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Medical laboratories — Practical guidance for the estimation of measurement uncertainty

Laboratoires médicaux — Lignes directrices pratiques pour l'estimation de l'incertitude de mesure

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Contents

Page

Foreword ..... **iv**
Introduction ..... **v**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Abbreviated terms and symbols **13**
5 Measurement uncertainty for medical laboratories **13**
5.1 Measurement uncertainty concept 13
5.2 Maximum allowable measurement uncertainty 16
5.3 Sources of measurement uncertainty 17
5.4 Expression of measurement uncertainty 18
5.5 Use of relative standard uncertainty for calculating uncertainty estimates 21
5.6 Reporting measurement uncertainty 22
6 Steps for estimating uncertainty of measurand values **22**
6.1 Measurand definition 22
6.2 Measurement precision 24
6.3 Effect of reagent and internal quality control lot changes on estimating uncertainty 24
6.4 Laboratories using multiple measuring systems for the same measurand 25
6.5 Uncertainty of end-user calibrator values (u_{cal}) 25
6.6 Measurement bias 26
6.7 Process overview for estimation of measurement uncertainty 26
6.8 Re-estimation of measurement uncertainty 27
6.9 Qualitative results based on numerical results 27
6.10 Uncertainty of counting entities 28
6.11 Limitations of measurement uncertainty estimates 28
Annex A (informative) **Worked examples of estimating measurement uncertainty** **29**
Annex B (informative) **Example of applying measurement uncertainty to result interpretation** **58**
Annex C (informative) **Supplementary information on some aspects of uncertainty** **60**
Bibliography **71**

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 212, *Clinical laboratory testing and in vitro diagnostic test systems*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Improved standardization and harmonization of medical laboratory practices worldwide benefits society as patients and healthcare professionals increasingly move within and between healthcare services in the global economy. To help achieve the objective of improved standardization among medical laboratories, ISO 15189 focuses on the application of the quality systems approach in the medical laboratory. Since the first version of ISO 15189 was published in 2003, this international standard has been increasingly adopted worldwide as a desirable (and in some cases mandatory) quality system standard for medical laboratories.

To ensure that measurement results are useful and safe in medical practice and to permit meaningful comparison with medical decision limits and previous results of the same kind in the same individual, medical laboratories require estimates for the overall variability in values reported by their measurement procedures. To achieve this, ISO 15189:2012, 5.5.1.4, requires that "…(medical laboratories)… shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples." Additionally, "Upon request, the laboratory shall make its estimates of measurement uncertainty available to laboratory users."

For medical laboratories and healthcare providers, measurement uncertainty (MU) estimates:

— indicate that multiple values are possible for a given measurement;

— provide evidence that the term 'true value' of a quantity is a theoretical concept;

— quantify the quality of a result relative to its suitability for use in making medical decisions;

— assume that known medically significant bias is eliminated;

— assist in identifying technical steps to reduce MU;

— allow combination with other sources of uncertainty;

— can be used to determine if medically allowable analytical performance specifications can be achieved;

— support interpretation of patient results close to medical decision limits.

To enable fulfilment of the requirement of ISO 15189 for estimation of MU, it is essential that medical laboratories be provided with a coherent, standardized, and best practice approach to the terminology, principles and statistical methods required for estimation of MU. *Evaluation of measurement data — Guide to the expression of uncertainty in measurement* (GUM) JCGM 100:2008, a definitive reference on the topic of MU, provides in-depth information regarding the mathematical and metrological considerations appropriate for a detailed estimation of elements to be considered in the estimation of MU for a broad range of measuring systems, across many disciplines in science and engineering. In the Scope, GUM subclause 1.2, states that "This document is primarily concerned with the expression of uncertainty in the measurement of a well-defined physical quantity that can be defined by an essentially unique value." GUM, Scope subclause 1.4, goes on to say that "…(GUM) provides general rules for evaluating and expressing uncertainty in measurement rather than detailed, technology-specific instructions. (GUM) … does not discuss how the uncertainty of a particular measurement result, once evaluated, may be used for different purposes, for example, to draw conclusions about the compatibility of that result with other similar results, to establish tolerance limits in a manufacturing process, or to decide if a certain course of action may be safely undertaken. Therefore, it may be necessary to develop particular standards based on (GUM) that deal with the problems peculiar to specific fields of measurement or with the various uses of quantitative expressions of uncertainty. These standards may be simplified versions of (GUM) but should include the detail that is appropriate to the level of accuracy and complexity of the measurements and uses addressed."

This document is therefore concerned with practical approaches to estimation of MU, to be applied in medical laboratory settings for the purpose of estimating MU of values produced by measurement procedures intended to measure a broad range of biological measurands. The measurands of interest

are subject to measurement for the purpose of providing medical diagnostic information to health care practitioners and are typically present in complex biological fluid and tissue matrices. In contemporary medical laboratory settings, the vast majority of these measurements are performed with commercial devices, including automated instrumentation and packaged reagent kits. Characterization of the performance of these measurement procedures in an end-user laboratory environment is typically limited to the gathering of empirical performance data using surrogate quality control samples designed to emulate the intended patient samples. Such data, commonly known as internal quality control (IQC) data, may be appropriate for characterization of repeatability and long-term imprecision of a given measurement procedure. Additional uncertainty information regarding higher order elements of the calibration hierarchy for a given measurement procedure should be available from the manufacturer, and should be accounted for in the medical laboratory's process for estimation of MU. As such, a GUM top down approach is appropriate, and a particular application for use in medical laboratories is outlined in Clause 6.

Medical laboratories — Practical guidance for the estimation of measurement uncertainty

1 Scope

This document provides practical guidance for the estimation and expression of the measurement uncertainty (MU) of quantitative measurand values produced by medical laboratories. Quantitative measurand values produced near the medical decision threshold by point-of-care testing systems are also included in this scope. This document also applies to the estimation of MU for results produced by qualitative (nominal) methods which include a measurement step. It is not recommended that estimates of MU be routinely reported with patient test results, but should be available on request.

NOTE See Annex B for an example of application of the MU.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
analyte
component represented in the name of a measurand

Note 1 to entry: Constituent of a sample with a measurable property.

EXAMPLE In the measurand (measured quantity) "mass of total protein in 24-hour urine", "total protein" is the analyte (and "mass" is the property.) In "amount of substance concentration of glucose in plasma", "glucose" is the analyte (and "amount of substance concentration" is the property.)

[SOURCE: ISO 18113-1:2009, modified]

Note 2 to entry: JCGM 200:2012, 5.4, states that a primary measurement standard may be "…prepared by dissolving a known amount of substance of a chemical component to a known volume of solution".

3.2
calibration
operation that, under specified conditions, in a first step, establishes a relation between the quantity values with associated measurement uncertainties provided by measurement standards (calibrators) and their corresponding indications and, in a second step, uses this relationship to establish a measurement result from an indication (for an unknown sample).

Note 1 to entry: A calibration may be expressed by a statement, calibration function, calibration diagram, calibration curve, or calibration table. In some cases, it may consist of an additive or multiplicative correction of the indication with associated MU.

Note 2 to entry: Calibration should not be confused with adjustment of a measuring system, often mistakenly called "self-calibration", nor with verification of calibration.

Note 3 to entry: Often, the first step alone in the above definition is perceived as being calibration.

[SOURCE: JCGM 200:2012, 2.39, modified]

3.3
calibrator
measurement standard used in calibration

[SOURCE: JCGM 200:2012, 5.12]

Note 1 to entry: In this document, calibrator is synonymous with calibration material.

Note 2 to entry: A calibrator is a measurement standard used in the calibration of a measuring system according to a specified measurement procedure.

3.4
commutability of a reference material
property of a reference material, demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two measurement procedures, and the relation obtained among the measurement results for other specified materials

Note 1 to entry: The reference material in question is usually a calibrator and the other specified materials are usually routine samples.

[SOURCE: JCGM 200:2012, 5.15]

Note 2 to entry: It is typical that there are more than two measurement procedures available and comparison among all applicable measurement procedures is desirable.

Note 3 to entry: The requirement for the closeness of agreement shall be appropriate for the intended use of the reference material.

Note 4 to entry: The commutability statement is restricted to the measurement procedures as specified in a particular comparison.

3.5
component
constituent of a mixture the amount or concentration of which can be varied independently

[SOURCE: International Union of Pure and Applied Chemistry (IUPAC) Compendium of Chemical Terminology (Gold Book) Version 2.3.3 2014-02-24, modified]

Note 1 to entry: See also *analyte* (3.1).

3.6
coverage factor
k
number larger than one by which a standard uncertainty value (u) is multiplied to obtain an *expanded uncertainty*, U (3.9)

Note 1 to entry: A coverage factor is usually symbolized k.

[SOURCE: JCGM 200:2012, 2.38, modified]

3.7
coverage interval
interval containing the set of true quantity values of a measurand with a stated probability, based on the information available

Note 1 to entry: A coverage interval does not need to be centred on the chosen measured quantity value (see JCGM 101:2008).

Note 2 to entry: A coverage interval should not be termed “confidence interval” to avoid confusion with the statistical concept (see GUM:1995, 6.2.2).

Note 3 to entry: A coverage interval can be derived from an expanded MU (see GUM:1995, 2.3.5).

[SOURCE: JCGM 200:2012, 2.36]

Note 4 to entry: The term ‘true’ is considered redundant by GUM. For this document the term ‘value of the measurand’ is used.

3.8
end-user calibrator
end-user in vitro diagnostic medical device (IVD MD) calibrator
reference material used as a measurement standard (calibrator) intended for use with one or more measurement procedures intended to examine a particular measurand in human samples

3.9
expanded measurement uncertainty
U
expanded uncertainty
(multiplication) product of a *u* by a (coverage) factor *k* larger than the number one

[SOURCE: JCGM 200:2012, 2.35, modified]

Note 1 to entry: A measured value $x \pm [k \times u(y)]$, with coverage factor $k = 2$, means that the laboratory believes (≈95 % level of confidence) that the value of the measurand lies in the interval of values defined by the following formula:

$$x \pm \left[k \times u(y)\right]$$

where

X	is the measured value;
K	is the coverage factor (usually 2 for ≈95 % confidence);
$u(y)$	is the standard uncertainty of a measured value, y.

3.10
external quality assessment
EQA
international, national or local program designed to provide regular, external, independent quality assessment of a medical laboratory’s analytical performance, and assist in detecting bias of reported results compared to other laboratories.

Note 1 to entry: Also known as Proficiency Testing (PT)[19-21].

Note 2 to entry: EQA is the term used in this document.

3.11
indication
quantity value provided by a measuring instrument or a measuring system

Note 1 to entry: An indication may be presented in visual or acoustic form or may be transferred to another device. An indication is often given by the position of a pointer on the display for analog outputs, a displayed or printed number for digital outputs, a code pattern for code outputs, or an assigned quantity value for material measures.

[SOURCE: JCGM 200:2012, 4.1]

3.12
intermediate precision condition of measurement
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 an extended period of time, but may include other conditions involving changes

Note 1 to entry: The changes can include new calibrations, calibrators, operators, and measuring systems.

Note 2 to entry: A specification for the conditions should contain the conditions changed and unchanged, to the extent possible.

[SOURCE: JCGM 200:2012, 2.22]

Note 3 to entry: For this document, the term long-term precision (u_{Rw}) is used to mean precision data for a given measurement procedure obtained over an extended period of time that at some point includes the effects of all or most changes in measuring conditions, for example, consumable lot changes, re-calibrations, etc. Such changes should be defined for each measurement procedure [see 3.33 repeatability condition of measurement (JCGM 200:2012, 2.20); see 3.40 uncertainty component under conditions of within-laboratory precision (u_{Rw})].

Note 4 to entry: Changed conditions may include instrument maintenance where appropriate.

Note 5 to entry: u_{Rw} is often the major contributor to the combined standard uncertainty of a measurement result in the medical laboratory.

3.13
internal quality control
IQC
set of procedures and specified materials used by laboratory staff for the repetitive monitoring of analytical performance of measuring systems

3.14
long-term precision
u_{Rw}
see 3.12, 3.40

Note 1 to entry: Both the term 'long-term precision' and the symbol u_{Rw} are used in this document when refering to an uncertainty estimate based on data observed under intermediate precision conditions of measurement.

3.15
maximum allowable measurement uncertainty
target measurement uncertainty
maximum fit for purpose MU for measurement results produced by a given measurement procedure, and specified as an upper limit based on an evaluation of medical requirements

[SOURCE: JCGM 200:2012, 2.34 and 4.26, modified]

Note 1 to entry: JCGM 200:2012, 4.26, defines maximum permissible measurement error. In modern English usage, the difference between the terms 'allowed' and 'permitted' is analogous to the difference between the concepts of tolerance (allowed) and authorization (permitted). Authorization implies a statutory, mandated, or legal requirement. For most measurands in laboratory medicine there are no legal limits of performance, therefore allowable is the preferred adjective in the context of this definition.

Note 2 to entry: The maximum allowable MU is considered to represent fit-for-purpose performance based on use of a measurement result for a medical decision.

3.16
measurand
quantity intended to be measured

Note 1 to entry: Specification of a measurand requires knowledge of the kind of quantity, description of the state of the phenomenon, body, or substance carrying the quantity, including any relevant component, and the chemical entities involved.

Note 2 to entry: In the second edition of the VIM and in IEC 60050-300:2001, the measurand is defined as the "particular quantity subject to measurement".

Note 3 to entry: The measurement, including the measuring system and the conditions under which the measurement is carried out, might change the phenomenon, body, or substance such that the quantity being measured may differ from the measurand as defined. In this case, adequate correction is necessary.

EXAMPLE The length of a steel rod in equilibrium at ambient Celsius temperature of 23 °C will be different from the length at the specified temperature of 20 °C, which is the measurand. In this case, a correction is necessary.

Note 4 to entry: In chemistry, 'analyte', or the name of a substance or compound, are terms sometimes used for 'measurand'. This usage is erroneous because these terms do not refer to quantities.

[SOURCE: JCGM 200:2012, 2.3]

Note 5 to entry: In laboratory medicine, the description of the measurand includes the name of the quantity (e.g. amount of substance concentration), the component/analyte (e.g. β-D-glucose), and the biological system in which it is found (e.g. blood plasma.)

[SOURCE: ISO 18113-1:2009, 3.39]

3.17
measurement
process of experimentally obtaining one or more quantity values that can be reasonably attributed to a quantity

Note 1 to entry: Measurement does not apply to nominal properties.

Note 2 to entry: Measurement implies comparison of quantities or counting of entities.

Note 3 to entry: Measurement presupposes a description of the quantity commensurate with the intended use of a measurement result, a measurement procedure, and a calibrated measuring system operating according to the specified measurement procedure, including the measurement conditions.

[SOURCE: JCGM 200:2012, 2.1]

Note 4 to entry: A measurand is the quantity intended to be measured by a medical laboratory. See 3.17.

3.18
measurement bias
estimate of a systematic measurement error

[SOURCE: JCGM 200:2012, 2.18]

Note 1 to entry: Difference between the accepted value of a commutable reference material and the mean value of replicate measurements produced under repeatability conditions by a medical laboratory measurement procedure.

Note 2 to entry: Difference between the mean value of replicate measurements produced by a reference measurement procedure and the mean value of replicate measurements produced under repeatability conditions by a medical laboratory measurement procedure.

Note 3 to entry: Because of measurement imprecision, values for measurement bias cannot be exactly known.

3.19
measurement error
measured quantity value minus a reference quantity value

[SOURCE: JCGM 200:2012, 2.16]

Note 1 to entry: In general, a measurement has imperfections that give rise to an error in the measurement result. Traditionally, an error is viewed as having two components, namely, a random component and a systematic component.

Note 2 to entry: Error is an idealized concept and errors cannot be known exactly.

[SOURCE: JCGM 100:2008; 3.2.1, Notes 1 and 2]

3.20
measurement method
generic description of a logical organization of operations used in a measurement

Note 1 to entry: Measurement methods may be qualified in various ways such as:

— substitution measurement method;

— differential measurement method;

— null measurement method;

— direct measurement method;

— indirect measurement method.

Note 2 to entry: See IEC 60050-300:2001.

[SOURCE: JCGM 200:2012, 2.5]

3.21
measurement precision
precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as variance, standard deviation (SD), or coefficient of variation (C_V) under the specified conditions of measurement.

Note 2 to entry: The 'specified conditions' can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or long-term conditions of measurement (see ISO 5725-1:1994).

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and long-term measurement imprecision.

[SOURCE: JCGM 200:2012, 2.15]

Note 4 to entry: Imprecision denotes the statistical measure or metric related to the degree of closeness or dispersion, such as SD, CV, range, etc. In this context, a measurement procedure is of good precision when the imprecision is low, and of bad precision when the imprecision is high relative to the precision needed to make medical decisions based on the value of the quantity measured.

3.22
measurement procedure
detailed description of a measurement according to one or more measurement principles and to a given measurement method, based on a measurement model and including any calculation to obtain a measurement result

Note 1 to entry: A measurement procedure is usually documented in sufficient detail to enable a competent operator to perform a measurement.

[SOURCE: Modified – added "competent"]

Note 2 to entry: A measurement procedure can include a statement concerning a target MU.

Note 3 to entry: A measurement procedure is sometimes called a standard operating procedure, abbreviated SOP.

[SOURCE: JCGM 200:2012, 2.6]

Note 4 to entry: Target MU as described in Note 2 of JCGM 200:2012, 2.6, is referred to as maximum allowable measurement uncertainty in this document. See 3.15.

3.23
measurement repeatability
measurement precision under a set of repeatability conditions of measurement

[SOURCE: JCGM:200:2012, 2.21]

Note 1 to entry: See 3.33.

3.24
measurement result
set of quantity values being attributed to a measurand together with any other available relevant information

Note 1 to entry: A measurement result generally contains 'relevant information' about the set of quantity values, such that some may be more representative of the measurand than others. This may be expressed in the form of a probability density function (PDF).

Note 2 to entry: A measurement result is generally expressed as a single measured quantity value and a MU. If the MU is considered to be negligible for some purpose, the measurement result may be expressed as a single measured quantity value. In many fields, this is the common way of expressing a measurement result.

[SOURCE: JCGM 200:2012, 2.9]

3.25
measurement standard
realization of the definition of a given quantity, with stated quantity value and associated MU, used as a reference

EXAMPLE 1 1 kg mass measurement standard with an associated u of 3 µg.

EXAMPLE 2 Set of reference solutions of cortisol in human serum having a certified quantity value with MU for each solution.

EXAMPLE 3 Reference material providing quantity values with measurement uncertainties for the mass concentration of each of ten different proteins.

Note 1 to entry: A "realization of the definition of a given quantity" can be provided by a measuring system, a material measure, or a reference material.

Note 2 to entry: A measurement standard (calibrator) is frequently used as a reference in establishing measured quantity values and associated MU for other quantities of the same kind, thereby establishing metrological traceability through calibration of other measurement standards, measuring instruments, or measuring systems.

Note 3 to entry: The term "realization" is used here in the most general meaning. It denotes three procedures of "realization". The first one consists in the physical realization of the measurement unit from its definition and is realization sensu stricto. The second, termed "reproduction", consists not in realizing the measurement unit from its definition but in setting up a highly reproducible measurement standard based on a physical phenomenon, as it happens, e.g. in case of use of frequency-stabilized lasers to establish a measurement standard for the metre, of the Josephson effect for the volt or of the quantum Hall effect for the ohm. The third procedure consists in adopting a material measure as a measurement standard. It occurs in the case of the measurement standard of 1 kg.

Note 4 to entry: A standard MU associated with a measurement standard is always a component of the combined standard MU (See JCGM 100:2008, 2.3.4) in a measurement result obtained using a procedure calibrated with the measurement standard. Frequently, this component is smaller when compared with other components of the combined standard MU.

Note 5 to entry: Quantity value and MU must be determined at the time when the measurement standard is used.

Note 6 to entry: Several quantities of the same kind or of different kinds may be realized in one device which is commonly also called a measurement standard.

Note 7 to entry: The word “embodiment” is sometimes used in the English language instead of “realization”.

Note 8 to entry: In science and technology, the English word “standard” is used with at least two different meanings: as a specification, technical recommendation, or similar normative document (in French « norme ») and as a measurement standard (in French « étalon »). This vocabulary is concerned solely with the second meaning.

[SOURCE: JCGM 200:2012, 5.1]

3.26
measurement uncertainty
MU
parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used.

Note 1 to entry: MU includes components arising from systematic effects, as in the case of corrections to the assigned quantity values of measurement standards. Sometimes estimated systematic effects are not corrected for, but instead, the associated MU components are incorporated.

Note 2 to entry: The parameter may be, for example, a SD called standard MU (or a specified multiple of it), or the half-width of an interval, having a stated coverage probability.

