ACCREDITATION SCHEME FOR LABORATORIES

Technical Guide 4

A Guide on Measurement Uncertainty in Medical Testing

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1.0 General

1.1 Introduction

This document provides guidance on how measurement uncertainty can be estimated and be applied in the field of medical testing. The aim is to provide a general overview of the measurement uncertainty concept, and practical guidelines to assist medical laboratories meet and comply with laboratory accreditation requirements of ISO 15189:2022 “Medical laboratories – Requirements for quality and competence”.

The objectives of this Guide are to:

(a) provide guidance on the processes of implementing the measurement uncertainty concept in medical testing;
(b) assist medical laboratories calculate the measurement uncertainty of their measured quantity values;
(c) describe practical approaches concerning the estimation, meaning and use of measurement uncertainties.

In providing these guidelines, the Work Group recognizes that the theoretical and practical aspects of measurement uncertainty in medical testing are still evolving and that this Guide will be continually updated.

1.2 Background

“When reporting the result of a measurement of a physical quantity, it is obligatory that some quantitative indication of the quality of the result be given so that those who use it can assess its reliability. Without such an indication, measurement results cannot be compared either among themselves or with a reference value given in a specification or standard”. [Introduction from GUM – “Uncertainty of measurement – Guide to the expression of uncertainty in measurement”.]

Measurement is a process of experimentally obtaining a value for a quantity using a measurement procedure comprising a logical set of operations. If the measuring system is sufficiently sensitive, repeated measurements on the same sample generally produces different values, even if measuring conditions are kept as constant as possible. Thus repeated measurements do not produce a single value for the measured quantity, and therefore there is uncertainty as to the true value of the measured quantity. Such result variability reflects the cumulative effect of unavoidable fluctuations in electro-mechanical performance, reagents, calibrators, laboratory environment etc. The expected dispersion of values obtained from repeated measurements on the same sample can be statistically described by calculation of the standard deviation of the values (standard measurement uncertainty) from the mean value. Measurement uncertainty is therefore a property of a measurement result, and provides a quantitative estimate of the reliability of the result.

Without knowledge of their associated uncertainties, it is not possible to determine if two numerically different measured values of the same measurand are also statistically different, and therefore a measurement result without its uncertainty cannot be meaningfully compared with a reference value or a previous result of the same type. Also, knowledge of the magnitude of measurement uncertainty is critical to assessing whether the measurement results produced by a measurement procedure are fit for clinical

applications. Estimating measurement uncertainty and ensuring that the uncertainty is fit-for-purpose are among the responsibilities of every medical laboratory.

1.3 GUM Principles

The GUM approach was developed primarily for measurement in physics, but the principles can also be applied to chemical and biological measurements. The principles of measurement uncertainty that ensures test outputs are fit for clinical purpose are based on the GUM approach, as follows:

(a) To specify what is being measured,
(b) To identify what causes the result to change,
(c) To quantify the uncertainty components - Types of uncertainties are as follows:
    i. Type A: evaluation of a component of measurement uncertainty by a statistical analysis of measured quantity values obtained under the defined measurement conditions;
    ii. Type B: evaluation of a component of measurement uncertainty determined by means other than a Type A evaluation e.g from a calibration certificate.
(d) To convert to standard uncertainties,
(e) To combine the uncertainties,
(f) To express as expanded uncertainty.

1.4 Scope

This Guide explains the fundamental concepts, estimation and application of measurement uncertainty in medical laboratories. The recommendations provided are consistent with the GUM approach and with ISO standards related to laboratory accreditation at the measurement (analytical) phase, but do not fully address the following important sources of uncertainty of the measurement result:

(a) Biological variation of the measurand,
(b) Pre- and post-measurement (analytical) processes.

In the analytical phase of a measurement result, the Guide discusses the sources of measurement uncertainty, the generation of statistical estimates of uncertainties and their combination, and the use of uncertainty estimates. The Guide applies only to quantitative measurements.

2.0 Principles of Measurement Uncertainty

2.1 What is Metrological Traceability?

Metrological traceability is defined as a property of a measurement result whereby the result can be related to a reference (usually national or international standards) through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

ISO 15189, Clause 6.5.3 requires that "*A programme for calibration of measuring systems and verification of trueness shall be designed and performed so as to ensure that results are traceable to SI units or by reference to a natural constant or other stated reference.*" Ensuring that laboratory measurement results are comparable requires the use of well-recognized reference materials for method validation, calibration, estimation of measurement uncertainty and quality control/quality assurance. The long-term clinical goal is to ensure comparability of measurement results produced by any laboratory at any time, i.e. two measurement results for the same analyte obtained by different measurement procedures at different times and different locations are comparable via their traceability to a common reference standard.

To achieve an improved accuracy for measurement results, the values assigned by manufacturers to calibrators and control materials supporting routine measurement procedures are required to be traceable to higher-order reference measurement procedures and reference materials. An example of a primary reference material is the Standard Reference Material (SRM) 914, which is crystalline creatinine available from The National Institute of Standards and Technology (NIST). In addition, the availability of a secondary commutable reference material, matrix-matched for human serum is critical for effective implementation of standardization of calibrators for routine measurement procedures (Figure 1). It should be noted that the uncertainty of the quantity value increases as it is transferred down the traceability chain to the routine calibrator.

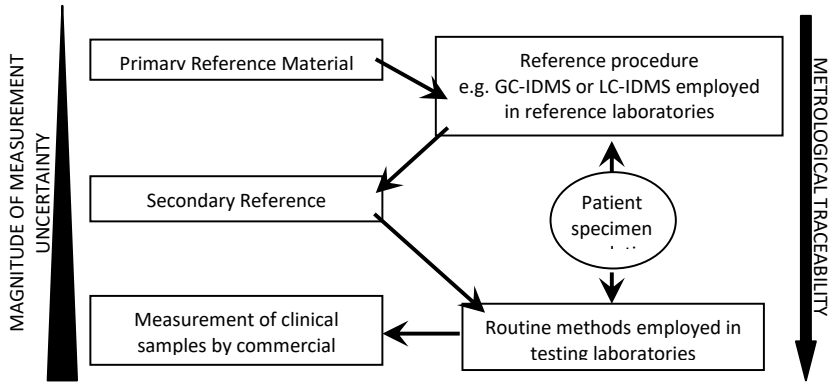

Figure 1: The reference measurement system for standardization of calibrators.

2.2 Why Measurement Uncertainty?

Measurement uncertainty is defined as a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. Measurement uncertainty is a property of the measurement result of a measurement, not a property of the measurement procedure. It provides quantitative estimates and thus represents the expected variability in a laboratory result produced by a measurement procedure control.

ISO 15189, Clause 7.3.4 requires that "*The laboratory shall determine the uncertainty of results, where relevant. Uncertainty components which are of importance shall be taken into account*". It is the responsibility of the laboratory that produces the results to evaluate measurement uncertainty and ensure that the test results are fit for their clinical purpose.

Measurement uncertainty estimates are essential for assessing whether methods are suitable for clinical use and for comparison of results of a similar type. The thorough assessment of the components contributing to the measurement uncertainty may also reveal aspects of a test method to which attention should be directed in order to improve procedures and accuracy of the measurement.

2.3 Sources of Measurement Uncertainty

Significant sources of measurement uncertainty shall be listed for each analyte. The following is a list of possible sources of uncertainty covering many types of measurand encountered in medical laboratories:

(a) Pre-measurement phase:
- i. differences in patient preparation,
- ii. specimen collection technique,
- iii. transport of sample,
- iv. storage time and storage temperature of sample,
- v. intra-individual variability (such as pregnancy, fasting/non-fasting, drug use, diurnal),
- vi. within individual biological variation.

(b) Measurement phase:
- i. environmental conditions of the laboratories, such as temperature, humidity and dust that may affect the measurements and sample stability,
- ii. operator bias, including interpretation of measurement procedure and reading of data from an equipment,
- iii. measuring systems, such as balances, glassware or thermometer calibrations,
- iv. reagents and calibrators, including batch variations,
- v. uncertainty of the calibrator values and dispensed volumes,
- vi. random variation in repeated observations of the measurand under identical conditions,
- vii. method recoveries and blank correction.

(c) Post-measurement phase:

i. software (including algorithms),
ii. reporting, i.e. number of significant figures .

Although it is very important to identify and minimize significant pre-measurement and post-measurement uncertainties, measurement uncertainty is concerned only with those sources that arise from within the measuring system i.e. from sample preparation to production of measurement result. Hence, pre- and post-measurement uncertainties are not included in the estimation of measurement uncertainty. However, if pre- or post-analytical factors are included in the definition of the measurand, sources of uncertainty associated with these should be evaluated and also made available.

2.4 Measurement Bias

Accuracy is a qualitative term referring to whether there is agreement between a measurement made on an object and its true (target or reference) value. Bias (systematic error) is a quantitative term describing the difference between the average of measurements made on the same object and its true value. Bias remains constant or varies in a predictable manner.

It is a general requirement of the ISO GUM that known significant bias should be eliminated or minimized e.g. by re-calibration. Next, the uncertainty associated with the value of the bias correction is evaluated and, if significant relative to the imprecision, combined with the latter to obtain the combined uncertainty of the measurement values.

Ideally, a routine procedure would be referenced to a commutable certified reference material (CRM) with an SI-traceable assigned value, but this option is presently available for only a small number of analytes. For those lacking SI-traceability, it is often useful to check the accuracy of the test results by estimating bias relative to conventional reference materials, reference methods, trueness controls, spiking studies, interlaboratory comparisons, external quality assessment schemes, etc. It is important to know whether the compared measurands are identical before drawing conclusions about bias estimates.

Whichever approach is used, the mean value generated by the routine method is compared with the reference value to assess if there is a significant bias by using the Student's *t* test. If the bias is small and insignificant at 95% confidence level, no correction factor is applied but an estimated uncertainty may be included in the combined uncertainty; otherwise, it should be investigated and if possible, eliminated or corrected by recalibration of the measurement procedure. The uncertainty of the bias value (combined uncertainty of the reference or target value used and the uncertainty of the mean value (standard error of the mean, SEM) of the reference obtained by the laboratory under repeatability conditions) or correction factor used should be evaluated to be included in the combined uncertainty.

2.5 Measurement Precision

The term “precision” is a qualitative term, defined as closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. The “specified conditions” can be, for example, repeatability conditions of measurement, intermediate conditions of measurement, or reproducibility conditions of measurement. It varies in an unpredictable manner. A higher degree of precision is achieved as random variations decrease. Figure 2 is a graphical presentation depicting the concept of bias and imprecision of measurement data.

