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In vitro diagnostic medical devices — Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples

Dispositifs médicaux de diagnostic in vitro — Exigences pour l'établissement d'une traçabilité métrologique des valeurs attribuées aux étalons, aux matériaux de contrôle de la justesse et aux échantillons humains

Reference number
ISO 17511:2020(E)

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Foreword

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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on 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 the following URL: 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*.

This second edition cancels and replaces the first edition (ISO 17511:2003), which has been technically revised. The main changes compared to the previous edition are as follows:

— incorporation of the special requirements for metrologically traceable calibration hierarchies for measurement of catalytic concentration of enzymes (previously covered in ISO 18153:2003);

— to clarify that final reported values on human samples shall be metrologically traceable to the highest order available reference, the title and scope were modified to include metrological traceability of values assigned to human samples;

— updated normative references to remove International Vocabulary of Basic and General Terms in Metrology, 2nd edition, ISO, Geneva (1993) and ISO Guide 35:1989, Certification of reference materials — General and statistical principles;

— revision of Clause 4 to clearly define requirements of a manufacturer of an in vitro diagnostic medical device in establishing and documenting metrological traceability of assigned values (for calibrators, trueness controls and human samples), while incorporating requirements previously addressed in Clauses 6, 7 and 8 (thus eliminating those sections);

— revision of Clause 5 to incorporate additional models of metrologically traceable calibration hierarchies, especially 5.3 for measurement of catalytic concentration of enzymes (where the measurand is defined by a primary RMP; previously addressed in ISO 18153:2003), and 5.6 for an overview of the concept of assigned values of materials for measurands with metrological traceability to international harmonisation protocols (addressed in detail in ISO 21151).

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.

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Introduction

In laboratory medicine, the objective of examining a measurand in a human sample is to produce laboratory results that will enable a clinician to assess the risk of a disease, or to diagnose and make treatment decisions for a medical condition. To be clinically useful, the results obtained from a given human sample examined by different laboratories or among different in vitro diagnostic medical devices (IVD MDs) within a single laboratory should be equivalent, regardless of the measurement procedure employed. Equivalent results allow uniform application of medical decision limits and reference intervals, which can reduce the risk of harm caused by medical decisions based on non-equivalent examination results. Equivalence of results among different IVD MDs for the same measurand is also important for the analysis of results in medical records for the purpose of supporting clinical decisions and for conducting epidemiological investigations.

Equivalent results for human samples for a measurand can be achieved by establishing metrological traceability of the values assigned to the calibrators for a measurement procedure (MP) to the highest available reference system component for the measurand. Metrological traceability describes the calibration hierarchy and the sequence of value assignments, demonstrating an unbroken linkage between the measurement result for a human sample up to the highest available reference system component in the calibration hierarchy. The point at which metrological traceability begins (i.e. the highest level of metrological traceability in the calibration hierarchy) depends on the availability of higher order reference measurement procedures (RMPs), reference materials (RMs) or harmonisation protocols for the stated measurand.

Limitations in implementing metrologically traceable calibrations occur when different IVD MDs intended for the same measurand do not measure the same or very closely related measurable quantities. Some measurands of medical interest may be well-defined elements or molecules. An increasing number of medical decisions depend on measurands that consist of complex and variable mixtures of chemical structures, molecular species and molecular complexes in varying proportions, e.g. glycoproteins with multiple isoforms, variant amino acid sequences, nucleic acid sequences, and other complex molecular forms. When the selectivity of an IVD MD is not fit-for-purpose, sample-specific influence quantities in human samples due to factors including disease, drugs or other pathological conditions may lead to erroneous values for the intended measured quantity. Even with metrological traceability to higher order reference system components, the selectivity of MPs at all levels in the calibration hierarchy for a given IVD MD can influence its ability to achieve results for human samples that are equivalent to the results obtained with other IVD MDs for the same measurand.

This document presents requirements for manufacturers of IVD MDs in documenting the calibration hierarchy for a measured quantity in human samples using a specified IVD MD. The document includes various model calibration hierarchies offering potential technical solutions for different kinds of measurands in establishing metrological traceability of assigned values for human samples, calibrators and trueness control materials. Use of this document as part of a broadly-based risk management program for manufacturers of IVD MDs is consistent with the requirements of ISO 14971 and is expected to assist in the reduction of the risk of harm to patients due to non-equivalence of results among different IVD MDs.

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In vitro diagnostic medical devices — Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples

1 Scope

This document specifies technical requirements and documentation necessary to establish metrological traceability of values assigned to calibrators, trueness control materials and human samples for quantities measured by IVD MDs. The human samples are those intended to be measured, as specified for each IVD MD. Metrological traceability of values for quantities in human samples extends to the highest available reference system component, ideally to RMPs and certified reference materials (CRMs).

All parties having a role in any of the steps described in a calibration hierarchy for an IVD MD are subject to the requirements described. These parties include but are not limited to manufacturers (of IVD MDs), RMP developers (see ISO 15193), RM producers (see ISO 15194), and reference/calibration laboratories (see ISO 15195) supporting calibration hierarchies for IVD MDs.

NOTE 1 Producers of RMs intended for use in standardization or calibration of IVD MDs include commercial and non-commercial organizations producing RMs for use by many end-users of IVD MDs and/or calibration laboratories, or for use by a single end-user medical laboratory, as in the case of a measurement standard (calibrator) intended to be used exclusively for calibration of a laboratory-developed MP.

This document is applicable to:

a) all IVD MDs that provide measurement results in the form of numeric values, i.e. rational (ratio) and/or differential (interval) scales, and counting scales.

b) IVD MDs where the measurement result is reported as a qualitative value established with a ratio of two measurements (i.e. the signal from a specimen being tested and the signal from a RM with a specified concentration or activity at the cut-off), or a counting scale, with corresponding decision threshold(s). This also includes IVD MDs where results are categorized among ordinal categories based on pre-established quantitative intervals for a quantity.

c) RMs intended for use as trueness control materials for verification or assessment of calibration of IVD MDs, i.e. some commutable CRMs and some external quality assessment (EQA) materials (if so indicated in the RM's intended use statement).

d) IVD MD-specific calibrators and trueness control materials with assigned values, intended to be used together with a specified IVD MD.

e) IVD MDs as described in a) and b), where no end-user performed calibration is required (i.e. when the manufacturer performs a factory calibration of the IVD MD).

This document is not applicable to:

a) calibrators and trueness control materials for IVD MDs which, due to their formulation, are known to have zero amount of measurand;

b) control materials that are used only for internal quality control purposes in medical laboratories to assess the imprecision of an IVD MD, either its repeatability or reproducibility, and/or for assessing changes in IVD MD results compared to a previously established calibration condition;

c) control materials that are used only for internal quality control purposes in medical laboratories and which are supplied with intervals of suggested acceptable values that are not metrologically traceable to higher order reference system components;

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d) properties reported as nominal scales and ordinal scales, where no magnitude is involved.

NOTE 2 Nominal scales are typically used to report e.g. identity of blood cell types, microorganism types, identity of nucleic acid sequences, identity of urine particles.

