Institute for Quality Management in Healthcare

Centre for Accreditation

# Guidance on Measurement Uncertainty for Medical Laboratories

December 2014, Version 1.0

By Dr. Lynn Allen and Mrs. Linda Crawford

Institute for Quality Management in Healthcare
1500–393 University Ave
Toronto, Ontario M5G 1E6
Canada

**TABLE OF CONTENTS**

## 1.0 Scope

The purpose of this document is to provide the following information for accredited medical laboratories:

- An understanding of measurement uncertainty;
- An explanation of what the IQMH Centre for Accreditation requires;
- How to determine measurement uncertainty; and
- Applications and uses for measurement uncertainty.

Ontario Laboratory Accreditation (OLA) first outlined its expectations with regard to traceability and measurement uncertainty in September 2007 ("Traceability and Uncertainty of Measurement for Medical Laboratories—OLA's Expectations").[1] In that document, it was stated that traceability and measurement uncertainty, in concert with method validation, quality control and quality assurance, ensure valid laboratory measurements. Appropriately established traceability and measurement uncertainty for quantitative and semi-quantitative tests or examinations are important factors in determining the "correctness" of laboratory methods and in assessing if results obtained by these methods are fit for intended use.

Previous versions of this guidance were published in 2009 and 2011, and are replaced with this 2014 IQMH document.

The use of appropriate reference materials is also important for method validation, calibration and quality control/quality assurance. In medical laboratory testing, traceability and measurement uncertainty are essential and usually overlapping components. The IQMH position statement on "Accreditation Traceability Requirements" can be found on the IQMH Website www.iqmh.org .[2]

## 2.0 Acknowledgements

The first guidance was released in 2009 and the second version in 2011. The contribution of Dr. Godfrey Moses in the preparation of these two documents is gratefully acknowledged.

This 2014 version incorporates advice from Dr. Douglas Gornall, and Scientific Committees serving the IQMH Centre for Proficiency Testing.

## 3.0 What is Measurement Uncertainty?

### *Concept*

Measurement uncertainty expresses the level of confidence a laboratory has in the utility or meaningfulness of a test result. It also provides a measure of the expected variability in a laboratory result when a test is performed on different occasions.

The concept of measurement uncertainty was originally developed for precise physical measurements but is now accepted world-wide as applicable to medical laboratory examinations that produce measured quantity values, and is included in the International Organization for Standardization's standard, ISO 15189:2012 Medical laboratories: Requirements for quality and competence (clause 5.5.1.4), on which IQMH bases its accreditation requirements.[3]

In clinical laboratory testing, the concept of error is widely accepted to set quality goals, e.g., allowable limits of error, total allowable error. However this approach might overestimate error and thus the concept of measurement uncertainty evolved. It assumes that laboratories take steps to minimise known bias and imprecision but recognizes that in practice, bias correction and replicate measurements can reduce potential error but not eliminate it altogether. Thus the true value of a measured quantity cannot be known exactly, and this is the fundamental thinking behind measurement uncertainty.

In the concept of measurement uncertainty, a measurement result comprises two uncertainties:

(1) Uncertainty due to imprecision ($u_{SD}$ or $u_{precision}$)

(2) Uncertainty associated with a bias correction ($u_B$ or $u_{bias}$)

When both these uncertainties are combined, they provide the combined standard uncertainty ($u_c$).

Although the combined standard uncertainty is used to express the uncertainty of many measurement results, for some commercial, industrial, and regulatory applications (e.g., when health and safety are concerned), what is often required is a measure of uncertainty that defines an interval about the measurement result within which the value of the measurand can be confidently asserted to lie. The measure of uncertainty intended to meet this requirement is termed expanded uncertainty, and is obtained by multiplying by a coverage factor.

Advice on the evaluation of uncertainty was first published in 1993 and refined in 1995 as the "Guide to the Expression of Uncertainty in Measurement" (commonly referred to as the GUM). The most recent version is a 2008 correction (GUM 1995 with minor correction) available from the Joint Committee for Guides in Metrology of the Bureau International des Poids et Mesures (BIPM).. [4, 5]

Because measurement uncertainty is concerned with analytical and not physiological variability, it includes the consideration of bias but does not consider or incorporate biological variation.

***Definitions of measurement uncertainty***

Here are four definitions of measurement uncertainty:

1. Parameter obtained from measurements, which serves, together with the measurement result, to characterize a range of values for the true value of the measurand. (Source: EuroLab Technical Report 2006). [6]
2. Estimated quantity intended to characterize a range of values which contains the reference value, where the latter may be either the true or expected value, depending on definition or agreement. (Source: EuroLab Technical Report 2006). [6]
3. Parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand (the quantity intended to be measured). (Source: National Pathology Accreditation Advisory Council *Requirements for the estimation of measurement uncertainty*).[7]
4. Non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used. (Source: VIM 3rd edition).[8]