A reasonable estimate of the imprecision for the whole measurement procedure is to be made in order to allow a realistic verification of the procedure. The measurement precision component includes the effect of:

(a) the within-laboratory variation for the routine measurement procedure,
(b) the variation between different laboratories pertaining to the same measurement procedure.

If the medical laboratory staff is interested only in measurement uncertainty estimates of its own routine measurement procedure, the within-laboratory precision estimates based on either patient pooled samples or internal quality control materials will provide sufficient data. In any situation that the medical decision point is close to the detection limit (e.g. Troponin, TSH or PSA) then pooled patient samples should be used.

For procedures already employed in routine laboratory service, the most efficient approach to estimating the imprecision of the results is to calculate the standard deviation (SD) or coefficient of variation (CV, CV%, RSD – relative standard deviation) of the results achieved for the appropriate internal quality control materials during a certain period of time. Reference materials or pooled patient samples should be chosen at two or more clinically relevant concentrations. A statistically valid number of results should be collected across all routine works that are reasonably expected to have a detectable influence on the results produced (e.g. calibrator and reagent batches, different operators, different equipment, etc).

To estimate the measurement reproducibility of one particular routine measurement procedure, data may be obtained by participating in collaborative comparison studies (i.e. external quality assessment schemes). It involves several equipment, laboratories, reagent lots and operators over a period of time which includes all the potential sources of imprecision. This approach represents the whole combined between-laboratory precision of the analytical procedure in routine practice.

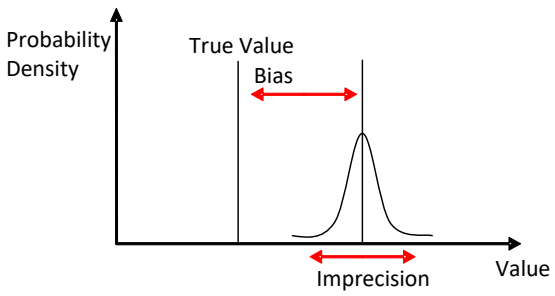

Figure 2: A graphical presentation showing accuracy and precision of Data.

2.6 Measurement Uncertainty in Medical Testing

The knowledge of uncertainty in test results by clinicians may reduce the potential for significant clinical misinterpretation. Although much of the measurement uncertainty data may appear to have no clinical value to the requesting doctors, it does have the potential to contribute to patient care in some specific clinical settings. Therefore, efforts should be taken for the laboratories to understand the clinical uses of the test results they report, and identify any major sources of uncertainty component that could significantly affect clinical interpretation. Such information should be readily available from the laboratory on request.

2.7 Role of Metrological Traceability

For measurement results produced by different measurement procedures for the same analyte to be comparable, they must be metrologically traceable to the same commutable reference material. The implementation of calibration traceability to higher-order reference methods and materials is the best approach to achieve the needed metrological comparability in biochemical measurement results, regardless of the measurement procedures used and/or the laboratories where the analyses are performed. The step towards standardization of clinical laboratory test results has been undertaken by an international consortium, the Joint Committee for Traceability in Laboratory Medicine (JCTLM). It promotes international comparability, reliability and equivalence of measurement results in clinical laboratories for the purpose of improving healthcare.

Without traceability to a reference measurement procedure or calibrator, physicians may receive results for the same patient from different laboratories with little or no information about the comparability of results.

3.0 Estimation of Measurement Uncertainty

3.1 Measurement Uncertainty Targets

A “measurement uncertainty target” is a quantitative measure used to describe the quality of the reference measurement value that the laboratory is aiming for. This concept is sometimes referred to as the “analytical goal”, and is based on fitness-for-purpose criteria. This term should not be confused with the “target value” often used in laboratory medicine to depict the “true value”.

There are three widely accepted levels of analytical goal for imprecision based on intra-individual biological variation:

Optimum: $CV_{imp} \leq 0.25\ CV_{intra}$
Desirable: $CV_{imp} \leq 0.50\ CV_{intra}$
Minimum: $CV_{imp} \leq 0.75\ CV_{intra}$

Where

CV_{imp} = Coefficient of variation derived from intermediate measurement precision (several runs, operators, reagent and calibrator batches)

CV_{intra} = Coefficient of variation derived from intra-individual biological variation of the specified measurand

A laboratory should initially set one or more measurement uncertainty targets before estimating measurement uncertainties. Such targets may be based on biological variation, international expert group recommendations, regulatory guidelines or local clinical laboratory judgment. The target for comparison should be relevant to the clinical application of the test result. A widely used approach to setting measurement uncertainty target is to define the upper acceptable limit for imprecision as a proportion of the intra-individual biological variation of the measurand. This approach is physiologically appropriate for some analytes (e.g. serum calcium, serum albumin, etc) but not others (e.g. urine sodium, which is significantly affected by dietary intake, hCG in pregnancy). The principle is that the uncertainty of the measurand result should not add significantly to the unavoidable variability of the analyte in the patient due to its biological variation. E.g. if $CV_{imp} = CV_{intra}$, then ~40% variability is added to that due to the biological variation alone, whereas if $CV_{imp} \leq 0.50\ CV_{intra,}$ then only ~12% is added.

For further information on biological variation data, refer to the webpage of Dr. James Westgard at http://westgard.com/biodatabase1.htm. As a general principle, it has been widely suggested that the analytical goal for precision of a test method remains below half the intra-individual biological variation ($CV_{imp} < 0.50\ CV_{intra}$). For example, CV_{intra} for cholesterol is 5.4 %, the desirable specification/goal for precision is calculated as 2.7 %. The goal is to demonstrate the preciseness in the method even though inherent biological variation may exist. This allows for a meaningful comparison of test results produced by any laboratory at any time.

The data obtained during the verification for measurement uncertainty shall be compared to the target measurement uncertainty. Checks should be performed regularly during routine operation. If the target is satisfied and the analytical variability is appropriately less than the biological variability, the test can be confidently used for clinical diagnosis and monitoring. If the target measurement uncertainty is exceeded, the uncertainty budget should be studied for major sources of uncertainty that might be reduced. If CV_{imp} reduction is unsuccessful or not feasible, it may be appropriate to consider a change of method.

There are three levels of analytical goal for bias based on biological variation:

Optimum: $B_{lab} \leq 0.125 (CV_{intra}^2 + CV_{inter}^2)^{1/2}$
Desirable: $B_{lab} \leq 0.250 (CV_{intra}^2 + CV_{inter}^2)^{1/2}$
Minimum: $B_{lab} \leq 0.375 (CV_{intra}^2 + CV_{inter}^2)^{1/2}$

where

B_{lab} = Laboratory measurement bias

CV_{intra} = Coefficient of variation derived from intra-individual biological variation of the specified measurand

CV_{inter} = Coefficient of variation derived from inter-individual biological variation, which shall not include CV_{intra}

3.2 Defining a Measurand

The measurand is the quantity intended to be measured, or a particular quantifiable property of the analyte used in the measuring system. Measurement uncertainty and the magnitude of its components depend mainly on the definition of the measurand which requires description of both the quantity intended to be measured and, if the measurand definition depends on the measurand procedure (e.g. catalytic activity concentration of enzymes; antibody specificity), how it is measured.

It should be noted that most measurement procedures do not directly measure the desired quantity. For example, a manual count of white blood cells in urine does directly measure the quantity (number) that is intended to be measured. In contrast, for the amount of calcium concentration in serum, the quantity intended to be measured is the number of calcium atoms in a specified volume of serum, but as this cannot be directly measured, another property of calcium is measured e.g. the amount of light absorbed at a specified wavelength when calcium complexes with a dye. Through measurement of the signal by a calibrator with a known concentration of calcium, we can relate the response of the 'surrogate' quantity in the unknown sample to the desired quantity via the measurement equation. If the amount-of-calcium concentration in serum is measured by atomic absorption spectrometry, the quantity actually measured is different, but the measurand has not changed.

The definition of any measurand requires description of:

(a) the system to be examined (e.g. plasma, urine, whole blood),
(b) the component in the system (e.g. glucose, leukocytes, coagulation process, number),
(c) the kind-of-quantity (e.g. amount-of-substance concentration, substance rate, activity concentration, number concentration).

For example, when measuring alkaline phosphatase in human serum, alkaline phosphatase catalytic activity is the quantity actually measured. Thus, alkaline phosphatase is the component (analyte), and alkaline phosphatase catalytic activity concentration in serum is the measurand. However, in this type of measurement,

definitions of the measuring conditions are critical because changes in pH, ionic strength, co-factors etc change the quantity being measured. Therefore, adequate description of the measurement procedure forms part of the measurand description.

Ideally, a well-defined analyte will have a quantity value that is metrologically traceable to the definition of an SI measurement unit and is assumed to be independent of the measurement procedure employed (rational method). For example, the amount-of-substance concentration of glucose in human serum is a well-defined measurand (i.e. single chemical entity of known molecular weight) with the measurement results metrologically traceable to the definition of the mole.

In most cases analytes are not sufficiently well-defined and measurements are not metrologically traceable to SI units e.g. arbitrary international units (IU). In some cases, the measurand has a quantity value that is metrologically traceable to a measurement unit, but only provided that a specific measurement procedure has been used (empirical method). In this scenario, the measurement procedure becomes an element in the definition of a measurand. For example, in the measurement of the concentration of a peptide hormone in plasma, the use of different antibodies from different manufacturers produces different results as different measurands may be measured. The reason is the exact structure of the targeted peptide hormone is not even known and different molecular entities (isoforms) of the peptide hormone are measured depending on the specificity of the antibodies. Therefore, in this situation, the definition of the measurand includes the measurement procedure with the prescribed use of a particular antibody.

In addition, definition of a measurand can determine the relevance of pre-analytical sources of variation. A measurand that is defined for a given person with sampling specification restricted to a particular state of the patient (e.g. fasting) or to a particular time (e.g. early morning) will have minimum pre-analytical sources of variation. For example, if the measurand is defined as cortisol in serum, S-Cortisol; amount-of-substance concentration (without respect to fasting state or time of day), the measurement uncertainty will reflect the variation due to changes in fasting status and diurnal variation of the hormone. However, if the measurand is defined as fasting early morning cortisol in serum, fS(08:30)-Cortisol; amount-of-substance concentration, the biological variation due to changes in the fasting state and diurnal variation will be removed from consideration.

A laboratory may use more than one of the same measuring system for the same measurand. For this purpose, SD (u_{Rw}) must be estimated separated for each measuring system, and then a pooled average can be calculated.

3.3 Number of Significant Digits

Laboratories should report test results to the number of significant digits consistent with the measurement uncertainty of the results. Laboratories need to be aware that patient results should be reported to the appropriate number of significant digits as use of an inappropriate number may adversely affect clinical interpretation.