NOTE 3 Ordinal scales are often applied to results differentiated into dichotomous groupings (e.g. 'sick' vs. 'healthy'), and occasionally to results differentiated into non-dichotomous categories where the result categories are rank-ordered but the rank-ordered categories cannot be differentiated in terms of relative degree of difference, e.g. negative, +1, +2, +3 for grading of presence of haemoglobin in urine specimens by visual observation.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 18113-2, *In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 2: In vitro diagnostic reagents for professional use*

ISO 15193, *In vitro diagnostic medical devices — Measurement of quantities in samples of biological origin — Requirements for content and presentation of reference measurement procedures*

ISO 15194, *In vitro diagnostic medical devices — Measurement of quantities in samples of biological origin — Requirements for certified reference materials and the content of supporting documentation*

3 Terms and definitions, symbols and abbreviated terms

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 measurable *quantity* (3.38)

EXAMPLE In the type of *quantity* (3.38) "mass of protein in 24-hour urine", "protein" is the analyte. In "amount of substance of glucose in plasma", "glucose" is the analyte. In both cases the long phrase represents the *measurand* (3.26).

3.2
analytical selectivity
selectivity of a measuring system
selectivity
property of a *measuring system* (3.29), used with a specified *MP* (3.27), whereby it provides measured *quantity* (3.38) values for one or more *measurands* (3.26) such that the values of each *measurand* (3.26) are independent of other *measurands* (3.26) or other *quantities* (3.38) in the phenomenon, body, or substance being investigated

EXAMPLE Capability of a *measuring system* (3.29) 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* (3.29) is usually obtained for *quantities* (3.38) 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.

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[SOURCE: ISO/IEC Guide 99:2007 4.13, modified — 'analytical selectivity' added as the preferred term. Included only Example 5 with abbreviated text and NOTES 3 and 4.]

3.3
measurement bias
bias
estimate of a systematic measurement error

Note 1 to entry: See ISO/IEC Guide 99:2007 2.17, systematic measurement error.

Note 2 to entry: This definition applies to quantitative measurements only.

[SOURCE: ISO/IEC Guide 99:2007 2.18, modified — Note 1 and 2 to entry have been added.]

3.4
calibration
operation that, under specified conditions, in a first step, establishes a relation between the *quantity* (3.38) values with *measurement uncertainties* (3.48) provided by *measurement standards* (3.28) and corresponding indications with associated *measurement uncertainties* (3.48) and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

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 *measurement uncertainty* (3.48).

Note 2 to entry: Calibration should not be confused with adjustment of a *measuring system* (3.29), often mistakenly called "self-calibration", or with *verification* (3.50) of calibration.

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

[SOURCE: ISO/IEC Guide 99:2007 2.39]

3.5
calibration hierarchy
sequence of *calibrations* (3.4) from a reference to the final *measuring system* (3.29), where the outcome of each *calibration* (3.4) depends on the outcome of the previous *calibration* (3.4)

Note 1 to entry: *Measurement uncertainty* (3.48) necessarily increases along the sequence of *calibrations* (3.4).

Note 2 to entry: The elements of a calibration hierarchy are one or more *measurement standards* (3.28) and *measuring systems* (3.29) operated according to *MPs* (3.27).

Note 3 to entry: A comparison between two *measurement standards* (3.28) may be viewed as a *calibration* (3.4) if the comparison is used to check and, if necessary, correct the *quantity* (3.38) value and *measurement uncertainty* (3.48) attributed to one of the *measurement standards* (3.28).

Note 4 to entry: In this document, a calibration hierarchy is defined as a detailed description of the process for assigning a value of a *measurand* (3.26) to a sample using a specified sequence of *MPs* (3.27) and *RMs* (3.39) (calibrated by higher order *RMs* (3.39) and/or *MPs* (3.27) for the same type of *quantity* (3.38), where available).

Note 5 to entry: For purposes of this definition, a sample includes human samples as well as *calibration materials* (3.6), EQA materials or other *RMs* (3.39).

[SOURCE: ISO/IEC Guide 99:2007 2.40, modified — excludes original Note 3. Note 3 to entry is Note 4 and Note 5 has been added.]

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3.6
calibrator
calibration material
measurement standard (3.28) used in *calibration* (3.4) of a *measuring system* (3.29) according to a specified *MP* (3.27)

[SOURCE: ISO/IEC Guide 99:2007 5.12, modified — “calibration material” has been added as an admitted term, "of a measuring system according to a specified MP" has been added at the end of the definition, NOTE has been deleted.]

3.7
catalytic activity
property of a component corresponding to the catalysed substance rate of conversion of a specified chemical reaction, in a specified *measuring system* (3.29)

Note 1 to entry: In this document the "component" is an enzyme.

Note 2 to entry: The *quantity* (3.38) "catalytic activity" relates to an amount of active enzyme, not its concentration; see 3.8.

Note 3 to entry: The coherent derived SI unit is "katal" (kat), equal to "mole per second" (mol s^{-1}).

Note 4 to entry: The *MP* (3.27) is an essential element of the definition of the *measurand* (3.26).

Note 5 to entry: In many instances, instead of the conversion rate of the substrate ascribed in the short name of the enzyme *analyte* (3.1), e.g. "creatine" in "creatine kinase", the conversion rate of an indicator substance as substrate of a combined reaction is measured. Then the *measurand* (3.26) should be defined as 'catalytic activity of the enzyme as measured by the conversion rate of an indicator substance in a specified system according to a given *MP* (3.27)', e.g. 'catalytic activity of creatine kinase as measured by the rate of conversion of NADP+ in the IFCC reference procedure in human serum'.

[SOURCE: ISO 18153:2003, 3.2]

3.8
catalytic-activity concentration
catalytic concentration
catalytic activity (3.7) of a component divided by volume of the original system

Note 1 to entry: The coherent derived SI unit is "katal per cubic metre" or "mole per second cubic metre" (kat m^{-3} = mol s^{-1} m^{-3}). In laboratory medicine, the unit of volume can be chosen to be "litre" (L).

Note 2 to entry: In this document the "component" is an enzyme and the "original system" can be, for example, the plasma of a blood sample.

[SOURCE: ISO 18153:2003, 3.3]

3.9
certified reference material
CRM
RM (3.39) accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated *uncertainties* (3.48) and *traceabilities* (3.31), using valid procedures

EXAMPLE Human serum with assigned *quantity* (3.38) value for the concentration of cholesterol and associated *measurement uncertainty* (3.48) stated in an accompanying certificate, used as a *calibrator* (3.6) or measurement *trueness control material* (3.46).

Note 1 to entry: ‘Documentation’ is given in the form of a ‘certificate’ (see ISO Guide 31).

Note 2 to entry: Procedures for the production and CRM certification are given in ISO 17034:2016 and ISO Guide 35:2017.

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Note 3 to entry: In this definition, "uncertainty" covers both '*measurement uncertainty*' (3.48) and 'uncertainty associated with the value of a nominal property', such as for identity and sequence. "Traceability" covers both '*metrological traceability* (3.31) of a quantity value' and 'traceability of a nominal property value'.

Note 4 to entry: Specified *quantity* (3.38) values of CRMs require *metrological traceability* (3.31) with associated *measurement uncertainty* (3.48)[25].

Note 5 to entry: ISO/REMCO has an analogous definition[25] but uses the modifiers "metrological" and "metrologically" to refer to both *quantities* (3.38) and nominal properties.

Note 6 to entry: Specific requirements for CRMs and the content of supporting documentation (in the field of in vitro diagnostic medical devices) are given in ISO 15194.

Note 7 to entry: For a specified material, a *calibration* (3.4) certificate provided by an accredited *calibration* (3.4) laboratory does not confer the status of CRM on these types of materials.

[SOURCE: ISO/IEC Guide 99:2007 5.14, modified — Note 6 and 7 to entry have been added.]