***Abbreviations Associated with Measurement Uncertainty (MU) and Calculations in this Document***

| Term | Abbreviation | Definition |
|---|---|---|
| Analytical Measurement Range | AMR | Analytical measurement range |
| Asterisk | * | Multiplication sign |
| Bias | B | Difference between the result and the expected or assigned or reference value |
| Coefficient of Variation | CV | Coefficient of variation |
| Coefficient of variation uncertainty; also termed relative standard measurement uncertainty | $u_{CV\ \%}$ | Standard measurement uncertainty ($u_{SD}$) divided by the absolute value of the measured quantity value. CV = SD * 100/Mean. Express as %<br>**Explanation**<br>Relative standard measurement uncertainty expressed as a coefficient of variation (CV) |
| Combined standard measurement uncertainty | $u_c$ | Standard measurement uncertainty that is obtained using the individual standard measurement uncertainties associated with the input quantities in a measurement model<br>**Explanation**<br>Like 1 SD or 1 CV |
| Confidence Interval | CI | Confidence interval |
| Coverage factor | k | Number larger than one by which a combined standard measurement uncertainty is multiplied to obtain an expanded measurement uncertainty<br>**Explanation**<br>Multiply combined standard measurement uncertainty (u) by k to obtain expanded measurement uncertainty (U)<br>k is taken to be either 1.96 or 2 [like 2 SD / 2CV] |
| Coverage Factor | K | Coverage factor |
| Expanded measurement uncertainty | U | Product of a combined standard measurement uncertainty and a coverage factor larger than the number one<br>**Explanation**<br>This means that $U = u_c * k$ or $U = ku_c$ (where k is 1.96 or 2)<br>Defines interval around a result of a measurement that is expected to encompass 95% of values. It includes k factor (coverage factor) which is 1.96 or 2 - that is, the uncertainty range includes 95% of results<br>Expanded uncertainty can be expressed in absolute reporting units or relative percent. |
| Measurement Uncertainty | MU | Measurement uncertainty |
| Range | R | Range |
| Root Mean Square of the Bias | RMS, RMSB | Root mean square of the observed bias, B |
| Square of a number | $N^2$, N^2, Nsquared | Square of a number |
| Square root of a number | SQRT(N), SQRT, Sqrt, $N^{0.5}$, $N^{1/2}$, N^0.5 | Square root of a number |
| **S**tandard Deviation | SD | Standard deviation |
| Standard Deviation Index | SDI | Mean bias divided by method SD |
| Standard Error of the Mean | SEM | Standard error of the mean |
| Standard measurement uncertainty | $u_{SD}$ | Measurement uncertainty expressed as a standard deviation (SD)<br>**Explanation**<br>Precision of the lab method calculated over 6 months |
| Standard Uncertainty | $u_s$ or u | Standard uncertainty |

| Term | Abbreviation | Definition |
|---|---|---|
| Standard Uncertainty associated with a specific reference | $SR_g$ | Standard uncertainty associated with a specific reference and estimated from all-method group reproducibility PT/EQA data |
| Standard Uncertainty associated with a reference material or calibrator or to an assigned/reference value | $u_{ref}$ | Standard Uncertainty associated with a reference material or calibrator or to an assigned/reference value |
| " | $u_{Bref}$ | Calibrator bias/uncertainty relative to a reference system |
| " | ucref | Standard uncertainty associated with reference material or calibrator traceable to a reference method (obtained from the manufacturer or certifying body) |
| Student's t value | t or t-value | Student's t value |
| Uncertainty associated with a bias correction | uB or ubias | Uncertainty associated with a bias correction |

***Components of Measurement Uncertainty***

Combined uncertainty of measurement = Uncertainty of precision and uncertainty of bias

$u_C = (u_{PRECISION}\text{^}2 + u_{BIAS}\text{^}2)\text{^}0.5$

Uncertainty of precision

$U_{PRECISION}$ = QC CV/100 (Your laboratory's quality control CV, calculated over six months)

Uncertainty of bias (assumes bias has been corrected)

$u_{BIAS} = ((RMS_{BIAS})\text{^}2 + ((SD/SQRT\ N_{GROUP})\text{^}2)\text{^}0.5$

Where $RMS_{BIAS}$ = uncertainty associated with your relative bias from all method mean or reference value

Standard error of group's reproducibility where $SD/SQRT\ N_{GROUP}$ = uncertainty associated with all method mean or reference value

For further information on the calculation of SEM, RMS and (SD/Sqrt N), see Appendices A, B, and C, respectively.

***How to Express Measurement Uncertainty***

The expanded standard uncertainty provides an interval of values within which the true value of the measured quantity is believed to lie, with a stated coverage probability.

Uncertainty of measurement for plasma glucose: 6.8 +/- 0.4 mmol/L (95% coverage probability)

Here are alternate ways of stating this same thing:

- This means the true value for the glucose concentration is believed to lie within the interval 6.4 and 7.2 mmol/L, with a probability of 95%
- It is the "range" that contains the "true value" of a test or examination result, with 95 % confidence
- If test result is 6.8 +/- 0.4 mmol/L , the actual result lies between 6.4 and 7.2 mmol/L (95% probability)

Key points to consider when estimating measurement uncertainty using current laboratory data:

- Not all data requires conversion or transformation.
- Do not convert uncertainty data to standard uncertainty if it is given as SD (use as is). Convert to standard uncertainty, $u_s$, if the uncertainty data is given as a confidence interval, an expanded uncertainty or a stated range.
- Combine different contributions, all expressed in the same format, to achieve an overall estimate of uncertainty.

***IQMH Accreditation Requirement (version 6.0, December 2013)***

**Requirement VI.8:**

> *The laboratory shall determine the uncertainty of results (measurement uncertainty), for each examination that includes measured quantity values. The laboratory shall define the performance requirements for the measurement uncertainty and regularly review measurement uncertainty calculations.*[9]
>
> **What to Look For Application Guidance**
> For quantitative measurements, has a measurement uncertainty been calculated?
> *Measurement uncertainty can be calculated by two different approaches:*
> *- bottom-up, also called GUM*
> *- top-down, which uses statistical principles by the evaluation of QC data*
> *In both cases, bias shall be considered when sufficient data is available.*
>
> Are measurement uncertainty calculations regularly reviewed?
> *The period of review shall be based on parameters defined by the laboratory.*
> Are the performance requirements for measurement uncertainty defined for each quantitative examination?
> Does the laboratory make its estimates of uncertainty available to laboratory users upon request?

The above guidance information states that the laboratory is expected to calculate the measurement uncertainty for quantitative results, and regularly review these calculations at a frequency defined by the laboratory. Performance requirements for measurement uncertainty are to be defined for each quantitative examination, and be made available to laboratory users upon request.

It clarifies that measurement uncertainty can be calculated by two different approaches:

(1) Bottom-up, also called GUM

(2) Top-down, which uses statistical principles based on the evaluation of QC data

In both cases, bias shall be considered when sufficient data is available.