Note 3 to entry: MU comprises, in general, many components. Some of these may be evaluated by Type A evaluation of MU from the statistical distribution of the quantity values from series of measurements and can be characterized by SD. The other components, which may be evaluated by Type B evaluation of MU, can also be characterized by SD or evaluated from probability density functions based on experience or other information.

Note 4 to entry: In general, for a given set of information, it is understood that the MU is associated with a stated quantity value attributed to the measurand. A modification of this value results in a modification of the associated uncertainty.

[SOURCE: JCGM 200:2012, 2.26]

Note 5 to entry: All measurements have bias and imprecision. For example, replicate measurements of a sample performed under repeatability conditions generally produce different values for the same measurand. Because the different values could all be reasonably attributed to the same amount of measurand, there is uncertainty as to which value should be reported as the value of the measurand.

Note 6 to entry: Based on available data about the analytical performance of a given measurement procedure, an estimation of MU provides an interval of values that is believed to include the actual value of the measurand, with a stated level of confidence.

Note 7 to entry: Available data about the analytical performance of a given measurement procedure typically comprise uncertainty of calibrator assigned values and long-term imprecision of IQC materials.

Note 8 to entry: In medical laboratories, most measurements are performed in singleton, and are taken to be an acceptable estimate of the value of the measurand, while the MU interval indicates other results that are also possible.

3.27
measuring system
set of one or more measuring instruments and often other devices, including any reagent and supply, assembled and adapted to give information used to generate measured quantity values within specified intervals for quantities of specified kinds.

Note 1 to entry: A measuring system may consist of only one measuring instrument.

[SOURCE: JCGM 200:2012, 3.2]

3.28
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 MU

Note 1 to entry: For this definition, a 'reference' can be a definition of a measurement unit through its practical realization, or a measurement procedure including the measurement unit for a non-ordinal quantity, or a measurement standard.

Note 2 to entry: Metrological traceability requires an established calibration hierarchy.

Note 3 to entry: Specification of the reference must include the time at which this reference was used in establishing the calibration hierarchy, along with any other relevant metrological information about the reference, such as when the first calibration in the calibration hierarchy was performed.

Note 4 to entry: For measurements with more than one input quantity* in the measurement model, each of the input quantity values should itself be metrologically traceable and the calibration hierarchy involved may form a branched structure or a network. The effort involved in establishing metrological traceability for each input quantity value should be commensurate with its relative contribution to the measurement result.

Note 5 to entry: JCGM 200:2012, 2.50 defines input quantity in a measurement model as the 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. Example: length of a steel rod at a specified temperature is the measurand, while the ambient temperature, the observed length of the steel rod, and the thermal expansion coefficient of the steel rod are the input quantities in the measurement model.

Note 6 to entry: Metrological traceability of a measurement result does not ensure that the MU is adequate for a given purpose or that there is an absence of mistakes in metrological traceability implementation.

Note 7 to entry: A comparison between two measurement standards may be viewed as a calibration if the comparison is used to check and, if necessary, correct the quantity value and MU attributed to one of the measurement standards.

Note 8 to entry: The International Laboratory Accreditation Cooperation (ILAC) considers the elements for confirming metrological traceability to be an unbroken metrological traceability chain to an international measurement standard or a national measurement standard, a documented MU, a documented measurement procedure, accredited technical competence, metrological traceability to the SI, and calibration intervals (see ILAC P-10:2002).

Note 9 to entry: The abbreviated term "traceability" is sometimes used to mean 'metrological traceability' as well as other concepts, such as 'sample traceability' or 'document traceability' or 'instrument traceability' or 'material traceability', where the history ("trace") of an item is meant. Therefore, the full term of "metrological traceability" is preferred if there is any risk of confusion.

[SOURCE: JCGM 200:2012, 2.41]

3.29
proficiency testing
PT
also known as External Quality Assessment (EQA)

Note 1 to entry: See 3.10.

3.30
property
attribute of a substance, body or phenomenon e.g. color, nucleotide sequence, length, mass, light emission wavelength

3.31
quantity
property of a phenomenon, body, or substance, where the property has a magnitude that can be expressed as a number and a reference

Note 1 to entry: The preferred IUPAC-IFCC format for designations of quantities in laboratory medicine is "*System — Component; kind-of-quantity*".

EXAMPLE 1 "Plasma (Blood) - Sodium ion; amount-of-substance concentration equal to 143 mmol/l in a given person at a given time".

[SOURCE: JCGM 200:2012, 1.1]

EXAMPLE 2 Number concentration of erythrocytes in blood sample (Whole Blood – erythrocytes; number concentration equal to 5×10^6/µl in a given person at a given time).

Note 2 to entry: "Quantity" is not to be confused with "analyte", see 3.1.

Note 3 to entry: Measurement procedures for which the measurement is expressed in a qualitative manner (e.g. "present" or "not present") against a ratio or counting scale with a pre-determined decision threshold, are consistent with this definition of the term quantity.

3.32
relative standard measurement uncertainty
u_{rel}
standard measurement uncertainty (u) divided by the absolute value of the measured quantity value

[SOURCE: JCGM 200:2012, 2.32]

Note 1 to entry: This general calculation is commonly termed a coefficient of variation (C_V).

Note 2 to entry: In this document, relative standard measurement uncertainty (u_{rel}) is used to distinguish it from other uses of CV.

3.33
repeatability condition of measurement
condition of measurement, out of a set of conditions that includes the same measurement procedure, same operator, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time

[SOURCE: JCGM 200:2012, 2.20]

Note 1 to entry: A repeatability study is usually conducted when verifying the analytical performance characteristics of a measurement procedure before introduction to service as it indicates the best precision achievable in the hands of the laboratory. A repeatability study may also be performed if an apparent bias of an in-service measurement procedure based on minimal replication requires further evaluation.

3.34
selectivity of a measuring system
property of a measuring system, used with a specified measurement procedure, whereby it provides measured quantity values for one or more measurands such that the values of each measurand are independent of other measurands or other quantities in the phenomenon, body, or substance being investigated

EXAMPLE 1 Capability of a measuring system to measure the amount-of-substance concentration of creatinine in blood plasma without being influenced by the other components present in the sample.

Note 1 to entry: In chemistry, selectivity of a measuring system is usually obtained for quantities with selected components in concentrations within stated intervals.

Note 2 to entry: Selectivity as used in physics is a concept close to specificity as it is sometimes used in chemistry.

[SOURCE: JCGM 200:2012, 4.13, modified]

EXAMPLE 2 Capability of a measuring system to measure the amount-of-substance concentration of creatinine in blood plasma by a Jaffé procedure without being influenced by the glucose, urate, ketone and protein concentrations.

3.35
standard deviation
SD
<for a series of measurements of the same measurand> quantity characterizing the dispersion of the results

[SOURCE: JCGM 100:2008, B.2.17, modified]

Note 1 to entry: A measure of the variability (dispersion or spread) of any set of numerical values about their arithmetic mean (average), defined as the positive square root of the variance.

Note 2 to entry: Quantitative measure of the variation or dispersion of values, from the same process, about the true but unknown value.

Note 3 to entry: SD, as used in this document, refers to long-term precision (see 3.21).

Note 4 to entry: SDs are used in many different situations to quantify the dispersion of values of different types of data sets. In the context of MU, the SD quantifying the dispersion of quantity values obtained from precision studies under repeatability or long-term precision conditions is termed standard MU (u) to distinguish it from other applications of SD.

Note 5 to entry: SDs cannot be added or subtracted. Such calculations require use of variances (see 3.39).

3.36
standard error
SE
quantitative measure of the variation or dispersion of sample means or sample averages

Note 1 to entry: Sometimes known as SD of the mean.

Note 2 to entry: A value of a measurand for example in a reference material requires calculation of a mean value using a small number of measured values obtained under repeatability conditions. If the repeatability study were to be repeated many times slightly different mean values would be obtained, so that the mean value also has a MU. Instead of performing multiple repeatability studies, the u of the mean value obtained from a single repeatability study can be quantified by calculating [see formula] the SD of the mean value (SD_{mean}):

$$SD_{mean} = SD / \sqrt{n}$$

where

SD_{mean} is calculated by dividing the SD of the n contributing observations in the series (from a repeatability study) by the square root of n.

EXAMPLE To evaluate the bias of a serum creatinine measurement procedure, a reference material for serum creatinine was measured 20 times under repeatability conditions.

Mean value 122,0 µmol/l, SD 0,63 µmol/l; $n = 20$

SD_{mean} $= 0{,}63/\sqrt{20} = u = 0{,}15$ µmol/l, $U = 0{,}30$, $k = 2$

Note 3 to entry: The expanded uncertainty, U, for the creatinine concentration in the reference material (with a mean value of 122,0 µmol/l) as measured by an end-user measurement procedure is: 122,0 ± 0,30 µmol/l (≈95 % level of confidence). It should be noted that the measurand values assigned to higher order reference materials also have stated uncertainties provided with their certificates that must be combined with the u of the mean value obtained as described above to correctly estimate the MU of the end-user measurement procedure.

Note 4 to entry: It can be seen that as n increases, the SD_{mean} decreases, providing a more reliable estimate of the mean value of the measurand in the reference material.

3.37
standard measurement uncertainty
u
standard uncertainty
measurement uncertainty expressed as a standard deviation

[SOURCE: JCGM 200:2012, 2.30]

Note 1 to entry: A value for *u* is non-negative i.e. stated without a sign, for example 0,14 mmol/l. See also 3.40.

3.38
trueness
closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value

[SOURCE: JCGM 200:2012, 2.14]

3.39
variance
square of the standard deviation (SD^2; u^2)

Note 1 to entry: Sufficient measurement results obtained from precision studies are dispersed in a way that generally approximates a Gaussian distribution. Like SD, variance (SD^2; u^2) is a statistical parameter of how far individual values are spread out from the mean value of all the contributing results. While the SD is the average distance from the mean value, the variance is the average of the squared distance to the mean value. A low variance means contributing values are close to the mean and to each other, while a high variance means values are far from the mean and from each other.

Note 2 to entry: The spread of values typically obtained from performing replicate measurements under repeatability or intermediate precision studies using IQC materials can be characterised by variance. Variance is calculated per formula below as the sum of the squares of the difference of each individual value from the mean value, divided by the degrees of freedom (total number of values minus one):

$$SD^2 = \left[\Sigma\left(x-\bar{x}\right)^2\right]/n-1$$

Note 3 to entry: The measurement unit of variance is impractical for laboratory use because its measurement unit is the square of the measurement unit applicable to the data (see 3.35). For laboratory calculation purposes, variance must first be converted either to a SD or to a standard uncertainty (u), calculated by the following formula:

$$SD = \sqrt{SD^2} \text{ or } u = \sqrt{u^2}$$

so that the dispersion of measurand values can be expressed in the same measurement unit.

Note 4 to entry: SD or u values cannot be added or subtracted. Such calculations using independent standard uncertainties (u) or relative standard uncertainties (u_{rel}) require the values to first be converted to their respective variances (SD^2; CV^2), and then summed per Formula (1):

$$\text{estimated total variance} = \left(u^2{}_1 + u^2{}_2 + \ldots u^2{}_n\right) \quad (1)$$

Once the total variance is calculated per Formula (1), the estimated total (combined) standard uncertainty is calculated per Formula (2):

$$\text{estimated total standard uncertainty}, u(y) = \sqrt{\ } \text{total variance [from Formula (1)]} \quad (2)$$

Similarly, the total relative variance may be calculated per Formula (3):

$$\text{total relative variance} = \left(u_1 / \text{value}_1\right)^2 + \left(u_2 / \text{value}_2\right)^2 + \ldots\left(u_n / \text{value}_n\right)^2 \quad (3)$$

and total relative standard uncertainty (u_{rel}) is calculated per Formula (4):

$$u_{rel} = \sqrt{\text{total relative variance. [from Formula (3)]}} \quad (4)$$

For worked examples see A.2.4.

3.40
uncertainty component under conditions of within-laboratory precision
u_{Rw}
estimate of standard uncertainty for a given measuring system in the same laboratory over an extended time period that includes routine changes to measuring conditions, for example, lot changes of reagents, calibrators, instrument maintenance

Note 1 to entry: Also termed long-term precision (which is used in this document; see 3.12, 3.21, 3.23)

4 Abbreviated terms and symbols

k	coverage factor applied to u to obtain an expanded confidence interval U
SD_{mean}	standard deviation of the mean value of a measurand obtained from a repeatability study. Taken to be the uncertainty of measured value y of measurand Y
u	measurement uncertainty expressed as a standard deviation
u_{bias}	u of a bias value
u_{cal}	u of the value assigned to an end-use calibrator
u_{ref}	u of the value assigned to a reference material
u_{Rw}	u for long-term imprecision of measured values obtained under defined conditions in same laboratory for a period sufficient to include all routine changes to measuring conditions, e.g., different lots of reagents, operators, and environmental conditions.
$u_{rel}(y)$	relative u of a measured value y [$u(y)/y$]; also expressed as a percentage % $u_{rel}(y)$. Used in this guide to distinguish from other uses of CV
$U_{rel}(y)$	relative expanded uncertainty of measured value y of measurand Y
% $U_{rel}(y)$	percent relative expanded uncertainty of the measured value y of measurand Y
$u_r(y)$	u of mean value of replicate measurements of Y performed under repeatability(r) conditions
$U(y)$	expanded uncertainty of the measured value y of measurand Y
$u(y)$	standard uncertainty of a measured value, y, of measurand Y
$\bar{x}$	mean value

5 Measurement uncertainty for medical laboratories

5.1 Measurement uncertainty concept

All measurement results include some error. Because of measurement imprecision, the magnitude of error in an individual result cannot be exactly known. The measurement uncertainty (MU) concept

recognises that a single measurement result is the best available value for a measurand and that other values are possible if the measurement were to be repeated on the same sample.

Based on the known analytical performance of the measurement procedure used, a measurement result of x units can be stated as having an uncertainty of u units.

This can be expressed as an interval of possible values for a measurand (see 5.4), with the interval defined as $x \pm u$ units. The value of the measurand is assumed by the laboratory to lie within the interval $x - u$ to $x + u$ units, with a stated level of confidence.

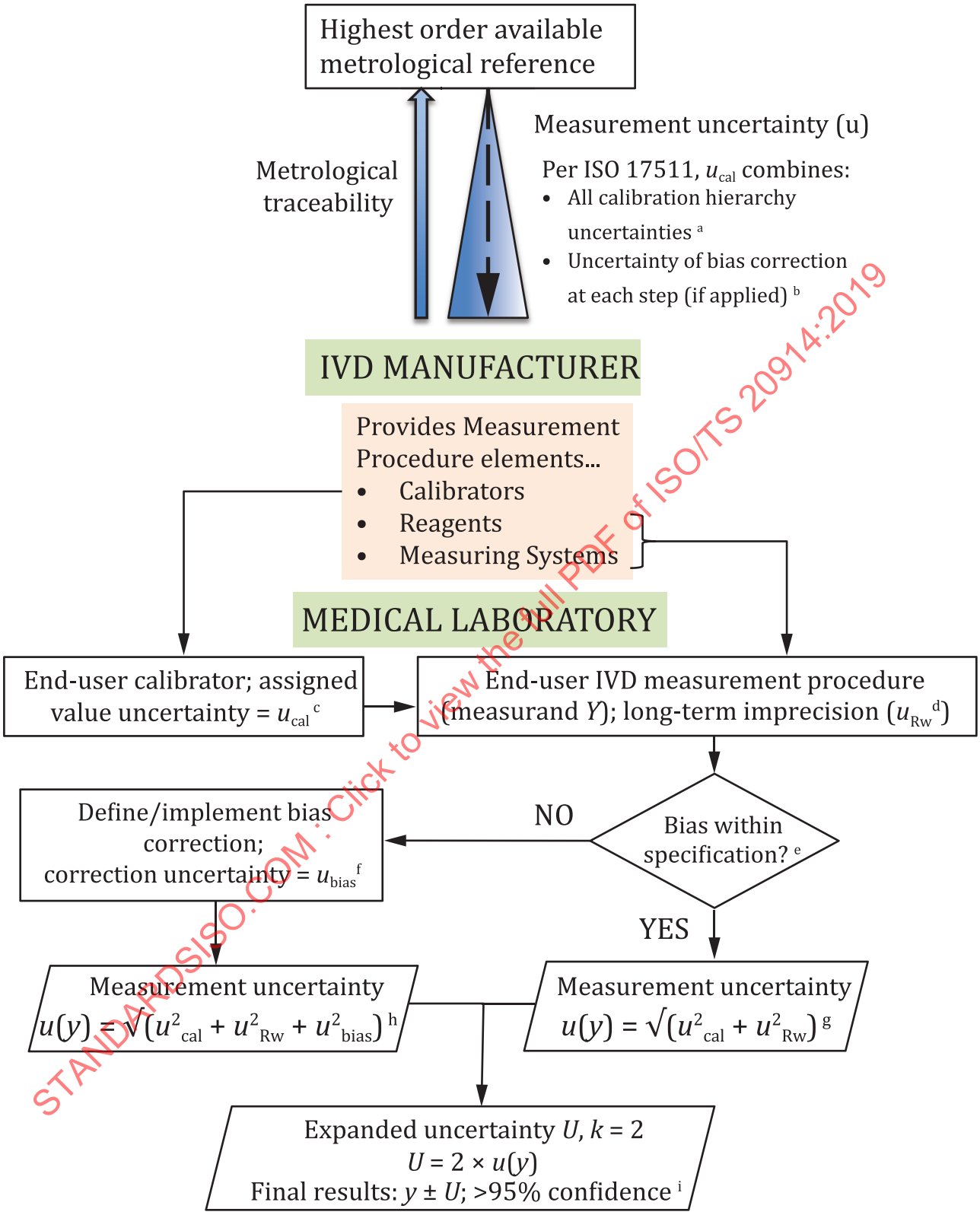
Medical laboratory measurement procedures are well-suited to utilizing internal quality control (IQC) and other available data to estimate MU without the need for measurement models and complex statistics. The following sections describe a practical method for medical laboratories to estimate u for their measurement procedures that utilizes available IQC data in addition to uncertainty associated with the assigned value of calibrators and the uncertainty of any bias corrections introduced.

Figure 1 is a flow chart that illustrates the overall process to be followed by a medical laboratory in estimation of MU, and identifies the inter-relationships among major sources of MU that should be accounted for by the laboratory when estimating MU for a given measuring system.

MU only considers uncertainties arising from sources within the technical bounds of a measuring system and assumes that:

— uncertainties due to pre- and post-analytical steps are minimised by standardising these processes;

— measured human samples are typical and do not have unusual sample-specific factors (e.g. interferences) affecting measurement procedures.



a *Calibration hierarchy uncertainties*: Depending on the measurand, there can be multiple value assignment steps and associated uncertainties from the end-user calibrator back to the highest available reference. See ISO 17511.

b *Uncertainty of calibrator bias correction*: Note that an end-user calibrator manufacturing process already includes correction of any medically significant measurement bias relative to the highest order reference used, and therefore estimation of a bias by the end-user laboratory and subsequent estimation of a u_{bias} is rarely required. If further bias correction is required by the end-user medical laboratory, then the uncertainty of the bias correction, $u_{bias,}$ needs to be combined with the manufacturer's estimate of u_{cal} prior to calculating $u(y)$. See 6.6, C.6 and NOTES 5, 6 and 8.

c *Uncertainty of the value assigned to an end-user calibrator*: Calibrator manufacturers need to provide their estimates of u_{cal} to end-users on request.

d *Calculation of long-term imprecision, u_{Rw}*: Short-term estimates of the imprecision of a measuring system can underestimate u because required information can be lacking. Long-term imprecision estimates from IQC data collected across important changes to measuring conditions is usually more complete. The time required to collect sufficient data is mainly governed by measuring system specific factors such as measurement frequency, calibration frequency, frequency of reagent and calibrator lot changes, shelf-life of consumables, variable environmental conditions, among others., equipment maintenance procedures. See 5.3.

e *Measurement bias verification*: The laboratory needs to ensure that the magnitude of any measurement bias does not exceed the acceptable specification for medical use. External quality assessment (EQA) performance is often used for this purpose, but caution needs to be exercised. In addition to accounting for the commutability of the EQA material, the EQA target value can itself be biased, depending on how the value is assigned to the material. Where there are doubts concerning validity of a bias observed for an EQA material, the laboratory needs to consider assessing bias with human samples.

f *Bias correction*: Appearance of a medically unacceptable measurement bias can be detected by EQA surveillance providing that the EQA schemes fulfil some mandatory requirements, as described[20] [21]. If unresolved by the manufacturer, the laboratory can introduce a correction factor. If so, the uncertainty of the correction factor, u_{bias}, needs to be estimated and included in the calculation of $u(y)$. Use of bias correction factors are not permitted by some national regulations.

g *Calculation of MU of a final result, u(y); bias within specification:* This calculation takes into account the uncertainty of the value assigned to the calibrator, $u_{cal,}$ and long-term precision, u_{Rw}.

h *Calculation of MU of a final result, u(y); bias exceeds specification:* This calculation takes into account the uncertainty of the value assigned to the calibrator, $u_{cal,}$ long-term precision, u_{Rw}, and the uncertainty of any bias corrections, u_{bias}, introduced by the end-user medical laboratory.

i *Human sample results*: It is usual practice to expand u by multiplying it by a coverage factor (k) of 2 to provide an expanded uncertainty, U, with a level of confidence of approximately 95 % when associated with human sample results. Uncertainties are not required to be routinely reported to medical practitioners with results, but should be available on request. See 5.4.6.