3.10
commutability of a reference material
commutability
property of a *RM* (3.39), demonstrated by the closeness of agreement between the relation among the measurement results for a stated *quantity* (3.38) in this material, obtained according to two *MPs* (3.27), and the relation obtained among the measurement results for other specified materials

Note 1 to entry: The *RM* (3.39) in question is usually a *calibrator* (3.6) and the other specified materials are usually routine samples.

Note 2 to entry: In commutability assessment of an *RM* (3.39), comparisons among all applicable *MPs* (3.27) is desirable.

Note 3 to entry: Closeness of agreement of measurement results is defined in terms of fitness for purpose as appropriate for the intended use of the *RM* (3.39).

Note 4 to entry: A commutability statement is restricted to the *MPs* (3.27) as specified in a particular comparison.

[SOURCE: ISO/IEC Guide 99:2007 5.15 modified — Note 2 and Note 3 have been deleted. Note 2 to entry to Note 4 to entry have been added.]

3.11
control material
substance, material or article intended by its *manufacturer* (3.22) to be used to verify the performance characteristics of an *IVD MD* (3.21)

[SOURCE: ISO 18113-1:2009, 3.13]

3.12
end-user IVD MD calibrator
end-user calibrator
RM (3.39) used as a *measurement standard* (3.28) intended for use with one or more *IVD MD* (3.21) *MPs* (3.27) intended to examine a particular *measurand* (3.26) in human samples

Note 1 to entry: End user calibrators includes *RMs* (3.39) or *calibrators* (3.6) applied internally by the *manufacturer* (3.22) to implement a final *calibration* (3.4) of the *IVD MD* (3.21), prior to the *IVD MD's* (3.21) release and delivery to the end-user, where end-user calibration is not required (i.e. 'factory calibration').

Note 2 to entry: Factory-generated *calibrations* (3.4) or *calibration* (3.4) functions include *calibration* (3.4) information (equations, formula, functions, parameters, data) stored, e.g., in electronic format, for use with a microprocessor as part of an *IVD MD* (3.21) *measuring system* (3.29) to transform "signal" generated in the course of measuring unknown human samples to an amount of substance or other final measured value.

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3.13
equivalence of measured values
equivalent results
agreement of measured values among different *IVD MDs* (3.21) intended to measure the same *measurand* (3.26), where the differences in measured values on the same human samples do not affect clinical interpretation

Note 1 to entry: A conclusion of equivalence of measured values for the same human samples among two or more *MPs* (3.27) is based on the differences in measured values being within a pre-defined margin or limit.

[SOURCE: Harmonization.net, modified — wording revised for clarity.]

3.14
higher order reference material
higher order RM
CRM (3.9) that meets internationally accepted quality requirement and provides a common metrological reference within the *calibration hierarchy* (3.5) to which *manufacturers* (3.22) can establish *metrological traceability* (3.31)

Note 1 to entry: Quality requirements for higher order RMs are laid out in ISO 15194.

Note 2 to entry: Higher order RMs include fit–for–purpose *primary RMs* (3.35), *primary calibrators* (3.37), *secondary calibrators* (3.42) and *international conventional calibrators* (3.17).

Note 3 to entry: Pure substances constitute the *primary measurement standard* (3.37) and ultimate source of higher-order *metrological traceability* (3.31) for most traceability chains in chemistry, thermometry and calorimetry in general and for the certification of solution and *matrix* (3.24) *RMs* (3.39) in particular (see ISO Guide 35:2017).

Note 4 to entry: According to Joint Committee for Traceability in Laboratory Medicine (JCTLM) FAQs[27], a higher order RM is a *CRM* (3.9), meeting internationally accepted quality requirements, to which other measurement results can be referenced, and its *measurement uncertainty* (3.48) is completely established. Metrologically, a higher order RM is a *RM* (3.39) deployed at a higher level in the *calibration hierarchy* (3.5). Certified, highest order RMs, where available, are used by *IVD MD* (3.21) *manufacturers* (3.22) to assign values to *working calibrators* (3.51). These *working calibrators* (3.51) are subsequently used by the *manufacturer* (3.22) to assign values to *measurands* (3.26) in *end-user IVD MD calibrators* (3.12) and *control materials* (3.11) for use with *IVD MDs* (3.21) in medical laboratories and other IVD testing environments. Higher order RMs are most commonly produced and distributed by national metrology institutes (NMIs), e.g. U.S. National Institute of Standards and Technology (NIST), European Commission Joint Research Centre (EU-JRC), LGC Standards (UK), World Health Organization (WHO), National Institute for Biological Standards and Control (UK), National Institute of Metrology (CN), National Metrology Institute of Japan (JP), Reference Material Institute for Clinical Chemistry Standards (JP), Japanese Industrial Standards Committee (JISC), Centro Nacional de Metrología (MX), etc. Some commercial sources also provide RMs listed by JCTLM[28].

3.15
higher order reference measurement procedure
higher order RMP
reference measurement procedure (RMP) (3.40) meeting internationally accepted quality requirements and providing a common metrological reference within the *calibration hierarchy* (3.5) to which *manufacturers'* (3.22) can establish *metrological traceability* (3.31) and accepted as providing measurement results fit for their intended use in assessing *measurement trueness* (3.47)

Note 1 to entry: Quality requirements for *higher order RMPs* (3.15) are defined in ISO 15193.

Note 2 to entry: For reasons of higher cost, equipment complexity and operator training requirements, higher order RMPs are typically performed in national *metrology* (3.32) institutes and/or accredited *calibration* (3.4) laboratories.

Note 3 to entry: In laboratory medicine, *RMPs* (3.40) that meet the requirements of ISO 15193 are considered to be higher order RMPs.

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Note 4 to entry: According to JCTLM FAQs[27], higher order RMPs are well documented, high accuracy *(MPs)* (3.27) used for assigning values to *calibration materials* (3.6). At the highest level (these *MPs*) (3.27) are frequently expensive to develop, too complicated for routine use and not suitable for high throughput analysis.

3.16
influence quantity
quantity (3.38) that, in a direct measurement, does not affect the *quantity* (3.38) that is actually measured, but affects the relation between the indication and the measurement result

EXAMPLE Amount-of-substance concentration of bilirubin in a direct measurement of haemoglobin amount-of-substance concentration in human blood plasma.

[SOURCE: ISO/IEC Guide 99:2007 2.52, modified — excludes 3 examples and 2 notes.]

3.17
international conventional calibrator
international conventional calibration material
international measurement standard
calibrator (3.6) whose *quantity* (3.38) value is not *metrologically traceable* (3.31) to the SI but is assigned by international agreement

Note 1 to entry: The *quantity* (3.38) is defined with respect to the intended clinical application.

3.18
international conventional reference measurement procedure
international conventional RMP
MP (3.27) yielding values that are not metrologically traceable to the SI but which by international agreement are used as reference values for a defined *quantity* (3.38)

Note 1 to entry: The *quantity* (3.38) is defined with respect to the intended clinical application.

3.19
international harmonisation protocol
description of a process implemented by an international body to achieve *equivalence of measured values* (3.13) within medically acceptable limits among two or more *IVD MDs* (3.21) intended for examination of the same *measurand* (3.26) for cases where there are no *higher order RMPs* (3.15) and no fit for purpose *CRMs* (3.9) or *international* conventional *calibrators* (3.17)

Note 1 to entry: A harmonisation protocol can be used to achieve standardization of measured values for a stated *measurand* (3.26) when there are no other higher order reference system components that are suitable for use.