According to GUM, it usually suffices to quote combined standard uncertainty, u_c and expanded uncertainty, U to at most two significant figures. The measurement value will have the same number of decimal places as the uncertainty. In reporting final results, it

is generally better to round uncertainties up rather than to the nearest digit. For example, if x = 21.272 mg with U = 1.1 mg, x should be rounded to 21.3 mg. In medical laboratories it is usual to round the uncertainty to the same significant figure as is used for the reported result.

Rounding may affect the statistical use of results (e.g. quality control data, comparison of results, clinical trials) and should be deferred until the final result is calculated.

3.4 Re-evaluation of Measurement Uncertainty

The measurement uncertainty is documented in an uncertainty budget, which is defined as a statement of measurement uncertainty, of the contributing components, their calculations and combinations.

The measurement uncertainty shall be re-evaluated if any source of uncertainty listed in Section 2.3 changes significantly. For example, in the analytical phase, important changes such as the measuring system, calibrators, reagents and/or measurement procedures are usually reflected in the internal quality control system. If these effects of change are significant, a review of the measurement uncertainty is necessary.

The uncertainty of a reagent value provided by a supplier can only be retained for a new batch if the supplier has validated that performance properties and storage stability of the new batch meet the specifications of the previous batch. Besides, if the uncertainty of a new batch of a calibrator value is changed as indicated in the certificate of analysis by a supplier, the uncertainty budget shall be reviewed.

4.0 Application of Measurement Uncertainty

For clinical laboratories the end-point of estimating measurement uncertainty is to define an interval of quantity values within which the true value of a measured quantity lies with a stated level of confidence. Measurement uncertainty can be estimated by two different approaches:

(a) The bottom-up approach according to GUM principles is based on a comprehensive categorization of the measurement in which each potential source of uncertainty is identified and quantified. The estimates of uncertainty, expressed as standard deviations (standard uncertainties), are assigned to individual components of the procedure which are then mathematically combined using propagation rules to provide the “combined standard uncertainty” of the result.

(b) The top-down approach uses available laboratory test performance information, such as method validation, intra-laboratory and inter-laboratory data, to calculate estimates of the overall uncertainty associated with the result produced by a given measuring system.

Other approaches involve various combinations and/or modifications of the top-down and bottom-up approaches. In both cases, bias needs to be addressed separately and the uncertainty in the estimate of bias, depending on its magnitude relative to other sources, is included in the combined standard uncertainty.

4.1 The Bottom-up Approach

4.1.1 Step 1: Specify the Measurand

The first step is to identify the measurand, which is a clear and unambiguous statement of what is intended to be measured. Also required is a quantitative expression (quantitative equation) relating the value of the measurand to the parameters on which it depends. All the information should be in the Standard Operating Procedure (SOP) or the manufacturers' instrument and method descriptions. Refer to Section 3.2 for requirements and considerations in defining a measurand.

4.1.2 Step 2: Identify the Sources of Uncertainty

A comprehensive list of relevant sources of uncertainty should be assembled. It is generally necessary to develop and record a list of sources of uncertainty relevant to an analytical method. In medical testing, sources of uncertainty are commonly grouped as pre-analytical, analytical and post-analytical phases as described in Section 2.3. This Guide considers only uncertainty sources that are directly related to the analytical phase itself.

In medical testing, it is common to minimize, where possible, the pre- and post-analytical uncertainties by implementing standardized protocols for patient preparation, staff training, specimen collection, transportation, storage and time limit to measurement. Note that blunders, spurious errors or any technical non-compliance shall not be considered as an element in an uncertainty budget.
It is convenient to start with the measurement equation used to calculate the measurand value. The relationship of the input quantities in the equation will determine how their uncertainties (such as SD or CV) are to be combined when calculating the combined uncertainty. All input quantities in the equation may have uncertainties associated with their values and will therefore contribute to the total uncertainty. Using a cause-and-effect diagram is helpful in listing the sources of uncertainty (see 7.0, Step 2). It helps to combine similar effects, avoid double counting of sources, delete sources that cancel or only have negligible effect.

4.1.3 Step 3: Quantify Standard Measurement Uncertainty

Once the input quantities and their relationships in a measurement model have been identified, the next step is to quantify the uncertainty arising from these sources. The information can be obtained from within the laboratory (method validation, internal quality control), from manufacturers of instruments (specifications and test reports), from certificates (e.g. for the calibrators and reagents), and from the scientific literature (e.g. biological variation).

All uncertainty components should be expressed as standard deviations (SD) or as relative standard deviations (CV). Type A uncertainties are typically estimated as the SD of repeated measurements. Type B uncertainties are based on literature, calibration

certificate, professional experience and so on. This requires information or assumptions on how values for the specific quantity are distributed (e.g. normal, rectangular or triangular).

For example, if the input quantity x_i is a result reported in a certificate, the result is normally presented in the form of $x_i \pm U$, where x_i is the best estimate and U is the expanded uncertainty calculating by multiplying the standard uncertainty $u(x_i)$ with a coverage factor k at a stated confidence level. The standard uncertainty shall therefore be calculated as

$$u(x_i) = U / k$$

If the manufacturer does not state the confidence level or the coverage probability, then an assumption on the distribution function has to be made. For example, a 10 mL Grade A volumetric flask is certified within ±0.2 mL, but without a statement as to whether the 0.2 mL represents a 68 %, 95 % or 99 % confidence, or a maximum error. In this situation a professional 'guess' needs to be made as to whether for such equipment the true volume delivered is likely to be close to 10 mL (a triangular distribution of probability), or there is an equal probability that the volume delivered could be anywhere between -0.2 to +0.2 mL of 10.0 mL (a rectangular distribution of probability). For the latter the standard uncertainty is calculated as $0.2 / \sqrt{3} \approx 0.12$ mL assuming a rectangular distribution. Refer to Appendix 2 for further information on three common distribution functions and transformation of Type B uncertainties.

Often, the result of a particular measured quantity value may be influenced by factors not included in the measurement equation, such as matrix effect, instrument sensitivity etc. To the extent that these factors can be identified and the uncertainty attributable to each factor quantified, an extended function can be defined.

4.1.4 Step 4: Calculate the Combined Standard Measurement Uncertainty

Following the estimation of individual or groups of components of uncertainty and expressing them as standard uncertainties, the next stage is to calculate the combined standard uncertainty using simple law of propagation rules. The general relationship between the combined standard uncertainty u_c of a value and the uncertainty of the independent parameters $x_1, x_2, \ldots x_n$ on which it depends is

Equation (1)
$$u_c^2(y) = \sum_{i=1}^{N} \left(\frac{\partial f}{\partial x_i} \right)^2 u^2(x_i)$$

where $u_c(y)$ = combined standard uncertainty
f = function describing the estimate of the measurand y in terms of x_i
$u(x_i)$ = standard uncertainty for each uncertainty component

The partial derivatives $\partial f / \partial x_i$ are called sensitivity coefficients which describe how the value y varies with changes in the values of the input values x_i. Wherever possible,

sensitivity coefficients are calculated for each component and incorporated in the calculation of a combined standard uncertainty and thus, an expanded uncertainty.

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

Rule 1

For models involving only a sum or different of quantities, e.g. $y = (a + b + c + ..)$ or y = (a + b) – (c + d), the combined standard uncertainty is given by

Equation (2) $$u_c(y(a,b,c..)) = \sqrt{u(a)^2 + u(b)^2 + u(c)^2 ....}$$

Rule 2

For models involving only a product or quotient, e.g. $y = (a \times b \times c \times ..)$ or $y = a/(b \times c \times \ldots)$, the combined standard uncertainty is given by

Equation (3) $$u_c(y) = y \times \sqrt{\left(\frac{u(a)}{a}\right)^2 + \left(\frac{u(b)}{b}\right)^2 + \left(\frac{u(c)}{c}\right)^2 ...}$$

where $(u(a)/a)$ etc are the uncertainties in the parameters, expressed as relative standard uncertainties.

In practice, a spreadsheet software is often used for the calculations..Alternatively, dedicated software such as GUM Workbench® which summarizes the result of the calculations in a simple uncertainty budget may also be used.

4.1.5 Step 5: Calculate the Expanded Measurement Uncertainty

The expanded uncertainty, U is obtained by multiplying the combined standard uncertainty $u_c(y)$ by a coverage factor, k.

Equation (4) $$U = ku_c(y)$$

The expanded uncertainty is required to provide an interval which may be expected to encompass a large fraction of the distribution of values attributed to the measurand. When the probability distribution characterized by y and $u_c(y)$ is approximately normal distribution, one can assume that taking $k = 2$ produces an interval having a level of confidence of approximately 95 % and that taking $k = 3$ produces an interval having a level of confidence of approximately 99 %.

4.1.6 Report the Measurement Uncertainty

When reporting the result of a measurement, at a minimum, one should

(a) Give a full description of how the measurand Y is defined,
(b) State the result of the measurement as $Y = y \pm U$ and give the units of y and U,
(c) Give the value of the coverage factor k used to obtain U,
(d) Give the approximate level of confidence associated with the interval $y \pm U$.

According to GUM, it usually suffices to quote combined standard uncertainty, $u_c(y)$, and expanded uncertainty, U, to at most two significant figures. For use in the medical laboratory, the expanded measurement uncertainty U should be rounded up to have the same number of decimal places as the reported measurement result.

It is advisable to prepare a full uncertainty budget which has detailed information on the measurement equation, all input values x_i, their standard uncertainties as well as the combined uncertainty.

For example, if the glucose concentration in human serum is reported to be 6.606 mmol/L and the expanded uncertainty was calculated as ± 0.094 mmol/L, the expanded uncertainty should be finally rounded to 0.1 mmol/L for practical use.

4.1.7 Summary of the Bottom-up Approach

1. **Step 1 - Specify the measurand**

   Clearly specify the quantity intended to be measured. This specification must include all of the factors that could significantly affect the measurement results. If relevant, express mathematically the relationship between the measurand and all the input quantities upon which the measurand depends.

2. **Step 2 - Identify the sources of uncertainty**

   List the possible sources of uncertainty. This will include sources that contribute to the uncertainty on the parameters in the relationship specified in Step 1. Using a cause-and-effect diagram provides one possible means of developing a suitable, structured analysis of uncertainty contributions.

3. **Step 3 - Quantify standard measurement uncertainty**

   Estimate the size of the uncertainty component associated with each potential source of uncertainty identified, either by the statistical analysis of repeated observations (Type A uncertainties) or by other means (Type B uncertainties) such as taking the uncertainty of a reference standard from a calibration certificate, estimating temperature effects on test results based on theoretical predictions, estimating the uncertainty of physical constants based on data in reference books and so on. Express each contribution as a standard uncertainty (SD).