Figure 1 — Process for estimation of measurement uncertainty for a typical in vitro diagnostics measuring system

5.2 Maximum allowable measurement uncertainty

The magnitude of MU should be suitable for a result to be used in a medical decision and ideally as small as technically possible. For a given measuring system, estimating the expanded uncertainty of the results produced is of very limited value unless it can be compared with an upper limit of allowable expanded uncertainty based on the quality of results required for medical use.

Such limits should be based on models defined by the 2014 European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) consensus conference[22] including clinical outcome studies, a selected proportion of biological variation, or, when information derived from the first two models are lacking, state-of-the-art of the measurement performance. The chosen approach is guided by factors such as the biological behaviour of the measurand, medical applications of results, and analytical performance characteristics of available measurement procedures. If a measurement procedure exceeds the upper allowable uncertainty limit, it may be possible to reduce the uncertainty by identifying and modifying a technical step that makes a relatively large contribution to the total uncertainty of results. If the uncertainty cannot be sufficiently reduced, a decision must be made as to whether the measurement procedure is to be changed or if the quality goal should be reconsidered.

The value selected by a laboratory for the maximum allowable MU and its justification forms part of the MU record for the measurement procedure.

5.3 Sources of measurement uncertainty

For each measurement procedure, it is important to identify the technical point from which uncertainty is to be estimated. Generally, this will be the point at which IQC materials enter the measurement process. If a procedure requires a pre-measurement sample preparation step and IQC materials also undergo this step, the laboratory must demonstrate that human samples and IQC material behave similarly. If IQC material does not undergo such a step, or is subjected to the pre-measurement sample preparation step, but behaves differently from human samples, the laboratory should design a study to estimate a typical uncertainty associated with the sample preparation process and combine it with the uncertainty of the measurement step.

Sources of uncertainty may arise from interfering substances that modify the interaction of the analyte with the measuring system and/or the signal generated by the measurement process. Examples include patient antibodies to an analyte or reagent, spectrophotometric interference by free haemoglobin, or cross-reactivity of structurally related molecules. These pre-measurement sources of uncertainty are generally individual sample-specific and not included in the estimation of MU for typical human samples.

Common sources of MU are:

— sample inhomogeneity;

— reconstitution procedures for lyophilised materials, e.g. calibrator and reagents;

— uncertainty of calibrator values, re-calibrations;

— instrumentation, e.g. electro-mechanical fluctuations, maintenance, parts replacement;

— reagent and calibrator instability;

— reagent and calibrator lot-to-lot variability;

— fluctuations in laboratory environment;

— operator bias introduced by reading analogue instrument indications;

— variable manual dexterity for manual and semi-automated methods;

— measurement bias vs. an accepted calibration hierarchy scheme;

— measurement formulae, e.g. approximations, assumptions, inexact values for constants, rounding of digits;

— more than one of the same measuring system for the same measurand;

— more than one measurement procedure for the same measurand that may have different analytical performance characteristics.

For most measuring systems in medical laboratories, the most significant uncertainty contributions to the overall MU are captured by:

— long-term imprecision data obtained for IQC materials for a period sufficient to include all changes to measuring conditions (u_{Rw});

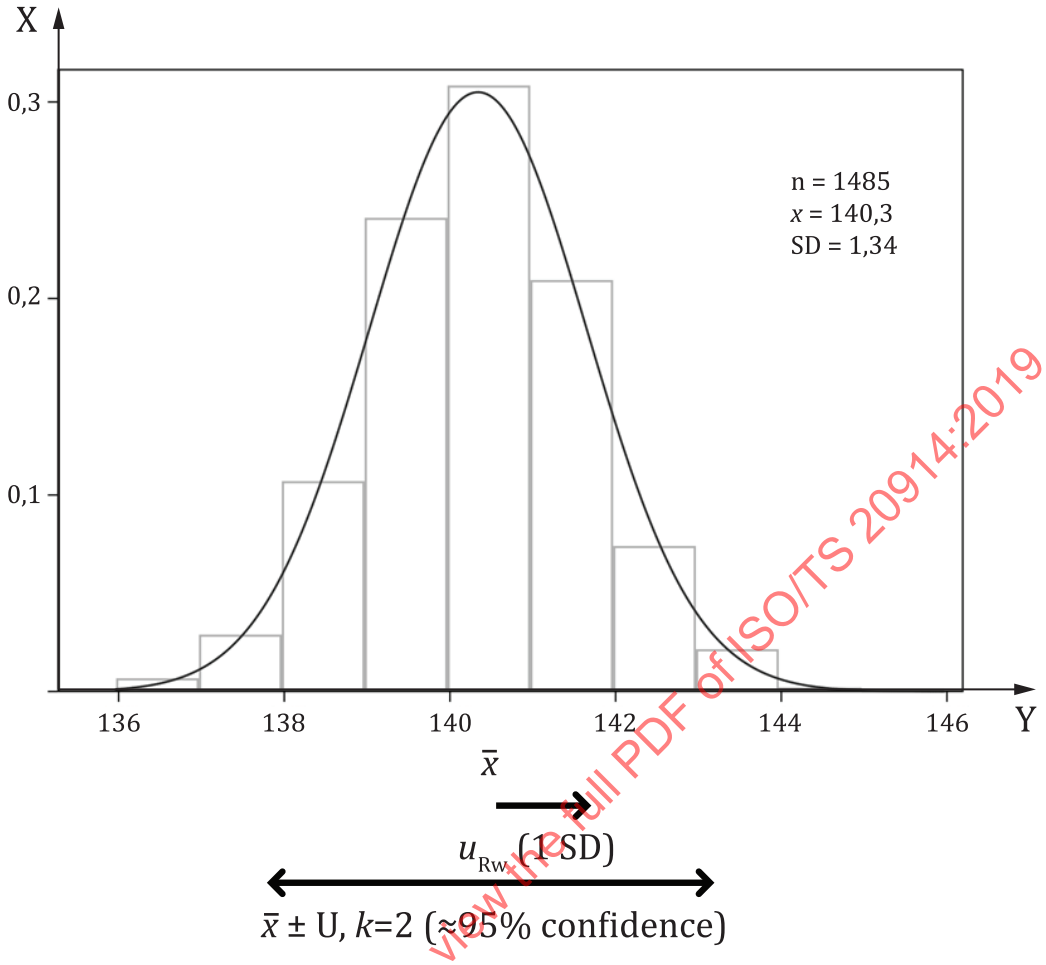
— uncertainty of end-user calibrator values (u_{cal}) – obtainable from the manufacturer or established by a laboratory that develops its own measuring system.

Occasionally a medically significant measurement bias may be corrected (if permitted by national regulations), in which case the uncertainty of the correction factor applied (u_{bias}) must be considered. Guidance in accounting for uncertainty associated with bias correction is provided in 6.6 and C.5 and C.6.

This document is not intended to describe guidance on performing an exhaustive accounting of all possible uncertainties, but is focused on the major foreseeable sources of MU for measuring systems used in medical laboratories, to provide practical estimates of MU that help ensure that patient results are fit for medical use. A record of uncertainties applicable to results produced by each applicable measuring system should be maintained by the medical laboratory, including data sources, assumptions and statistical processes used in calculations, maximum allowable MU and general action to be taken if the maximum allowable MU is exceeded (see 5.2).

5.4 Expression of measurement uncertainty

Shown in Figure 2 is a histogram of 1485 successive reported values for serum sodium ion concentration measured in an internal quality control (IQC) material, tested within a single laboratory using a single measuring system for a time period sufficient to include all anticipated routine changes in measuring conditions. The calculated mean of the reported values ($\bar{x}$ = 140,3 mmol/l) is considered to be the best available estimate of the amount of the measurand (sodium ion amount of substance concentration) in this IQC material. All of the individual IQC values reported represent possible values for the measurand in this material, but with lesser probability than the calculated $\bar{x}$.



Key

X fraction

Y sodium [mmol/l]

Figure 2 — Frequency of reported values for a measurand (serum sodium ion concentration, mmol/l) in an internal quality control (IQC) material

When repeatability or long-term precision data for a well-controlled measurement procedure are plotted in a histogram as shown in Figure 2, the dispersion of values is approximately Gaussian (normal). Assuming Gaussian behaviour of the data, the magnitude of the dispersion of values around the mean value ($\bar{x}$) can be quantified by calculation of the standard deviation (SD), in this case ±1,34 mmol/l. An SD obtained from such long-term precision data is termed a standard uncertainty (u), to distinguish it from other uses of SD. If the data are obtained for a period sufficient to include all routine changes to measuring conditions, the u is designated u_{Rw}, or the standard uncertainty under within-laboratory conditions.

Standard uncertainty, u, is always a positive value and has the same units of measurement as the measurand. When attached to a measurement result it has a sign of ± to define an interval of possible reported values for the measurand with a stated level of confidence. The interval, $\bar{x} \pm u_{Rw}$, defines a range of possible measurand values distributed around the mean value, including approximately 68 % of possible values for the measurand. Standard uncertainty, u, can be multiplied by a coverage factor k, typically 2, to provide an expanded uncertainty U, so that $\bar{x} \pm U$ provides a coverage interval that includes approximately 95 % of possible values for the measurand in this IQC material, with the stated measuring system.

In the same way that SD can be expressed as a percentage relative to the mean value as a percent coefficient of variation (% C_V), it is also of practical use to express standard uncertainty, u, as a percentage relative to the mean value (% u_{rel}), and expanded uncertainty, U, as percentage relative expanded uncertainty (% U_{rel}).

The measurement uncertainty of the measurement procedure determines the number of significant figures in the reported values of the measurand. The measurement uncertainty of a measurement procedure should be consistent with the medical requirements. In all cases, the number of significant digits in the reported values for a given measurement procedure shall not imply a smaller uncertainty than warranted by the uncertainty of the measurement procedure.

When making statistical calculations such as estimation of measurement uncertainty, it is often necessary to round the numbers, since it may not be possible, practicable, or reasonable to record all numbers calculated to the last available digit. To simplify presentation, statistical series are often rounded to make the information easily understood and comparable to other data. In this context, data rounding is acceptable provided that an adequate level of accuracy is retained and a set of rounding rules is established and consistently applied.

When performing a set of sequential calculations, as is typical when estimating standard measurement uncertainty, expanded uncertainty and relative uncertainty, rounding should be performed only on the final calculated estimates; never on intermediate calculated values that comprise inputs to subsequent and final calculations.

To simplify reporting of data, biostatistics experts recommend observing the following conventions with respect to reporting significant digits[23]:

a) When calculating a mean, use only the number of decimal digits in the final reported value plus one additional decimal digit.

   EXAMPLE Reported values (for serum sodium ion concentration) for five replicate determinations on the same sample are given only in whole integer numbers such as 122, 123, 124, 122, 125. The calculated mean value (n = 5) is 123,2 (i.e., the mean is reported with one more significant digit than the individual reported values.)

b) For reporting calculated SD's, use the number of decimal digits in the reported values plus two. For % CV, one decimal digit (for example, 3,1 %) should be sufficient.

There are three accepted approaches applicable to data rounding[24]. These approaches include:

Option A (even/odd method) – avoids bias in mean values over the long term.

EXAMPLE 1 1,35 is rounded to 1,4; 1,25 is rounded to 1,2.

Option B (computer/automated spreadsheet approach, e.g. Microsoft Excel).

The right-most digit to be rounded is not changed if followed by a digit less than 5. If the right-most digit to be rounded is followed by a digit greater than or equal to 5, it is increased by one. This approach is also known as conventional rounding.

EXAMPLE 2 1,25 is rounded as 1,3; 1,35 is rounded to 1,4. 1,23 is rounded to 1,2.

Option C (round-up method) – the most conservative approach; has advantages for evaluating compliance of uncertainties against maximum permissible errors, tolerances, or risk assessments.

EXAMPLE 3 1,23 is rounded to 1,3.

Because many medical laboratories are likely to use automated spreadsheets to perform the types of calculations described in this technical specification, it is recommended for simplicity that laboratories select Option B as their rounding convention when calculating measurement uncertainty. Regardless of which rounding option is selected, each laboratory should document the data rounding method they use to perform calculations of the estimated measurement uncertainties.

EXAMPLE 4

For the IQC frequency data displayed in Figure 2, the measuring system reports values in whole numbers with three significant digits and no decimal places; applicable units are mmol/l. The arithmetic mean [sodium] of 1 485 consecutive reported values is 140,3 mmol/l. Based on these data, u for [sodium] is 1,34 mmol/l. For a coverage factor $k = 2$, the expanded uncertainty, U, is therefore 2 times 1,34 mmol/l, or 2,68 mmol/l. The coverage interval, $\bar{x} \pm U$, is [140,3 − 2,68 mmol/l] to [140,3 + 2,68 mmol/l]; or $\bar{x} \pm U$ = 137,62 to 142,98 mmol/l.

Because the coverage factor, $k = 2$, was applied in estimating the expanded uncertainty, we are 95 % confident that the true mean value lies in the interval [137,62 to 142,98] mmol/l.

Allowing for rounding to one significant digit to the right of the decimal point the 95 % coverage interval for accurate reporting of sodium in human samples should be revised to 137,6 to 143,0 mmol/l. Or, in recognition that reported values for serum sodium are provided by the measuring system with only 3 significant digits and no decimal places, the ≈95 % coverage interval to describe the overall accuracy of a reported serum sodium value with the stated measuring system can be rounded (per Option B rounding rules as specified above) to 138 to 143 mmol/l.

In lieu of reporting the 95 % coverage interval as a range of absolute values, the coverage interval may be reported as the relative percentage expanded uncertainty (% U_{rel}). Using the absolute value of the expanded uncertainty (2,68 mmol/l) based on $k = 2$ and the mean value for the IQC material (140,3 mmol/l), % U_{rel} is calculated as follows:

% U_{rel} = $(U_{Rw}/\bar{x})$ × 100 = [(2,68 mmol/l)/(140,3 mmol/l)] × 100 = 1,91 % (rounded to 1,9 %) or, $\bar{x} \pm$ % $U_{rel} = \bar{x} \pm$ 1,9 %, $k = 2$, ≈95 % confidence.

To avoid misunderstanding, it is recommended that a statement be included in all presentations of data where data rounding might lead to discrepancies between calculations performed with and without rounding[25].

Example (statement): *Data rounding: Figures presented in tables have been rounded, and discrepancies may occur between sums of component items and totals. All percentages have been calculated using unrounded figures.*

5.5 Use of relative standard uncertainty for calculating uncertainty estimates

Uncertainty can be estimated using the absolute SD (u), the relative SD_{rel} (u_{rel}) or the % C_V (% u_{rel}) of the IQC values at a particular concentration. The choice to use SD or % C_V should be made as appropriate to the uncertainty information required as applicable to a particular test result.

Relative uncertainties should be used under two conditions:

a) when the % C_V is approximately constant over a substantial portion of the measuring interval, the estimated MU can be extrapolated to all concentrations in that portion of the measuring interval (see A.8);

b) when the measurand is a calculated result and the components of the calculation are multiplied or divided (see A.2.4, Rule 2, Examples 1 and 2).

Because MU can be estimated at only the concentrations at which IQC values are available, extrapolating the available MU estimates to other concentrations requires an assumption that the uncertainty or relative uncertainty is approximately constant over all or a portion of the measuring interval. To construct a precision profile, it may be necessary to augment routine IQC samples with additional IQC samples at other concentrations to obtain % C_V or SD estimates over the full measuring interval of interest.

With data sufficient to construct the precision profile for a measuring system, the question of whether the % C_V or SD is constant over a concentration interval can be assessed using one of two possible methods. In the first method, measurement procedure comparison data can be plotted using a difference plot as described in CLSI EP09c, 3rd edition. In the second method, data from an imprecision study can be used to create a precision profile as described in CLSI EP05-A3.

5.6 Reporting measurement uncertainty

MU estimates provide a quantitative indication of the reliability of results for human samples based on available information. MU is not routinely reported with patient test results, but MU information should be made available to laboratory users on request. In some medical situations it may be important for the laboratory to communicate the degree of MU associated with a test result.

6 Steps for estimating uncertainty of measurand values

6.1 Measurand definition

Definition of a measurand requires at least three pieces of information:

— system containing the analyte, e.g. venous whole blood, urine, red blood cells, renal stone;

— identity of the analyte, e.g. rubella antibody, digoxin, subunit of human chorionic gonadotrophin, HIV-1 RNA, CCG tri-nucleotide;

— quantity, e.g. amount of substance concentration, number, mass concentration, number concentration, number fraction, amount of substance rate concentration.

An example of a measurand is the number concentration of white blood cells in whole venous blood. For additional examples of definitions of measurands, see Table 1.

Biological analytes can be complex (isoforms, fragments); therefore, the definition of a measurand may additionally depend on the specific measurement procedure used. For example, the catalytic activity concentration of a serum enzyme is affected by changes in the temperature, pH and co-factors used in performing the measurement. In such cases, identification of the measurement procedure must be included in the measurand definition.

EXAMPLE 1 Table 1: serum enzyme - alanine aminotransferase: catalytic activity concentration by IFCC reference measurement procedure.

EXAMPLE 2 Table 1: serum IgG paraprotein: reagent kit manufacturer Y.

Another example is the different epitope selectivity of antibodies used by different commercial measurement procedures to measure the 'same' glycoprotein hormone e.g. different antibodies may recognise different isoforms, or bind them to different extents. The differences in measurand may be captured by providing the particular measurement procedure used, e.g. tumor marker X, measured by the measurement procedure of manufacturer Y.

Although not part of the formal definition of a measurand, it is usual practice to identify the measurement unit.

Table 1 — Example definitions of measurands with key elements

Biological System	Analyte	Quantity	Measurand Name	Unit
Serum	Alanine aminotransferase	Catalytic activity concentration	Catalytic activity concentration of alanine aminotransferase in serum; X Pty.Ltd measurement procedure.	µkat/l
Arterial blood	Calcium ion (unbound)	Amount-of-substance concentration	Amount of calcium ion concentration at pH 7,40 in arterial blood; X Pty. Ltd. Blood gas analyser calcium ion specific electrode	mmol/l
Urine	Calcium	Amount-of-substance	Amount of calcium in 24 h urine collection; X Pty.Ltd total calcium measurement procedure.	mmol/l (conventional unit: mol/24 h)
Liver tissue	Iron	Mass	Mass fraction of iron in dry weight of liver tissue; validated in-house measurement procedure.	µg/g
Serum	IgG paraprotein	Mass concentration	Mass of IgG paraprotein concentration in serum by agarose gel electrophoresis X Pty.Ltd measurement procedure.	g/l
Serum	Specific IgE to avocado	Reactivity concentration	Reactivity concentration of avocado-specific IgE concentration in serum. (XYZ IRP 76/50) X Pty.Ltd measurement procedure.	kU_A/l
Serum	Rubella virus antibody (IgG)	Reactivity of IgG antibodies concentration in serum to rubella virus antigen	Reactivity concentration of IgG antibodies in serum to rubella virus antigen. X Pty Ltd. measurement procedure.	kU/l
Plasma	HIV-1	Number concentration	Number of HIV-1 RNA copies concentration in plasma; X Pty.Ltd measurement procedure.	copies/ml
Random urine	White blood cells	Number concentration	Number concentration of white blood cells in random urine; in-house manual procedure No. 39	cells/µl
Venous blood	CD4 positive cells	Number concentration	Number of CD4 cells concentration in venous blood; in-house flow cytometry – see method manual.	cells/l
Bone marrow	B-lymphocytes (immature)	Number	Number fraction of immature B-lymphocytes in bone marrow; in-house flow cytometry – see method manual.	Dimensionless
Venous blood	Haemoglobin	Mass concentration	Amount of haemoglobin concentration in venous blood; X Pty.Ltd measurement procedure.	g/l
Plasma	Biological activity of clotting factors VII, X, V, II, fibrinogen	International Normalised Ratio (INR)	Tissue factor-induced relative time to form clot in citrated plasma; X Pty.Ltd measurement procedure.	Dimensionless
Maternal whole blood	Foetal red cells	Number	Number fraction of foetal red cells in maternal venous whole blood. Acid dilution test; in-house flow cytometry	Dimensionless

6.2 Measurement precision

Ideally, measurement conditions should be kept constant at all times, but in practice changes are unavoidable (see 5.3). Within-laboratory imprecision during a period sufficient to include most changes to measuring conditions (u_{Rw}) is, in the majority of cases, the largest contributor to the uncertainty of measurement results.

