TECHNOPATH
CLINICAL DIAGNOSTICS

Guide to Uncertainty of Measurement

THE QUALITY CONTROL COMPANY

Contents

Background - Why this is necessary

In Clinical Laboratory Diagnostics, patient care and the integrity of patient test results is our primary concern. In any metrology process where there can be some uncertainties in a test result we need to understand and act on this.

The ISO 15189:2012 standard contains enhanced expectations regarding measurement of uncertainty and the ISO 17025 standard specifies requirements for reporting and evaluating uncertainty of measurement.

15189:2012 & 17025:2017 Requirements

ISO 15189: 2012:
Section 5.5.1.4: "The laboratory shall determine measurement uncertainty for each measurement procedure, in the examination phases used to report measured quantity values on patients' samples. The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of measurement uncertainty".

Section 5.6.2: "Upon request, the laboratory should make its estimate of measurement of uncertainty available to laboratory users".

17025: 2017:
Section 7.6.1: "Laboratories shall identify the contributions to measurement uncertainty. When evaluating measurement uncertainty, all contributions that are of significance, including those arising from sampling, shall be taken into account using appropriate methods of analysis".

Section 7.6.3: "A laboratory performing testing shall evaluate measurement uncertainty. Where the test method precludes rigorous evaluation of measurement uncertainty, an estimation shall be made based on an understanding of the theoretical principles or practical experience of the performance of the method".

Section 7.8.3: "Test Reports: where applicable, the measurement uncertainty presented in the same unit as that of the measurand or in a term relative to the measurand (e.g. percent) when:

- it is relevant to the validity or application of the test results;
- A customer's instruction so requires, or
- The measurement uncertainty affects conformity to a specification limit".

MU Guidance Summary

Laboratories subject to accreditation programs (such as CAP) satisfy most of what is necessary in regards of ISO 15189 MU clause through the following ongoing routines:

- Quality Control (QC)
- Proficiency testing (PT)
- Calibration
- PEER comparison
    - Multi-instrument comparison
    - Method comparison
- Generation of data supporting the analytical measurement range as defined by the medical director.

It is recommended that laboratories ensure that they have access to confidence levels for their tests, derived from QC and other analytical processes, and be able to supply a proceudre that describes the quality routines that support the validity of the stated confidence levels [CAP Measurement of uncertainty guide, 2014].

Measurement Uncertainty Requirements Summary

Definition of Measurement of Uncertainty: Uncertainty of measurement is defined by ISO 15189 as “a parameter associated with the result of a measurement that characterises the dispersion of values that could reasonably be attributed to the measurand”.

Uncertainty is a property of a test result.
The preferred form of reporting is:

Result: (x ± U) units

With the adoption of the International Organization for Standardization (ISO) laboratory standard Medical Laboratories – Particular Requirements for Quality and Competence (ISO 15189), clinical pathology laboratories have been required to provide estimates of measurement uncertainty for all quantitative test results.

Uncertainty of measurement (UM, also referred to as measurement uncertainty, MU), traceability and numerical significance are inter-related concepts that affect both the format and the information conveyed by a quantitative result. As every measurement

is prone to error, it is often stated that a measurement result is complete only when accompanied by a quantitative statement of its uncertainty. This uncertainty assessment is required in order to decide if the result is adequate for its intended purpose (fit for purpose) and to ascertain if it is consistent with other similar or previous results. The development of strategies for setting quality goals in laboratory medicine and procedures for assessing fitness for purpose have been well covered in the clinical biochemistry literature. In particular, quality specifications based on biological variation have been discussed in detail. The accuracy, precision and fitness for purpose of medical laboratory results rely on the basic metrological concepts of a common system of units, traceability of measured values, and uncertainty of measurement and commutability of results within a calibration hierarchy.

Uncertainty of Measurement and Measurement Error

The result of any quantitative measurement has two essential components:

- A numerical value (expressed in SI units as required by ISO 15189) which gives the best estimate of the quantity being measured (the measurand). This estimate may well be a single measurement or the mean value of a series of measurements.