4. **Step 4 - Calculate the combined standard measurement uncertainty**

   Determine the combined standard uncertainty of the measurement result from the standard uncertainties. Evaluate the sensitivity coefficients for all input quantities either directly by differentiation of the mathematical function or indirectly by experiment, and combine the uncertainties using Equation 1. Alternatively, use appropriate rules to calculate the combined uncertainty.

5. **Step 5 - Calculate the expanded measurement uncertainty**

Multiply the combined standard uncertainty by the appropriate coverage factor associated with the desired level of confidence to give an expanded uncertainty.

6. **Report the measurement uncertainty**

   Unless otherwise required, report the measurement results and the expanded uncertainty based on an appropriate coverage factor.

4.2 The Top-down Approach

4.2.1 Measurement Uncertainty Using Intra- and Inter-laboratory Data

In the top-down approach, a combined standard uncertainty of the measurement is directly estimated from repeated measurements of selected samples. This approach is preferred in routine medical laboratories partly because of the availability of matrix-matched QC materials, and the practical problem that many measurement procedures are closed 'black box' systems where components are not accessible for uncertainty evaluation. The preferred method is to use performance data from both internal quality control (intra-laboratory) and external proficiency testing (inter-laboratory), assuming that the QC materials behave similarly to the patient samples.

The measurement data should be collected from a minimum period of six months but this is dependent on the frequency of analysis. This ensures that variations due to different operators, reagents and calibrator lots, recalibrations, routine instrument maintenance are captured. For new methods, a minimum of 30 replicate determinations of appropriate control or reference material is required to calculate an interim standard deviation. If bias is significant or known, it should be eliminated or minimized e.g. by re-calibration. The uncertainty of the value used for any bias adjustment should be estimated, and if large relative to the imprecision, included in the calculation of the combined standard uncertainty. Also, precision and accuracy data from method validation studies can be used, as long as there are no significant changes in the procedure following validation.

A top-down approach is generally more practical than the bottom-up approach for estimating the measurement uncertainty. However, it is the laboratory's decision to use the method most appropriate for their circumstances and supported by the available data.

4.2.2 Step 1: Specify the Measurand

Specify the measurand which includes the system containing the component (analyte) of interest, e.g. whole blood, plasma etc. The measurand description must also identify the kind-of-quantity being examined (e.g. amount-of-substance concentration, amount-of-substance activity, number concentration). Where possible, it is important to identify sources of uncertainty or develop an uncertainty budget so as to better understand the important sources of uncertainty and their contribution to the combined uncertainty.

4.2.3 Step 2: Imprecision of Measurement

The imprecision, expressed as u_{prec}, is an estimate of the uncertainty due to the random effects of the whole procedure over time. It is essential to estimate imprecision across as many unavoidable standard operating procedure variables as possible, e.g. calibrator and reagent batch changes, instrument maintenance, different operators, environment (intermediate condition). The intermediate precision *(u_{prec})* may be required to be calculated for at least two levels of QC across the reportable range.

Equation (5a)
$$u_{prec} = \sqrt{\frac{\left[SD_{L1}^2 \times (n_{L1} - 1) + SD_{L2}^2 \times (n_{L2} - 1)\right]}{n_{L1} + n_{L2} - 2}}$$

where

SD_{L1} and SD_{L2} = SD of each control level

n_{L1} and n_{L2} = number of data for each control level

If u_{prec} is evaluated from the RSD of the results obtained in the intermediate precision studies, the following equation is used to calculate u_{prec}.

Equation (5b)
$$u_{prec} = \sqrt{\frac{\left[RSD_{L1}^2 \times (n_{L1} - 1) + RSD_{L2}^2 \times (n_{L2} - 1)\right]}{n_{L1} + n_{L2} - 2}}$$

If more than two levels are used, calculate the u_{prec} as follows:

Equation (6a)
$$u_{prec} = \sqrt{\frac{\left[(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2 + \ldots\ldots + (n_k - 1)SD_k^2\right]}{(n_1 - 1) + (n_2 - 1) + \ldots\ldots + (n_k - 1)}}$$

or

Equation (6b)
$$u_{prec} = \sqrt{\frac{\left[(n_1 - 1)RSD_1^2 + (n_2 - 1)RSD_2^2 + \ldots\ldots + (n_k - 1)RSD_k^2\right]}{(n_1 - 1) + (n_2 - 1) + \ldots\ldots + (n_k - 1)}}$$

u_{prec} is the combined standard uncertainty associated with the result if bias is negligible.

Equation 5 shows the calculation of the average precision for the two control levels and is commonly applicable to methods where control levels describe performance across the entire analytical measuring ranges of the methods. For some methods, such as some immunoassays, with varying precision at different clinical decision limits or cut-points, measurement uncertainty based on u_{prec} must be calculated for each decision level.

Equation (7)
$$u_{prec(L1)} = SD_{L1} \text{ for QC Level 1;}$$
$$u_{prec(L2)} = SD_{L2} \text{ for QC Level 2.}$$

4.2.4 Step 3: Bias of Measurement

If bias is significant and has been estimated, usually by replicate measurements of a commutable reference material, correcting a measured value for this bias will increase the combined uncertainty. The uncertainty for bias comprises of:

(a) uncertainty in the reference value assigned to the reference materials used to assess the bias ($u(C_{ref})$ (available from accompanying certificate)

(b) standard error of the mean, of the replicate analyses of the reference materials (u_{rep}) where

(u_{rep} = SDM = $SD/\sqrt{n}$).

Hence, the bias of a procedure = Bias value ± u_{bias}

The uncertainty of bias (u_{bias}) is calculated by combining the two uncertainties:

Equation (8) $$u_{bias} = \sqrt{u(C_{ref})^2 + u_{rep}{}^2}$$

An appropriate reference material or reference procedure is not available, then alternative approaches may be used, e.g. external quality assessment data or inter-laboratory comparisons. If a dilution or concentration is performed, the appropriate uncertainty should be included to the combined uncertainty.

4.2.5 Step 4: Calculate the Combined Measurement Uncertainty

u_{bias} should be assessed for significance relative to the procedure imprecision (u_{prec}). The Student's *t* test can be used to objectively assess the relative significance of u_{bias}, or sometimes a subjective decision is made, e.g. ignore u_{bias} if it is <10 % of u_{prec}.

The significance of the bias is tested by a one-tailed Student's *t* test at 95% confidence. The *t* value is calculated as:

Equation (9) $$t = Bias / u_{bias}$$

The calculated *t* value can be compared with the 95 % critical *t* value, If $t > t_{crit}$, the null hypothesis that bias is not significant is rejected.

Using Rule 1, independent uncertainties are combined as variances according to Equation (2).

Case 1. Bias ignored or not evaluated

If the procedure has not been adjusted for bias, or if u_{bias} is <10 % of u_{prec}, or if the bias is not evaluated, then the estimated MU of the procedure is the intermediate precision expressed as:

Equation (10) $$u_c = u_{prec}$$

Case 2. Bias evaluated

If u_{bias} is assessed as being significant relative to u_{prec} (e.g. u_{bias} > 10 % u_{prec}), then the combined standard uncertainty:

Equation (11) $$u_c = \sqrt{u_{bias}{}^2 + u_{prec}{}^2}$$

This top-down approach is generally recognized as a direct estimate of the combined standard uncertainty of the whole procedure (u_c) using the GUM approach. This should be combined with other identified uncertainties which are major contributors to the combined uncertainty, such as dilution factor, temperature effects, matrix effect etc.

4.2.6 Step 5: Calculate the Expanded Measurement Uncertainty

The expanded uncertainty, *U,* is obtained by multiplying the combined standard uncertainty $u_c(y)$ by a coverage factor, *k* , as shown by Equation 4.

4.2.7 Report the Measurement Uncertainty

Report the measurement result and the expanded measurement uncertainty as described in Section 4.1.6.

4.3 Clinical Uses of Uncertainty Information

A summary of the measurement uncertainty information for all quantitative routine methods should be available within the laboratory and made available to its clients of the laboratory service when required.

The clinical uses of the uncertainty become important as data accumulate and the laboratory information systems become capable of comparing new results with previous results. Statistically, two results need to be greater than 2.77 CV_{imp} apart (that is, $1.96 \times \sqrt{2} \times CV_{imp}$) before there can be 95 % confidence that the two results are significantly different from each other. Either CV or SD can be used for these calculations.

For example, the sodium amount-of-substance concentration in plasma was found to be 150 mmol/L, with a standard uncertainty (u) of 1 mmol/L. A new sample was measured a few hours later. The minimal difference (MD) that would be considered significant is:

$$2.77 \times 1 = 2.77 \text{ mmol/L} = 3 \text{ mmol/L}$$

i.e. the laboratory would have ~95 % confidence that 147 and 153 mmol/L are measurably different from 150 mmol/L using their measurement procedure.

Therefore, if the two results differ by 3.7 % or more (or equivalent to 5.5 mmol/L), there is 95 % confidence that the two results are different.

When we wish to find out if two results from ~~a~~ the same patient are significantly different, from a biological point of view, biological variation of the two results needs to be considered as well as the measurement uncertainty of both results. The two results being compared need to be more than 2.77 analytical and biological CV apart (that is, $2.77 \sqrt{[CV_{imp}^2 + CV_{intra}^2]}$) before there can be 95 % confidence that the two results are different both measurably and biologically i.e. the difference between the two results are greater than can be explained by the combined effect of measurement uncertainty and intra-individual biological variation . It should be noted that such calculations are based on the assumption that the measurands behave the same biological variation in healthy and ill individuals.

For example, CV_{imp} determined from mean precision of long-term internal QC for creatinine amount-of-substance is 1.2 %. Creatinine intra-individual biological variation (CV_w from Westgard website): CV_{intra} = 5.3 %.

Sum of analytical and biological variations as CV:

$$CV_T = \sqrt{\left((1.2)^2 + (5.3)^2\right)} = 5.4\,\%$$

If the two results are analytically and biologically different, they need to differ by

$$2.77 \times \sqrt{\left(CV_{imp}{}^2 + CV_{intra}{}^2\right)}, (\text{for } 95\,\% \text{ confidence})$$
$$2.77 \times 5.4\,\% = 15\,\%$$

Thus, the two results would have to differ by at least 15 % to be 95 % confidence that they are both analytically and biologically different.

If we wish to decide if a patient result is significantly different from say an upper reference value e.g. PSA upper limit of 4.0 ng/L, then we are interested in only one side of the normal distribution (i.e. one-tailed), so in this case ~95 % confidence is $\sqrt{(1.65^2)}$ x SD = 2.7225 x SD. So if SD = 0.1, then a result needs to be > ~4.2 ng/L to be measurably different from 4.0 with ~95 % confidence. In this example, intra-individual BV is very large relative to MU, and should be included in the same way as the earlier example above.