IQC materials should, where possible, be used for the collection of imprecision data. Use of EQA data for calculation of u_{Rw} is not recommended due to the relatively small number of values obtainable in a typical EQA cycle. IQC protocols capture a much larger number and broader range of uncertainty events so that, particularly for large workload testing, fewer uncertainty events are missed. The IQC data must be collected for a sufficiently long-time interval to reflect most of the important sources of variability mentioned in 5.3. In addition, IQC data must be partitioned and treated separately to avoid including variability that only affects IQC results and does not reflect typical variability expected for results from human samples.

It is generally assumed that for a given measurement procedure the magnitude of imprecision for both IQC and typical human samples is similar, so that a standard uncertainty calculated for an IQC material is considered applicable to human samples with similar measurand values. This assumption should be validated by a performing a precision study of representative human samples and relevant IQC material(s) and their variances compared (F-test). If statistically significant differences are not detected, equivalent performance is confirmed. Use of IQC materials for long-term imprecision estimates is encouraged.

The assumption that IQC and typical human samples behave similarly with respect to measurement precision should also be carefully considered where a pre-treatment step is required for human samples but not for IQC materials, for example, haemoglobin A1c assays, where clinical samples require haemolysis, but IQC material can be provided as a haemolysate.

NOTE 1 MU is ideally estimated using IQC materials with mean measurand values close to important medical decision limits.

NOTE 2 Calculation of MU only uses IQC data produced when the measuring system is meeting analytical performance criteria. IQC data generated when a technical error or deviation from the SOP has occurred need to be excluded.

NOTE 3 More than one level of IQC can be used to monitor precision across a measuring interval, in which case the uncertainty for each level needs to be calculated and compared to decide if the uncertainties are essentially the same across the measuring interval, or sufficiently different as to be only applicable to the interval of results monitored by each IQC concentration level. Relative uncertainty (u_{rel}) can be more consistent across concentration intervals than is absolute uncertainty (u).

NOTE 4 Dissolving a lyophilised IQC material introduces a spurious contribution to the estimation of MU. The laboratory should introduce adequate procedures to minimise this uncertainty contribution.

Additional attributes to be considered in selection of suitable IQC materials for estimating u_{Rw} include but are not limited to:

— material provided preferably by a third-party (i.e. different from that used to check the alignment of the measuring system);

— material that closely resembles authentic clinical samples (ideally a commutable material);

— material(s) with an amount of substance (measurand concentration) appropriate to the intended medical application of the analyte[26].

6.3 Effect of reagent and internal quality control lot changes on estimating uncertainty

The assumption that human samples and IQC materials behave similarly in a measuring system with each different lot of reagent is not always valid. Differences are typically due to changes in the matrix-related influence of IQC with different reagent lots that is not observed for human samples[27]. In

addition, different IQC lots can have different amounts of a measurand caused by IQC manufacturing processes.

A lot change of either IQC or reagent that causes a significant shift in IQC absolute values without a matching change in human sample results may produce an over-estimate of MU not applicable to human samples. Such an over-estimation occurs if the IQC values obtained before and after such a lot change are treated as a single data set for calculation of u_{Rw}. Therefore, in this situation, the IQC values obtained before and following such a lot change must be collected separately and the u_{Rw} for each calculated separately, and only if assessed as compatible, can they be combined to obtain an overall u_{Rw} (see A.3). It is therefore essential to demonstrate that both IQC and human sample results behave similarly following a reagent lot change. Further guidance on verifying performance for human sample results following reagent lot changes is available in CLSI documents C24, 4th edition and EP26-A.

MU is typically estimated for:

— Precision under repeatability conditions (u_r). Usually undertaken during verification of IVD manufacturer claims for analytical performance, or validation of performance for a laboratory developed measuring system, and documents the smallest uncertainty achievable by the measuring system when operated by the laboratory (see A.2.3). This uncertainty will be an underestimate of the actual MU expected during daily or regular use of a measuring system.

— Within-laboratory precision for a period sufficient to include most changes to measuring conditions (u_{Rw}), also called long-term precision. This uncertainty will be a suitable estimate of the uncertainty expected during daily or regular use of a measuring system. It may take some time to accumulate sufficient IQC data to capture the uncertainty effects of most changes in measuring conditions. In such situations interim estimates are appropriate, but will require re-calculation as further data become available (see A.2.2).

6.4 Laboratories using multiple measuring systems for the same measurand

A busy laboratory may use more than one of the same measuring system for the same measurand, so that human samples can be analysed on any one of them. It is reasonable to estimate an average u that can be applied to the results irrespective of the measuring system used. For this purpose, u_{Rw} must be estimated separately for each measuring system, and then a pooled average can be calculated (see A.3). Medically significant systematic bias in results for human samples between several measuring systems should be eliminated, and the uncertainty of the mean residual bias should be combined with the imprecision uncertainty (see C.6 and C.5 and C.6).

6.5 Uncertainty of end-user calibrator values (u_{cal})

End-user calibrator values have uncertainty (u_{cal}) because their assignment requires the use of measurement procedures (see C.4). Values for u_{cal} should be obtainable on request from IVD manufacturers. In cases where the laboratory is the manufacturer, the laboratory is responsible for determining u_{cal}.

Where a manufacturer provides a value for u_{cal}, it must be a combination of all uncertainties introduced by the selected calibration hierarchy for the measurand beginning with the highest available reference (calibrator or measuremement procedure) down to the assigned value of the calibrator for the end-user IVD medical device.

A change of the IVD-medical device calibrator lot may systematically change IQC mean values due to lot-to-lot variability of the assigned value of the calibrator, resulting in an increase in the overall MU which should be captured by u_{Rw}. Generally, the values obtained for human samples will similarly change with a change in an IVD-medical device calibrator lot that results in a shift of IQC values. The u_{cal} provided by manufacturers typically does not include the calibrator lot-to-lot variability, consequently, the IQC values obtained before and after several calibrator lot changes have to be treated as a single data set to ensure that the estimate of u_{Rw} includes the calibrator lot-to-lot component of uncertainty. In some cases, a single lot of calibrator may be used for an extended time interval and the initial estimate of u_{Rw} for the measurement procedure is based on available IQC data without a calibrator lot change. In such

cases, the value of u_{Rw} may be underestimated and should be revised after one or more calibrator lot changes have occurred that reflect similarly shifted results for human samples.

NOTE 1 If calibrator lot-to-lot variability has been included in the u_{cal} value provided by the manufacturer, MU can be calculated as in example A.3

NOTE 2 For some calibrators a comprehensive u_{cal} is technically unavailable. For example, some international conventional biological reference materials are characterized by a specified biological activity, and their assigned values are expressed in arbitrary measurement units, e.g. International Units (IU), without a stated uncertainty, which may produce an underestimate of the applicable MU.

6.6 Measurement bias

IVD manufacturers are responsible for ensuring that their end-user measurement procedures exhibit minimal, medically acceptable measurement bias relative to the appropriate reference. Laboratories should regularly monitor measurement bias by participation in an appropriate EQA scheme where available[21].

It is important to consider how results are used medically for a given measurand, as this can help guide how comprehensive the MU estimates need to be. If results are intended to monitor changes in an individual over time, or are interpreted relative to in-house determined reference intervals, then bias correction may be unnecessary for appropriate medical interpretation. If results are interpreted relative to externally determined biological reference or decision limits, then elimination of medically significant measurement bias is important to ensure appropriate medical interpretation.

Any result variability due to small changes in bias that may occur in laboratory service over time will be captured by u_{Rw}. If ongoing EQA surveillance indicates the introduction of a medically significant measurement bias, it is the responsibility of the IVD manufacturer, or the laboratory in the case of laboratory-developed measurement procedures, to take immediate corrective action. If the manufacturer is unable to rectify an unacceptable bias, the laboratory may, if local regulations permit, manage such measurement bias by applying a correction factor to the results or by re-assigning a calibrator value. Bias correction is always imperfect because estimates of the magnitude of bias have uncertainty (u_{bias}). When the laboratory implements a correction for a medically significant measurement bias, the laboratory should estimate and account for u_{bias} in the estimate of $u(y)$. This circumstance is discussed further in C.5 and C.6.

NOTE Some national regulations require measurement procedures to be performed exactly according to manufacturer's instructions and therefore may prohibit the adjustment of end-user calibrator values or the use of correction factors for the purpose of eliminating medically significant measurement bias.

6.7 Process overview for estimation of measurement uncertainty

Combining the concepts introduced in 6.1 to 6.6, provides a stepwise procedure for estimating MU as shown in Figure 3.

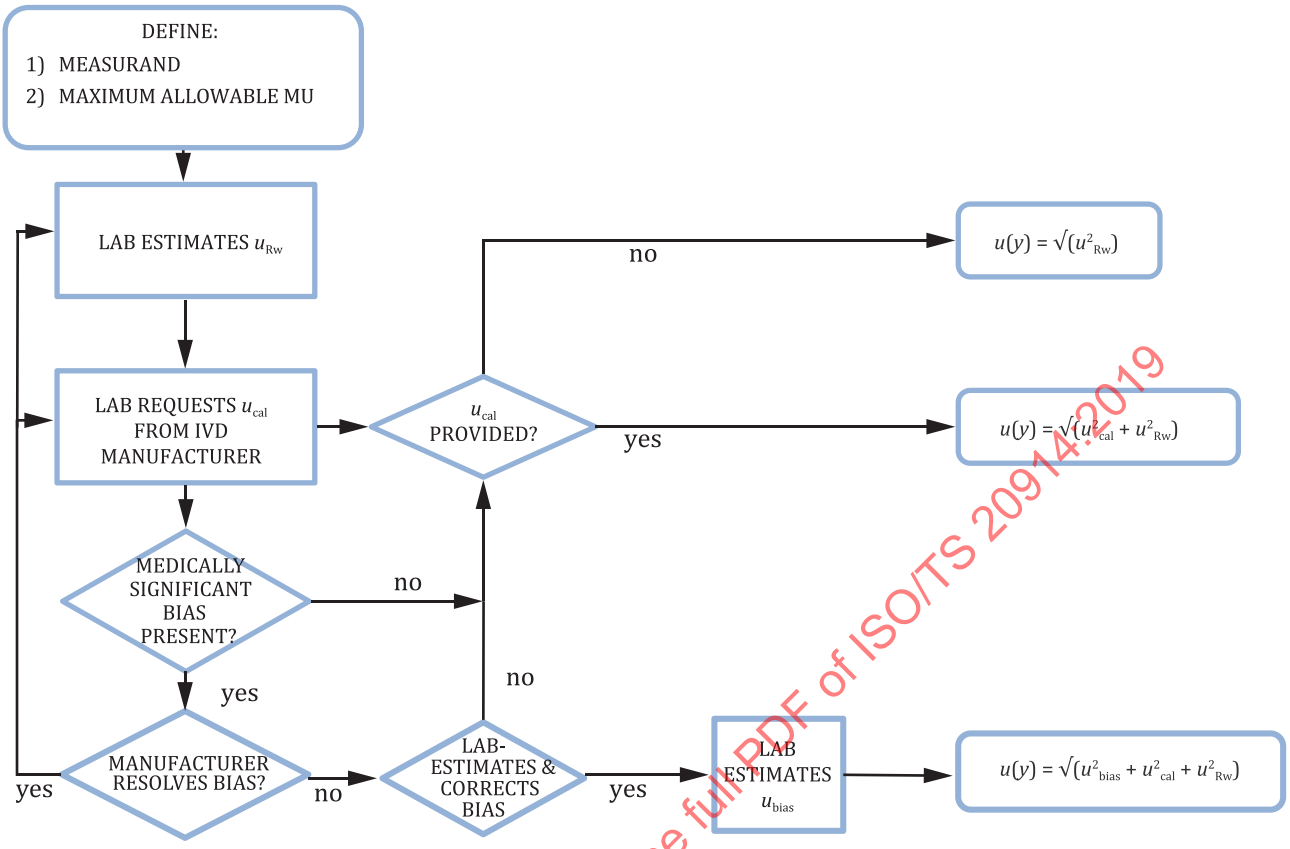


Figure 3 — Overview — Typical pathway for estimating measurement uncertainty

6.8 Re-estimation of measurement uncertainty

Apart from updating interim estimates, there is no need to re-estimate MU if IQC and EQA performance remain within specifications. Re-estimation of MU should be undertaken if there has been a significant change in a measuring system or a new measurement procedure is introduced.

6.9 Qualitative results based on numerical results

Some measurement procedures incorporate a measurement step that generates a measured value that is compared with a cut-off limit, the outcome of which is reported as text; e.g. rate of fluorescent product formation by a human sample relative to that by an index calibrator, expressed as a ratio, to determine if the sample is reported as positive or negative for serum hepatitis B surface antigen. For estimation of imprecision (u_{Rw}) of the measurement step, the output data generated by the appropriate IQC is handled in the same way as described for procedures that report quantitative results. The calculated expanded uncertainties may be used to delineate 'uncertainty zones' around the negative/positive cut-offs, e.g. negative, probably negative, probably positive, positive. In these cases, estimating uncertainty at measurement signal values other than those near the decision limits is unnecessary.

6.10 Uncertainty of counting entities

Counting of entities is a measurement (JCGM 200:2012, 2.1, Note 2). As such, MU associated with counting of entities should be estimated, e.g. number concentration of particular types of blood cells per volume of blood; number of tissue cells per amount of sample.

EXAMPLE If a microscope with a counting chamber is used to manually perform a differential count of white cells in a stained blood smear, the counts obtained may conform to a Poisson rather than a Gaussian distribution if the cell type is a small proportion (usually <10 %) of the total cells counted. In such situations, the cells to be counted are randomly distributed in the counting squares, so that each count of a specific cell type is assumed to occur randomly in space and time and to not interact with each other e.g. clumping. For a Poisson distribution, the variance of a count is equal to the count. Therefore, the $SD = \sqrt{\text{count}}$.

6.11 Limitations of measurement uncertainty estimates

The magnitude of the estimated MU, $u(y)$, for results produced by a given measuring system may be under-estimated due to incomplete information. A $u(y)$ known to be underestimated can still be used for comparing results produced by that specific measuring system, but should not be used to compare results produced by different measuring systems for the same measurand.

Annex A
(informative)

Worked examples of estimating measurement uncertainty

A.1 Introduction

Following are worked examples for the estimation of measurement uncertainty (MU) for measurement procedures typically undertaken by medical laboratories across a range of medical laboratory disciplines. The examples selected were chosen because they are illustrative of commonly encountered challenges in performing calculations to estimate MU in medical laboratories. The included examples are not intended, nor is it possible, to provide in this technical specification a comprehensive overview of MU calculations across the full range of medical laboratory disciplines and technologies. The alignment between concepts discussed in Clause 6 and the worked examples in Annexes A and C is shown in Figure A.1.

As stated in 5.4, rounding should be performed only on the final calculated estimates; never on intermediate calculated values that comprise inputs to subsequent and final calculations. All final percentages (estimated relative uncertainties and estimated relative expanded uncertainties) as reported in the following worked examples were calculated using un-rounded figures. Due to rounding of the final percentages, discrepancies may therefore occur between sums of component items and totals reported for final relative uncertainties. To clearly illustrate the proper calculation of component items in the worked examples, component uncertainties have been reported in the worked example tables with additional decimal places to the extent necessary to eliminate the impact of intermediate rounding on final uncertainty calculations.

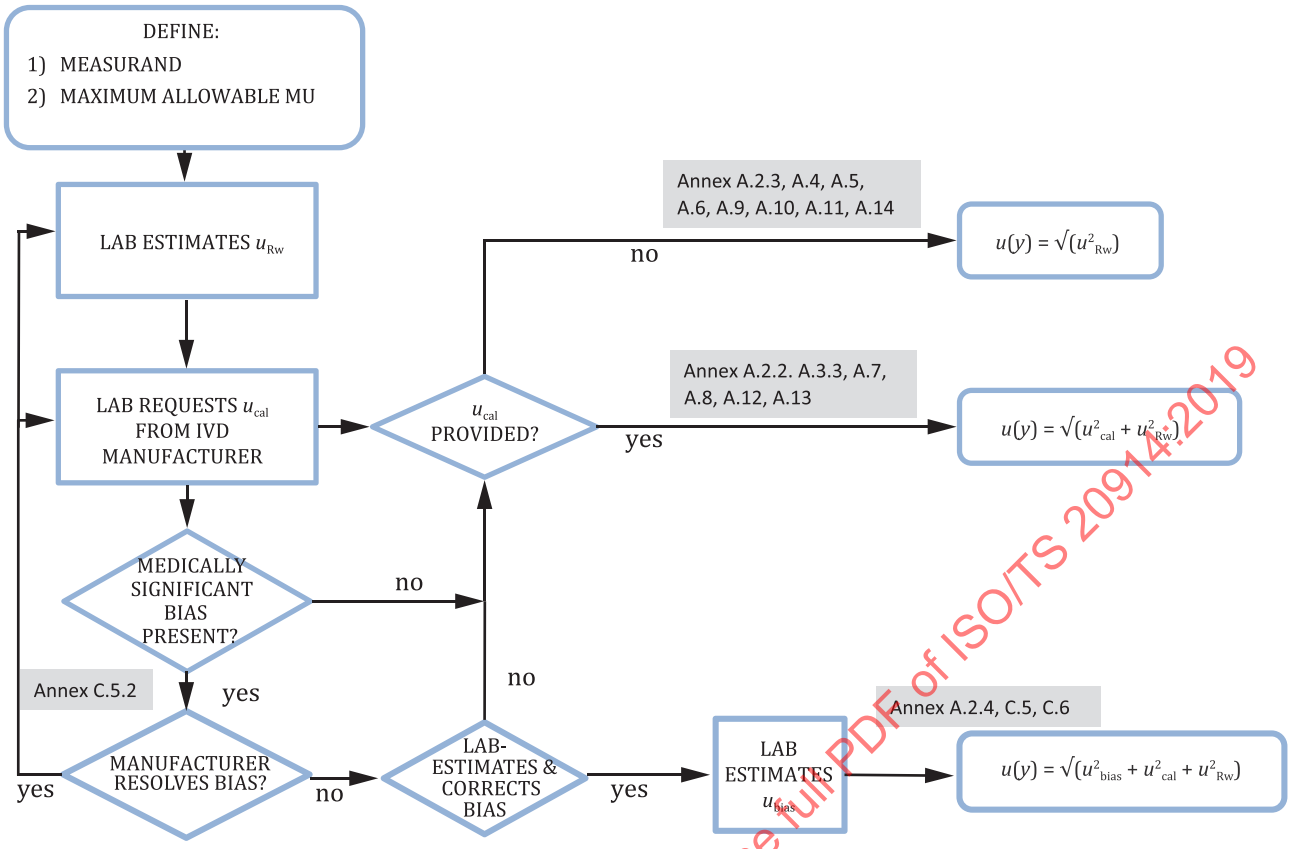


Figure A.1 — Overview of typical pathway for estimating measurement uncertainty with reference to specific worked examples in Annexes A and C

It should be noted that to ensure a consistent approach the worked examples all commence with obtaining the basic parameter of the standard measurement uncertainty (u) without a sign.

As stated in 5.5, users may find it more practical to perform MU calculations by direct use of relative standard uncertainty (u_{rel}) for each contributing standard uncertainty (See worked examples A.7, A.8, A.9 and C.7).

An estimate of expanded uncertainty, U, provides an interval of values that are possible for a measurand, centred on an actual measurement result and based on the information available about the analytical performance of the measurement procedure used. Estimates must be accompanied by a stated level of confidence, typically 'approximately 95 % confidence' when $k = 2$. The distribution of possible values is considered Gaussian, so that a measurement result is assumed to have the highest probability of representing the value of the measurand, and other values in the interval have lower probabilities although one of them could be correct. Uncertainties may be under-estimated if required information is incomplete.

The following clauses illustrate the basic statistics required for estimating uncertainties, and their application in a variety of commonly encountered medical laboratory situations. It is not possible to cover all combinations of uncertainty sources that arise in medical laboratories, however the principles illustrated below can be applied as required.

It is important to note that meaningful comparison of results produced by different measurement procedures for the same measurand may not be possible due to differences in the metrological traceability of the values reported on patient samples for the different measurement procedures.

A.2 Basic calculations

A.2.1 Standard deviation and variance (see 3.36, 3.40)

Variance (σ^2) is a measure of the spread between numerical values in a data set, indicating how far each is from the mean, and is calculated by taking the difference between each value and the mean of the data set, squaring each difference (to make them positive) and then dividing the sum of the squares by the number of values in the set minus 1 (degrees of freedom).

The square root of variance is termed the SD, which has the practical advantage of having the same measurement unit as the data used for its calculation. SDs cannot be added, subtracted, divided or multiplied together, and therefore SD^2 (variance, σ^2) is the necessary calculation when combining different components of MU.