Thus, the expectation is that accredited laboratories will:

- Define the performance requirements for measurement uncertainty
- Calculate the measurement uncertainty for each examination that produces quantitative results, by either the bottom-up or top-down approach. In either, bias must be considered when sufficient data is available.
- Determine a period of review, and review those calculations at this frequency
- Make the measurement uncertainty for any quantitative examination available to users of the laboratory upon request.

IQMH's expectations are consistent with the guidelines of measurement uncertainty (GUM) concept that, for completeness, the result of a measurement must have a quantitative statement of its uncertainty.[4, 5, 6] Calculations of the measurement uncertainty of the result of a given laboratory test or examination have several uses, including that pertaining to regulation, but key to the medical laboratory is its use in assessing if results obtained by the method are fit for intended use. The concept of measurement uncertainty is relatively new to the medical laboratory. Currently, there is no standardized process for calculating measurement uncertainty in the medical laboratory.

Therefore, what follows is a practical guide for medical laboratory personnel on how to calculate measurement uncertainty. It is focused on how to convert existing laboratory information to standard uncertainty and how to combine standard uncertainty to obtain expanded uncertainty.

Measurement uncertainty of a result for a given laboratory test or examination arises from several contributions, which are combined to give an overall uncertainty. In general, when uncertainty components are combined to produce an overall uncertainty, individual components must be expressed in the same form. When expressed as standard deviations, it is equivalent to the standard uncertainty.

This document does not define or classify which laboratory tests or examinations require determination of measurement uncertainty. However, ISO 15189:2012 states that measurement uncertainty applies to each measurement procedure used to report measured quantity values. IQMH accepts that this means measurement uncertainty pertains to all quantitative and semi-quantitative tests or examinations in which numerical values are produced as the final results of the measurements.

It is the laboratory's responsibility to define and classify its test or examination procedures/methods, as well as identify and determine measurement uncertainty for those falling into the quantitative and semi-quantitative category.

***Uses***

Measurement uncertainty is useful because:

- It provides quantitative evidence that measurement results meet clinical requirements for reliability
- It can be used to compare results with previous results using the same measurement procedure or with reference values, especially where clinical decision level or cut-offs are independent of the method, e.g., glucose, PSA, $HbA_{1c}$, eGFR
- It can provide insight into which technical steps might be improved, thereby reducing overall measurement uncertainty
- It is an essential component for achieving standardized and harmonized measurement results through metrological traceability.

Measurement uncertainty does not necessarily need to be reported to users of the laboratory, but shall be available if requested. Situations where this information is helpful are:

- If a clinician questions whether two laboratory results in a patient differ
- If a clinician questions whether a laboratory result in a patient differs from a target value in a consensus document
- Clinical trials
- Clinical research

## 4.0 How to Determine/Calculate Measurement Uncertainty in the Medical Laboratory – An Overview

Laboratories may select one or a combination of the following for quantitative and semi-quantitative measurements:

a) The bottom-up approach as per GUM principles, based on estimates of uncertainty, expressed as standard deviations (SDs), that are assigned to individual steps or components of the test, examination or procedure used to produce the result and combined to provide an expanded uncertainty associated with the specific result. [4, 5, 10]

   **Note:** This approach may not be the method of choice for routine medical laboratories.

b) The top-down approach, using available laboratory test performance information, such as method validation, intra-laboratory quality control (QC) data and proficiency testing (PT) data, to calculate estimates of the standard uncertainty associated with the result produced by overall testing procedure/method.

   **Note:** This approach is preferred by many routine medical laboratories. The process is described briefly below. A more detailed account is given in the National Association of Testing Authorities (NATA) Technical Note.[11]

c) Other approaches involve various combinations and/or modifications of the components in (a) and (b), personal experience and information on certified reference methods or materials.

***Determining Measurement Uncertainty by the Top-Down Approach Using Intra- and Interlaboratory Data***

The preferred method is to use performance data from both internal QC (intralaboratory) and proficiency testing (PT) programs (interlaboratory). For stable and well-established methods or procedures, imprecision is equivalent to the standard measurement uncertainty, assuming negligible or no bias. A minimum of six months' data is recommended (in order to ensure that variations due to multiple users, reagents and calibrator lots 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 (SD). If bias is significant or known, calculate the combined standard uncertainty as demonstrated in the following five steps. 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; this must be checked when sufficient data has been accumulated.

***Steps***

1. Calculate the overall SD of the method from monthly SDs (imprecision) for at least two levels of QC using Equation 1:

**Equation 1:**

$$SD = \{[(SD)_{L1}^2 + (SD)_{L2}^2] / 2\}^{1/2}$$

Where $(SD)_{L1}$ and $(SD)_{L2}$ are the average SD of each control level, respectively, for the past 6 months.

**Notes:** If more than two levels are used depending on QC requirements and clinical decision limits for the method, calculate the average SD as follows:

$SD = [(n_1\ SD_1^2 + n_2\ SD_2^2 + \ldots n_x\ SD_x^2) / (n_1 + n_2 + \ldots n_x)]^{1/2}$, where n and x are the numbers of monthly QC points and levels, respectively and SD is the average SD or the standard uncertainty, $u_s$.

Equation 1 shows the calculation of the average imprecision for the two control levels and is generally applicable to methods or measurement procedures (tests or examinations), where control levels depict comparable performance across the entire analytical measurement ranges (AMR). For some methods, such as some immunoassays, with varying imprecision at different clinical decision limits or control levels, measurement uncertainty must be calculated at each decision or control level.

If result of the laboratory test or examination is outside of the AMR and is re-obtained following dilution or concentration, the uncertainty associated with dilution or concentration process must be in combined with that obtained in Equation 1.

2. Calculate the standard uncertainty associated with the method, $u_s$:

$u_s$ = SD (average SD)

Calculate the bias, B associated with the method (intralaboratory and interlaboratory bias):

Bias, B = Test Result – Reference value

**Note:** An acceptable reference value includes any assigned value obtained from a higher order reference measurement procedure, from a mutually accepted national and international reference method or laboratory network, from peer group and all-methods' mean values from PT program.