3.20
international measurement standard
measurement standard (3.28) recognized by signatories to an international agreement and intended to serve worldwide as the basis for assigning values to other standards for the same *quantity* (3.38)

EXAMPLE 1 The international prototype of the kilogram.

EXAMPLE 2 ERM®-DA470k/IFCC for the *calibration* (3.4) of immunoassay-based in-vitro diagnostic devices or control products for the proteins certified. European Commission — Joint Research Centre (JRC), Geel, Belgium.

EXAMPLE 3 Triple point of water — the single combination of pressure and temperature at which liquid water, solid ice, and water vapour coexist in a stable equilibrium, occurring at exactly 273,16 K (0,01 °C; 32,02 °F) at a partial vapour pressure of 611,657 pascals (6,116 57 mbar; 0,006 036 59 atm).

[SOURCE: ISO/IEC Guide 99:2007 5.2, modified — Example 2 and Example 3 have been deleted. New Example 2 and Example 3 have been added]

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3.21
in vitro diagnostic medical device
IVD medical device
IVD MD
device, whether used alone or in combination, intended by the *manufacturer* (3.22) for the in vitro examination of specimens derived from the human body solely or principally to provide information for diagnostic, monitoring or compatibility purposes and including reagents, *calibrators* (3.6), *control materials* (3.11), specimen receptacles, software, and related instruments or apparatus or other articles

[SOURCE: ISO 18113-1:2009, 3.27]

3.22
manufacturer
entity with responsibility for design, manufacture, fabrication, assembly, packaging or labelling of an *IVD MD* (3.21), for assembling a *measuring system* (3.29), or adapting an *IVD MD* (3.21) before it is placed on the market and/or put into service, regardless of whether these operations are carried out by that entity or on their behalf by a third party

Note 1 to entry: An entity includes but is not limited to an individual, a corporation (or other legally established business), an association, an institution, or a medical laboratory. An entity should be identifiable in terms of a separate and distinct existence and objective reality.

Note 2 to entry: The manufacturer has ultimate legal responsibility for ensuring conformance with all applicable regulatory requirements for the *IVD MD* (3.21) in the countries or jurisdictions where it is intended to be made available or sold, unless this responsibility is specifically imposed on another entity by the Regulatory Authority (RA) within that jurisdiction.

Note 3 to entry: The manufacturer's responsibilities are described in other GHTF guidance documents. These responsibilities include meeting both pre-market requirements and post-market requirements, such as adverse event reporting and notification of corrective actions.

Note 4 to entry: 'Design and/or manufacture', as referred to in the above definition, may include specification development, production, fabrication, assembly, processing, packaging, repackaging, labelling, relabelling, sterilization, installation, or remanufacturing of an *IVD MD* (3.21); or putting a collection of *IVD MDs* (3.21), and possibly other products, together for a medical purpose.

Note 5 to entry: Any entity that assembles or adapts an *IVD MD* (3.21) that has already been supplied by a manufacturer for purposes of an examination to be performed on a human sample in accordance with the instructions for use, is not the manufacturer, provided the assembly or adaptation does not change the intended use of the *IVD MD* (3.21).

Note 6 to entry: Any entity who changes the intended use of, or modifies, an *IVD MD* (3.21) without acting on behalf of the original manufacturer and who makes it available for use under their own name, should be considered to be the manufacturer of the modified device.

Note 7 to entry: An authorised representative, distributor or importer who only adds its own address and contact details to the *IVD MD* (3.21) or the packaging, without obscuring or changing the existing labelling, is not considered a manufacturer.

Note 8 to entry: To the extent that an accessory is subject to the regulatory requirements of (an *IVD MD* (3.21)), the entity responsible for the design and/or manufacture of that accessory is considered to be a manufacturer.

[SOURCE: ISO 18113-1:2009, 3.36, modified — Replaced 'natural or legal person' and 'person' with 'entity'; source Notes are excluded; new Note 1 to entry is introduced; Notes to entry 2-8 added and sourced (with minor modifications to ensure consistency in terminology as given in this definition) from GHTF/SG1N055:2009, 5.1.]

3.23
matrix effect
influence of a property of the sample, independent of the presence of the *analyte* (3.1), on the measurement and thereby on the measured *quantity* (3.38) value

Note 1 to entry: A specified cause of a matrix effect is an *influence quantity* (3.16).

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Note 2 to entry: The term 'matrix effect' is sometimes erroneously used in cases of non-commutability of a material due to causes such as, e.g. a denatured *analyte* (3.1) or an added non-genuine component (surrogate *analyte* (3.1)) intended to simulate the *measurand* (3.26).

[SOURCE: ISO 15194:2009, 3.7, modified — Excluded NOTE 2 and Example; added Note 2 to entry.]

3.24
matrix
system matrix
<material> components of a material system, except the *analyte* (3.1)

Note 1 to entry: The biological system excluding the *analyte* (3.1) is the matrix of the material.

[SOURCE: ISO 15194:2009, 3.6, modified — added <material> as domain; added synonym 'system matrix'; added Note 1 to entry.]

3.25
maximum allowable measurement uncertainty
Umax(y)
maximum fit for purpose *measurement uncertainty* (3.48) for measurement results produced by a given *MP* (3.27), and specified as an upper limit based on an evaluation of medical requirements

Note 1 to entry: ISO/IEC Guide 99:2007 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* (3.26) 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: In this document, the *maximum allowable measurement uncertainty* (3.25) specification for an *IVD MD* (3.21) is abbreviated *Umax(y).*

3.26
measurand
quantity (3.38) intended to be measured

Note 1 to entry: Specification of a measurand requires knowledge of the kind of *quantity* (3.38), description of the state of the phenomenon, body, or substance carrying the *quantity* (3.38), 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 "*quantity* (3.38) subject to measurement".

Note 3 to entry: The measurement, including the *measuring system* (3.29) and the conditions under which the measurement is carried out, could change the phenomenon, body, or substance such that the *quantity* (3.38) being measured can 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'* (3.1), 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* (3.38).

Note 5 to entry: In laboratory medicine, the description of the measurand includes the name of the *quantity* (3.38) (e.g. amount of substance concentration), the component/*analyte* (3.1) (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, modified — Note to entry 3 and 5 added, example added]

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3.27
measurement procedure
MP
detailed description of a measurement according to one or more measurement principles and to a given *measurement method* (3.30), based on a measurement model and including any calculation to obtain a measurement result

Note 1 to entry: An MP is usually documented in sufficient detail to enable an operator to perform a measurement.

Note 2 to entry: An MP can include a statement concerning a target *measurement uncertainty* (3.48).

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

[SOURCE: ISO/IEC Guide 99:2007 2.6]

3.28
measurement standard
standard
realization of the definition of a given *quantity* (3.38), with stated *quantity* (3.38) value and associated *measurement uncertainty* (3.48), used as a reference

EXAMPLE 1 1 kg mass measurement standard with an associated standard *measurement uncertainty* (3.48) of 3 µg.

EXAMPLE 2 Set of reference solutions of cortisol in human serum having a certified quantity value with *measurement uncertainty* (3.48) for each solution.

EXAMPLE 3 *RM* (3.39) providing *quantity* (3.38) values with *measurement uncertainties* (3.48) for the mass concentration of each of ten different proteins.

Note 1 to entry: A "realization of the definition of a given *quantity* (3.38)" can be provided by a *measuring system* (3.29), a material measure, or a *RM* (3.39).