The calculation of a variance is performed according to Formula (A.1).

$$SD^2 = \sigma^2 = \left[\Sigma \left(x_i - \bar{x} \right)^2 \right] / (n - 1) \tag{A.1}$$

where

n = sample size (number of values in the series);

$\bar{x}$ = sample mean;

x_i = individual values of x in the data set;

σ^2 = variance;

Σ = summation of all values in the series.

A variance of 0 indicates that all the values in the data set are identical, while a large variance means some of the individual values are far from the data mean and from each other. The use of variance is limited by the fact that the unit associated with variance is the square of the measurement unit of the data used. However, the statistical advantage of variances is that they can be added, subtracted, divided and multiplied together.

A.2.2 Example — Calculation of u_{Rw} under long-term precision conditions

A given measurement procedure operated within manufacturer's specifications, using the same lots of reagent and calibrator, produced 342 values over several months for the same lot of IQC material. Having demonstrated that the IQC material behaves similarly to typical human samples in the measurement procedure, we are interested in quantifying the dispersion of the 342 results obtained by repeatedly measuring the same lot of IQC material under long-term precision conditions. Because the laboratory has evidence that the measurand in human samples and the measurand in the IQC material behave very similarly in the employed measurement procedure, the SD calculated for the IQC values can reasonably be taken as the u of results produced for typical human samples. Calculation of u to be applied to human sample results is presented in Table A.1.

NOTE The SD is used for the IQC results as a measure of the uncertainty expected for human samples.

Table A.1 — Example calculation of u under long-term precision conditions

Component (Analyte)	Sodium
Measurands	Amount of sodium concentration in serum, plasma, urine
Measurement unit	mmol/l
Measurement method	sodium selective electrode – Indirect
Measurement procedure	Model P2, consumables: X Pty Ltd. See Quality Manual.

Table A.1 *(continued)*

Component (Analyte)	Sodium		
Calibrator traceability	SI traceable – SRM 919b		
Calibrator uncertainty (u_{cal})	0,71 mmol/l		
Bias	Assessed by EQA- commutable material with target value set by ISO 15195 accredited reference laboratory: Average bias not medically significant		
Intermediate precision	Plasma IQC Level 1 Lot 576	Plasma IQC Level 2 Lot 586	Urine IQC Lot 884
Data collection period	2012-02-09 to 2013-05-13	2012-02-09 to 2013-05-13	2012-02-09 to 2013-05-13
N	342	338	122
Mean value mmol/l	134,8	149,8	86,4
u_{Rw}(Na) mmol/l	0,85	0,87	0,99
u(Na) = $\sqrt{(u^2_{Rw} + u^2_{cal})}$ mmol/l	$\sqrt{(0,85^2 + 0,71^2)}$ = 1,11	$\sqrt{(0,87^2 + 0,71^2)}$ = 1,12	$\sqrt{(0,99^2 + 0,71^2)}$ = 1,22
U(Na); k = 2; mmol/l	2,22	2,24	2,44
% U_{rel}(Na)	1,6 %	1,5 %	2,8 %
Applied to patients' results (≈95 % confidence)	±1,6 %	±1,5 %	±2,8 %

A.2.3 Example — Calculation of $u_r(y)$ under repeatability conditions

A medical application for estimation of u_r under repeatability conditions is presented in Table A.2. For the intra-operative comparison of parathyroid hormone (PTH) results for patients undergoing parathyroidectomy, results for the same patient on sequential independent samples can be compared within the same analytical run. Three different concentrations of IQC material are measured to obtain the u_r under repeatability conditions. Bias evaluation is not required for comparing results from the same patient produced in the same analytical run.

20 aliquots of each of three levels of matrix-matched IQC material were evaluated. The mean and SD of the 20 IQC values were calculated for each IQC level. The SD value is taken as the standard uncertainty under repeatability conditions, u_r(PTH), for each level. The estimated value for u_r(PTH) was considered by the laboratory to apply to the same intervals of PTH values as the measurand ranges monitored by the respective levels of IQC.

Table A.2 — Estimation of $u_r(y)$ under repeatability conditions for a new plasma parathyroid hormone measurement procedure

Component (Analyte)	Parathyrin — Intact (Parathyroid hormone; PTH)		
Measurand	Amount of intact parathyrin concentration in serum		
Measurement unit	pmol/l		
Measurement method	Two site sandwich chemiluminescence immunoassay		
Measurement procedure	Manufacturer Z Ltd.; Instrument AB, Manufacturer Z Ltd. See Procedure Manual for antibody specificity.		
Calibrator traceability	Assigned value traceable to WHO 1st IRP 95/646		
Calibrator uncertainty (u_{cal})	Not required for comparing several results from same patient obtained in same analytical run		
Bias	Not medically significant based on EQA performance		
Repeatability study: Human serum IQC measured in same analytical run			
	Level 1	**Level 2**	**Level 3**
IQC monitors interval: pmol/l	<10,0	10 to <50	≥50
N	20	20	20

Table A.2 *(continued)*

Component (Analyte)	Parathyrin — Intact (Parathyroid hormone; PTH)		
Mean: pmol/l	2,1	21,4	122,0
u_r(PTH), pmol/l	0,044	0,40	3,15
U_r(PTH) pmol/l; k = 2	0,088	0,80	6,30
% U_{rel}(PTH); k = 2	4,2 %	3,7 %	5,2 %
Applied to patients' results (≈95 % confidence)	±4,2 %	±3,7 %	±5,2 %

The repeatability standard uncertainty, u_r, provides useful initial guidance on the result MU when evaluating if the measuring system will be suitable for wider medical use. Because the value of the MU under repeatability conditions does not include variability from additional expected sources of variability in routine use, the $u(y)$ estimated in this example is not applicable to the uncertainty for long-term precision conditions for other medical uses of PTH values.

A.2.4 Combining independent standard measurement uncertainties

Two or more different sources of uncertainty within a measurement process may significantly contribute to the overall MU of a result, so there can be a need to combine two or more standard uncertainties to estimate the overall (combined) uncertainty of a measurement result.

SDs (u) cannot be summed, subtracted, multiplied or divided. To combine the standard uncertainties arising from different components of the overall measurement process the standard uncertainties of each of the contributing components must first be converted to variances, where variance = SD^2 (u^2). Note that u values are always positive.

Standard measurement uncertainties are combined according to one of two rules, depending on how the contributing measurements relate to each other in the formula that calculates the desired result.

Rule 1: Combining independently estimated absolute standard uncertainties

The combined uncertainty is calculated as the square root of the sum of the contributing variances.

EXAMPLE 1 Calculation of the overall standard uncertainty, $u(y)$, for measurand Y comprising uncertainties determined for the calibrator (u_{cal}), a bias correction (u_{bias}) and imprecision under long-term precision conditions (u_{Rw}): The contributing uncertainties are first expressed as variances prior to their addition according to Formula (A.2).

$$u(y) = \sqrt{\left(u^2_{cal} + u^2_{bias} + u^2_{Rw}\right)} \quad \text{(A.2)}$$

where

$u(y)$ is the overall standard measurement uncertainty;

u_{cal} is the uncertainty of the value assigned to the calibrator;

u_{bias} is the uncertainty of any bias correction;

u_{Rw} is the imprecision of the measurement procedure under long-term precision conditions.

For a given measurement procedure let: u_{cal} = 0,11; u_{bias} = 0,090; u_{Rw} = 0,43 mmol/l. Performing the calculation per Formula (A.2):

$$u(y) = \sqrt{(0{,}11^2 + 0{,}090^2 + 0{,}43^2)}$$

$$u(y) = \sqrt{(0{,}012\,1 + 0{,}008\,1 + 0{,}184\,9)} = \sqrt{0{,}205\,1}$$

$u(y)$ = 0,45 mmol/l

EXAMPLE 2 Standard uncertainty of a measurand value calculated by addition and/or subtraction of several measurand values – e.g. Plasma anion gap (AG), where AG is calculated per Formula (A.3):

$$\text{AG mmol/l} = \left(\left[Na^+\right] \text{mmol/l} + \left[K^+\right] \text{mmol/l}\right) - \left(\left[Cl^-\right] \text{mmol/l} + \left[HCO_3^-\right] \text{mmol/l}\right) \quad \text{(A.3)}$$

Let plasma:

— [Na+] = 143 mmol/l

— [K+] = 4,0 mmol/l

— [Cl-] = 104 mmol/l

— [HCO3-] = 22 mmol/l

AG = (143 + 4,0) − (104 + 22) = 21 mmol/l

Let:

— u(Na+) = 0,90 mmol/l

— u(K+) = 0,040 mmol/l;

— u(Cl-) = 0,78 mmol/l

— $u(HCO_3^-)$ = 1,22 mmol/l

NOTE The u value for each electrolyte should include u_{Rw} and u_{cal}, and u_{bias} if relevant.

Step 1. Each contributing u is first expressed as a variance (u^2).

$u^2(Na^+)$ = 0,81; $u^2(K^+)$ = 0,001 6; $u^2(Cl^-)$ = 0,608 4; $u^2(HCO_3^-)$ = 1,488 4

Step 2. The variances are added together, regardless of whether the absolute measurand values are added or subtracted in the calculation of AG. The sum of the variances gives the combined variance for the calculated AG.

Step 3. The square root of the combined variance gives the overall u for the calculated anion gap, u(AG).

u(AG) = √(0,81 + 0,001 6 + 0,608 4 + 1,488 4) = √2,908 4 = 1,705 4 mmol/l

Step 4. If coverage factor k = 2 is selected, then expanded uncertainty (≈95 % confidence) is determined as: U(AG)= 2 × 1,705 4 = 3,41 mmol/l

Applied to patients' results: U = ±3,41 mmol/l = ±16,2 % at an AG = 21 mmol/l

Result: AG = 21 ± 3,41 mmol/l or ± 16,2 % = [21 − 3,41 to 21 + 3,41] = [17,59 to 24,24] = [18 to 24] (k = 2; ≈95 % level of confidence)

EXAMPLE 3 Calculated plasma osmolality, where plasma osmolality (Osmo) is calculated per Formula (A.4):

$$\text{Osmo/l} = \left[2 \times Na^+ \text{mmol/l}\right] + \left[\text{urea mmol/l}\right] + \left[\text{glucose mmol/l}\right] + 9\ \left(\text{mOsmo/l}\right) \quad \text{(A.4)}$$

Measured values and standard MU values (u) for each component in human sample A:

Plasma Na = 130 mmol/l, u(Na) = 0,98 mmol/l; plasma urea = 6,5 mmol/l, u(urea) = 0,19 mmol/l; plasma glucose = 5,2 mmol/l, u(glu) = 0,090 mmol/l

Plasma $Osmo_{calc}$ = (2 × 130) + 6,5 + 5,2 + 9 = 280,7 = 281 mOsmo/l

The contributing MUs for each measured component propagate through to the final measured values and the calculation of Osmo according to their mathematical relationship in the formula for the calculated results. For the calculation of u(Osmo):

Step 1. Convert each component u to its variance (u^2)

where

u^2(Na) = $0{,}98^2$ = 0,960 4;

u^2(urea) = $0{,}19^2$ = 0,036 1;

u^2(glu) = $0{,}090^2$ = 0,008 1.

Step 2. Calculation of plasma osmolality per Formula (3) multiplies the sodium concentration by 2 to account for matching anions, but the variance for sodium is not similarly treated because the sodium concentration cannot be independent of itself, and therefore the variance u^2(Na) is multiplied by 2^2. The additional factor 9 is assumed to have no uncertainty. Therefore, $u(Osmo_{calc})$ is calculated per Formula (A.5).

$$u\left(Osmo_{calc}\right) = \sqrt{\left(2^2 \times u^2\left(Na\right) + u^2\left(urea\right) + u^2\left(glu\right)\right)}$$

$$u\left(Osmo_{calc}\right) = \sqrt{\left(2^2 \times 0{,}960\,4\right) + 0{,}036\,1 + 0{,}008\,1} = \sqrt{3{,}885\,8} = 1{,}971\,2\ \text{mOsmo/l} \quad \text{(A.5)}$$

$$U\left(Osmo_{calc}\right) = 3{,}94\ \text{mOsmo/l}\ \left(k = 2;\ \approx 95\,\%\ \text{level of confidence}\right)$$

$$\%\,U_{rel}\left(Osmo_{calc}\right) = \left(3{,}94 / 280{,}7\right) \times 100 = 1{,}4\,\%$$

Human sample A, $Osmo_{calc}$ = 280,7 ± 3,94 mOsmo/l or ± 1,4 % = [280,7-3,94 to 280,7 + 3,94] = [276,76 to 284,64] = [277 to 285] (k = 2; ≈95 % level of confidence)

Rule 2: Combining independently estimated absolute standard uncertainties, when the measurement formula requires the component uncertainties to be multiplied or divided.

If a measurement formula requires contributing independent standard uncertainties to be multiplied or divided, the component uncertainties must be expressed as relative standard uncertainties before applying rule 1.

NOTE 1 When users are comfortable combining contributing standard uncertainties to obtain an overall uncertainty, such calculations can be simplified by direct use of the relative standard uncertainty for each contributing standard uncertainty (See worked example A.9).

EXAMPLE 1 Calculation of a standard uncertainty involving division (ratio) of two independent measurand values.

Spot urine calcium to urine creatinine ratio is calculated per Formula (A.6) as:

$$\text{urine calcium to urine creatinine ratio} = \left[U_{ca}\right] / \left[U_{crea}\right] \quad \text{(A.6)}$$

where

$[U_{ca}]$ is the urine calcium amount of substance concentration in mmol/l;

$[U_{crea}]$ is the urine creatinine amount of substance concentration in mmol/l.

Patient sample: $[U_{ca}]$ = 6,40 mmol/l; $[U_{crea}]$ = 2,30 mmol/l, $[U_{ca}]/[U_{crea}]$ = 2,78

Because the calculation involves division, the two contributing uncertainties are expressed as % u_{rel}:

Let:

— $u[U_{ca}]$ = 0,040 mmol/l (calculated for IQC mean concentration 2,71 mmol/l)

— % $u_{rel}[U_{ca}]$ = ($u[U_{ca}]/[U_{ca}]$) × 100 = (0,040 0/2,71) × 100 = 1,476 0 % = 1,5 %

— $u[U_{crea}]$ = 0,150 mmol/l (calculated for IQC mean concentration 6,17 mmol/l)

— % $u_{rel}[U_{crea}]$ = ($u[U_{crea}]/[U_{crea}]$) × 100 = (0,150/6,17) × 100 = 2,431 1 % = 2,4 %

— % $u_{rel}[U_{ca}]/[U_{crea}]$ = $\sqrt{(1{,}467\ 0^2 + 2{,}431\ 1^2)}$ = 2,839 4 % = 2,8 %

— % U_{rel} $[U_{ca}]/[U_{crea}]$ = (2,839 4 %) × 2 = ± 5,7 %, k = 2 (≈95 % level of confidence)

For a human sample result, expanded percent uncertainty, % U for U_{ca}/U_{crea} = 2,78 ± 5,7 % (≈95 % level of confidence), or if the absolute expanded uncertainty value is required:

Conversion from % $u_{rel}[U_{ca}]/[U_{crea}]$ to $u[U_{ca}]/[U_{crea}]$ is performed as follows:

— $u[U_{ca}]/[U_{crea}]$ = ((% $u_{rel}[U_{ca}]/[U_{crea}]$)/100) × $[U_{ca}]/[U_{crea}]$ for the human sample = (2,839 4/100) × 2,78 = 0,078 94;

— $U[U_{ca}]/[U_{crea}]$; k = 2, = 0,078 94 × 2 = 0,157 9 = 0,158;

— Human sample result: $U[U_{ca}]/[U_{crea}]$ = 2,78 ± 0,158 (≈95 % level of confidence);

— % U_{rel} $[U_{ca}]/[U_{crea}]$ = (0,158/2,78) × 100 = 5,683 = 5,7 % (≈95 % level of confidence).

EXAMPLE 2 Calculation of combined standard uncertainty involving multiplication and division of contributing independent uncertainties.

Creatinine clearance is calculated from measurements of serum/plasma and urine creatinine concentrations in a timed (usually 24 h) urine collection as shown in Formula (A.7).

$$\text{Creatinine clearance } (Cl_{crea}) = \frac{[U_{crea}] \times U_{vol}}{[P_{crea}] \times t} \text{ ml/min} \tag{A.7}$$

Let:

— $[P_{crea}]$ = 146 µmol/l

— $[U_{crea}]$ = 2 900 µmol/l

— U_{vol} = 2 421 ml

— t = 24 h × 60 min = 1 440 min

— $Cl_{crea} = \frac{2\,900 \times 2\,421}{146 \times 1\,440} = 33{,}4$ ml/min

Step 1. Calculation of creatinine clearance involves multiplication and division of the measured values; the standard uncertainties of the measurand values must first be expressed as relative standard uncertainties (u_{rel})

Let the standard uncertainty (u) from IQC data for each measured value be:

P_{crea}:

— $u(P_{crea})$ = 1,438 µmol/l for mean IQC value of 70 µmol/l; % $u_{rel}(P_{crea})$ = (1,438/70) × 100 = 2,05 %

U_{crea}:

— $u(U_{crea})$ = 138,0 µmol/l for mean IQC value of 6 060 µmol/l; % $u_{rel}(U_{crea})$ = (138,0/6 060) × 100 = 2,28 %

U_{vol}:

— Measurement error subjectively assessed by laboratory as 2 421 ml ± 100 ml

The above estimate of volume error of ±100 ml (range of 200 possible values) is a professional 'estimate' by the laboratory and not a standard uncertainty by experimentation. Therefore, a decision is required as to whether the actual measured volume is likely to be:

— any value between −100 ml and +100 ml of measured value (rectangular distribution), or

— more likely to be close to the stated measured value (triangular distribution).

A more complete discussion of rectangular and triangular distributions is available in EURACHEM/CITAC Guide CG4:2012.

The laboratory considers the first option to be more credible.

The SD of a rectangular distribution = (range of possible values/2)/√3

$u(U_{vol})$ = (200/2)/√3 = 57,7 ml; % $u_{rel}(U_{vol})$ = 57,7/2 421 = 2,40 %

(If a triangular distribution is thought more likely, calculation would be (200/2)/√6).

t (time): Error assessed by laboratory as 24 h ± 0,5 h, assumed rectangular distribution

0,5 h = 30 min, range = 60 min $u(t)$ = (60/2)/√3 = 17,32 min

% $u_{rel}(t)$ = 17,32/1 440 = 1,20 %

$u_{rel}(Cl_{crea}) = \sqrt{[u^2_{rel}(P_{crea}) + u^2_{rel}(U_{crea}) + u^2_{rel}(U_{vol}) + u^2_{rel}(t)]} = \sqrt{(2{,}05^2 + 2{,}28^2 + 2{,}40^2 + 1{,}20^2)}$ = 4,074 4 % = 4,1 %

% $U_{rel}(Cl_{crea})$ = 8,148 8 % = 8,1 %, k = 2

$U(Cl_{crea})$ = 33,4 × (8,148 8/100) = 2,721 7 ml/min = 2,72 ml/min

Patient result: 33,4 ± 2,72 ml/min (≈95 % level of confidence).

A.3 Measurement uncertainty across changes to measuring conditions

A.3.1 General approach to calculation

To collect adequate IQC data under long-term precision conditions it is likely that one or more lots of reagent, calibrator and IQC are exhausted and new lots are introduced. It is essential that uncertainty estimates should account for the impact of such changes to measuring conditions.

Laboratories with large workloads may use two or more identical measuring systems for the same measurand, so that human samples could be analysed on any one system. In such situations, it is useful to estimate a MU that is applicable to all results regardless of which measuring system is used.

A change in measuring conditions that introduces a significant measurement variation relative to values obtained under previous conditions that is not also observed for human samples could inappropriately increase the SD if the IQC values were treated as a single set.

When a new IQC lot is introduced, it is likely to have a mean value different from the previous lot. Therefore, when IQC lots are changed, the IQC data must be collected, calculated and evaluated separately prior to combining the data with that of previous IQC lots.

When a new lot of reagent(s) is introduced, it is essential to check that if a change in IQC values occurs it is matched by a similar magnitude of change in values obtained for a panel of typical human samples. If the values for both IQC and the human sample panel are not significantly changed, or show the same magnitude of shift in values, then the IQC values obtained for the old and new reagent lots can be

treated as a single data set. However, if only the IQC values show a significant change, then the old and new IQC values must be collected and SDs calculated separately prior to determining how the data should be pooled.

Introduction of a new lot of calibrator is expected to cause only small changes in measurement results for both human and IQC samples within a specified interval. Therefore, separate collection and calculation of IQC data is not required unless the calibrator manufacturer introduces a significant change in absolute values for human samples through a process of re-standardisation of the calibrator value. Calculation of the IQC data as a single set across the calibrator lot change will capture, as a random error, the variability of human sample results due to the calibrator change.