Then calculate the standard uncertainty associated with the bias using Equation 2:

**Equation 2:**

$$u_B = [(SEM^2)\ \text{or}\ (RMS)^2 + (SR_g)^2 + (u_{ref}^2)]^{1/2}$$

| Higher order reference measurement procedure, mutually accepted national and international reference method or laboratory network | Peer group and all-methods' mean values from PT program. |
|---|---|
| $u_B = [(SEM^2) + (SR_g)^2 + (u_{ref}^2)]^{1/2}$ | $u_B = [(RMS)^2 + (SR_g)^2 + (u_{ref}^2)]^{1/2}$ |

3. Calculate the combined standard uncertainty of the method, $u_c$ using Equations 3 or 4:

**Equation 3:**

$$u_c = [(u_s)^2 + (u_B)^2]^{1/2}$$

**Equation 4:**

$$u_c = [(u_s)^2 + (SEM^2) \text{ or } (RMS)^2 + (SR_g)^2 + (u_{ref}^2)]^{1/2}$$

| Higher order reference measurement procedure, mutually accepted national and international reference method or laboratory network | Peer group and all-methods' mean values from PT program. |
|---|---|
| $u_c = [(u_s)^2 + (SEM^2) + (SR_g)^2 + (u_{ref}^2)]^{1/2}$ | $u_c = [(u_s)^2 + (RMS)^2 + (SR_g)^2 + (u_{ref}^2)]^{1/2}$ |

**Notes:** $u_{ref}$ is the standard uncertainty associated with the reference material or calibrator traceable to a reference method (obtained from the manufacturer or certifying body, respectively, as available and/or applicable).

SEM is the uncertainty of the observed mean derived from in-laboratory replicate measurements of a reference material; RMS is the uncertainty associated with the observed bias derived from peer means using PT performance data.

$SR_g$ is the standard uncertainty associated with the assigned or reference value using the all-method group reproducibility in PT.

Medical laboratories can purchase certified reference materials and calculate the SEM by performing replicate analyses (minimum of N=10) for each test or examination as available; or calculate the RMS from peer group means in PT performance data from at least three different surveys, each with multiple levels.

If a dilution or concentration is performed, the appropriate uncertainty should be added to the combined uncertainty.

4. Calculate the expanded uncertainty of the method, U using Equation 5:

**Equation 5:**

$$U = u_c \times 1.96 \ (\sim 2)$$

**Note:** The expanded uncertainty is the combined uncertainty x k-value, which is taken to be either 2 or 1.96 and represents a 95% confidence level.

5. Express the expanded uncertainty as Measured Results +/– U, (units).

## 5.0 Converting Laboratory Uncertainty Information into Standard Uncertainty – An Explanation

Uncertainty information in the medical laboratory is available in different forms:

1) as standard deviation (SD);

2) as confidence interval (CI);

3) as expanded uncertainty (U) and

4) as a range (R).

Therefore, some conversion is often required.

***MU as SD***

No conversion is required. For tests or examinations for which the uncertainty is required for results at a single level, precision data (the standard deviation) derived from replicate measurements at the specified level over an extended time period is the method standard uncertainty.

For tests or examinations for which uncertainty is required for results at different levels, the weighted or pooled SD, or the SDs obtained at each level is the standard uncertainty of the method, respectively.

If the SD is the standard deviation of the mean (e.g. SD from six or more monthly means), the method SD is the standard error of the mean (SEM), which is the SD divided by the square-root of the number of means ($N^{0.5}$).

***MU as a CI***

This is usually stated in the form: **Value ± CI**

The standard uncertainty ($u_s$) is determined from the following: $\mathbf{u_s \times (SD/N^{0.5}) = CI/t}$

Where t is the Student's t value (obtained from statistical tables) and N is the number of observations.

**Example:**

A frozen human serum reference material is stated by the supplier to contain 0.346 ± 0.007 mmol/L creatinine.

0.007 is the expanded uncertainty. Divide this by the coverage factor.

$u_s$ = 0.007/1.96 mmol/L

***MU as Expanded Uncertainty, U***

Standard uncertainty ($u_s$) = U/k (coverage factor)

**Example:**

A calibration solution is stated by the manufacturer to contain 2.5 ± 0.5 mmol/L glucose (expanded uncertainty).

Divide the expanded uncertainty by the coverage factor.

$u_s$ = 0.5/1.96 mmol/L

Note: Because the coverage factor, k is used, and the confidence interval is not stated, the confidence level is taken to be 95%.

***MU as a Range***

This is usually stated in the form: **Value ± a**

If a confidence level or distribution type is not stated and values close to the mean are no more likely than those at the extreme (e.g., pipette volumes, digital balance), the uncertainty range is considered to be distributed in a rectangular fashion and the standard uncertainty is derived as follows:

$u_s = a/(3^{0.5})$

**Note 1**: The Nordtest Handbook gives an example of rectangular distribution. [12]

If on the other hand, values close to the mean are more likely than those at the extreme (e.g., temperature settings using a thermostat), the distribution is considered to be triangular and the standard uncertainty is:

$u_s = a/(6^{0.5})$

**Note 2**: The above are examples of type B uncertainty and are applicable to semi-quantitative methods such as drugs of abuse and viral hepatitis marker screening, in which estimates of uncertainty are determined based on assigned cut-off values of the test or examination. The approach is also useful in the GUM bottom-up approach where one needs to identify all potential sources of uncertainty, e.g., calibration, weighing, pipetting, temperature, instrument fluctuations, counting. Estimate the amount of error (uncertainty) as an SD by experiment or from available information and then combine the contributing uncertainties mathematically. This process is unwieldy but may be required in non-quantitative areas of the clinical laboratory, e.g., microbiology, hematology cell counts.

**Note 3**: Rectangular versus triangular distribution is discussed by the LABNETWORK online. [13]

**Example:**

The specification listed for a 1 mL automatic pipette is 1±0.01 mL (uncertainty as a range but no confidence level and distribution stated). The actual volume of the liquid taken up or dispensed by the pipette during operation is anywhere from 0.99 to 1.01 mL.