Note 2 to entry: *A* measurement standard is frequently used as a reference in establishing measured *quantity* (3.38) values and associated *measurement uncertainties* (3.48) for other *quantities* (3.38) of the same kind, thereby establishing *metrological traceability* (3.31) through *calibration* (3.4) of other measurement standards, measuring instruments, or *measuring systems* (3.29).

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* (3.28) of 1 kg.

Note 4 to entry: A standard *measurement uncertainty* (3.48) associated with a measurement standard is always a component of the *combined standard measurement uncertainty* (3.33) in a measurement result obtained using the measurement standard (see ISO/IEC Guide 98-3:2008 — GUM, 2.3.4). Frequently, this component is small compared with other components of the *combined standard measurement uncertainty* (3.33).

Note 5 to entry: *Quantity* (3.38) value and *measurement uncertainty* (3.48) must be determined at the time when the measurement standard is used.

Note 6 to entry: Several *quantities* (3.38) of the same kind or of different kinds may be realized in one device which is commonly also called a measurement standard.

[SOURCE: ISO/IEC Guide 99:2007 5.1, modified — Example 2 to Example 4 and Note 7 to Note 9 have been deleted.]

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3.29
measuring system
measurement 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* (3.38) values within specified intervals for *quantities* (3.38) of specified kinds

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

[SOURCE: ISO/IEC Guide 99:2007 3.2]

3.30
measurement method
method of measurement
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.

See IEC 60050-300:2001.

[SOURCE: ISO/IEC Guide 99:2007 2.5]

3.31
metrological traceability
property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of *calibrations* (3.4), each contributing to the *measurement uncertainty* (3.48)

Note 1 to entry: For this definition, a 'reference' can be a definition of a measurement unit through its practical realization, or a *MP* (3.27) including the measurement unit for a non-ordinal *quantity* (3.38), or a *measurement standard* (3.28).

Note 2 to entry: Metrological traceability requires an established *calibration hierarchy* (3.5).

Note 3 to entry: Specification of the reference must include the time at which this reference was used in establishing the *calibration hierarchy* (3.5), along with any other relevant metrological information about the reference, such as when the first *calibration* (3.4) in the *calibration hierarchy* (3.5) was performed.

Note 4 to entry: For measurements with more than one input *quantity* (3.38) in the measurement model, each of the input *quantity* (3.38) values should itself be metrologically traceable and the *calibration hierarchy* (3.5) involved may form a branched structure or a network. The effort involved in establishing metrological traceability for each input *quantity* (3.38) value should be commensurate with its relative contribution to the measurement result.

Note 5 to entry: Metrological traceability of a measurement result does not ensure that the *measurement uncertainty* (3.48) is adequate for a given purpose or that there is an absence of mistakes.

Note 6 to entry: A comparison between two *measurement standards* (3.28) may be viewed as a *calibration* (3.4) if the comparison is used to check and, if necessary, correct the *quantity* (3.38) value and *measurement uncertainty* (3.48) attributed to one of the *measurement standards* (3.28).

Note 7 to entry: The ILAC considers the elements for confirming metrological traceability to be an unbroken metrological traceability chain to an *international measurement standard* (3.20) or a national *measurement standard* (3.28), a documented *measurement uncertainty* (3.48), a documented *MP* (3.27), accredited technical competence, metrological traceability to the SI, and *calibration* (3.4) intervals (see ILAC P10:01/2013).

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Note 8 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.

Note 9 to entry: Regarding Note 4 to entry above, VIM , 2.50, defines input *quantity* (3.38) in a measurement model as the *quantity* (3.38) that must be measured, or a *quantity* (3.38) the value of which can be otherwise obtained, in order to calculate a measured quantity value of a *measurand* (3.26).

EXAMPLE Length of a steel rod at a specified temperature is the *measurand* (3.26), while the ambient temperature, the observed length of the steel rod, and the thermal expansion coefficient of the steel rod are the input *quantities* (3.38) in the measurement model.

[SOURCE: ISO/IEC Guide 99:2007 2.41, modified — Note 9 to entry and EXAMPLE have been added.]

3.32
metrology
science of measurement and its application

Note 1 to entry: Metrology includes all theoretical and practical aspects of measurement, whatever the *measurement uncertainty* (3.48) and field of application.

[SOURCE: ISO/IEC Guide 99:2007 2.2]

3.33
combined standard measurement uncertainty
combined standard uncertainty
$u(y)$
standard *measurement uncertainty* (3.48) that is obtained using the individual standard measurement uncertainties associated with the input *quantities* (3.38) in a measurement model (see 4.7)

[SOURCE: ISO/IEC Guide 99:2007 2.31, modified — Note to entry has been deleted.]

3.34
precision of measurement
closeness of agreement between indications or measured *quantity* (3.38) 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 standard deviation, variance, or coefficient of variation 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 reproducibility conditions of measurement (see ISO 5725-1:1994).

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement reproducibility.

Note 4 to entry: Sometimes "measurement precision" is erroneously used to mean measurement accuracy.

[SOURCE: ISO/IEC Guide 99:2007 2.15]

3.35
primary reference material
primary RM
high purity material of the *analyte* (3.1), certified for the mass/mole fraction of the *analyte* (3.1) in the material, and which constitutes the realization of the International System of Units (SI) for the *analyte* (3.1) of interest

Note 1 to entry: A primary reference material has its value assigned either directly by a *primary RMP* (3.36) or indirectly by determining the impurities of the material by appropriate analytical methods (e.g. mass balance method).

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3.36
primary reference measurement procedure
primary RMP
reference measurement procedure (RMP) (3.40) used to obtain a measurement result without relation to a *measurement standard* (3.28) for a *quantity* (3.38) of the same kind

EXAMPLE The volume of water delivered by a 50 mL pipette at 20 °C is measured by weighing the water delivered by the pipette into a beaker, taking the mass of beaker plus water minus the mass of the initially empty beaker, and correcting the mass difference for the actual water temperature using the volumic mass (mass density).

Note 1 to entry: The term '*primary RMP*' (3.36) as used here refers to a fully detailed set of measurement instructions whereas the term 'primary *method of measurement*' (3.30) as defined by the Consultative Committee for Amount of Substance (CCQM) is a generic description of a measurement principle or a *measurement method* (3.30) covering various procedures.

[SOURCE: ISO/IEC Guide 99:2007 2.8, modified — Note 1 and Note 2 have been deleted and Note 1 to entry has been added.]

3.37
primary measurement standard
primary standard
primary calibrator
measurement standard (3.28) established using a *primary RMP* (3.36), or created as an artefact, chosen by convention

EXAMPLE 1 Primary measurement standard of amount-of-substance concentration prepared by dissolving a known amount of substance of a chemical component to a known volume of solution.

EXAMPLE 2 Primary measurement standard for pressure based on separate measurements of force and area.

EXAMPLE 3 Primary measurement standard for isotope amount-of-substance ratio measurements, prepared by mixing known amount-of-substances of specified isotopes.

EXAMPLE 4 Triple-point-of-water cell as a primary measurement standard of thermodynamic temperature.

EXAMPLE 5 The international prototype of the kilogram as an artefact, chosen by convention.

[SOURCE: ISO/IEC Guide 99:2007 5.4]

3.38
quantity
property of a phenomenon, body, or substance, where the property has a magnitude that can be expressed as a number and a reference

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

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

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

Note 2 to entry: "Quantity" is not to be confused with "*analyte*"(3.1).