Appendix A: Definitions

1. **Analyte**
   the substance or specific component that is the subject of measurement.

2. **Certified reference material**
   a reference material, accompanied by certification or documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures.

3. **Coefficient of variation**
   the standard deviation divided by the mean value of the parameter measured.

4. **Combined standard measurement uncertainty / combined standard uncertainty**
   standard uncertainty that is obtained using the combined individual standard measurement uncertainties associated with the input quantities in a measurement model. The symbol for a combined standard uncertainty is u_c

5. **Commutability of a reference material**
   property of a reference material (usually a calibrator), demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material and the relation obtained among the measurement results for other specified materials (usually routine samples).

6. **Coverage factor**
   numerical factor larger than one by which a combined standard uncertainty is multiplied to obtain an expanded uncertainty.

7. **Distribution function**
   function giving, for every value x, the probability that the random variable X be less than or equal to x.

8. **Expanded measurement uncertainty / expanded uncertainty**
   product of a combined standard uncertainty and a factor larger than the number one. The symbol is U.

9. **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.

10. **Internal quality control**
    set of procedures undertaken by laboratory staff for the continuous monitoring of operation and the results of measurements in order to decide whether results are reliable enough to be released.

11. **Input quantity in a measurement model / input quantity**
    quantity that must be measured, or a quantity, the value of which can be otherwise obtained, in order to calculate a measured quantity value of a measurand.

12. **Intermediate measurement precision / intermediate precision**
    measurement precision under a set of intermediate precision conditions of measurement.

13. **Intermediate precision condition of measurement / intermediate precision condition**
    condition of measurement, out of a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over and extended period of time, but may include other conditions involving changes such as calibrations, calibrators, operators and measuring systems.

14. **Measurand**
    quantity intended to be measured/ a particular quantifiable property of the analyte used in the measuring system. For example, the 'mass of protein in 24-hour urine from a given person at a given time' is a measurand. The component 'protein' is termed "analyte". The term "calcium" can be referred to either of the measurands 'amount-of-substance concentration of total calcium in serum of a given person at a given time' or 'amount-of-substance concentration of ionized calcium in serum of a given person at a given time'. In the first case, total calcium includes free calcium ions (ionized calcium) and bound calcium (complex bound calcium and protein bound calcium).

15. **Measured quantity value/ measured value of a quantity/ measured value**
    quantity value representing a measurement result.

16. **Measurement**
    process of experimentally obtaining one or more quantity values that can reasonable be attributed to a quantity.

17. **Measurement accuracy / accuracy of measurement / accuracy**
    closeness of agreement between a measured quantity (a test result) and a true value of a measurand.

18. **Measurement bias / bias**
    estimate of a systematic measurement error. The difference between the expectation of the test results and an accepted reference value.

19. **Measurement error / error of measurement / error**
    measured quantity value minus a reference quantity value.

20. **Measurement model / model of measurement / model**
    mathematical relation among all quantities known to be involved in a measurement.

21. **Measurement precision / precision**
    closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions (can range from repeatability to reproducibility conditions of measurement).

    Note 1: Precision 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, variance of relative standard deviation of the test results.

22. **Measurement procedure**
detailed description of a measurement according to one or more measurement principles and according to a given measurement method. A measurement procedure is sometimes called a standard operating procedure (SOP).

23. **Measurement repeatability / repeatability**
measurement precision under a set of repeatability conditions of measurement.

24. **Measurement reproducibility / reproducibility**
measurement precision under reproducibility conditions of measurement.

25. **Measurement result / result of measurement**
set of values attributed to a measurand, obtained by measurement.

26. **Measurement trueness / trueness of measurement / trueness**
closeness of agreement between the average of an infinite number of replicate measured values and a reference quantity value.

Note 1: Measurement trueness is inversely related to systematic measurement error, but not related to measurement error.

Note 2: "Measurement accuracy" should not be used for "measurement trueness" and vice versa

27. **Measurement uncertainty / uncertainty of measurement / uncertainty**
non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.

28. **Measuring system**
set of one or more measuring instruments and often other devices, including any reagent and supply, assembled to give information used to generate measured values

29. **Metrological traceability**
property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty.

Note 1: Metrological traceability of a measurement result does not ensure that the measurement uncertainty is adequate for a given purpose or there is an absence of mistakes.

Note 2: The abbreviated term "traceability" is sometimes used for 'metrological traceability'. However, "metrological traceability" is preferred to distinguish from other concepts, such as "sample traceability' or 'document traceability' or 'instrument traceability".

30. **Output quantity in a measurement model / output quantity**
quantity, the measured value of which is calculated using the values of input quantities in a measurement model.

31. **Post-examination procedures / post-analytical phase**
processes following the examination including systematic review, formatting and interpretation, authorization for release, reporting and transmission of the results, and storage of samples of the examinations.

32. **Pre-examination procedures / pre-analytical phase**
steps starting, in chronological order, from the clinician's request and including the examination requisition, preparation of the patient, collection of the primary sample, and transportation to and within the laboratory, and ending when the analytical examination procedure begins.

33. **Proficiency testing**
Evaluation of participating laboratory's performance against pre-established criteria by mean of inter-laboratory comparisons. Following the ISO/IEC Guide 17043 (Clause 3.7), the term "proficiency testing" is taken in its broadest sense to include quantitative, qualitative, sequential, simultaneous, single occasion, and continuous schemes.

34. **Quantity value / value of a quantity / value**
number and reference together expressing magnitude of a quantity.

Example: "Plasma (Blood) – Sodium ion; amount-of-substance concentration equal to 143 mmol/L in a given person at a given time'

35. **Random measurement error / random error of measurement / random error**
component of measurement error that in replicate measurements varies in an unpredictable manner.

36. **Reference material**
material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties, such as calibration of an apparatus, the assessment of trueness of a measurement method.

37. **Relative standard deviation**
standard deviation divided by the absolute value of the quantity value.

38. **Relative standard measurement uncertainty**
standard measurement uncertainty divided by the absolute value of the measured quantity value. Relative standard uncertainty may be symbolized as $u(x)/x$ or $u_{rel}(x_i)$.

39. **Repeatability condition of measurement / repeatability condition**
condition of measurement that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time.

40. **Reproducibility condition of measurement / reproducibility condition**
condition of measurement that includes different locations, operators, measuring systems, and replicate measurement on the same or similar objects.

41. **Sample**
one or more parts taken from a system/ population and intended to provide information on the system/ population, often to serve as a basis for decision on the system.

42. **Sensitivity coefficient**
change in calculated output quantity value, caused by an isolated small change in a given input quantity value, divided by this change.

43. **Significant digit in a quantity value / significant digit**
digit in a quantity value that carries meaning with regard to measurement uncertainty of that quantity value.

44. **Standard measurement uncertainty / standard uncertainty**
measurement uncertainty expressed as a standard deviation. The standard uncertainty is symbolized u_x or $u(x)$.

45. **Standard deviation**
measure of the spread of the data about the mean value.

46. **System**
part or phenomenon of the perceivable or conceivable world consisting of a demarcated arrangement of a set of elements and a set of relationships or processes between these elements.

Note 1: The concept "system" is used both in the sense of a phenomenon, body, or substance, such as a person or a blood sample, carrying a property, and in the combination of measuring instruments, reagents and supplies constituting a measuring system.

47. **Systematic measurement error / systematic error of measurement / systematic error**
component of measurement error that in replicate measurements remains constant or varies in a predictable manner

48. **True quantity value / true value of a quantity / true value**
quantity value consistent with the definition of a quantity.

Note 1: In the Error Approach to describing measurement, a true quantity value is considered unique and, in practice, unknowable. The Uncertainty Approach is to recognize that, owing to the inherently incomplete amount of detail in the definition of a quantity, there is not a single true quantity value but rather a set of true quantity values consistent with the definition. However, this set of values is, in principle and in practice, unknowable. Other approaches dispense altogether with the concept of true quantity value and rely on the concept of metrological compatibility of measurement results for assessing their validity.

49. **Type A evaluation of measurement uncertainty / Type A evaluation**
evaluation of a component of measurement uncertainty by a statistical analysis of measured quantity values obtained under defined measurement conditions.

50. **Type B evaluation of measurement uncertainty / Type B evaluation**
evaluation of a component of measurement uncertainty determined by means other than a Type A evaluation.

Examples: Evaluation based on information:
- associated with authoritative published quantity values,
- associated with the quantity value of a certified reference material,
- obtained from a calibration certificate
- obtained from limits deduced through personal experience.

51. **Uncertainty budget**
statement of a measurement uncertainty, of the components of that measurement uncertainty, and of their calculation and combination.

Appendix B: Distribution Functions

The following are some common probability distribution functions that can be used to calculate a standard uncertainty. 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.

(a) Normal Distribution

The normal probability distribution is by far the most common and most important continuous probability distribution. 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:

The expanded uncertainty, *U*, is obtained by dividing the quoted uncertainty by an appropriate coverage factor, *k*, for normal distribution.

Confidence Level	*k* factor (coverage factor)
95	1.96 (round up to 2)
99	2.575 (round up to 3)

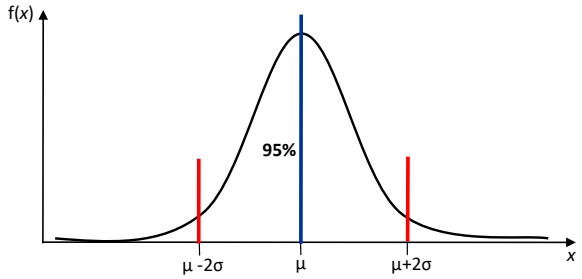

Figure 3: The normal distribution

For example, a calibration certificate shows that the certified concentration of calcium in human serum is 2.218 ± 0.016 mmol/L where the reported uncertainty is an expanded uncertainty, calculated using a coverage factor of k=2, which gives a level of confidence of approximately 95%. The standard uncertainty for the concentration is thus 0.016/2 mmol/L = 0.008 mmol/L.

(b) Rectangular Distribution

It is used when uncertainties are given by maximum bound within which all values are equally probable. For example, a certificate or other specification gives limits without specifying a level of confidence. The standard uncertainty is computed by dividing the half interval 'a' by squared root of 3, i.e. √3.