- A measure of the uncertainty associated with this estimated value. In clinical biochemistry this may well be the variability or dispersion of a series of similar measurements (for example, a series of quality control specimens) expressed as a standard uncertainty (standard deviation) or combined standard uncertainty.

By definition, the term error (or measurement error) is the difference between the true value and the measured value. The most likely or 'true' value may thus be considered as the measured value including a statement of uncertainty which characterises the dispersion of possible measured values. As the measured value and its uncertainty component are at best only estimates, it follows that the true value is indeterminate. Uncertainty is caused by the interplay of errors which create dispersion around the estimated value of the measurand; the smaller the dispersion, the smaller the uncertainty.

Even if the terms error and uncertainty are used somewhat interchangeably in everyday descriptions, they actually have different meanings. They should not be used as synonyms. The ± (plus or minus) symbol that often follows the reported value of a measurand and the numerical quantity that follows this symbol, indicate the uncertainty associated with the particular measurand and not the error.

If repeated measurements are made of the same quantity, statistical procedures can be

used to determine the uncertainties in the measurement process. This type of statistical analysis provides uncertainties which are determined from the data themselves without requiring further estimates. The important variables in such analyses are the mean, the standard deviation and the standard uncertainty of the mean (also referred to as the standard deviation of the mean or the standard error of the mean).

Measuring Uncertainty Methodology

Measurement of uncertainty in the clinical pathology laboratory can be determined by 'Type A evaluation '(of uncertainty): any method for evaluating uncertainty using statistical analysis of a series of observations. Using Internal Quality Control material is a common practice when certain assumptions are made.

- The Internal Control Material represents a similar sample matrix to clinical samples
- Analyte concentrations are representative of levels found routinely in clinical samples
- Both controls and clinical samples share a common analysis pathway and are treated in an identical manner
- The method of analysis is stable and remains consistently under control.
- Current guidelines suggest at least 6 months data is recommended when calculating uncertainty

For clinical pathology laboratories to measure uncertainty, certain basic statistical analysis of Internal Control material must first be completed. To begin with Intra assay precision within a run must be determined. This is normally calculated by running repeated replicates of the same sample at the same time to determine the precision within a run and will identify any random uncertainties. Inter assay precision calculation on the same material refers to precision over a number of different runs, it is normally measured by running replicates of the sample over several days e.g. one replicate every day for 30 days. This process will identify any systematic uncertainties. To measure uncertainty (u) the clinical pathology laboratory must first calculate the standard error of mean (SEM) of the intra assay precision (A) and the SD of the inter assay precision (B).

Once calculated, both A and B now need to be squared, add together and then a final calculation of the square root (see below).

$$u = \sqrt{(A^2 + B^2)}$$

Expanded Uncertainty

Once clinical pathology laboratories have determined the uncertainty they may then

want to re-scale the result. The standard uncertainty may be thought of as equivalent to 'one standard deviation', but we may wish to have an overall uncertainty stated at another level of confidence, e.g. 95 percent. This re-scaling can be done using a *coverage factor, k*. Multiplying the *standard uncertainty, u* , by a *coverage factor* gives a result which is called the *expanded uncertainty*, usually shown by the symbol *U*.

A particular value of coverage factor gives a particular confidence level for the expanded uncertainty. Most commonly, we scale the overall uncertainty by using the coverage factor k = 2, to give a level of confidence of approximately 95 percent. (k = 2 is correct if the combined standard uncertainty is normally distributed).

Some other coverage factors (for a normal distribution) are:
k = 1 for a confidence level of approximately 68 percent
k = 2.58 for a confidence level of 99 percent
k = 3 for a confidence level of 99.7 percent

U = k.u
k = coverage factor, usually 2

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

Factors Affecting Uncertainty

When calculating uncertainty for laboratory assays it is important that we consider bias. Bias must be measured and, if it is significant, removed or minimised when calculating uncertainty. If we do not remove it the uncertainty of the bias, correction must be calculated and included in the overall uncertainty measurement. To calculate this we must first determine the uRef, uncertainty of the analyte value assigned to the reference material / EQA, and uRep, uncertainty of the analyte value in the reference material / EQA when measured in replicate in the Clinical Laboratory. The uncertainty bias is then calculated using the following formula: **uBias = √(Ref2 + uRep2)**

Clinical Laboratories can investigate the bias of assays by measuring them against the following:

- An assayed QC material
- Unassayed QC material alongside a peer group reporting programme
- External Quality Assessment or Proficiency Testing scheme

- Calibration or reference materials

Sources of Uncertainty

Sources of uncertainty can be due to analytical error, according to J. Hammerling (2012) there are three phases during the analytical process when error can occur; pre-analytical, analytical and post- analytical.