Note 3 to entry: *MPs* (3.27) 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.

[SOURCE: ISO/IEC Guide 99:2007 1.1, modified — Note 1 to Note 6 have been deleted, and Example 2, Note 2 to entry and Note 3 to entry have been added.]

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3.39
reference material
RM
material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

EXAMPLE 1 Examples of RMs embodying *quantities* (3.38):

a) water of stated purity, the dynamic viscosity of which is used to calibrate viscometers;

b) human serum without an assigned *quantity* (3.38) value for the amount-of-substance concentration of the inherent cholesterol, used only as a measurement precision *control material* (3.11).

EXAMPLE 2 Examples of RMs embodying nominal properties:

a) colour chart indicating one or more specified colours;

b) DNA compound containing a specified nucleotide sequence;

c) urine containing 19-androstenedione.

EXAMPLE 3 Substance of known triple-point in a triple-point cell.

EXAMPLE 4 Glass of known optical density in a transmission filter holder.

EXAMPLE 5 Spheres of uniform size mounted on a microscope slide.

EXAMPLE 6 Human serum with an assigned quantity value for cholesterol (amount of substance) concentration and associated *measurement uncertainty* (3.48), used as a *calibrator* (3.6) or measurement *trueness control material* (3.46).

Note 1 to entry: Examination of a nominal property provides a nominal property value and associated uncertainty. This uncertainty is not a *measurement uncertainty* (3.48).

Note 2 to entry: RMs with or without assigned *quantity* (3.38) values can be used for measurement precision control whereas only RMs with assigned *quantity* (3.38) values can be used for *calibration* (3.4) or measurement *trueness control* (3.46).

Note 3 to entry: 'RM' comprises materials embodying *quantities* (3.38) as well as nominal properties.

Note 4 to entry: A RM is sometimes incorporated into a *measuring system* (3.29).

Note 5 to entry: Some RMs have assigned *quantity* (3.38) values that are *metrologically traceable* (3.31) to a measurement unit outside a system of units. Such materials include vaccines to which International Units (IU) have been assigned by the WHO[29].

Note 6 to entry: In a given measurement, a given RM can only be used for either *calibration* (3.4) or quality assurance.

Note 7 to entry: The specifications of a RM should include its material traceability, indicating its origin and processing[25].

Note 8 to entry: ISO/REMCO has an analogous definition[25] but uses the term "measurement process" to mean 'examination' (see ISO 15189:2012), which covers both measurement of a *quantity* (3.38) and examination of a nominal property.

Note 9 to entry: A RM, accompanied by documentation issued by an authoritative body and referring to valid procedures used to obtain a specified property value with associated *measurement uncertainty* (3.48) and *metrological traceability* (3.31), is called a *CRM* (3.9).

Note 10 to entry: Requirements for the specifications of RMs intended for *calibration* (3.4) of *RMPs* (3.40) are described in ISO 15194.

Note 11 to entry: Uses of RMs include the *calibration* (3.4) of a *measuring system* (3.29), assessment of a *MP* (3.27), assigning values to other materials, and quality control. See also *measurement standard* (3.28).

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Note 12 to entry: Example of a RM that embodies a *quantity* (3.38): Blood plasma containing a stated mass fraction of glucose, intended for use as a *calibrator* (3.6).

[SOURCE: ISO/IEC Guide 99:2007 5.13, modified — Note 3 to entry, excludes EXAMPLE 1.c; Note 4 to entry, replaced "…specially fabricated device" with "…measuring system".]

3.40
reference measurement procedure
RMP
MP (3.27) accepted as providing measurement results fit for their intended use in assessing measurement *trueness* (3.47) of measured *quantity* (3.38) values obtained from other *MPs* (3.27) for *quantities* (3.38) of the same kind, in *calibration* (3.4), or in characterizing *RMs* (3.39).

Note 1 to entry: Requirements for RMPs for use in *calibration hierarchies* (3.5) supporting *IVD MDs* (3.21) are described in ISO 15193.

[SOURCE: ISO/IEC Guide 99:2007 2.7, modified — Note 1 to entry has been added.]

3.41
reference measurement system
measuring system (3.29) accepted as fit for its intended purpose in assessing or establishing measurement *trueness* (3.47) for quantity values obtained from other *MPs* (3.27) for the *measurand* (3.26); comprised of (1) a unit of measurement, (2) a definition of the *measurand* (3.26), (3) *RMP(s)* (3.40), (4) *RM(s)* (3.39) and (5) one or more laboratories providing reference measurement services.

Note 1 to entry: Definition is taken from Reference [30].

3.42
secondary measurement standard
secondary standard
secondary calibrator
measurement standard (3.28) established through *calibration* (3.4) with respect to a *primary measurement standard* (3.37) for a *quantity* (3.38) of the same kind

Note 1 to entry: *Calibration* (3.4) may be obtained directly between a *primary measurement standard* (3.37) and a secondary measurement standard or involve an intermediate *measuring system* (3.29) calibrated by the *primary measurement standard* (3.37) and assigning a measurement result to the secondary measurement standard.

Note 2 to entry: A *measurement standard* (3.28) having its quantity value assigned by a ratio *primary RMP* (3.36) is a secondary measurement standard.

Note 3 to entry: An alternate applicable term for a secondary standard or *calibrator* (3.6), not included in VIM 5.5, is 'secondary reference material.'

[SOURCE: ISO/IEC Guide 99:2007 5.5, modified — Note 3 to entry has been added.]

3.43
manufacturer's selected measurement procedure
manufacturer's selected MP
MP (3.27) that is calibrated by one or more *primary* (3.37) or *secondary calibrators* (3.42) when available

Note 1 to entry: Throughput and other desired "productivity" features can make a given selected *MP* (3.27) less desirable for use in a setting requiring higher volume and faster turnaround times. A selected *MP* (3.27) can also be one with established clinical validity, in addition to having known (and acceptable) analytical performance attributes. Selected *MPs* (3.27) are sometimes used by *manufacturers* (3.22)as an internal benchmark to support research and development of new *MPs* (3.27) (intended to be commercialized by the *manufacturer* (3.22)), and are often used to support assignment of values to "*working*" or "*master*" *calibrators* (3.51) in support of routine value assignment of "product" *end-user IVD-MD calibrators* (3.12) for use by one or more *IVD-MDs* (3.21).

Note 2 to entry: The manufacturer's selected MP can be based on the same principle and *measurement method* (3.30) as the end-user's *IVD MD* (3.21), but operated under more precisely controlled conditions (e.g., a larger number of replicates and/or a stricter control system) so as to reduce *measurement uncertainty* (3.48) in the value of the *quantity* (3.38) measured.

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Note 3 to entry: The manufacturer's selected MP can be based on the same principle and *measurement method* (3.30) as that of a *higher order RMP* (3.15) for the *measurand* (3.26).

3.44
manufacturer's standing measurement procedure
manufacturer's standing MP
MP (3.27), calibrated with a *RM* (3.39) or with a *manufacturer's working calibrator* (3.51), used to assess or assign values to the *end-user's calibrator* (3.12)

Note 1 to entry: The manufacturer's standing MP can be based on the same principle and *measurement method* (3.30) as the end-user's *IVD MD* (3.21), but operated under more precisely controlled conditions (e.g., a larger number of replicates and/or a stricter control system) so as to reduce *measurement uncertainty* (3.48) in the value of the *quantity* (3.38) measured.

