226	0.011101	0.000123
104	2.017033	0.029094	0.000846	106	2.025306	0.045180	0.002041
95	1.977724	-0.010215	0.000104	91	1.959041	-0.021084	0.000445
89	1.949390	-0.038549	0.001486	99	1.995635	0.015510	0.000241
Mean	**1.987939**				**1.980125**		
Sum			**0.002716**				**0.005421**
SD SQD			**0.000679**				**0.001355**
Sp SQD =	(4 x 0.000679) + (4 x 0.001355) / (5 + 5 - 2) equals				0.001017		
Sp					0.0318919 7		
Sp x SQRT (1/5 + 1/5)					0.020170		
t =	(1.987939 - 1.980125) / 0.03189197 x SQRT(0.2 + 0.2)						
t =	0.007813 / 0.020170 equals				0.3873719 2		
F =	0.000679 / 0.001355				0.501047690		

The tabulated 95% critical value is 2.306 for 8 degrees of freedom (i.e. 5 + 5 – 2).

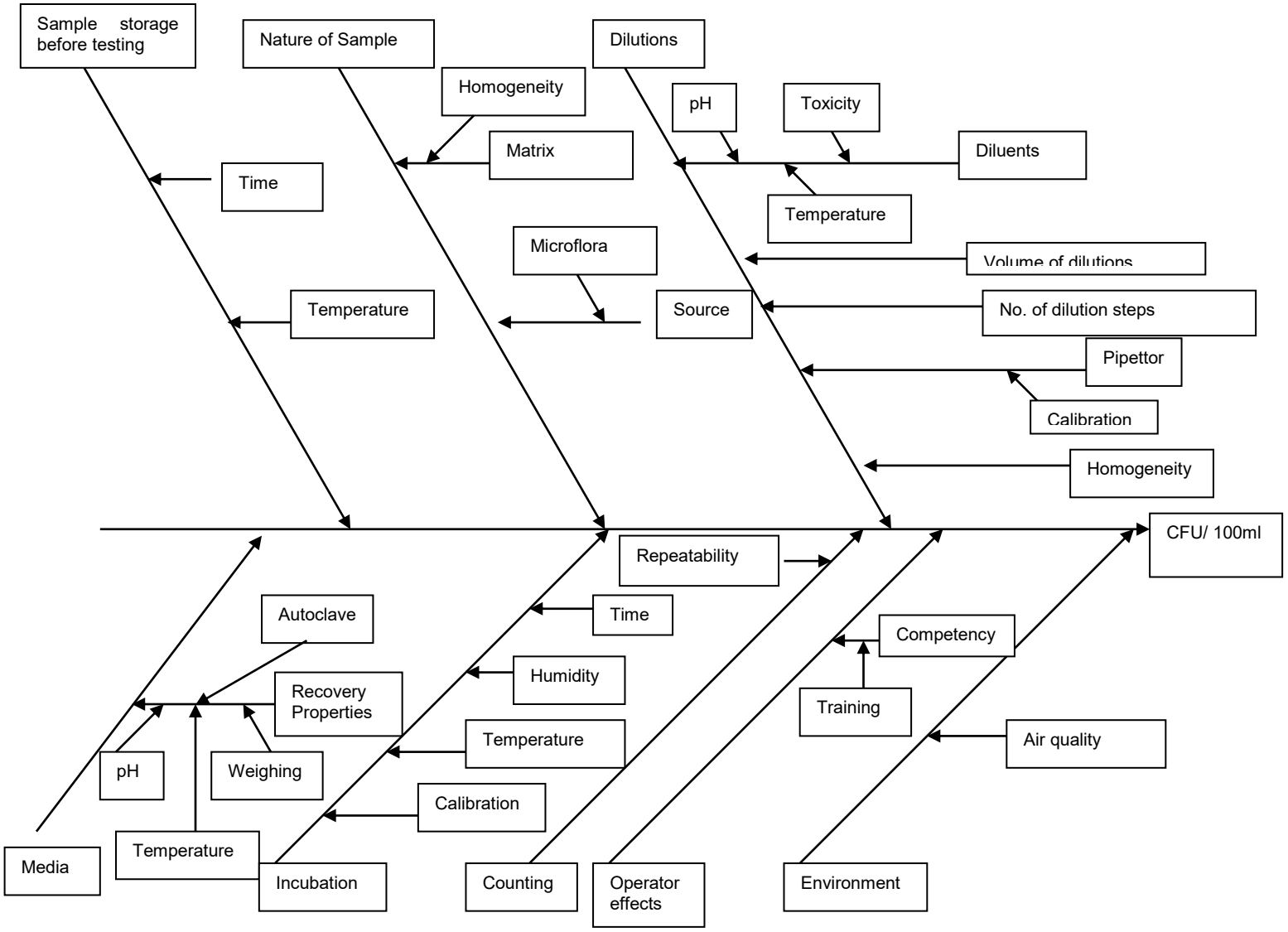
This critical value exceeds the calculated value of 0.3874; therefore H_0 is not rejected and there is no difference between the means of results obtained. There are no significant differences between the growth promotion performances of the two batches of medium.

The general equation used for the above Student – t statistic for the comparison of two sets of data is as follow:

$$t = \frac{(\overline{X}_1 - \overline{X}_2)}{S_p \sqrt{(1/n_1 + 1/n_2)}}$$

where $S_p^2 = [\ s_1^2(n_1 - 1) + s_2^2(n_2 - 1)\ ] / (n_1 + n_2 - 2)$

Diagram 1: Cause and Effect Diagram on MU Estimate of Total Coliform Count of Water from Reservoir



APPENDIX G
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