$u_s = 0.01/(3^{0.5})$

$= 0.006$ mL (for a rectangular distribution)

***Combining MU***

Either variances or relative variances can be used depending on the parameters associated with the uncertainties. In cases where the final calculation involves addition or subtraction, uncertainties are combined as variances ($SD^2$).

**Example:**

Mean serum creatinine from three independent EQA surveys is 69 umol/L; method long-term SD from six months' in-house QC data is 2.24 umol/L; method calibrated and regularly verified with calibration solution of expanded uncertainty of 0.5 relative to an certified international standard as per manufacturer; in-house method uncertainty of bias relative EQA peer or from certified reference material is 0.024 umol/L; method uncertainty of bias relative to all-methods' mean value or an assigned reference value is 0.030 umol/L.

$u_c(\text{creatinine}) = [u_s(SD_m)^2 + u_s(U_{cal}/2)^2 + u_s(B_m)^2 + u_s(B_{ref})^2]^{0.5}$

$u_c(\text{creatinine}) = [(2.24)^2 + (0.5/2)^2 + (0.024)^2 + (0.030)^2]^{0.5}$

$= 2.25$ umol/L

U, expanded uncertainty is 2.25 × 2.96 = 6.66 umol/L or 9.7 % (6.66/69 × 100).

Mean serum creatinine results is 69 +/–7 umol/L or (uncertainty estimate rounded to one significant digit).

**Note**: In cases where the final calculation involves a product or quotient, uncertainties are combined as CV (coefficient of variation).

**Example:**

Albumin:Creatinine Ratio (ACR),Urine

ACR,Urine = Albumin (mg/L) / Creatinine (mmol/L); units: mg/mmol

$u_c(\text{ACR,urine}) = [u_s(\text{Alb})^2 + u_s(\text{Creat})^2]^{0.5}$

$u_s(\text{Alb}) = [u_s(\%CV_m)^2 + u_s(\%U_{cal}/2)^2 + u_s(\%B_m)^2 + u_s(\%B_{ref})^2]^{0.5}$

$u_s(\text{Creat}) = [u_s(\%CV_m)^2 + u_s(\%U_{cal}/2)^2 + u_s(\%B_m)^2 + u_s(\%B_{ref})^2]^{0.5}$

$u_c(\text{ACR, urine \%}) = \{[1.9^2\ \% + (1.0/2)^2\ \% + 5.3^2\ \% + 0.3^2\ \%]^2 + [3.2^2\ \% + (1.0/2)^2\ \% + 2.4^2\ \% + 3.0^2\ \%]^2\}^{0.5}$

$= \{[3.61 + 0.25 + 2.81 + 0.09]^2 + [10.24 + 0.25 + 5.76 + 9.0]^2\}^{0.5}$

$= \{2.60 + 5.02\}^{0.5}$

$= 2.76$ % (or 0.03)

Thus

U (ACR,urine) = 7.57 x 1.96 % (14.8) or 0.15

Mean urine albumin and urine creatinine values of specimen are 29 g/L & 69 mmol/L, respectively

ACR,urine = 0.42 +/– 0.15 mg/mmol

**Note**: Intra- and interlaboratory uncertainty of bias applies the bias relative to peer and all-method groups, respectively. It is not the actual bias. The actual bias is used to determine whether to include or exclude the uncertainty of bias attributable to the all-method group reproducibility in the combined estimate. If the actual bias is equal to or less than twice the method SD, then it is acceptable to exclude the all-method group reproducibility term for uncertainty of bias from the combined calculation.

For more complex cases where the final calculation involves derived values, such as in the estimated glomerular filtration rate (eGFR), calculated creatinine clearance and fractional excretion rates, in which the results of the test or examination are multiplied by a factor (with zero uncertainty) or raised to an exponent, the standard uncertainties are expressed as follows:

$u_{(s)} = b * u_{(x)}$ (measured value multiplied by a factor b)

$u_{(s)} = n^q$ (measured value raised to the power q)

***A Top-Down Alternative Approach using the Uncertainty Calculator***

This is an alternative approach to the modeling method of ISO using laboratory performance data. It is a process based on GUM principles and utilizes basic statistical equations to calculate type A and B standard and expanded uncertainties. [8, 9]

Internal quality control data for each test and quality control level, as well as external PT survey results, are transferred to an MS Excel® spreadsheet, termed the "Uncertainty Calculator."

Standard uncertainties are calculated for various components (intra-laboratory method imprecision and bias, as well as bias relative to a reference or other laboratory) and combined as described above to provide estimates of expanded uncertainties.

The procedure and steps involved in determining measurement uncertainty by the top-down alternative method are described in detail in the "Uncertainty Calculator," which is available from: http://www.gamma-dynacare.com/content/media/publications.aspx?expandable=4.[14]

IQMH thanks Gamma-Dynacare Medical Laboratories for sharing this tool.

When using this tool, a modified approach to the above-noted steps is:

1. Select the test
2. Determine precision from QC data
3. Consider bias
    a. Determine bias from PT/EQA results
    b. Ignore or correct bias in the test/examination procedure, as applicable
    c. Express actual bias as SDI (Z-value or SD ratio; SDI is mean bias divided by method SD)
    d. Test for significant bias using SDI…if bias is small, exclude SEM
        i. If SDI $\leq$ 2, include only RMS of your method in calculation of uncertainty associated with bias
        ii. If SDI > 2, include both RMS of your method **and** interlaboratory SD/Sqrt N **or** $U_{ref}$ (N is number of laboratories – all method) –

            in the calculation of uncertainty associated with the bias
4. Calculate combined uncertainty

5. Calculate expanded uncertainty by multiplying combined uncertainty by coverage factor
6. Express expanded uncertainty in absolute reporting units or relative percent

***Measurement Uncertainty for Some Common Medical Laboratory Tests – Examples***

The values used in the following examples are not real results and are used for illustrative purposes only.