It is therefore essential:

— for each different condition of measurement, to determine if IQC values require separate treatment

— to calculate the mean and SD for each separate IQC data set if IQC values among different conditions of measurement require separate treatment

— to calculate the SD (u_{Rw}) either by using a single data set comprised of all IQC values or by calculating SD (u_{Rw}) by pooling the individual SDs from the IQC data for each condition to obtain an average u_{Rw} if IQC values among different conditions of measurement do not require separate treatment

EXAMPLE

Over time a measuring system had two changes of IQC lot, and one lot change of reagent that caused a change in measured values for IQC but not for the human samples. A change in calibrator lot and a preventive maintenance of the measuring system did not affect IQC or human sample values. In summary, there were a total of five changes to measuring conditions, three of which required separate collection of IQC data.

Let:

— IQC Lot 1 = u_1

— IQC Lot 2 = u_2

— Reagent Lot change = u_3

— Calibrator Lot change, maintenance

If the number of IQC values obtained for each measuring condition (n_1, n_2, n_3) are reasonably similar (balanced), Formula (A.8) can be used to calculate the average standard uncertainty across the changing measuring conditions:

$$\text{Pooled average } u_{Rw} = \sqrt{\left(\frac{u^2{}_1 + u^2{}_2 + u^2{}_3}{3}\right)} \tag{A.8}$$

Under circumstances where there are very large differences in the number of IQC data points (n) between different measuring conditions (e.g. among multiple instruments, among multiple IQC lots, among multiple reagent lots, etc.), calculation of the pooled average u_{Rw} may be performed with weighting of 'n' for each logical sub-group of data. In this circumstance, alternative approaches may be applicable.

A.3.2 Estimation of $u(y)$ using multiple lots of reagent, where internal quality control shifts are observed without a corresponding shift in patient values

EXAMPLE

An immunoassay for intact parathyroid hormone (iPTH) was evaluated prior to each new lot of reagent entering service, using both a panel of human samples and IQC materials, and including both the outgoing and new reagent lots. At each reagent lot changeover it was found that results for patient panels were not significantly changed, but the IQC values were at times significantly changed. To guard against obtaining a misleading estimate of the IQC SD by analysing as a single IQC data set across all reagent lots, the IQC data were collected and analyzed separately for each reagent lot, as shown in Table A.3. Since the number of IQC data points, n, obtained for each reagent lot was similar, the contribution of each IQC data set to the pooled average variance was treated equally (balanced) in calculating the u_{Rw}, without weighting for n, and calculated according to Formula (A.8).

Table A.3 — Evaluation of IQC data among multiple reagent lots, with no significant shift in patient data

Component (Analyte)	Parathyrin — Intact (Parathyroid hormone; iPTH)		
Measurand	Amount of iPTH concentration in serum		
Measurement unit	pmol/l		
Measurement method	Two site sandwich chemiluminescence immunoassay		
Measurement procedure	Manufacturer Z Pty Ltd. See procedure manual for antibody selectivity and limitations		
Calibrator traceability	Assigned value traceable to WHO 1st IRP 95/646		
Calibrator uncertainty (% U_{cal}), k = 2	2,1 %		
Bias	Not medically significant relative to EQA peer group		
IQC matrix: Human serum	**Level 1**	**Level 2**	**Level 3**
IQC monitors interval: pmol/l	<10,0	10,0 – <50,0	>50,0
Reagent Lot no. 66	**Data collection: 5/03/15-29/11/15**		
N	138	118	106
Mean: pmol/l	2,13	16,85	58,45
u_{Rw}(66) pmol/l	0,094 0	0,504	1,726
Reagent Lot no. 67	**Data collection: 30/11/15-21/05/16**		
N	142	139	142
Mean: pmol/l	2,11	18,08	62,00
u_{Rw}(67) pmol/l	0,088 0	0,558	2,033
Reagent Lot no. 68	**Data collection: 22/05/16-03/09/16**		
N	129	126	120
Mean: pmol/l	2,17	18,69	64,26
u_{Rw}(68) pmol/l	0,092 0	0,643	2,157
Pooled average u_{Rw}(PTH) pmol/l	Level 1: $\sqrt{[(0{,}094\ 0^2 + 0{,}088\ 0^2 + 0{,}092\ 0^2)/3]} = 0{,}091\ 37 = 0{,}091\ 4$ Level 2: $\sqrt{[(0{,}504^2 + 0{,}558^2 + 0{,}643^2)/3]} = 0{,}571\ 2 = 0{,}571$ Level 3: $\sqrt{[(1{,}726^2 + 2{,}033^2 + 2{,}157^2)/3]} = 1{,}980\ 3 = 1{,}980$		
U_{Rw} pmol/l, k = 2 (≈95 % confidence) = 2 × u_{Rw}	0,182 74 = 0,183	1,142 4 = 1,142	3,960 6 = 3,961
% U_{Rw}, k = 2, ≈95 % confidence = (U_{Rw}/ $\bar{x}_{avg}$) × 100	8,552 5 % = 8,6 %	6,391 6 % = 6,4 %	6,432 7 %= 6,4 %
% U(PTH), k =2, ≈95 % confidence = $\sqrt{(\%\ U^2_{cal} + \%\ U^2_{Rw})}$	$\sqrt{(2{,}1^2 + 8{,}552\ 5^2)}$ 8,806 5 % = 8,8 %	$\sqrt{(2{,}1^2 + 6{,}391\ 6^2)}$ 6,727 7 % = 6,7 %	$\sqrt{(2{,}1^2 + 6{,}432\ 7^2)}$ 6,766 8% = 6,8 %

A.3.3 General approach for estimation of u_{Rw} using multiple lots of internal quality control

The general approach to calculation of u_{Rw} when IQC data has been accumulated across multiple lots of IQC material and the number of results for each lot is approximately the same is the same as described in A.3.1. An example of a u_{Rw} calculation of this type is shown below.

IQC Level 1: Lot 47: Feb-May: $n = 168$, $\bar{x} = 4{,}32$ mmol/l, $u_{Rw} = 0{,}230$ mmol/l

Lot 48: June-Sept: $n = 186$, $\bar{x} = 4{,}43$ mmol/l, $u_{Rw} = 0{,}270$ mmol/l

Lot 49: Oct –Mar: $n = 172$, $\bar{x} = 3{,}96$ mmol/l, $u_{Rw} = 0{,}210$ mmol/l

Average $\bar{x}$: 4,24 mmol/l

$$u_{Rw}\left(\text{lots } 47,48,49\right) = \sqrt{\left(\frac{0{,}230^2 + 0{,}270^2 + 0{,}210^2}{3}\right)} = 0{,}237\,977\,59 \text{ mmol/l} \approx 0{,}238 \text{ mmol/l}$$

U_{Rw}(lots 47,48,49) = 2 × u_{Rw} = 0,475 955 18 = 0,476 mmol/l; k = 2, ≈95 % confidence

% U_{Rw} = (U_{Rw}/ $\bar{x}_{avg}$) × 100 = (0,475 955 18/4,236 7) × 100 = 11,234 1 % ≈ 11,2 %; k =2, ≈95 % confidence

The pooled average value of u_{Rw} can be combined with the standard uncertainty estimate for the calibrator assigned value (u_{cal}), if available, to calculate the overall U and %U_{REL}.

A.4 Pooled average standard uncertainty among several identical measuring systems, accounting for different internal quality control mean values

Laboratories with large workloads may use several copies of the same measuring system for the same measurand, so that a human sample could be analysed with any particular measuring system. In such situations, it is useful to estimate a single $u(y)$ that can reasonably be applicable to results produced by any of the several measuring systems.

The several measuring systems will usually be monitored by the same IQC lot at the same time. The u_{Rw} value is calculated separately for each of the several measuring systems. There is the possibility of a different IQC mean value being obtained by each measuring system for the same IQC lot. Therefore, the standard uncertainty of the mean values for the IQC lot across the several measuring systems must be calculated and included in the calculation of the pooled average uncertainty.

EXAMPLE

IQC level 1 lot 50 is concurrently used on three identical measuring systems A, B, C. It is assumed that measurement bias for human samples between the three systems is medically irrelevant, being monitored by regular checks using a panel of typical human samples, and that IQC and human samples have similar imprecision in the several measuring systems.

Step 1: For IQC data obtained under long-term precision conditions of use, calculate for each measuring system the mean, $\bar{x}$ (A), $\bar{x}$ (B), $\bar{x}$ (C)) and u_{Rw}(A), u_{Rw}(B), u_{Rw}(C) of the IQC values produced by each of the three systems. For each measuring system (A,B,C), tabulate the IQC mean ($\bar{x}$), number of data points collected (n), and SD values (u_{Rw}), as shown in Table A.4.

Table A.4 — Example matrix for tabulation of internal quality control data among multiple identical measuring systems

Measuring system	A	B	C
n	n_A	n_B	n_C

Table A.4 *(continued)*

Measuring system	A	B	C
$\bar{x}$	$\bar{x}_A$	$\bar{x}_B$	$\bar{x}_C$
SD	u_{Rw}(A)	u_{Rw}(B)	u_{Rw}(C)

Step 2: From the SDs, based on the data obtained for IQC lot 50 for each measuring system (A,B,C), calculate the variances [u^2_{Rw}(A), u^2_{Rw}(B), u^2_{Rw}(C)] for each system (A,B,C).

Step 3: Calculate the variance, u^2(A,B,C), of the three mean values among the three measuring systems (A,B,C) for IQC lot 50.

Step 4: Combine the variance of the three mean values u^2(A,B,C) with the pooled average imprecision variance, u^2_{Rw}(A,B,C),

where

pooled average combined variance = u^2(A,B,C) + u^2_{Rw}(A,B,C), and

u(pooled) = $\sqrt{(u^2(A,B,C) + u^2_{Rw}(A,B,C))}$.

u(pooled) is then combined with the relevant u_{cal} to calculate the overall combined $u(y)$.

Worked example — Pooled average standard uncertainty among several identical measuring systems, accounting for different internal quality control mean values

Steps 1 & 2: Data matrix and calculated variances are shown in Table A.5.

Table A.5 — Worked example — Data matrix and calculated variances (steps 1 & 2) - pooled average standard uncertainty among several measuring systems, accounting for IQC mean differences

	QC level 1, lot 50		
Measuring system:	**A**	**B**	**C**
n	280	190	400
$\bar{x}$ (mmol/l)	5,15	4,93	5,28
SD	0,160	0,190	0,200
u^2_{Rw}	0,025 6	0,036 1	0,040 0

Step 3: Calculate variances for (a) among-measuring system differences in mean values and (b) pooled average imprecision variance

a) Calculation of the variance, u^2(A,B,C), among the three measuring system's (A, B, C) mean values (5,15, 4,93, 5,28 mmol/l) for IQC lot 50 is performed as follows:

— $\bar{x}$ (A,B,C) = (5,15 + 4,93 + 5,28)/3 = 5,12

— SD(A,B,C) = $\sqrt{[\sum(x - \bar{x})^2]/n - 1} = \sqrt{\sum[(5,15 - 5,12)^2 + (4,93 - 5,12)^2 + (5,28 - 5,12)^2]/2}$

— = 0,176 918 = 0,177 mmol/l = u(A,B,C)

— u^2(A,B,C) = (0,176 918)^2 = 0,0313

b) Calculation of the variance, u^2_{Rw}(A,B,C) for pooled average imprecision within measuring system is performed as follows:

— u_{Rw}(A,B,C) = $\sqrt{((0,160^2 + 0,190^2 + 0,200^2)/3)} = \sqrt{0,034}$ = 0,184 12 mmol/l

— u^2_{RW}(A,B,C) = 0,184 12^2 = 0,033 9

Step 4: Combine the variances of the among-measuring system mean values, u^2(A,B,C), and the pooled average imprecision variance, u^2_{Rw}(A,B,C)

Calculation of the combined variance is performed as follows:

— u(pooled) = $\sqrt{(u^2(A,B,C) + u^2_{Rw}(A,B,C))}$ = $\sqrt{(0{,}031\ 3 + 0{,}033\ 9)}$ = 0,255 343 = 0,255 mmol/l

— % u_{rel}(pooled) = [u(pooled)/ $\bar{x}$ (A,B,C)] × 100 = (0,255 343/5,12) × 100 = 4,987 2% = 5,0 %

— % U_{rel}(pooled) = 2 × % u_{rel}(pooled) = 9,974 3 % = 10,0 %; k = 2, ≈95 % confidence

For an estimate of the overall uncertainty, u(pooled) can now be combined with u_{cal} and u_{bias} (if relevant), and is applicable to results monitored by IQC level 1, irrespective of whether measured on measuring system A, B or C.

A.5 Uncertainty of anion gap (AG) results using internal quality control data

An alternative to combining contributing uncertainties illustrated earlier (A.2.4, Example 2) is to use IQC electrolyte values obtained under long-term precision to calculate AG in the same way as for human samples, which may better reflect the uncertainty of AG results as reported by a medical laboratory.

Step 1: IQC data is accumulated for serum/plasma electrolytes (sodium, potassium, chloride, total CO_2) for a time period sufficient to include all changes to measuring conditions.

Step 2: The laboratory's selected formula for AG calculation and all sets of IQC electrolyte values are entered into an appropriate spreadsheet capable of calculating AG results from the IQC electrolyte values.

The sample spreadsheet below (Table A.6) shows the first six of 1 374 entries for IQC level 1 electrolyte values and the formula used by the laboratory information technology (IT) system for calculation of human sample anion gaps. This approach will include, for example, small uncertainties introduced by automatic rounding of values by a laboratory IT system.

Table A.6 — First six of 1374 IQC level 1 electrolyte data entries for calculated AG values

H5 =(D5+E5)-(F5+G5)

A	B	C	D	E	F	G	H
	seq no	date	serum QC level 1				
			Na	K	Cl	HCO3	anion gap
	1	28/07/2014	123.3	4.09	85.9	18.4	23.09
	2	29/07/2014	125.6	4.17	86.6	16.9	26.27
	3	29/07/2014	124.0	4.11	86.2	16.5	25.41
	4	31/07/2014	123.3	4.09	85.9	16.4	25.09
	5	29/07/2014	123.1	4.08	85.9	16.5	24.78
	6	30/07/2014	124.2	4.13	86.2	17.2	24.93

Step 3: Calculate the mean and imprecision for successive AG values for the IQC material.

Using the successive AG values shown in Table A.6, Column H, calculate the mean and imprecision uncertainty u_{Rw}(AG). The resulting calculated parameters are shown in Table A.7.

Table A.7 — Mean anion gap value, u_{Rw}(AG) and U (AG) for internal quality control level 1

Time period	28/07/14 - 21/08/15
IQC level 1	
N	1 374
Mean AG (mmol/l)	25,8
u_{Rw}(AG) mmol/l	1,45
U (AG); k = 2, mmol/l	3,0

The expanded uncertainty (U) for an AG result is ±3,0 mmol/l.

A.6 Uncertainty of results for estimated glomerular filtration rate (eGFR)

Estimated glomerular filtration (eGFR), calculated using an estimation formula requiring serum/plasma creatinine concentration, age, sex and ethnicity (Caucasian or African-American) may improve detection of asymptomatic chronic kidney disease (CKD). The current CKD-EPI formulae for the calculation of eGFR[28] used by laboratory computing systems are shown in Table A.8.

Table A.8 — CKD-EPI formulae for calculating eGFR, by ethnicity and sex

Ethnicity	Sex	Serum creatinine (S_{cr}), µmol/l (mg/dl)	eGFR formula (S_{cr} in mg/dl) ml/min per 1,7373 m^2
Black	Female	≤62 (≤0,7)	$166 \times (S_{cr}/0,7)^{-0,329} \times (0,993)^{Age}$
Black	Female	>62 (>0,7)	$166 \times (S_{cr}/0,7)^{-1,209} \times (0,993)^{Age}$
Black	Male	≤80 (≤0,9)	$163 \times (S_{cr}/0,9)^{-0,411} \times (0,993)^{Age}$
Black	Male	>80 (>0,9)	$163 \times (S_{cr}/0,9)^{-1,209} \times (0,993)^{Age}$
White or other	Female	≤62 (≤0,7)	$144 \times (S_{cr}/0,7)^{-0,329} \times (0,993)^{Age}$
White or other	Female	>62 (>0,7)	$144 \times (S_{cr}/0,7)^{-1,209} \times (0,993)^{Age}$
White or other	Male	≤80 (≤0,9)	$141 \times (S_{cr}/0,9)^{-0,411} \times (0,993)^{Age}$
White or other	Male	>80 (>0,9)	$141 \times (S_{cr}/0,9)^{-1,209} \times (0,993)^{Age}$

A.6.1 Estimation of uncertainty of estimated glomerular filtration rate, u(eGFR)

The uncertainty of eGFR results, u(eGFR), as determined with any of the published estimating formulae is due predominately to the measurement of serum/plasma creatinine. Medical laboratories may therefore choose to estimate u(eGFR) by combining the uncertainties for u_{Rw}(crea) and u_{cal} (crea). Alternatively, laboratories may choose to directly estimate u(eGFR) as calculated and reported to reflect the expected variability of eGFR results as reported by the laboratory. This approach will require IQC values for serum or plasma creatinine measurements to first be converted to eGFR values, which will capture the uncertainty of creatinine measurement under long-term precision conditions, u_{Rw}(crea), as well as the uncertainty of the values assigned to the creatinine calibrator, u_{cal}(crea), and will also capture any uncertainties caused by the mathematical calculation of results and how the laboratory IT system handles number rounding.

An estimate of the laboratory measurement contribution to u(eGFR) can be achieved by utilising serum/plasma creatinine IQC data applied to a 'phantom' individual. An estimate of u(eGFR) can be obtained by:

— Selecting one of the above eGFR formulae (Table A.8) and creating a 'phantom' subject by randomly choosing ethnicity, gender and age;

— Enter the selected formula and 'phantom' subject parameters into an appropriate spreadsheet;

— Copy across from the IQC record sufficient serum/plasma creatinine values obtained for a selected IQC level obtained under long-term precision conditions;

— Calculate the eGFR for all the IQC serum/plasma creatinine values;

— Calculate the mean and SD of the eGFR values. Take the SD as being the u_{Rw}(eGFR);

— Combine u_{Rw}(eGFR) with u_{cal}(crea) in the usual manner.

This approach does not consider contributing uncertainties associated with determining the eGFR formula itself, such as subject age and effect of multiplier terms applied in the eGFR formula. The formulae to calculate eGFR also have an uncertainty for an individual result when compared to a measured GFR that is in addition to the uncertainty from the creatinine measurement in the medical laboratory. Inspection of the data for individual subjects measured GFR values used to estimate the best-fit function to calculate eGFR reveals very significant scatter, so it should be understood by medical practitioners that for individual human subjects that eGFR values could be up to approximately ±30 % of the measured GFR value. Consequently, the uncertainty of the eGFR for the laboratory measurement contribution to a given eGFR value on an individual subject underestimates the overall uncertainty in that it does not account for the uncertainty of the estimating formula.

A.6.2 Examples of estimated glomerular filtration formulas in a format suitable for processing with a typical commercial spreadsheet

For the complex calculation of u(eGFR), the process is best conducted using a commercial spreadsheet, an example of which is shown in Table A.9. For specific table columns referenced in the worked examples below, also refer to Table A.9.

eGFR formula for females (caucasian):

IF(C2<63, 144*(C2*0,011 3/0,7)^−0,329*0,993^40, 144*(C2*0,011 3/0,7)^−1,209*0,993^40) (A.9)

Formula (A.9) calculates eGFR for a caucasian female aged 40 years where a set of IQC serum/plasma creatinine concentrations are ≤62 µmol/l, and separately calculates for a set of IQC serum/plasma creatinine concentrations >62 µmol/l (the first part of each formula is triggered if the creatinine concentration is <stated value; otherwise the second part of the formula is activated).

eGFR formula for males (caucasian):

IF(C2<81, 141*(C2*0,011 3/0,9)^−0,411*0,993^40, 141*(C2*0,011 3/0,9)^−1,209*0,993^40) (A.10)

Formula (A.10) calculates eGFR for a caucasian male aged 40 years where a set of IQC serum/plasma creatinine concentrations are ≤80 µmol/l, and separately for a set of IQC plasma/serum creatinine concentrations >80 µmol/l (the first part of each formula is triggered if the creatinine concentration is <stated value; otherwise the second part of the formula is activated).

Explanatory Notes – Table A.9

a) If serum/plasma creatinine concentration is reported in:

   — µmol/l, use Formulae (A.9), (A.10) as presented here

   — mg/dl, omit factor 0,011 3 from Formulae (A.9) and (A.10)

b) C2*0,011 3 = conversion of IQC creatinine values (expressed as µmol/l) located in each cell of column C by factor 0,0113 to express creatinine value as mg/dl for use with the other components of the formula.

c) Formula notation C2 in the below example identifies the data cell that happens to contain the first result of a set of IQC serum/plasma creatinine concentrations in µmol/l.

d) Column C: The spreadsheet was set up to calculate the eGFR for each of 1 699 IQC level 1 creatinine values collected over approximately one year for a 'phantom' female subject aged 40 with a serum/plasma creatinine ≥63 µmol/l.

e) Column D: The eGFR calculated for the first five IQC creatinine values is shown.

f) Column E: The formula was replaced (not shown) by that for males (Caucasian) aged 40 years, and the eGFR calculated for the same IQC data set.

g) Column G: 1741 values for serum/plasma creatinine IQC level 2.

h) Column H: Calculated eGFR values using the same formula as for column D.

i) Column I: Calculated eGFR values using the same formula as for column E.