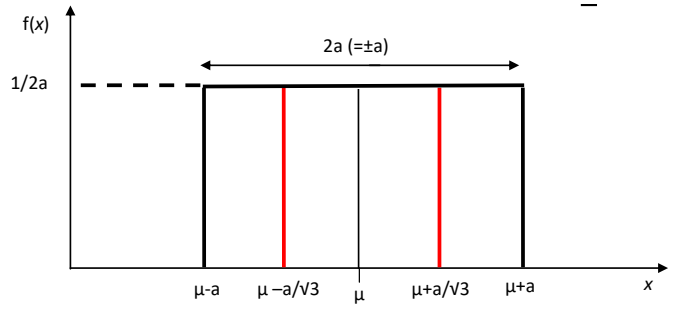

Figure 4: The rectangular distribution models cases where the probability of obtaining any value between two limits is equal to the probability of obtaining any other value

For example, a 5 mL class bulb pipette has tolerance of ± 0.03 mL. Tolerance means that a particular pipette has a volume between 4.97 mL to 5.03 mL at 20 °C. One can assume that the true volume in the pipette has an equal probability of being any value in the range 4.97 mL to 5.03 mL. The rectangular distribution shows that there is constant probability throughout the tolerance range and zero probability outside the range. The standard uncertainty for the volume is thus 0.03/√3 mL = 0.017 mL.

(c) Triangular Distribution

A triangular distribution is an appropriate model where a value is more likely to be in the centre of its range than towards the outside. The standard uncertainty is computed by dividing the half-interval 'a' by squared root of 6, i.e. √6.

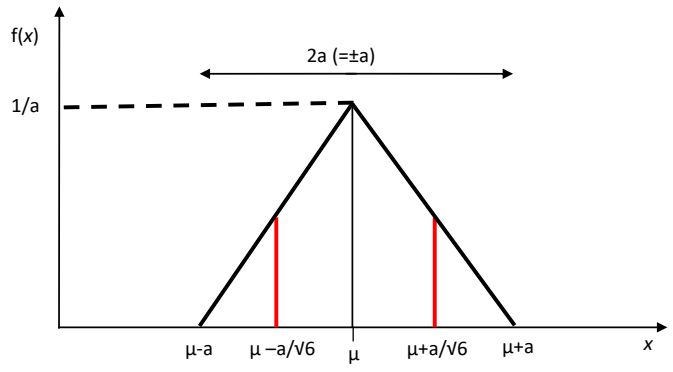

Figure 5: The triangular distribution models cases where the values are more likely to be near the mean than at the extremes.

For example when temperature is to be controlled at 20 ± 2 ºC using thermostat which is calibrated, the thermostat will hold the temperature very close to 20 ºC. The triangular distribution shows that the most likely value for the temperature is 20 ºC with decreasing probability across the range 18 ºC to 22 ºC and zero probability outside the range. The standard uncertainty for the temperature read by the thermostat assuming triangular distribution is thus 2/√ 6 ºC = 0.82 ºC.

Appendix C - The Uncertainty Estimation Process Based on Bottom-up Approach

Step 1:	Specify Measurand
Step 2:	Identify Uncertainty Sources
Step 3:	Simplify by grouping sources covered by existing data Quantify grouped components of uncertainty Quantify remaining components of uncertainty Convert components to standard uncertainties
Step 4:	Calculate combined standard uncertainty Review and if necessary re-evaluate large components
Step 5**:**	Calculate expanded uncertainty
	Report

Appendix D - The Uncertainty Estimation Process Based on Top-down Approach

Step 1: Specify Measurand

Step 2: Imprecision of measurement
Calculate u_{prec} for at least two levels of QC across the reportable range

Step 3: Bias of measurement
Calculate u_{bias} from $u(C_{ref})$ and u_{rep}

Step 4: Calculate combined standard uncertainty
If bias is not considered or $u_{bias} < 10\ \% \ u_{prec}$,
$u_c = u_{prec}$; otherwise $u_c = \sqrt{u_{bias}^2 + u_{prec}^2}$
Review and if necessary re-evaluate large components

Step 5**:** Calculate expanded uncertainty

Report

Appendix E - Example of the Certificate of Analysis (COA) from National Institute of Standards & Technology (NIST)

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 909b

Human Serum

This Standard Reference Material (SRM) is a lyophilized human serum material primarily intended for use in evaluating the accuracy of higher order clinical methods for the determination of specified constituents in human serum. It can also be used to validate working or secondary reference materials. Because of matrix effects associated with serum that has been lyophilized, some routine clinical methods may exhibit a bias in results between fresh (or fresh-frozen) serum and lyophilized serum. A unit of SRM 909b consists of six bottles of lyophilized human serum (three bottles each or two different analyte concentration levels) and six bottles of deionized, autoclaved water (11.5 mL each). Before use, the serum in each bottle is to be reconstituted with 10.00 mL of the water provided.

Certified Concentration Values: The certified concentrations of the serum analytes were determined using high order reference measurement procedures [1-12] calibrated with NIST high purity SRMs. This calibration provides direct traceability to the mole for the certified analytes in this SRM. The concentrations and their uncertainties for the two analyte concentration levels (SRM 909b-I and SRM 909b-II) are listed in Tables 1, 1a, and 2. The certified concentrations apply only to reconstituted serum at room temperature (20 °C to 25 °C). See "Instructions for Use".

Reference Concentration Values: Reference concentration values for total bilirubin are provided in Table 3. The reference concentrations were derived from results reported by two collaborating laboratories and NIST. The reference values are noncertified values that **DO NOT** meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple methods.

Information Values: Information values for the activity of selected enzymes and pH are provided in Table 4. The analytical measurements were made by the supplier of the material.

Expiration of Certification: The certification of this SRM lot is valid, within the measurement uncertainties, until the date stamped on the outer box label **(DO NOT DISCARD)**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of the analyses were under the chairmanship of M.J. Welch of the NIST Analytical Chemistry Division.

Analytical measurements were performed in the NIST Analytical Chemistry Division by R.G. Christensen, S.A. Margolis, K.E. Murphy, P.J. Paulsen, C.S. Phinney, K.W. Pratt, M.S. Rearick, L.T. Sniegoski, T.W. Vetter, and R.D. Vocke and by P. Ellerbe and S.E. Long, College of American Pathologists Research Associates at NIST. Technical advice was provided by R. Schaffer, consultant to the SRM Program. Technical advice and coordination for the total bilirubin measurements were provided by B. Doumas of the Medical College of Wisconsin (Milwaukee, WI). Analytical measurements for the determination of total bilirubin were performed in the NIST Analytical Chemistry Division by Y.Y. Davidson and L.T. Sniegoski and by scientists at the Wisconsin State Laboratory of Hygiene (Madison, WI) and the Children's Hospital of Wisconsin (Milwaukee, WI).

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Appendix F - Worked Examples

F1 - Estimation of MU for Glucose in Human Serum Using Bottom-up Approach

Step 1: Specify the Measurand

Measurand	Amount-of-substance concentration of glucose in human serum
Units	mmol/L
Measurement procedure	The concentration of glucose is determined using a spectrophotometric procedure
Traceability	NIST S M 965b

From the manufacturers' instrument and method descriptions, the values of glucose concentration are calculated from a two-point calibration curve and depend on the absorbance of the sample and calibration solutions, and the concentration of the calibration solutions.
The unknown concentration is described by the following model equation

$$c_x = c_0 + \frac{A_s - A_0}{A_{cal} - A_0} \cdot (c_{cal} - c_0)$$

where

c_x	=	Total concentration of glucose in the sample solution [mmol/L]
c_0	=	Total concentration of glucose in solution used to establish the zero-point of the calibration curve [mmol/L]
A_s	=	Normalized and blank-corrected absorbance signal of sample solution in the cuvette [AU]
A_0	=	Absorbance signal from reagents [AU]
A_{cal}	=	Normalized and blank-corrected absorbance signal of calibrator solution in the cuvette [AU]
c_{cal}	=	Total concentration of glucose in the calibrator [mmol/L]

Everything that appears to the right of the equation is referred to as "input quantities". In addition, there may be other uncertainty sources ("influence quantities"), e.g. temperature variations, that do not appear in the expression but still affect the result.

Figure 6: Outline of the measurement procedure. A ready-to-use liquid serum material with assigned values is used for the method calibration. It is assumed that the analytical conditions (volume and incubation times) in the mixing and measurement steps are the same for the samples and the calibrators. The dilution step is performed when the concentration of the analyte in the sample exceeds the measurement range. (Below)

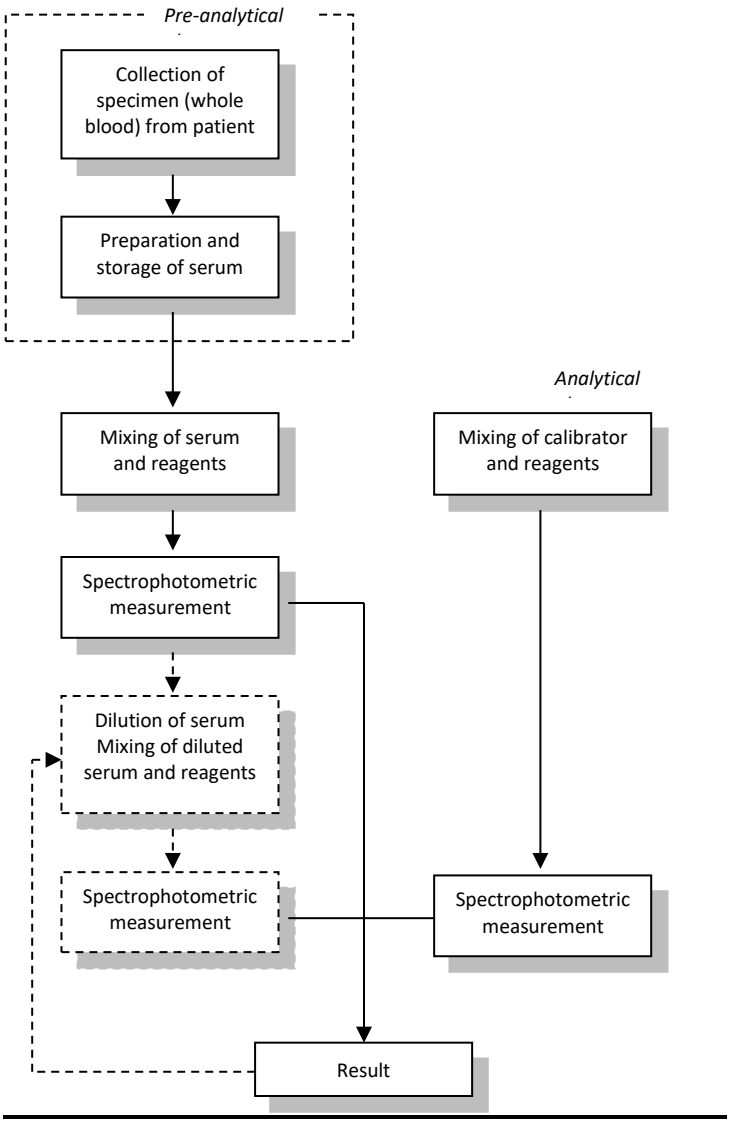
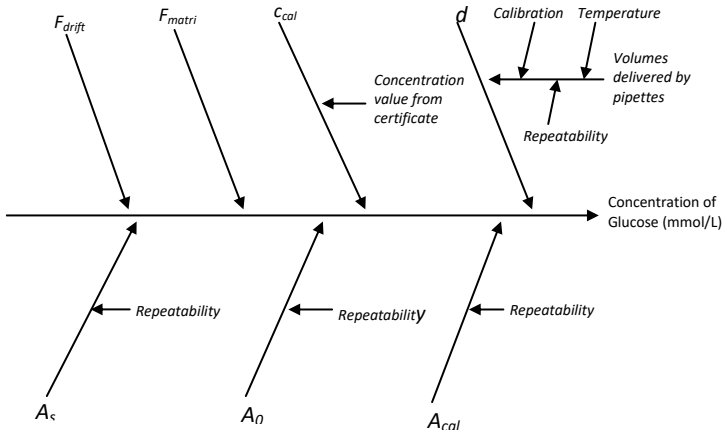


Figure 6: Outline of the measurement procedure. A ready-to-use liquid serum material with assigned values is used for the method calibration. It is assumed that the analytical conditions (volume and incubation times) in the mixing and measurement steps are the same for the samples and the calibrators. The dilution step is performed when the concentration of the analyte in the sample exceeds the measurement range.