***Plasma Glucose (Fasting Plasma Glucose)***

Fasting plasma glucose (FPG) (indicative of impaired glucose utilization) = 6.8 mmol/L; CV = 2.0% or SD = 0.14 mmol/L; $u_{Bref}$ (calibrator bias/uncertainty relative to a reference system) = 2.0% or 0.1 mmol/L; SEM (method bias/uncertainty of the test method) = 2.0 % or 0.1 mmol/L; acceptable sources are peer group data from EQA or PT programs, replicate analyses of certified reference material or accuracy surveys with frozen serum samples and manufacturers' and reference laboratories.

The combined uncertainty of fasting plasma glucose is:

$u_{sFPG}$ = Standard Uncertainty = $[(uSD)^2 + (u_B)^2]^{1/2}$ mmol/L]

= $[(SD)^2 + (SEM)^2 + (u_{Bref})^2]^{1/2}$

= $[(0.14)^2 + (0.1)^2 + (0.1)^2]^{1/2}$

= 0.2 mmol/L

The expanded uncertainty of fasting plasma glucose, $U_{FPG}$ is:

$U_{FPG} = 0.2 \times 1.96$ or $U_m = 0.2 \times 2 = 0.4$ mmol/L, representing a 95% confidence interval of uncertainty.

At a fasting plasma glucose of 6.8 mmol/L, the $U_{FPG}$ = 0.4 mmol/L

i.e. the fasting plasma glucose = 6.8 +/– 0.4 mmol/L (95% CI = 6.4 – 7.2 mmol/L).

***Anion Gap (AG)***

Estimates of measurement uncertainty derived from other measurement results (often termed inputs) are combined uncertainties of the independent inputs. If inputs interact by addition or subtraction, SD must be used. However, if they interact by multiplication or division, CV must be used in the determination (see next example).

For routine use, the anion gap, AG is derived as follows: AG = [Na – (Cl + HCO3)] mmol/L.

AG result is 14 mmol/L derived from [Na 140 – (Cl 105 + HCO3 21)] = 14 mmol/L.

Inputs interact by addition and subtraction, standard uncertainty is SD: SDs for Na, Cl and HCO3 for level 1 or level 2 QC derived from Equation 1 are: Na+ = 1.0 mmol/L; Cl = 1.2 mmol/L; HCO3 = 0.9 mmol/L.

The standard uncertainty of the anion gap, $uSD_{AG}$ is: $uSD_{AG} = [(1.0^2 + 1.2^2 + 0.9^2)]^{1/2} = 2$ mmol/L.

The expanded uncertainty of the anion gap, $U_{AG}$, is: $U_{AG} = uSD_{AG} \times 1.96$ or $uSD_{AG} \times 2 = 4$ mmol/L, representing a 95% confidence interval of uncertainty.

Note: The number 1.96 or 2 is called the coverage factor as previously described. $U_{AG}$ is equivalent to the total error (TE) in the absence of a systematic error (SE). At an AG of 14 mmol/L, the $U_{AG}$ = 4 mmol/L, i.e. the anion gap = 14 +/– 4 mmol/L (95% CI = 10 – 18 mmol/L).

***Creatinine Clearance (Cr-Cl)***

For routine use, creatinine clearance (Cr-Cl) is derived as follows:

Cr-Cl = [$U_{cr}$ (umol/L) × Vol (mL)] / [t(min) × $S_{cr}$(umol/L)] mL/min.

Cr-Cl is the creatinine clearance, $U_{cr}$, the urine creatinine, Vol, the volume of urine, $S_{cr}$, the serum creatinine and t, the collection time. Because inputs interact by multiplication or division, CV must be used in determining measurement uncertainty here.

Typical CV are $U_{cr}$ = 2.3%; Vol = 10%; $S_{cr}$ = 3.5%; t = 1%.

The standard uncertainty of creatinine clearance, $uCV_{CCL}$ is: $uCV_{CCL} = [(2.3^2 + 10^2 + 3.5^2 + 1^2)]^{1/2} = 11\%$

$SD_{CCL} = uCV_{CCL} \times$ Cr-Cl

$= 11\% \times 80 = 8.8$ mL/min

The expanded uncertainty of creatinine clearance, $U_{CCL}$ is: $U_{CCL} = SD_{CCL} \times 1.96$ or $SD_{CCL} \times 2 = 18$ mL/min.

At a CCL of 80 mL/min, the $U_{CCL}$ = 18 mL/min, i.e. the creatinine clearance = 80 +/– 18 mL/min (95% CI = 62 – 98 mL/min).

***Erythrocyte (Erc) or Red Blood Cell (RBC) Count***

Mean Erc or RBC for a borderline low specimen is $4.12 \times 10^9$/L; method SD and % CV are 0.24 and 0.6, respectively; method bias (RMS) is 0.009; all-method group variability is negligible.

Units are cells x $10^9$/L throughout

$u_c = [(SD^2) + RMS^2]^{0.5}$

$= [0.24^2 + 0.009^2]^{0.5}$

$= 0.240169$

$U = 0.24 \times 1.96 = 0.47 \times 10^9$/L

Erc or RBC = 4.12 +/- 0.47 $\times 10^9$/L

## 6.0 Conclusion

In order to comply with accreditation requirements, medical laboratories must establish traceability and estimate the measurement uncertainty associated with all quantitative measurement results. The laboratory needs to acquire calibration certificates stating traceability to a standard or reference value or establish its own chain to the highest available reference. When this is not possible, the laboratory must use other means to provide confidence in the method.

Measurement uncertainty is to be expressed in this form:

Measurand (result of laboratory test or examination): Value +/– U units

U is the expanded uncertainty and units are typically SI or internationally accepted non-SI units.

The expanded uncertainty, U, represents the range of values within which the true value produced by the measurement method lies. U is calculated by multiplying the combined standard uncertainty by a coverage factor, k. The magnitude of the coverage factor depends on the confidence level assigned to the range of values. For most medical laboratory procedures, k is taken as 1.96 or 2, signifying a 95% confidence limit.

This document explains different options on how to calculate measurement uncertainty.