Table A.9 — Example spreadsheet for calculating *u*(eGFR)

SUM =IF(C2<63,144*(C2*0.0113/0.7)^-0.329*0.993^40,144*(C2*0.0113/0.7)^-1.209*0.993^40)

	A	B	C	D	E	F	G	H	I	J	K	L
1	seq no	date	serum IQC creat Level 1	eGFR female >62 sCr	eGFR male >80 sCr		serum IQC creat Level 2	eGFR female >62 sCr	eGFR male >80 sCr			
2	1	28/07/2014	79.9	79.9	106.3		552.4	7.7	10.2			
3	2	29/07/2014	82.3	77.1	102.3		537.0	8.0	10.6			
4	3	29/07/2014	82.2	77.2	102.5		540.2	7.9	10.5			
5	4	29/07/2014	82.9	76.4	101.4		542.5	7.9	10.5			
6	5	29/07/2014	80.9	78.7	105.8		540.1	7.9	10.5			

Table A.10 shows the mean serum/plasma creatinine values and the mean of the eGFR values calculated as above from 1699 estimations of creatinine IQC level 1 and 1741 estimations of creatinine IQC level 2 obtained under long-term precision conditions over approximately one year.

Table A.10 — Mean creatinine and mean eGFR values for IQC level 1 and level 2, with associated uncertainties

	IQC level 1			IQC level 2		
Period	**28Jul2014-21Aug2015**					
N	1699			1 741		
	Plasma creatinine	eGFR female, 40 yrs	eGFR male, 40 yrs	Plasma creatinine	eGFR female, 40 yrs	eGFR male, 40 yrs
Mean	82,0 µmol/l	77,5 ml/min 1,73 m^2	103,0 ml/min 1,73 m^2	533,9 µmol/l	8,0 ml/min 1,73 m^2	10,7 ml/min 1,73 m^2
u_{Rw}	1,80 µmol/l	2,00 ml/min 1,73 m^2	2,70 ml/min 1,73 m^2	9,10 µmol/l	0,20 ml/min 1,73 m^2	0,20 ml/min 1,73 m^2
U_{Rw}(eGFR) ml/min 1,73 m^2; k = 2, ≈95 % confidence		4,00	5,00		0,40	0,40
% u_{Rel}		2,00/77,5 × 100 = 2,580 65 % = 2,6 %	2,7/103,0 × 100 = 2,621 36 % = 2,6 %		0,20/8,0 × 100 = 2,500 00 % = 2,5 %	0,20/10,7 × 100 = 1,869 16 % = 1,9 %
% U_{Rel}(eGFR) ml/min 1,73 m^2; k = 2, ≈95 % confidence		5,161 13 % = 5,2 %	5,242 72 % = 5,2 %		5,000 00 % = 5,0 %	3,738 31 % = 3,7 %,

The results of the example are listed below.

Patient (female, caucasian):

eGFR result = 80 ml/min 1,73 m^2.

U(eGFR) = 80 × 5,161 3/100 = ±4,129 0 ml/min 1,73 m^2 = ±4,13 ml/min 1,73 m^2 (k = 2; ≈95 % level of confidence)

Patient (male, caucasian):

eGFR result = 11 ml/min 1,73 m^2.

U(eGFR) = 11 × 3,738 31/100 = ±0,411 2 ml/min 1,73 m^2 = ±0,41 ml/min 1,73 m^2 (k = 2; ≈95 % level of confidence)

As noted above, this estimate of uncertainty only represents the contribution of the laboratory measurement itself and does not include uncertainty from the formula parameters derived from a large population, which would contibute significant additional uncertainty when applied to an individual subject. Estimation of $u(y)$ for eGFR based on creatinine IQC values alone is a minor component of the overall uncertainty of eGFR as used for medical applications.

A.7 Estimation of expanded uncertainty for number concentration of white blood cells in whole blood

Table A.11 shows a worked example for the calculation of estimated uncertainty of white blood cell counts in whole blood.

Table A.11 — Worked example – Uncertainty of white blood cell counts

Component (Analyte)	White blood cells (WBC)		
Measurand	Number concentration of white blood cells in whole blood		
Measurement unit	10^9/l		
Measurement method	Impedance		
Measurement procedure	Instrument 2; TY Manufacturer, see Method manual		
Calibrator traceability	ICSH recommended reference measurement procedure		
Calibrator uncertainty u_{cal}	0,038 × 10^9/l – provided by manufacturer		
Bias:	Assessed by EQA: average bias within target		
Long-term precision			
IQC material:	Stabilised WBC, supplied by XYZ IVD manufacturer		
	Level 1	**Level 2**	**Level 3**
Period	August 2015		
IQC lot	880200	870100	868800
N	66	62	60
Mean; 10^9/l	9,1	19,7	3,8
u_{Rw}, 10^9/l	0,105	0,280	0,115
u(WBC) = $\sqrt{(u^2_{cal} + u^2_{Rw})}10^9$/l	0,111 66 = 0,112	0,282 57 = 0,283	0,121 12 = 0,121
Period	September 2015		
IQC lot	880800	870700	869100
N	64	66	69
Mean, 10^9/l	9,0	21,2	3,5
u_{Rw}, 10^9/l	0,125	0,275	0,130
u(WBC) = $\sqrt{(u^2_{cal} + u^2_{Rw})}10^9$/l	0,130 66 = 0,131	0,277 613 = 0,278	0,135 44 = 0,135
Period	October 2015		
IQC lot	889900	879700	869700
N	63	68	65
Mean, 10^9/l	9,2	20,4	3,5
u_{Rw}, 10^9/l	0,130	0,255	0,115
u(WBC) = $\sqrt{(u^2_{cal} + u^2_{Rw})}10^9$/l	0,135 44 = 0,135	0,257 82 = 0,258	0,121 11= 0,121
Pooled average uncertainty (unweighted)			
u(WBC): Aug, Sept, Oct: 10^9/l	0,112, 0,131, 0,135	0,283, 0,278, 0,258	0,121, 0,135, 0,121

Table A.11 *(continued)*

Component (Analyte)	White blood cells (WBC)		
Variance = u^2(WBC)	0,012 468; 0,017 072; 0,018 344	0,079 846; 0,077 069; 0,066 471	0,014 670; 0,018 344; 0,014 668
Sum of variances	0,047 884	0,223 386	0,047 682
Average variance	0,015 961	0,074 462	0,015 894
Pooled average u(WBC) = √average variance	0,126 34 × 10^9 /l	0,272 88 × 10^9 /l	0,126 07 × 10^9 /l
% u_{rel} at IQC among lot (n = 3) mean values of 9,1; 20,4 and 3,6	(0,126 34/9,1) × 100 = 1,388 35 = 1,4 %	(0,272 88/20,4) × 100 = 1,337 64 = 1,3 %	(0,126 07/3,6) × 100 = 3,501 98 = 3,5 %
Allowable u_{rel}(WBC) based on within-individual biological variation (CV_W) ≤12,0 %.	Meets optimum performance specification of <3,0 %	Meets optimum performance specification of <3,0 %	Meets desirable performance specification of <6,0 %
Pooled U, k = 2	0,126 34 × 2 = 0,252 68 = 0,253	0,272 88 × 2 = 0,545 76 = 0,546	0,126 07 × 2 = 0,252 14 = 0,252
% $U_{rel;}$ k = 2, ≈5 % level of confidence	1,388 35 × 2 = 2,77 67 = 2,8 %	1,337 64 × 2 = 2,675 28 = 2,7 %	3,501 98 × 2 = 7,003 96 = 7,0 %
Applied to patients' results	±2,8 %	±2,7 %	±7,0 %

A.8 Estimation of uncertainty of amount of substance concentration of albumin in serum/plasma – comparing uncertainty estimates using relative uncertainties vs. standard uncertainties

Caution should be exercised when combining standard uncertainties, where the amount of substance is significantly different among the uncertainty components being combined. As illustrated in A.8.1, Table A.12 and A.8.2, Table A.13, different uncertainty estimates % U_{rel} for measurement of serum/plasma albumin are obtained when uncertainty is calculated by combining standard uncertainties compared to combining relative uncertainties. The differences in the estimated % U_{rel} are especially noticeable for albumin >35,0 g/l (±4,7 % when estimated using standard uncertainty vs. ±6,2 % when using relative uncertainties).

In this example, the calibrator's assigned value (≈24 g/l) is much closer to the Level 1 IQC (≈28 g/l) than the Level 2 IQC (≈42 g/dl). When combining the standard uncertainties (Table A.12) instead of the relative uncertainties (Table A.13), the same absolute standard uncertainty associated with the calibrator (≈0,58 g/l) is combined with the standard uncertainty for both levels of IQC, even though the amount of substance is quite different. Combining standard uncertainties from different uncertainty components across significantly different concentrations of substance assumes that absolute variation is approximately constant across the range of concentrations. Combining relative uncertainties instead assumes that relative variation is approximately constant. With the relative uncertainty method of estimation as shown in Table A.13, a better estimate of overall uncertainty is achieved because the $u_{rel(Rw)}$ is similar (0,020 and 0,019 g/l respectively) at both levels of IQC, suggesting that the measurement procedure exhibits a constant CV. The standard uncertainty method (Table A.12), which assumes consistent absolute variation at all concentrations, underestimates the contribution of the uncertainty of the calibrator at higher albumin concentrations, leading to underestimation of the % U_{rel} for albumin >35,0 g/l.

A.8.1 Uncertainty of amount of substance concentration of albumin in serum/plasma calculated with standard uncertainties

Table A.12 describes a method for estimation of % U_{rel} for the amount of substance concentration of albumin in serum/plasma using standard uncertainties, u.

Table A.12 — Estimating % U_{rel} for serum/plasma albumin using standard uncertainties, u

Component (Analyte)	Albumin	
Measurand	Amount of albumin concentration in serum/plasma	
Measurement unit	g/l	
Measurement method	Colorimetric: Bromocresol purple end-point spectrophotometry	
Measurement procedure	Instrument 2 and commercial kit from manufacturer Y	
Calibrator traceability	ERM-DA470k/IFCC reference preparation	
Bias (g/l)	Assessed by EQA using commutable material with target value set by accredited reference laboratory: average bias within target (<0,2 g/l): no correction required	
Long-term precision		
Period	February 2014 - March 2015	
Calibrator lot; concentration	4570; 23,7 g/l	
Calibrator uncertainty u_{cal}	0,583 g/l (from manufacturer)	
IQC	Level 1	Level 2
Monitors interval (g/l)	≤35,0	>35,0
IQC lot	6792	5389
N	1 390	1 414
Mean albumin value, g/l	28,32	42,16
u_{Rw} g/l	0,586	0,780
$u(\text{Alb}) = \sqrt{(u^2_{cal} + u^2_{Rw})}$ g/l	$\sqrt{(0{,}583^2 + 0{,}586^2)} = 0{,}826\ 61 = 0{,}827$	$\sqrt{(0{,}583^2 + 0{,}780^2)} = 0{,}973\ 80 = 0{,}974$
Period	April 2015 - March 2016	
Calibrator lot; concentration	4779; 23,8 g/l	
Calibrator uncertainty u_{cal}	0,574 g/l (from manufacturer)	
IQC lot	6913	5876
N	1 216	1 290
Mean albumin value, g/l	27,14	41,22
u_{Rw} g/l	0,624	0,811
$u(\text{Alb}) = \sqrt{(u^2_{cal} + u^2_{Rw})}$ g/l	$\sqrt{(0{,}574^2 + 0{,}624^2)} = 0{,}847\ 85 = 0{,}848$	$\sqrt{(0{,}574^2 + 0{,}811^2)} = 0{,}993\ 58 = 0{,}994$
Pooled average uncertainty u(Alb) – unweighted		
Pooled average u(Alb) g/l	$\sqrt{((0{,}82\ 661^2 + 0{,}847\ 85^2)/2)}$ $= 0{,}837\ 30 = 0{,}837$	$\sqrt{((0{,}973\ 80^2 + 0{,}993\ 58^2)/2)} = 0{,}983\ 74 = 0{,}984$
% u_{rel} at IQC mean value	$(0{,}837\ 30/27{,}7) \times 100$ $= 3{,}022\ 74 = 3{,}0$ %	$(0{,}983\ 74/41{,}69) \times 100 = 2{,}359\ 65 = 2{,}4$ %
Allowable % u_{rel} (Alb) based on ≤75 % of within-individual biological variation (CV_I) ≤3,2 % = ≤2,4 %	Not acceptable	Borderline acceptable
% U_{rel}, k = 2; ≈95 % confidence	$3{,}02\ 274 \times 2$ $= 6{,}045\ 48 = 6{,}0$ %	$2{,}359\ 65 \times 2 = 4{,}719\ 30 = 4{,}7$ %
Applied to patients' results	±6,0 %	±4,7 %

A.8.2 Uncertainty of amount of substance concentration of albumin in serum/plasma calculated using relative standard uncertainties, u_{rel}

Table A.13 describes a method for estimation of % U_{rel} for the amount of substance concentration of albumin in serum or plasma using relative standard uncertainties, u_{rel}.

Table A.13 — Estimating % U_{rel} for serum/plasma albumin using relative standard uncertainties, u_{rel}

Component (Analyte)	Albumin	
Measurand	Amount of albumin concentration in serum/plasma	
Measurement unit	g/l	
Measurement method	Colorimetric: Bromocresol purple end-point spectrophotometry	
Measurement procedure	Instrument 2 and commercial kit from manufacturer Y	
Calibrator traceability	ERM-DA470k/IFCC reference preparation	
Bias	Assessed by EQA using commutable material with target value set by accredited reference laboratory; average bias within target (≤0,2 g/l); no correction required	
Long-term precision		
IQC	Level 1	Level 2
Monitors interval – (g/l)	≤35,0	>35,0
Period	**February 2014 - March 2015**	
Calibrator lot; concentration	4570; 23,7 g/l	
Calibrator uncertainty u_{cal}	0,583 g/l (from manufacturer)	
Relative standard uncertainty of calibrator $u_{rel(cal)}$	0,583/23,7 = 0,024 599 = 0,024 6	
IQC lot	6792	5389
n	1 390	1 414
Mean albumin value, g/l	28,32	42,16
u_{Rw} g/l	0,586	0,780
Relative standard uncertainty $u_{rel(Rw)}$	0,586/28,32 = 0,020 692 = 0,020 7	0,780/42,16 = 0,018 501 = 0,018 5
$u_{rel}(Alb) = \sqrt{(u_{rel(cal)}^2 + u_{rel(Rw)}^2)}$	$\sqrt{(0,024\,599^2 + 0,020\,692^2)}$ = 0,032 145 = 0,032 1	$\sqrt{(0,024\,599^2 + 0,018\,501^2)}$ = 0,030 779 8 = 0,030 8
Period	**April 2015-March 2016**	
Calibrator lot; concentration	4779; 23,8 g/l	
Calibrator uncertainty u_{cal}	0,574 g/l (from manufacturer)	
Relative standard uncertainty of calibrator $u_{rel(cal)}$	0,574/23,8 = 0,024 118 = 0,024 1	
IQC lot	6913	5876
n	1 216	1 290
Mean albumin value, g/l	27,14	41,22
u_{Rw} g/l	0,624	0,811
Relative standard uncertainty $u_{rel(Rw)}$	0,624/27,14 = 0,022 992 = 0,023 0	0,811/41,22 = 0,019 675 = 0,019 7
$u_{rel}(Alb) = \sqrt{(u_{rel(cal)}^2 + u_{rel(Rw)}^2)}$	$\sqrt{(0,024\,118^2 + 0,022\,992^2)}$ = 0,033 321 = 0,033 3	$\sqrt{(0,024\,118^2 + 0,019\,675^2)}$ = 0,031 125 = 0,031 1
Pooled average uncertainty u_{rel}(Alb) – unweighted		
Pooled average % u_{rel} (Alb)	$\sqrt{((0,032\,145^2 + 0,033\,321^2)/2)}$ = 0,032 738 × 100 = 3,273 8 = 3,3 %	$\sqrt{((0,030\,779\,8^2 + 0,031\,125^2)/2)}$ = 0,030 952 9 × 100 = 3,095 29 = 3,1 %
Allowable % u_{rel} (Alb) based on ≤75 % of within-individual biological variation (CV_I) ≤3,2 % = ≤2,4 %	Not Acceptable	Not Acceptable
% U_{rel}, k = 2; ≈95 % confidence	6,547 6 = 6,5 %	6,190 6 = 6,2 %
Applied to patients' results	±6,5 %	±6,2 %

A.9 Calculating % U_{rel} for international Normalised Ratio

A worked example for calculation of % U_{rel} for international normalised ratio (INR) measurements is given in Table A.14. For the example shown in Table A.14, the following notations apply:

— INR = (Sample prothrombin time/Mean Normal Prothrombin time)^{ISI}

— $u(INR) = \sqrt{[ISI^2 \times (u^2(MNCT) + u^2_{Rw})]}$

— Relative standard uncertainty: $u_{rel}(INR) = \sqrt{[ISI^2 \times (u^2_{rel}(MNCT) + u^2_{rel(Rw)})]}$. [see A.2.4, Rule 2.]

Table A.14 — Worked example — % U_{rel} for INR measurements

Component (Analyte)	Biological activity of clotting factors VII, X, V, II, fibrinogen	
Measurand	Tissue factor-induced relative time to form clot in citrated plasma	
Measurement unit	Second	
Measurement method	Mechanical clot detection	
Measurement procedure	Instrument 1; commercial kit from manufacturer T	
Allowable standard measurement uncertainty derived from prothrombin time (PT) within-individual biological variation in adults	Minimum ≤ % CV_I performance specification ≤2,4 %	
Calibrator traceability	Human recombinant thromboplastin WHO 4th IS 2009	
Period	**July 2013- Jan 2014**	
Thromboplastin lot no.	1112	1113
International Sensitivity Index (ISI)	1,31	
Bias	EQA derived: within allowable specification: no action	
Mean Normal Clotting Time (MNCT); sec	n = 20 13,3	
u(MNCT), sec	0,53	
% u_{rel}(MNCT)	(0,53/13,3) × 100 = 3,984 96 = 4,0 %	
Long-term precision		
IQC	Level 1	Level 2
Monitors interval – sec	≤20	>20
IQC lot	3167	3168
n	1 682	1 671
Mean PT time; sec	13,2	25,3
u_{Rw}(PT); sec	0,30	0,48
% $u_{rel(Rw)}$(PT) Goal: ≤2,4 %	(0,30/13,2) ×100 = 2,272 73 = 2,27 % Acceptable	(0,48/25,3) × 100 = 1,897 23 = 1,90 % Acceptable
u^2_{rel}(INR) = $ISI^2 \times [u^2_{rel}(MNCT) + u^2_{rel}]$	$1{,}31^2 \times (3{,}984\ 96^2 + 2{,}272\ 73^2)$ = 36,115 68	$1{,}31^2 \times (3{,}984\ 96^2 + 1{,}897\ 23^2)$ = 33,428 58
% u_{rel}(INR)	√36,115 68 = 6,009 63 % = 6,0 %	√33,428 58= 5,781 75 = 5,8 %
% U(INR), k = 2	12,001 93 = 12,0 %	11,563 50 =11,6 %
Applied to patients' results	±12,0 %	±11,6%
Period	**Feb 2014-March 2015**	
Thromboplastin lot no.	1245	1246
International Sensitivity Index (ISI)	1,26	
Bias	EQA derived: within allowable specification: no action	

Table A.14 *(continued)*

Component (Analyte)	Biological activity of clotting factors VII, X, V, II, fibrinogen	
Mean Normal Clotting Time (MNCT); sec	n = 20 13,0	
u(MNCT); sec	0,51	
% u_{rel}(MNCT)	(0,51/13,0) × 100 = 3,923 08 = 3,92 %	
Long-term precision		
IQC	Level 1	Level 2
Monitors interval – sec	≤20	>20
IQC lot	3336	3338
n	2 865	2 854
Mean PT time (sec)	13,1	25,0
u_{Rw}(PT); sec	0,32	0,51
% $u_{rel(Rw)}$(PT) %	(0,32/13,1) × 100 = 2,44 275 = 2,44 % Borderline acceptable	(0,51/25,0) × 100 =2,040 00 = 2,04 % Acceptable
u^2_{rel}(INR) = ISI^2 × [u^2_{rel}(MNCT) + u^2_{rel}]	1,26^2 × (3,923 08^2 + 2,442 75^2) = 33,907 30	1,26^2 × (3,923 08^2 + 2,040 00^2) = 31,041 00
u_{rel}(INR) % = $\sqrt{u^2_{rel}}$(INR)	$\sqrt{33,907\ 30}$ = 5,823 00 = 5,8 %	$\sqrt{31,041\ 00}$ = 5,571 45 = 5,6 %
% U(INR), k = 2	11,646 00 = 11,6 %	11,142 90 % = 11,1 %
Applied to patients' results	±11,6 %	±11,1 %

A.10 Uncertainty for a human immunodeficiency virus type 1 viral load measurement

Table A.15 shows a worked example for calculation of the estimated uncertainty of human immunodeficiency virus type 1 (HIV-1) viral load measurements.