3.45
true value of a quantity
true value
quantity value consistent with the definition of a *quantity* (3.38)

Note 1 to entry: In the (total) Error Approach to describing measurement, a true quantity value is considered unique and, in practice, unknowable. The Uncertainty Approach is to recognize that, owing to the inherently incomplete amount of detail in the definition of a *quantity* (3.38), there is not a single true quantity value but rather a set of true quantity values consistent with the definition. However, this set of values is, in principle and in practice, unknowable. Other approaches dispense altogether with the concept of true quantity value and rely on the concept of metrological compatibility of measurement results for assessing their validity.

Note 2 to entry: In the special case of a fundamental constant, the *quantity* (3.38) is considered to have a single true quantity value.

Note 3 to entry: When the definitional uncertainty associated with the *measurand* (3.26) is considered to be negligible compared to the other components of the *measurement uncertainty* (3.48), the *measurand* (3.26) may be considered to have an "essentially unique" true quantity value. This is the approach taken by the GUM and associated documents, where the word "true" is considered to be redundant.

Note 4 to entry: The concept of a true value recognizes that, due to inherent *measurement uncertainty* (3.48), the true value can never be known.

[SOURCE: ISO/IEC Guide 99:2007 2.11, modified —Note 4 to entry has been added.]

3.46
trueness control material
trueness control
RM (3.39) that is used to assess the *measurement bias* (3.3) of a specified *quantity* (3.38) in a specified *measuring system* (3.29)

Note 1 to entry: Trueness control materials are often prepared in a *matrix* (3.24) designed to emulate the *matrix* (3.24) of the intended human samples.

Note 2 to entry: Trueness control materials should be evaluated to establish their *commutability* (3.10) with human samples.

Note 3 to entry: Trueness control materials may be made available by their *manufacturers* (3.22) as *CRMs* (3.9).

3.47
trueness of measurement
measurement trueness
trueness
closeness of agreement between the average of an infinite number of replicates measured quantity values and a reference quantity value

Note 1 to entry: Measurement trueness is not a quantity and thus cannot be expressed numerically, but measures for closeness of agreement are given in ISO 5725-1.

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Note 2 to entry: Measurement trueness is inversely related to systematic measurement error but is not related to random measurement error.

Note 3 to entry: "Measurement accuracy" should not be used for 'measurement trueness'.

Note 4 to entry: For qualitative examinations, trueness of measurement (closeness of agreement) can be expressed in terms of concordance (i.e. percent agreement with a reference examination).

Note 5 to entry: Trueness is a property of the *MP* (3.27) that reflects the *bias* (3.3) of the measurements from the expected or target value. It is described qualitatively as good or bad. A *MP* (3.27) has good trueness if the *bias* (3.3) of the measurements is low.

Note 6 to entry: The measure of trueness is usually expressed in terms of *bias* (3.3). Trueness has sometimes been referred to as "accuracy of the mean."

[SOURCE: ISO/IEC Guide 99:2007 2.14, modified — Note 3 and Note 6 to entry have been added. Note 6 is taken from ISO 16577: 2016, 3.105.]

3.48
uncertainty of measurement
measurement uncertainty
non-negative parameter characterizing the dispersion of the quantity values being attributed to a *measurand* (3.26), based on the information used

Note 1 to entry: Measurement uncertainty includes components arising from systematic effects, as in the case of corrections to the assigned quantity values of *measurement standards* (3.28). Sometimes estimated systematic effects are not corrected for, but instead, the associated measurement uncertainty components are incorporated.

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

Note 3 to entry: Measurement uncertainty comprises, in general, many components. Some of these may be evaluated by Type A evaluation of measurement uncertainty from the statistical distribution of the quantity values from series of measurements and can be characterized by standard deviations. The other components, which can be evaluated by Type B evaluation of measurement uncertainty, may also be characterized by standard deviations, 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 measurement uncertainty is associated with a stated quantity value attributed to the *measurand* (3.26). A modification of this value results in a modification of the associated uncertainty.

Note 5 to entry: Type A evaluation of measurement uncertainty is defined as evaluation of a component of measurement uncertainty by a statistical analysis of measured quantity values obtained under defined measurement conditions [adapted from VIM, 2.28].

Note 6 to entry: Type B evaluation of measurement uncertainty is defined as evaluation of a component of measurement uncertainty determined by means other than a Type A evaluation. This may include standard deviations (a) obtained from information associated with authoritative published quantity values, (b) associated with quantity values of *CRMs* (3.9), (c) obtained from a *calibration* (3.4) certificate, (d) obtained from experience or other means [adapted from VIM, 2.29].

[SOURCE: ISO/IEC Guide 99:2007 2.26]

3.49
validation
verification (3.50), where the specified requirements are adequate for an intended use

EXAMPLE 1 A *MP* (3.27), ordinarily used for the measurement of mass concentration of nitrogen in water, may be validated also for measurement of mass concentration of nitrogen in human serum.

EXAMPLE 2 An *MP* (3.27) for creatinine (mass) concentration in human serum can also be validated for the measurement of creatinine (mass) concentration in human urine.

EXAMPLE 3 An *MP* (3.27) for the measurement of PSA (mass) concentration in serum to aid in the diagnosis of prostate cancer in males older than 40 years.

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Note 1 to entry: ISO 9000 defines validation as confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled.

[SOURCE: ISO/IEC Guide 99:2007 2.45, modified — Example 2, Example 3 and Note 1 to entry have been added. Example 2 and Note 1 to entry have come from ISO 18113-1:2009, 3.72.]

3.50
verification
provision of objective evidence that a given item fulfils specified requirements

EXAMPLE 1 Confirmation that a given *RM* (3.39) as claimed is homogeneous for the quantity value and *MP* (3.27) concerned, down to a measurement portion having a mass of 10 mg.

EXAMPLE 2 Confirmation that performance properties or legal requirements of a *measuring system* (3.29) are achieved.

EXAMPLE 3 Confirmation that a target *measurement uncertainty* (3.48) can be met.

Note 1 to entry: When applicable, *measurement uncertainty* (3.48) should be taken into consideration.

Note 2 to entry: The item may be, e.g. a process, *MP* (3.27), material, compound, or *measuring system* (3.29).

Note 3 to entry: The specified requirements may be, e.g. that a *manufacturer's* (3.22) specifications are met.

Note 4 to entry: Verification in legal *metrology* (3.32), as defined in OIML V1:2013, and in conformity assessment in general, pertains to the examination and marking and/or issuing of a verification certificate for a *measuring system* (3.29).

Note 5 to entry: Verification should not be confused with *calibration* (3.4). Not every verification is a *validation* (3.49).

Note 6 to entry: In chemistry, verification of the identity of the entity involved, or of activity, requires a description of the structure or properties of that entity or activity.

Note 7 to entry: Verification is the process by which the lab confirms that the established performance claims of an IVD (e.g. accuracy, precision, reportable range) can be replicated in the lab before human sample testing is performed.

Note 8 to entry: Verification may be sufficient to implement a new IVD under circumstances where the test is performed and used in the manner as directed in the package insert.

[SOURCE: ISO/IEC Guide 99:2007 2.44, modified — Note 7 to entry and Note 8 to entry have been added.]

3.51
working measurement standard
working standard
manufacturer's working calibrator
manufacturer's master calibrator
measurement standard (3.28) that is used to calibrate or verify measuring instruments or *measuring systems* (3.29)

Note 1 to entry: A working measurement standard is usually calibrated (value assigned) with reference to a reference *measurement standard* (3.28).