- bottom-up (GUM) [4, 5, 10]
- top-down [11]
- top-down alternative [14]

The latter two include using QC and PT data.

The approach taken by laboratories may depend on resources available.

Generally, the "top-down" approach using laboratory method performance data is recommended, and described in detail. If resources are available, laboratories may choose to develop their own equations to calculate measurement uncertainty. However, if resources are limited, use of the "top-down" alternative Uncertainty Calculator in MS Excel® format from Gamma-Dynacare Medical Laboratories is an acceptable alternative. [12]

When using any top-down approach to determine measurement uncertainty for stable and well-established methods, it is important to note that this approach does not include uncertainty associated with the pre-analytical process, such as patient preparation or sampling.

## 7.0 References

1. Crawford L, Moses G. Traceability and Uncertainty of Measurement for Medical Laboratories—OLA's Expectations. QMP–LS News, No. 118. Toronto (ON): QMP–LS QView. c2007 [2007 Sept; cited 2011 Dec 08].

2. Institute for Quality Management in Healthcare (IQMH). Position Statement – Traceability Accreditation Requirements. 2014. Available from: https://www.iqmh.org.

3. International Organization for Standardization. ISO 15189:2012 Medical laboratories: Requirements for quality and competence; 5.5.1.4. Geneva: International Organization for Standardization.

4. International Organization for Standardization. ISO/IEC 98:1995 Guide to the expression of uncertainty in measurement (GUM). Geneva: International Organization for Standardization; 1995. Available at http://www.bipm.org/en/publications/guides/.

5. Joint Committee for Guides in Metrology. Evaluation of measurement data — Guide to the expression of uncertainty in measurement (GUM 1995 with minor corrections). Available at http://www.bipm.org/en/publications/guides/.

6. EuroLab Technical Report. Guide to the Evaluation of Measurement Uncertainty for Quantitative Test Results, 2006. Available from http://www.eurolab.org/documents/EL_11_01_06_387%20Technical%20report%20-%20Guide_Measurement_uncertainty.pdf.

7. National Pathology Accreditation Advisory Council (NPAAC). Requirements for the estimation of measurement uncertainty. Canberra: Commonwealth of Australia, Australian Government Department of Health & Ageing; 2007. ISBN 1-74186-164-0. Available from http://www.health.gov.au/internet/main/publishing.nsf/Content/health-npaac-emu.htm.

8. Joint Committee for Guides in Metrology (JCGM) International Vocabulary of Metrology (VIM) – Basic and General Concepts and Associated Terms, 3rd edition, 2012. Available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2012.pdf

9. Quality Management Program—Laboratory Services. Ontario Laboratory Accreditation Division. Ontario Laboratory Accreditation Requirements and Guidance Information, 2013 Version 6.0. Ontario (Canada): QMP-LS; 2013.

10. International Laboratory Accreditation Cooperation (ILAC). ILAC P10:01/2013 ILAC Policy on the Traceability of Measurement Results. Available from https://www.ilac.org/documents/ILAC_P10_01_2013.pdf

11. National Association of Testing Authorities (NATA). Technical Note #33 - Guidelines for estimating and reporting measurement uncertainty of chemical test results. Australia; 2013. Available from https://www.health.gov.au/internet/main/publishing.nsf/Content/717802D80CFD8A4ACA257BF0001BDBB7/$File/technical_note_33.pdf.

12. NORDTEST. Nordtest Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories, 3rd edition, 2012. Available from http://www.nordtest.info/images/documents/nt-technical-reports/nt_tr_537_ed3_1_English_Handbook%20for%20Calculation%20of%20Measurement%20uncertainty%20in%20environmental%20laboratories.pdf

13. LABNETWORK. Chemical Lab Uncertainty of Measurement. Available from: http://www.labnetwork.org/en/chemical-lab-recent/112-uncertainty-of-measurement.

14. Gamma Dynacare Medical Laboratories. http://www.gamma-dynacare.com/content/media/publications.aspx?expandable=4; Under Presentations (Uncertainty of Measurement in the Canadian Laboratory, click on Uncertainty Measurement Tool Limited License [PDF], Complete the license form (click box ☐ to give ☒), save and send it, with your contact information, via email to: uofmcalculator@gamma-dynacare.com **or** to Dr. Hui Li at LiH@gamma-dynacare.com.

## 8.0 Recommended Reading

1) Physics Laboratory Physical Reference Data [database on the Internet]. Gaithersburg (MD): National Institute of Standards and Technology (NIST). Recommendation INC-1 (1980) – Expression of experimental uncertainties. Available from: http://physics.nist.gov/cuu/index.html.

2) International Organization for Standardization (ISO). ISO 17511:2003 In vitro diagnostic medical services – Measurement of quantitative biological samples – Metrological traceability of values assigned to calibrators and control materials. Geneva: International Organization for Standardization. Available from: http://www.iso.org.

3) Asia Pacific Laboratory Accreditation Cooperation. General Information on Uncertainty of Measurement, APLAC TC 010, 09/2010. Available at https://www.aplac.org/documents/tc/aplac_tc_010_issue_2.pdf

4) White GH. Basics of estimating measurement uncertainty. Clin Biochem Rev. 2008;29 Suppl i:S53–S60. Available at http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=2556585&blobtype=pdf

5) CLSI. Expression of Measurement Uncertainty in Laboratory Medicine; Approved Guideline. CLSI Document EP29-A, January 2012.