Table A.15 — Worked example — Uncertainty of HIV-1 viral load measurements

Component (Analyte)	Human immunodeficiency virus type 1 (HIV-1) RNA		
Measurand	Number of HIV-1 RNA copies concentration in plasma		
Measurement unit	Log_{10} HIV-1 RNA copies/ml		
Measurement method	cDNA PCR amplification of cDNA and quantitation by detection by intensity of fluorescence emission		
Measurement procedure	HIV-1 Test, D Diagnostics Pty Ltd.		
Allowable standard measurement uncertainty	< ± 0,5 log_{10} result		
Calibrator traceability	WHO 1st International Standard for HIV-1 RNA for nucleic acid-based techniques (NIBSC 97/656)		
Calibrator uncertainty, u_{cal}	Not stated		
Bias	≤0,25 log_{10} EQA peer group weighted mean: - acceptable		
Reported values	Whole number		
Reference values	<1,602 1 (40 copies/ml) not detected 1,602 1 to 1,00E+7 (40 copies –10 million copies/ml) result value reported >1,00E+7 (10 million copies/ml) reported as >10 million copies/ml		
Long-term precision			
	Level 1	Level 2	Level 3

Table A.15 *(continued)*

Component (Analyte)	Human immunodeficiency virus type 1 (HIV-1) RNA		
IQC lot No.	443	453	463
N	34	73	19
Mean $\log_{10}$ copies/ml Copies/ml	2,66 457	3,18 1 514	4,21 16 218
$\text{Log}_{10}u$	0,120	0,160	0,230
$\text{Log}_{10}u_{\text{rel}}$	0,120/2,66 = 0,045	0,160/3,18 = 0,050	0,230/4,21 = 0,055
U, $\log_{10}$ copies/ml, *k* = 2, ≈95 % level of confidence *U*, copies/ml	0,120 × 2 = 0,240 2,66 ± 0,240 = 2,42 – 2,90 263 – 794	0,160 × 2 = 0,320 3,18 ± 0,320 = 2,86 – 3,50 724 – 3 162	0,23 × 2 = 0,46 4,21 ± 0,460 = 3,75–4,67 5 623 – 46 774
Allowable *U*: < ± 0,5 $\log_{10}$ of mean IQC Range $\log_{10}$ copies/ml Copies/ml	2,66 ± 0,5 2,16–3,16 145–1 445	3,18 ± 0,5 2,68–3,68 479 – 4 786	4,21 ± 0,5 3,71–4,71 5 129 – 51 286
Fit for purpose	Acceptable	Acceptable	Acceptable

A.11 Uncertainty of *BCR-ABL1* measurement using one lot of internal quality control material

BCR-ABL1 is an abnormal gene fusion whose protein product is an activated tyrosine kinase that causes chronic myeloid leukaemia. Measurement of the *BCR-ABL1* RNA transcript level during treatment has medical relevance for patient outcome and the *BCR-ABL1* level is used for change of treatment decisions.

The following worked example for MU estimation (Table A.16) is for a molecular test measuring BCR-ABL1 fusion gene transcripts on an international reporting scale (IS) by qRT-PCR. The measurement procedure involves extraction of RNA, conversion of RNA to complementary DNA (cDNA) using reverse transcription and quantitative polymerase chain reaction (qRT-PCR). *BCR-ABL1* RNA is extracted from white cells derived from peripheral blood or bone marrow. *BCR-ABL1* transcripts rapidly degrade and RNA stabilisation is required to limit degradation after sample collection.

BCR-ABL1 transcripts are reported as a percentage ratio to a second transcript termed a control gene. A control gene is measured to compensate for differences in the degradation status between samples and for differences in the reverse transcription process. IQC samples undergo all processes required to generate the reportable *BCR-ABL1* value except for lysis of red cells, which is not applicable since cells are used that are pre-stabilised in an RNA stabilisation solution. However, the major sources of variability related to characterisation, potential between-unit heterogeneity, and potential degradation during transport and long-term storage were combined to estimate the relative expanded (*k* = 2) uncertainty (% U_{rel}) and are captured by including QC in every batch.

Table A.16 — Worked example — Uncertainty of *BCR-ABL1* gene transcript measurement

Component	*BCR-ABL1* fusion gene transcripts (RNA)
Measurand	% ratio of BCR-ABL1 fusion gene transcripts to a control gene (ABL1) transcript level in peripheral blood or bone marrow
Measurement unit	% ratio on the International Scale (IS)
Measurement method	Reverse transcription followed by fluorescence-based quantitative real-time PCR of cDNA
Measurement procedure	Manufacturer X BCR-ABL1 assay kit

Table A.16 *(continued)*

Component	*BCR-ABL1* fusion gene transcripts (RNA)	
Allowable standard measurement uncertainty: BCR-ABL1 biological variation-based	≤2-fold at the medically relevant level of 0,1 % IS, which is a major molecular response; ≤5-fold at lower levels	
Calibrator traceability	Certified reference materials: ERM®-AD623a, ERM®-AD623b, ERM®-AD623c, ERM®-AD623d, ERM®-AD623e, ERM®-AD623f	
Calibrator uncertainty [cp/µl]	Not applicable. Calibrator contains the sequence for both of the genes in order to measure the raw transcript values. The calibrator uncertainty cannot be expressed as a ratio and is omitted from the measurement uncertainty calculation.	
Bias	Corrected to the IS by kit manufacturer and traceable to the 1st WHO International Genetic Reference Panel for quantitation of *BCR-ABL* translocation by RQ-PCR (NIBSC code: 09/138WHO). Bias medically irrelevant based on EQA performance	
Reported values	Deep molecular responses are reported according to recommended control gene cut-off values[29]	
Reference values	The dynamic range of the IS is 10 % IS and below. According to international recommendations, values below 10 % IS, 1 % IS and 0,1 % IS at 3, 6 and 12 months of tyrosine kinase inhibitor therapy indicate an optimal response and a change of therapy is not required	
IQC	Level 1 low control	Level 2 high control
Period	1/12/12 to 9/6/13	
Lot No.	L/11	H/11
N	150	150
Mean (BCR-ABL1/ABL1 % IS)	0,06	15,00
u(BCR-ABL1/ABL1 % IS)	0,012	2,200
U(BCR-ABL1/ABL1 % IS) *k* = 2, ≈95 % level of confidence	0,024	4,400
% U_{rel}	20,0 %	14,7 %
Fit for purpose	Acceptable	Acceptable

A.12 Uncertainty of rubella IgG antibody measurement

For rubella IgG antibody measurement, values obtained for each successive IQC lot were separately collected for calculation of u_{Rw}(Rub). The full worked example and calculations for the uncertainties are shown in Table A.17.

Table A.17 — Worked example — Estimation of uncertainty of rubella IgG antibody measurement

Component (Analyte)	IgG antibodies to rubella virus
Measurand	Reactivity of IgG antibodies in plasma/serum to rubella virus antigen
Measurement method	Sandwich immunoassay with detection by direct chemiluminescence
Measurement procedure	Manufacturer E, according to manufacturer's instructions – see method manual
Measurement unit	kIU/l – arbitrary units
Calibrator traceability	WHO 1st IS for human anti-rubella immunoglobulin (RUBI-1-94)
[a] Selected % *U* value applied dependent on patient result relative to IQC interval covered.	

Table A.17 *(continued)*

Component (Analyte)	IgG antibodies to rubella virus		
Bias	0,28 kIU/l – assessed relative to EQA peer group mean (cycle 1/01/15-30/06/15). Acceptable – No action		
Reference values	<5,0 IU/ml – Not detected; 5,0-9,9 IU/ml – Equivocal; ≥10,0 IU/ml – Detected		
Maximum allowable standard measurement uncertainty	Within best 20 % of EQA peer group		
Long-term precision			
Period	11/12/15- 14/03/16		
Calibrator lot 378 Reagent lot 640	Cal 1: 7,0, U_{cal} = 0,188 kIU/l, k = 2, % U_{cal} = 2,685 71 = 2,7 % Cal 2: 400, U_{cal} = 5,607 kIU/l, k = 2, % U_{cal} =1,401 75 = 1,4 %		
IQC	Level 1	Level 2	Level 3
Monitors	5,0-9,9 kIU/ml	10,0-50,0 kIU/ml	>50,0 kIU/ml
IQC lot	51333	53655	54778
N	68	68	68
Mean, kIU/ml	8,11	19,97	122,33
u_{Rw}(Rub) kIU/ml	0,610	1,440	8,440
U_{Rw}; k = 2	1,220	2,880	16,880
% U_{Rw}	(1,22/8,11) × 100 = 15,043 16 = 15,0 %	(2,88/19,97) × 100 = 14,421 63 = 14,4 %	(16,88/122,33) × 100 = 13,798 74 = 13,8 %
% U(Rub) = $\sqrt{(\%U^2_{cal} + \% U^2_{Rw})}$ ≈95 % level of confidence	$\sqrt{(2,685\ 71^2 + 15,043\ 16^2)}$ = 15,281 00 % = 15,3 %	$\sqrt{(2,685\ 71^2 + 14,421\ 63^2)}$ = 14,669 58 = 14,7 %	$\sqrt{(1,401\ 75^2 + 13,798\ 74^2)}$ = 13,869 76 = 13,9 %
Period	**15Mar16-13Jun16**		
Calibrator lot 413 Reagent lot 765	Cal 1: 7,4, U_{cal} = 0,198 kIU/l, k = 2, % U_{cal} = 2,675 68 = 2,7 % Cal 2: 357, U_{cal} = 5,311 kIU/l, k = 2, % U_{cal} = 1,487 68 = 1,5 %		
IQC lot	52661	53690	54394
N	85	85	76
Mean, kIU/ml	7,5	21,3	118,9
u_{Rw}(Rub) kIU/ml	0,53	1,75	10,36
U_{Rw}; k = 2	1,06	3,50	20,72
% U_{Rw}	(1,06/7,5) × 100 = 14,133 33 = 14,1 %	(3,5/21,3) × 100 = 16,431 92 = 16,4 %	(20,72/118,9) × 100 = 17,426 41 = 17,4 %
% U(Rub) = $\sqrt{(\%\ U^2_{cal} + \%\ U^2_{Rw})}$ ≈95 % level of confidence	$\sqrt{(2,675\ 68^2 + 14,133\ 33^2)}$ = 14,384 38 = 14,4 %	$\sqrt{(2,675\ 68^2 + 16,431\ 92^2)}$ = 16,648 34 = 16,6 %	$\sqrt{(1,487\ 68^2 + 17,426\ 41^2)}$ = 17,489 80 = 17,5 %
Pooled % U for 2 reagent Lots % U(Rub)	$\sqrt{[(15,281\ 00^2 + 14,384\ 38^2)/2]}$ = 14,839 46 = 14,8 %	$\sqrt{[(14,669\ 58^2 + 16,648\ 34^2)/2]}$ = 15,690 18 = 15,7 %	$\sqrt{[(13,869\ 76^2 + 17,489\ 80^2)/2]}$ = 15,783 91 = 15,8 %
Applied to patients' results[a]	±14,8 %	±15,7 %	±15,8 %

[a] Selected % U value applied dependent on patient result relative to IQC interval covered.

NOTE The statistical treatment of u_{cal} is complex when a calibration curve is based on more than a single calibrator value. For practical purposes, as in the above case, a professional judgement can be made on selecting the calibrator that is considered to be most applicable to each level of IQC. It shows that u_{cal} makes a trivial contribution to % U(Rub).

A.13 Uncertainty of hepatitis B surface antigen measurement

This automated measurement procedure for hepatitis B surface antigen (HBsAg) uses matched calibrator and reagent lots which, depending on workload, are changed at 2 to 4 month intervals. In this worked example (Table A.18), there is one IQC lot change for each level, necessitating the separate collection and calculation of IQC values. If the same IQC lot had been used for both lots of calibrator and reagent, the IQC data could have been treated as a single data set.

Table A.18 — Worked example — Estimation of uncertainty for Hepatitis B surface antigen measurement

Component (Analyte)	HBsAg	
Measurand	Reactivity of HBsAg in serum/plasma to hepatitis B virus surface antibody	
Measurement unit	Sample/Cut-off ratio (S/Co) = Index value	
Measurement method	Sandwich immunoassay with detection by direct chemiluminescence	
Measurement procedure	Manufacturer F	
Calibrator traceability	Traceable to WHO IRS 00/588	
Bias, Index value	0,17 - assessed relative to EQA peer group mean (cycle 1/03/15-30/11/15): Acceptable – No action	
Reference values	S/Co <1 Negative ≥1 to ≤50 – Positive	
Allowable standard measurement uncertainty	Within best 20 % of EQA peer group	
Long-term precision		
Period	**09 Mar 15-15 Jun 15**	
Calibrator lot 431 Reagent lot 431	% U_{cal} = 4,0 %, k=2	
IQC	Level 1	Level 2
Lot no.	327	337
N	73	73
Mean Index value	1,38	5,48
u_{Rw}(HBsAg)	0,080	0,360
U_{Rw}; k = 2	0,160	0,720
% U_{Rw}	(0,160/1,38) × 100 = 11,594 20 = 11,6 %	(0,720/5,48) × 100 = 13,138 69 = 13,1 %
% U(HBsAg) = $\sqrt{(\%\ U^2_{cal} + \%\ U^2_{Rw})}$ ≈95 % level of confidence	$\sqrt{(4{,}0^2 + 11{,}594\ 20^2)}$ = 12,264 81 = 12,3 %	$\sqrt{(4{,}0^2 + 13{,}138\ 69^2)}$ = 13,734 09 = 13,7 %
Period	**16 Jun 15-15 Sep 15**	
Calibrator lot 501 Reagent lot 501	% U_{cal} = 3,9 %, k = 2	
IQC	Level 1	Level 2
Lot no.	378	388
N	81	81
Mean Index value	1,40	5,37
u_{Rw}(HBsAg)	0,100	0,310
U_{Rw}; k = 2	0,200	0,620
% U_{Rw}	(0,200/1,40) × 100 = 14,285 71 = 14,3 %	(0,620/5,37) × 100 = 11,545 62 = 11,5 %

Table A.18 *(continued)*

Component (Analyte)	HBsAg	
% U(HBsAg) = $\sqrt{(\%\ U^2_{cal} + \%\ U^2_{Rw})}$ ≈95 % level of confidence	$\sqrt{(3{,}9^2 + 14{,}285\ 71^2)}$ = 14,808 49 = 14,8 %	$\sqrt{(3{,}9^2 + 11{,}545\ 62^2)}$ = 12,186 52 = 12,2 %
Pooled % U for 2 reagent lots; % U(HBsAg)	$\sqrt{[(12{,}264\ 81^2 + 14{,}808\ 49^2)/2]}$ = 13,596 27 = 13,6 %	$\sqrt{[(13{,}734\ 09^2 + 12{,}186\ 52^2)/2]}$ = 12,983 38 = 13,0 %
Applied to patient results	±13,6 %	±13,0 %

A.14 Uncertainty of number concentration of red blood cells and total WBC in urine using a manual method

A manual examination of cell counts in urine may be undertaken by more than one operator. Because the reliability of manual counting procedures is dependent on operator skill, it is important to estimate between-operator uncertainty[30][31].

As shown in Table A.19, twelve operators on the same day performed a red cell and total WBC count on each of two urines, and a total WBC count on a third urine. Each operator loaded a counting grid with sample, randomly used one of five microscopes and was blind to results obtained by other operators. Between-operator estimations of the number of total red cell or WBC concentrations in urine conform to a Gaussian distribution, so that u is calculated in the usual manner.

Table A.19 — Worked example — Estimation of uncertainty of urine red blood cell (RBC) and WBC counts

Operator	Urine 1		Urine 2		Urine 3
	RBC/µl	WBC/µl	RBC/µl	WBC/µl	WBC/µl
1	11	13	116	96	250
2	13	23	77	88	153
3	26	20	119	101	137
4	27	22	113	113	348
5	28	30	108	118	290
6	28	18	103	110	230
7	16	10	155	128	240
8	13	16	121	117	280
9	14	9	130	118	248
10	12	15	175	130	297
11	25	10	130	95	255
12	13	10	110	118	230
Mean cells/µl	18,833 33 = 18,8	16,333 33 = 16,3	121,416 67 = 121,4	111,000 00 = 111,0	246,500 00 = 246,5
u (between operator), cells/µl	7,171 07 = 7,17	6,513 39 = 6,51	24,999 85 = 25,00	13,307 55 = 13,31	58,210 43 = 58,21
U, k = 2, cells/µl, ≈95 % level of confidence	14,342 14 = 14,34	13,026 78 = 13,03	49,999 70 = 50,00	26,615 10 = 26,62	116,420 86 = 116,42
% U_{rel}	76,152 96 = 76,2 %	79,755 81 = 79,8 %	41,180 26 = 41,2 %	23,977 57 = 24,0 %	47,229 56 = 47,2 %

Within-operator uncertainty should also be estimated by each individual performing the measurement in a short time period (a repeatability study) by preparing and counting 10 or more samples from the same urine specimen.

The combined uncertainty for each individual operator (u) can then be calculated as:

$$u = \sqrt{[u^2(\text{between operator}) + u^2(\text{operator})]}$$

NOTE The highly variable uncertainty in counting low numbers of cells suggests uniform sampling of urines is a major limitation that can or cannot impact medical interpretation.

Annex B
(informative)

Example of applying measurement uncertainty to result interpretation

When human sample results are close to medical decision limits, or changes in results over time are relatively small in a monitored subject, consideration of MU can aid interpretation.

EXAMPLE 1 Determining if a measured value exceeds a medical decision limit.

Human sample C: Serum prostate specific antigen (PSA) concentration = 4,3 µg/l.

Medical interpretation question: Is there a >95 % level of confidence that the result is measurably higher than the medical decision limit of 4,0 µg/l for a 61 year old patient?

u(PSA) was estimated to be 0,14 µg/l based on the combined uncertainties for u_{cal} (provided by the manufacturer) and u_{Rw} obtained for an IQC mean value of 3,6 µg/l.

If the measurement of human sample C were performed many times under repeatability conditions, would >5 % of results fall below 4,0 µg/l? This is termed a one-sided distribution, for which a confidence level of ≈95 % requires a z-score of 1,65 (see Reference [32]).

The laboratory would have a confidence level of ≈95 % that the result is measurably higher than 4,0 µg/l if the result is >4,0 + (1,65 × 0,14) = >4,23 µg/l. To ensure >95 % confidence, the uncertainty is rounded up to the next significant digit, i.e. 4,23 is rounded up to 4,3. The PSA measurement procedure can reliably distinguish between measurand values of 4,0 (the medical decision limit) and ≥4,3 µg/l.

NOTE This example only considers the uncertainty of the measured values. Within-individual biological variation must also be considered when determining if the two values are physiologically different (see below).

EXAMPLE 2 Determining if sequential measured values on a single patient are different.

Two PSA results for Patient C one year apart were 4,4 and 4,8 µg/l. Is there a >95 % level of confidence that these two measured PSA values are different?

NOTE This example only considers the uncertainty of the measured values. Within-individual biological variation must also be considered when determining if two sequential values for the same subject are physiologically different (see Example 3).

The remeasured value has an equal chance of changing up or down, so the distribution of other possible values for each result is bi-directional. The z-score for a bi-directional distribution at a level of confidence of ≈95 % is 1,96. Since u(PSA) for each result is the same, the two measured results will be different (≈95 % level of confidence) if their values differ by > z ×√2 × u(PSA). See Reference [32] for additional background.

As for Example 1, u(PSA) was estimated to be 0,14 µg/l.

Solving the formula, [z ×√2 × u(PSA)] = 1,96 × √2 × 0,14 µg/l = 0,39 µg/l.

Therefore, the second result must be ≥4,4 + 0,39 = ≥4,79 µg/l to be measurably different from the first value. The two results are measurably different (≈95 % level of confidence).

EXAMPLE 3 Combining measurement and pre-measurement uncertainties.

For some measurands it is useful for optimal result interpretation to take account of both MU and within-individual biological variation. For this purpose, the $u(y)$ is combined (see A.2.4) with the SD of the within-individual biological variation (CV_I).