Step 2: Identify the Sources of Uncertainty



a) Absorbance measurements, A_s, A_0, A_{cal}
The absorbances as measured for the sample and calibration solutions appear as input quantities in the model. Any variation in these signals contributes to the uncertainty of the glucose concentration.

b) Glucose concentration in calibrators, c_0, c_{cal}

The measurement standards should be accompanied by a certificate with information about the assigned property value, e.g. c_{cal} and their uncertainties. The lower point of the calibration curve is derived from pure water having no deliberate content of glucose.

c) Sample dilution, d
As shown in Figure 1, the analytical phase may include a dilution of a sample. This is done by the analyst before the sample is introduced into the instrument. To calculate the concentration in the sample, a dilution factor d needs to be multiplied.

$$d = \frac{V_1 + V_2}{V_1}$$

where V_1 and V_2 are the volumes of sample and diluent (solvent) respectively.

d) Matrix effects, F_{matrix}
It is difficult to have calibrators with exactly the same properties (commutability) as the patient sample. Any difference in composition between sample and calibrator can result in difference in instrument response. The process of freeze-drying and reconstitution of human serum is, e.g. known to affect some component. This is described as “matrix effects” or that sample and calibrator are not “commutable”.

e) Changes in instrument sensitivity, F_{drift}

f) Calibration and control samples may affect changes in instrument sensitivity, $F_{drift.}$
The information from the control samples is fed into a chart as part of the internal quality control procedures. Changes in the instrument sensitivity (“drift”) between calibrations are

directly proportional to changes in concentration and constitute a source of uncertainty. The stability of the instrument is tested by the manufacturer during installation based on given specifications, and/or by the laboratory during the instrument validation. A factor F_{drift} is introduced in the model to handle the uncertainty associated with the allowed drift.

Extending the measurement model

Our model is modified so that it includes the highlighted uncertainty sources which are important. The factors are included by assigning a value of 1 (in the absence of effects on the result) but they may have an associated uncertainty.

$$c_x = \left[c_0 + \frac{A_s - A_0}{A_{cal} - A_0} \cdot (c_{cal} - c_0) \right] \cdot d \cdot F_{matrix} \cdot F_{drift}$$

where

c_x	=	Total concentration of glucose in the sample solution [mmol/L]
d	=	Factor describing possible sample dilution
F_{matrix}	=	Factor describing the contribution from possible matrix effects (difference in commutability of sample and calibrator)
F_{drift}	=	Factor describing the contribution from an allowed drift in instrument sensitivity

Step 3: Quantify standard measurement uncertainty

All uncertainty components should be expressed as standard uncertainties. This requires information on how values for the specific quantity are distributed (e.g. normal, rectangular, and triangular).

a) c_0
A blank solution (distilled/deionized water) is used to establish the zero-point of the calibration curve. The uncertainty contribution of c_0 is considered negligible.

b) A_s
This absorbance value comes from the sample while the uncertainty was estimated during the method validation.

Mean = 0.1153 AU Pooled standard deviation = 0.50 %
No. of data = 20 Standard uncertainty = 5.765×10^{-4} AU

Value	Standard uncertainty	Type of uncertainty	Probability distribution
0.1153 AU	5.765×10^{-4} AU	Type A	Normal

c) A_0

This absorbance value comes from the water-filled cuvette while the uncertainty was estimated during the method validation.

Mean = -1.15×10^{-3} AU Pooled standard deviation = 16 %
No. of data = 20 Standard uncertainty = 1.84×10^{-4} AU

Value	Standard uncertainty	Type of uncertainty	Probability distribution
-1.15×10^{-3} AU	1.84×10^{-4} AU	Type A	Normal

d) A_{cal}

This absorbance value comes from the calibrator while the uncertainty was estimated during the method validation.

Mean = 0.26565 AU Pooled standard deviation = 0.4 %
No. of data = 20 Standard uncertainty = 1.0626×10^{-3} AU

Value	Standard uncertainty	Type of uncertainty	Probability distribution
0.26565 AU	1.0626×10^{-3} AU	Type A	Normal

e) c_{cal}

The value and expanded uncertainty (10.5 ± 0.10 mmol/L) at 95 % confidence level come from a certificate provided by the supplier. The values were obtained by applying a reference method (isotope dilution mass spectrometry) together with reference materials (NIST SRM 917a and SRM 965).

Value	Standard uncertainty	Type of uncertainty	Probability distribution
10.5 mmol/L	0.05 mmol/L	Type B	Normal

f) F_{matrix}

The certificate informs that the calibrator was prepared from a pool of fresh frozen human serum without any additives and the material may not be commutable with natural human serum in all routine glucose measurement procedures. The analyst has on some occasions noted a small effect from similar materials and wishes to take this into account in the form of an extra uncertainty contribution.
The halfwidth of the limits which corresponds to the relative uncertainty is estimated as 0.1 %. A rectangular distribution is assumed for the variation of matrix effects.

Value	Standard uncertainty	Type of uncertainty	Probability distribution
1	$0.001/\sqrt{3} = 5.774 \times 10^{-4}$	Type B	Rectangular

g) F_{drift}

The laboratory allows a maximum sensitivity drift between calibrations of ±1 %. A rectangular distribution is assumed for the variation of instrument sensitivity.

Value	Standard uncertainty	Type of ncertainty	Probability distribution
1	$0.01/\sqrt{3} = 5.774 \times 10^{-3}$	Type B	Rectangular

h) *d*

A sample volume, V_1 of 50 µL is manually diluted by an addition of 450 µL of 0.9% NaCl, V_2 outside the instrument. The uncertainties of the volumes come from information provided by the manufacturer of the pipettes and were confirmed in internal validation work.

There are three sources of uncertainty in the volume of the pipettes:-

i. The uncertainty in the certified internal volume of the pipettes
ii. Variation in filling the pipettes to the mark
iii. The pipettes and solution temperatures differing from the temperature at which the volume of the pipettes were calibrated

***V_1 - 50 µL*:**

i. *Calibration*: Expanded uncertainty from manufacturer's certificate = 0.6 %

The standard uncertainty is calculated assuming a rectangular distribution:

(0.6 % x 50 µL)/ √3 = 0.1732 µL

ii. *Repeatability:* The uncertainty due to variations in filling can be estimated from a repeatability experiment. A series of 10 fill and weigh experiments had a standard deviation of 0.065 µL. This can be used directly as a standard uncertainty.

iii. *Temperature:* According to the manufacturer, the pipette has been calibrated at a temperature of 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 the temperature range and the coefficient of the volume expansion.
Coefficient of the volume expansion of water is $0.00021^{\circ}C^{-1}$.
Therefore, uncertainty of temperature effect on volume expansion (with the assumption of a rectangular distribution for the temperature variation):

$$\frac{0.00021^{\circ}C^{-1} \times 50\,\mu L \times 4\ ^{\circ}C}{\sqrt{3}} = 0.0242\,\mu L$$

The three contributions are combined using Rule 1 to give the standard uncertainty of V_1:

$$u(V_1) = \sqrt{0.1732^2 + 0.065^2 + 0.0242^2} = 0.1866\,\mu L$$

***V_2 - 450 µL*:**

i. *Calibration*: Expanded uncertainty from manufacturer's certificate = 0.6 %

The standard uncertainty is calculated assuming a rectangular distribution:

(0.6 % x 450 µL)/ √3 = 1.5588 µL

ii. *Repeatability:* The uncertainty due to variations in filling can be estimated from a repeatability experiment. A series of 10 fill and weigh experiments had a standard deviation of 0.560 µL. This can be used directly as a standard uncertainty.

iii. *Temperature:* According to the manufacturer, the pipette has been calibrated at a temperature of 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 the temperature range and the coefficient of the volume expansion.
Coefficient of the volume expansion of water is $0.00021°C^{-1}$.
Therefore, uncertainty of temperature effect on volume expansion (with the assumption of a rectangular distribution for the temperature variation):

$$\frac{0.00021°C^{-1} \times 450\,\mu L \times 4\ °C}{\sqrt{3}} = 0.2182\,\mu L$$

The three contributions are combined using Rule 1 to give the standard uncertainty of V_1:

$$u(V_2) = \sqrt{1.5588^2 + 0.560^2 + 0.2182^2} = 1.6706\,\mu L$$

$$d = \frac{V_1 + V_2}{V_1}$$

The expression is broken down to two elements: (V_1 + V_2) and V_1.