Pre-analytical Errors

Results can be affected before the patient sample reaches the laboratory. Sample collection, storage and transportation, as well as the patient's state can all affect testing. Examples of pre-analytical error include; incorrect tests ordered, samples labelled incorrectly, in proper sample collection and incorrect sample storage. According to Hammerling (2012) this is the stage at which most errors occur.

Post-analytical Errors

This is the final stage of the analytical process. When Clinical laboratories release results to the clinician the interpretation of the results provided will affect how they move forward with patient care, therefore your report format and LIS/Middleware should be considered. In order to detect and minimise these sources of error in the analytical process there should be procedures in place to govern every stage.

Analytical Errors

"The analytical phase begins when the patient specimen is prepared, and ends when the test result is interpreted and verified by the technologist in the laboratory" (Hammerling, 2012:43). Whilst the pre-analytical stage is completely out of the Clinical Laboratory hands, any errors that occur at this stage will occur in the Clinical laboratory. This can be due to how the reagents are stored and prepared, the performance of your instruments, operator performance and calibration of the instruments.

Because QC already manages all these areas of uncertainty in the Clinical laboratory's analytical processes, we can use it to calculate the measurement uncertainty

Additional Factors

Note: When calculating combined uncertainties for analytes that are calculated using addition or subtraction, e.g. Anion gap, the SD or 'u' value can be used. On the other hand when calculating combined uncertainties for analytes that are calculated using division and multiplication, e.g. creatinine clearance, the SD or 'u' must first be converted to CV.

Introducing IAMQC® PEER MU Report

Technopath's **IAMQC PEER software** will generate a Measurement of Uncertainty report in MS-Excel format from your online IAMQC PEER account.

To calculate the MU for your instruments the report uses both the Intra-assay and Inter-assay precision.

1. Intra assay precision refers to precision within a single run; it is normally measured by running 20 or more replicates of the same sample at the same time and calculating the Standard Error of the Mean (SEM). The SEM is calculated using the formula, SEM =((SD of your run) divided by (square root of the number of replicated)).
2. Inter assay precision refers to precision over multiple runs. IAMQC Peer uses your labs running SD to calculate the Inter Assay precision of your analytes.

For further detail on using IAMQC PEER software to generate the MU report, please review our **IAMQC MU report guide**.

Support Services and Training

Technopath Clinical Diagnostics provides a full suite of Quality Control training services supported by our training materials.

- Our support team will guide you through the process of establishing your Measurement of Uncertainty programme.
- Whether you are just getting started or seeking to advance to six sigma verification within your laboratory, we have the solution to support your needs.

- To learn more about Technopath Clinical Diagnostics and our customer value, please visit the **"Why Technopath"** section of our website.
- For more information visit our **Knowledge Centre** where you can access our technical libraries for our QC materials and IAMQC Data Management software tutorials and detailed user guides.
- To contact our QC specialists and for more information on how to get started please email **QCSupport@technopathcd.com**.

Conclusion

Uncertainty of a measurement refers to the doubt, which exists for the result of any measurement within the laboratory. There are a number of factors which must be considered when calculating uncertainty, including the chosen method, Bias, analytical errors and so on. If uncertainty is quantified it is no longer uncertainty but the confidence interval within which the results fall. Uncertainty should be assessed regularly and attempts made to improve the value.

References

Uncertainty of Measurement: A review of the Rules for Calculating Uncertainty Components through Functional Relationships.Clin Chem Rev2012 May:33(2) 49-75

A Beginners guide to Uncertainty of Measurement Issue 2 NPL March 2001