## 9.0 Appendix A: Calculation of SEM – Example Using Reference Material

### Calculation of SEM – example, using reference material

SEM is the relative SD or standard error of the mean of replicate measurements of the reference material or calibrator. Medical laboratories can purchase certified reference materials and calculate the SEM by performing replicate analyses (minimum of N=10) for each analyte (test/examination; SEM = $SD/N^{½}$ ).

| **Analyte** | **Reference Material** | **Calibrator** | **SI Units** | **Value** | **Uncertainty = U (k=2)** | **u or uCref** This is standard uncertainty |
|---|---|---|---|---|---|---|
| Creatinine | NIST 914a | Level 1<br>Level 2 | µmol/L<br>µmol/L | 81.3<br>702.8 | 0.37<br>1.63 | 0.185 or **0.228%**<br>0.815 or 0.116% |
| **uCref** | Standard uncertainty associated with reference material or calibrator traceable to a reference method (obtained from the manufacturer or certifying body) | | | | | |

One needs the target value for the reference material from the certificate, above.

| Day / Measurement | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 79.1 | 80.1 | 80.9 | 80.9 | 80.7 | 80.5 | 79.6 | 79.2 | 80.5 | 79.6 |
| 2 | | 79.9 | 78.1 | 80.7 | 80.6 | 80.7 | 79.9 | 78.2 | 78.4 | 80.2 | 79.9 |
| 3 | | 78.4 | 78.8 | 78.4 | 80.0 | 80.4 | 78.7 | 79.9 | 78.7 | 80.3 | 78.8 |
| 4 | | 80.3 | 79.1 | 78.8 | 80.1 | 79.5 | 80.5 | 80.1 | 78.4 | 78.2 | 79.6 |
| 5 | | 78.5 | 80.0 | 80.8 | 79.9 | 80.5 | 78.8 | 81.0 | 79.5 | 78.9 | 80.5 |
| Mean of 50 results | | 79.66 | | | | | | | | | |
| Creatinine reference value | | 81.3 | | | | | | | | | |
| B | Bias = Mean of 50 results - Certified Reference Value | -1.64 | | | | | | | | | |
| B% | % of reference value | -2.02 | | | | | | | | | |
| SD | SD of the 50 results | 0.872 | | | | | | | | | |
| SEM | SEM = SD / SQRT(50) | 0.123 | | | | | | | | | |
| SEM as % | % of Certified Reference Value | 0.152 | | | | | | | | | |

## 10.0 Appendix B: Calculation of RMS – Example Using PT DATA

**Data Example** - Creatinine QMP-LS Results over 3 surveys

| Survey | Vial # | Result Obtained umol/L | Reference Value (Assigned Value) umol/L | # of Laboratories/ Survey | Reference Value SD |
|---|---|---|---|---|---|
| 1 | A | 86 | 88 | 39 | 5.4 |
| | B | 236 | 235 | 39 | 6.7 |
| | C | 83 | 85 | 38 | 6.1 |
| 2 | A | 279 | 277 | 73 | 8.3 |
| | B | 254 | 246 | 73 | 6.9 |
| | C | 83 | 84 | 72 | 7.3 |
| 3 | A | 676 | 665 | 78 | 18.4 |
| | B | 94 | 91 | 77 | 5.4 |
| | C | 98 | 97 | 78 | 4 |

**Calculation of RMS**

$RMS_{Bias}$ = uncertainty of the lab's bias. Calculation - square % bias, sum, sum/N, SQRT, where N is # of challenges

$RMS_{Bias}$ = (Sum of SQ relative bias to reference value/N)^0.5

1. Take absolute or relative differences from target or reference (bias)

2. Square differences

3. Sum square differences

4. Divide sum of square differences by N (number of survey results of comparable values)

5. Take square-root of mean of squared differences

| **Assigned Value** umol/L | **Result** umol/L | **Bias** = Result - Assigned Value | **% Bias** = Bias *100/ Assigned Value | **% Bias$^2$** |
|---|---|---|---|---|
| 88 | 86 | -2 | -2.27 | 5.17 |
| 235 | 236 | 1 | 0.43 | 0.18 |
| 85 | 83 | -2 | -2.35 | 5.54 |
| 277 | 279 | 2 | 0.72 | 0.52 |
| 246 | 254 | 8 | 3.25 | 10.58 |
| 84 | 83 | -1 | -1.19 | 1.42 |
| 665 | 676 | 11 | 1.65 | 2.74 |
| 91 | 94 | 3 | 3.30 | 10.87 |
| 97 | 98 | 1 | 1.03 | 1.06 |
| | | | Sum (% Bias$^2$) | 38.08 |
| | | | # challenges | 9 |
| | | | Sum (% Bias$^2$)/N | = 38.08/9 = 4.23 |
| | | | $RMS_{Bias}$ % | = SQRT(4.23) = 2.057 |

## 11.0 Appendix C: Calculation of (SD/Sqrt N) group – Example Using PT Data

SD/SqrtN = standard error of groups' reproducibility; uncertainty associated with All Method Mean (AMM)

(SD/Sqrt N)group = ((Sum of (Reference Value SD/SqrtN expressed as value relative to reference value)^2)/Count of # reference value SDs)^0.5)

| | | | | | | | | (SD/Sqrt N)group |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 0.007135541 |
| Survey | Vial | N | Reference Value | SD | $SD^2$ = Variance | Reference Value SD/SqrtN | SD/SqrtN expressed as value relative to the reference value | SQ SD/SqrtN expressed as value relative to the reference value |
| 1 | A | 39 | 88 | 5.4 | 29.16 | 0.86469203 | 0.009826046 | 9.65512E-05 |
| | B | 39 | 235 | 6.7 | 44.89 | 1.07285863 | 0.004565356 | 2.08425E-05 |
| | C | 38 | 85 | 6.1 | 37.21 | 0.98955067 | 0.011641773 | 0.000135531 |
| 2 | A | 73 | 277 | 8.3 | 68.89 | 0.97144152 | 0.003507009 | 1.22991E-05 |
| | B | 73 | 246 | 6.9 | 47.61 | 0.80758392 | 0.003282861 | 1.07772E-05 |
| | C | 72 | 84 | 7.3 | 53.29 | 0.86031325 | 0.010241824 | 0.000104895 |
| 3 | A | 78 | 665 | 18.4 | 338.56 | 2.08338974 | 0.003132917 | 9.81517E-06 |
| | B | 77 | 91 | 5.4 | 29.16 | 0.61538711 | 0.006762496 | 4.57313E-05 |
| | C | 78 | 97 | 4 | 16 | 0.45291081 | 0.004669184 | 2.18013E-05 |