First, the uncertainty of (V_1 + V_2) is calculated using Rule 1:

$$\begin{aligned} u(V_1 + V_2) &= \sqrt{u(V_1)^2 + u(V_2)^2} \\ &= \sqrt{0.1866^2 + 1.6706^2} \\ &= 1.6810\,\mu L \end{aligned}$$

Then, the uncertainty of *d* is calculated using Rule 2:

$$u(d) = d \times \sqrt{\left(\frac{u(V_1 + V_2)}{(V_1 + V_2)}\right)^2 + \left(\frac{u(V_1)}{V_1}\right)^2}$$
$$= 10 \times \sqrt{\left(\frac{1.6810}{500}\right)^2 + \left(\frac{0.1866}{50}\right)^2}$$
$$= 0.05023$$

Summary of uncertainty components for glucose in human serum

Parameters	Glucose in human serum	
	Value	Standard Uncertainty
c_0	0.0	-
A_s	0.1153	5.765×10^{-4}
A_0	-1.15×10^{-3}	1.84×10^{-4}
A_{cal}	0.26565	1.0626×10^{-3}
c_{cal}	10.5	0.05
F_{matrix}	1	5.774×10^{-4}
F_{drift}	1	5.774×10^{-3}
d	10	0.05023

Step 4: Calculate the Combined Standard Measurement Uncertainty

$$c_x = \left[c_0 + \frac{A_s - A_0}{A_{cal} - A_0} \cdot (c_{cal} - c_0)\right] \cdot d \cdot F_{matrix} \cdot F_{drift}$$
$$= \left[\frac{0.1153 - (-1.15 \times 10^{-3})}{0.26565 - (-1.15 \times 10^{-3})} \cdot (10.5)\right] \cdot 10 \cdot 1 \cdot 1$$
$$= 45.8293 \text{ mmol/L}$$

Using Rule 1, first combine the following uncertainty components:

a) $A_s - A_o$

$A_s - A_o = 0.1165$

$$u(A_s - A_0) = \sqrt{u(A_s)^2 + u(A_0)^2}$$
$$= \sqrt{\left(5.765 \times 10^{-4}\right)^2 + \left(1.84 \times 10^{-4}\right)^2}$$
$$= 6.0515 \times 10^{-4}$$

b) $A_{cal} - A_o$

$A_{cal} - A_o = 0.2668$

$$u(A_{cal} - A_0) = \sqrt{u(A_{cal})^2 + u(A_0)^2}$$
$$= \sqrt{\left(1.0626 \times 10^{-3}\right)^2 + \left(1.84 \times 10^{-4}\right)^2}$$
$$= 1.0784 \times 10^{-3}$$

c) c_{cal} - c_o

c_{cal} - c_o = 10.5

$u(c_{cal} - c_o)$ = 0.05

Using Rule 2, combine all uncertainty components.

$$u(c_x) = c_x \times \sqrt{\left(\frac{u(A_s - A_0)}{(A_s - A_0)}\right)^2 + \left(\frac{u(A_{cal} - A_0)}{(A_{cal} - A_0)}\right)^2 + \left(\frac{u(c_{cal} - c_0)}{(c_{cal} - c_0)}\right)^2 + \left(\frac{u(d)}{d}\right)^2 + \left(\frac{u(F_{matrix})}{F_{matrix}}\right)^2 + \left(\frac{u(F_{drift})}{F_{drift}}\right)^2}$$

$$= 45.8293 \times \sqrt{\left(\frac{6.0515 \times 10^{-4}}{0.1165}\right)^2 + \left(\frac{1.0784 \times 10^{-3}}{0.2668}\right)^2 + \left(\frac{0.05}{10.5}\right)^2 + \left(\frac{0.05023}{10}\right)^2 + \left(\frac{5.774 \times 10^{-4}}{1}\right)^2 + \left(\frac{5.774 \times 10^{-3}}{1}\right)^2}$$

$$= 0.5122 \text{mmol/L}$$

Step 5: Calculate the Expanded Measurement Uncertainty

$U = ku_c(y)$

$U = 2 \times 0.5122$

$U = 1.0244$ mmol/L

Report the Measurement Uncertainty

Reporting value = 45.8293 mmol/L
Expanded uncertainty = 1.0244 mmol/L

The measurement result of glucose for the given person = 45.8 ± 1.0 mmol/L, the reported uncertainty in an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95 %.

F2 - Estimation of MU for Creatinine in Human Serum Using Top-down Approach

Step 1: Specify the Measurand

Measurand	Amount-of-substance concentration of creatinine in human serum
Units	mmol/L
Measurement procedure	Spectrophotometric procedure
Traceability	NIST SRM 967a

Step 2: Imprecision of Measurement

QC Level 1:

Mean = 0.0687 mmol/L

SD_{L1} = 0.0018 mmol/L

RSD_{L1} = 2.62%

QC Level 2:

Mean = 0.4041 mmol/L

SD_{L2} = 0.0121 mmol/L

RSD_{L2} = 2.99%

Imprecision data for intermediate conditions included different batch of reagents and calibrators, several operator changes and instrument routine maintenance. Data were obtained for at least six months with *n* = *200*.

$$u_{prec} = \sqrt{\frac{\left[RSD_{L1}^2 \times (n_{L1} - 1) + RSD_{L2}^2 \times (n_{L2} - 1)\right]}{n_{L1} + n_{L2} - 2}}$$
$$= \sqrt{\frac{\left[2.62^2 \times (200 - 1) + 2.99^2 \times (200 - 1)\right]}{200 + 200 - 2}}$$
$$= 2.81\%$$

Step 3: Bias of Measurement

NIST SRM 967a was used to estimate the bias of measurement.

Table 1. Certified concentration value for Creatinine[(a)]

Concentration Level	mmol/L
Level 2	0.3427 ± 0.0072

(a) The uncertainty in the certified value, calculated according to the method described in the ISO Guide, is expressed as an expanded uncertainty, *U*. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent the standard uncertainty of the mean concentration. The coverage factor, *k*, is determined from the Student's *t*-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence.

I. Determine the standard uncertainty of the concentration value of creatinine in the SRM, $u(C_{ref})$ by dividing the expanded uncertainty with coverage factor, k =2.

$u(C_{ref})$ = 0.0072/2
= 0.0036 mmol/L

$u(C_{ref})$ = (0.0036/0.3427) x 100 %
= 1.05 % [expressed as relative standard uncertainty]

II. Determine the repeatability standard deviation, u_{rep}, from 10 measurements of SRM creatinine using routine procedure under repeatability conditions (N.B. artificial data for illustration only).
Mean = 0.3518 mmol/L

SD = 0.0076 mmol/L

u_{rep} = $SDM = SD/\sqrt{n}$
= 0.0076 / √10
= 0.0024 mmol/L

u_{rep} = (0.0024/0.3518) x 100 %
= 0.68 % [expressed as relative standard uncertainty]

III. Determine the uncertainty of bias (u_{bias})
Bias = Mean of replicate measurements by routine procedure minus SRM assigned value
= 0.3518 – 0.3427 mmol/L
= 0.0091 mmol/L

$$u_{bias} = \sqrt{u(C_{ref})^2 + u_{rep}{}^2}$$
$$= \sqrt{(0.0036)^2 + (0.0024)^2}$$
$$= 0.0043 \text{ mmol/L}$$

Express u_{bias} as relative standard uncertainty:

$$u_{bias} = \sqrt{u(C_{ref})^2 + u_{rep}{}^2}$$
$$= \sqrt{(1.05)^2 + (0.68)^2}$$
$$= 1.25\%$$

Step 4: Calculate the Combined Measurement Uncertainty

I. u_{bias} should be assessed for significance relative to the procedure imprecision (u_{prec}).

The significance of the bias is tested by a one-tailed Student's *t* test at 95% confidence.

$$t = Bias / u_{bias}$$
$$= 0.0091/0.0043$$
$$= 2.116$$

t_{crit} = 1.83 with 9 degrees of freedom.

Since $t > t_{crit}$, bias is considered significant.

In addition, u_{bias} is compared to u_{prec}.

u_{bias} / u_{prec} = 1.25% / 2.81%
= 0.445

u_{bias} = 44.5 % u_{prec}

Since u_{bias} is of significant magnitude relative to imprecision, therefore u_{bias} is included in the estimation of combined uncertainty.

II. Determine the combined uncertainty
Since u_{bias} is assessed as being significant relative to u_{prec} (e.g. u_{bias} > 10% u_{prec}), then the combined standard uncertainty:

$$u_c = \sqrt{u_{bias}^2 + u_{prec}^2}$$
$$= \sqrt{(1.25)^2 + (2.81)^2}$$
$$= 3.075\%$$

Step 5: Calculate the Expanded Measurement Uncertainty

$U = ku_c(y)$
$U = 2 \times 3.075\%$
$U = 6.15\%$

Report the Measurement Uncertainty

Measured result/reporting value = 0.1453 mmol/L
Expanded uncertainty = 6.15% x 0.1453 = 0.0089 mmol/L

The measurement result of creatinine for the given person = 0.1453 ± 0.0089 mmol/L, the reported uncertainty in an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%.

Appendix G - Bibliography

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Appendix H - The Joint Committee for Traceability in Laboratory Medicine (JCTLM)

The Joint Committee for Traceability in Laboratory Medicine (JCTLM) was established in 2002 under the auspices of the Bureau International des Poids et Mesures (BIPM), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and the International Laboratory Accreditation Cooperation (ILAC). The goal of the JCTLM is to support worldwide comparability, reliability and equivalence of measurement results in clinical laboratories for the purpose of improving healthcare.

The JCTLM utilizes a series of review teams to provide the resources and expertise to identify acceptable reference materials, methods and laboratories. These specialties of these review teams comprise a range of analytes which are as listed below.

- Blood Gases
- Blood Groupings
- Coagulation Factors
- Drugs
- Electrolytes
- Enzymes
- Metabolites-Substrates
- Microbiology Serology
- Non-Electrolyte Metals
- Non-Peptide Hormones
- Nucleic Acids
- Proteins
- Quality Systems
- Vitamins

In laboratory medicine, many hundreds of different analytes are measured or determined. With regards to the implementation of traceability, is it important to differentiate between:

a. Type A (JCTLM List 1) analytes, those for which well-recognised reference materials and methods exist and can be traced to the International System (SI) units. For example, electrolytes (e.g. sodium), minerals (e.g. calcium), metabolic products (e.g. cholesterol, glucose, creatinine, etc), steroid hormones and vitamins;

b. Type B (JCTLM List 2) analytes, which are rather heterogeneous in human samples and are not directly traceable to SI units. For example, coagulation factors, blood cell counting tests, tests for nuclear materials and immunoassays (including those for cardiac markers, tumour markers, viral markers).

Two lists of higher order reference materials and reference measurement procedures are published (http://www.bipm.org/jctlm/home.do).

Table 1: Traceability and analyte classification

Type A analytes:

- Well defined compounds;
- Traceable to SI units;
- Results are not method-dependent;
- Approx. 65 analytes (e.g. metabolites, electrolytes, drugs etc)
- Full traceability chains.

Type B analytes:

- Not well defined (often heterogeneous mixtures);
- Analytes can be bound or in free state;
- Not traceable to SI units, but to arbitrary units (e.g. WHO International Units);
- Immunochemical procedures show inherent variability;
- 400 – 600 analytes (e.g. tumour markers, viral antigens, coagulation factors etc)
- Full traceability chains frequently not available.