Note 2 to entry: In relation to *verification* (3.50), the terms "check standard" or "control standard" are also sometimes used.

Note 3 to entry: A *manufacturer* (3.22) may choose to prepare a manufacturer's working calibrator, which is intended to transfer *trueness* (3.47) by means of *calibration* (3.4) to *end-user IVD-MD calibrators* (3.12).

Note 4 to entry: A working measurement standard is sometimes implemented as a surrogate *RM* (3.39) in lieu of a more expensive *higher order RM* (3.14).

[SOURCE: ISO/IEC Guide 99:2007 5.7, modified — Note 3 to entry and Note 4 to entry have been added.]

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4 General requirements to be fulfilled by a manufacturer for establishing, validating and documenting metrological traceability of human sample values determined with a specified IVD MD

4.1 Requirements for documenting metrological traceability of measured quantity values

A manufacturer shall document the complete calibration hierarchy and identify the highest metrological reference to which the resulting measured quantity values are traceable, in conformance with the requirements set out in this document.

The manufacturer's documentation concerning the metrological traceability of measured quantity values in human samples with a specified IVD MD shall include:

a) a description of the reference measurement system, including the following elements:

   i. applicable system of units (for example SI, IU, arbitrary or other) and definition of the measurand;

   ii. highest order MP, if applicable, or protocols for establishing a metrologically traceable calibration for the IVD MD;

   NOTE 1 'Protocols' in 'ii.' include those defined by a mandated body or by other authoritative body (e.g., an international professional body.)

   iii. (if applicable) RMs for calibration of any MP in 'ii';

   iv. reference laboratories and/or laboratory networks, designated by national metrology institutes, professional bodies, accreditation bodies or other authoritative body to be capable of providing fit for purpose examinations of the measurand in the intended human samples.

   NOTE 2 Laboratories within the scope of 'iv.' include calibration or reference laboratories operated by (or on behalf of) a manufacturer.

b) a description of the calibration hierarchy, usually consisting of alternating pairs of MPs and RMs, establishing an unbroken sequence of value transfers, starting with the highest order reference system element available (see 4.1.a) and culminating in measured quantity values for human samples using the IVD MD.

c) specifications for the *Umax(y)* for the IVD MD (i.e. the measurement uncertainty upper specification limit, see 3.25). The estimated combined expanded measurement uncertainty, U(y) (see 4.3.2), shall be documented to not exceed the *Umax(y).* This assessment shall include an estimate of the *combined standard uncertainty*, *u(y)* (3.33), of the final measured values on human samples for the specified IVD MD. The estimation of *u(y)* (3.33) shall account for (and document) the u_{cal} of value(s) assigned to any calibrators used to calibrate the IVD MD, regardless of whether the final calibration of the IVD MD is performed by the end-user of the IVD MD or by the IVD MD manufacturer (sometimes called "factory calibration").

d) a summary description of the validation study(s) supporting the claim of metrological traceability of final measured quantity values assigned to human samples, using the specified IVD MD.

4.2 Definition of the measurand

The measurand shall be defined and described per the following characteristics, and included in the manufacturer's documentation:

a) name of the analyte (e.g. β-D-glucose).

b) biological system (e.g. human plasma). The intended medical use with regard to a particular medical decision shall be taken into account.

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EXAMPLE Human chorionic gonadotropin (total β-hCG) in human blood plasma, either for pregnancy detection or for tumour detection and monitoring.

c) kind of quantity (e.g. amount-of-substance).

d) unit of measurement (e.g. mmol/L).

e) in cases where a measurand is defined by a particular MP, measurement protocol, or a group of MPs (i.e. an operationally defined measurand), the MP or protocols shall be stated. MPs, calibrators or protocols that are essential to the definition of a particular measurand shall be available for general access and use by appropriately qualified laboratory personnel.

4.3 Specifications for maximum allowable expanded measurement uncertainty, *Umax(y)*

4.3.1 General requirements

The *Umax(y)* for an IVD MD shall be established by the manufacturer for measurements using the IVD MD in its intended setting with the intended human samples, and at minimum within the measurement intervals where medical decisions are made. Specifications for *Umax(y)* shall be included in the manufacturer's documentation of the calibration hierarchy for the IVD MD.

4.3.2 Scope of the specification

The *Umax(y)* specification established by the manufacturer of the IVD MD shall account for the combined measurement uncertainty associated with all steps in the calibration hierarchy for the IVD MD, down to and including the value assignment of end-user IVD MD calibrators in addition to the expected uncertainty contribution due to routine use of the IVD MD, at minimum under repeatability conditions.

NOTE 1 The *Umax(y)* specification for an IVD MD is the specification for the combined expanded (k=2) maximum allowable measurement uncertainty covering all steps in the calibration hierarchy, including the final measurement on human samples. Strategies for setting the *Umax(y)* for an IVD MD have been the central theme of various international conferences [31]–[34].

4.4 Defining the calibration hierarchy

4.4.1 General requirements

The calibration hierarchy shall be defined as a sequence of consecutive calibrations and value assignments, alternating between fit-for-purpose MPs and RMs (measurement standards or calibrators), beginning with a measurement standard and/or MP and ending with values for the measurand in the intended human samples as determined with the end-user IVD MD. The technical documentation of the calibration hierarchy shall include a graphic representation (i.e. a figure or other illustration) describing the linkage from the final results on human samples examined with the specified IVD MD up to the highest available metrological reference.

NOTE 1 Depending on the availability of higher order references (materials and MPs) for a given measurand, various calibration hierarchies and value transfer models are available (see Clause 5).

NOTE 2 The outcome (result) of each successive calibration in the hierarchy depends on the outcome (result) of the previous calibration (see Clause 5).

NOTE 3 For certain measurands, the quantity being measured changes at various steps throughout the calibration hierarchy.

EXAMPLE 1 For certain proteins in serum, the quantity being measured might be the amount of substance of a defined peptide derived from the protein of interest, or the amount of substance of a functional epitope.

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EXAMPLE 2 For β-D-glucose in serum, the quantity being measured might be a mass fragment of a derivative of β-D-glucose determined with mass spectrometry, or the product of the enzymatic degradation of β-D-glucose (e.g. H_2O_2 when using a glucose oxidase procedure).

4.4.2 Measured quantity

For each step in the defined calibration hierarchy where practical, the quantity being measured in the applicable RM (or human samples, in the case of the final measurements with an IVD MD) shall be identified, and the relationship between the measured quantity (or quantities) and the measurand shall be established.

4.4.3 Highest level of metrological traceability

For a given measurand, the metrologically highest placed MP, measurement protocol or calibration material in the calibration hierarchy shall be identified and shall define the highest level of metrological traceability for the stated measurement system.

4.4.4 Traceability to SI

For an IVD MD that claims metrological traceability of reported values for human samples to the SI, the defined calibration hierarchy shall be supported by available higher order references, including either or both RMs (requirements of ISO 15194 shall apply) and RMPs (requirements of ISO 15193 shall apply) that enable realization of the appropriate SI unit of measure for the corresponding measurand.

4.4.5 Non-SI traceable IVD MDs

To claim metrological traceability for a calibration and reported values (e.g. arbitrary or International conventional units) using a non-SI traceable IVD MD, the calibration hierarchy for the IVD MD shall be defined in a way that enables consistent realization of the corresponding (non-SI) units of measure.

4.4.6 Number of levels in the specified hierarchy

The number of levels (i.e. number of consecutive pairs of MPs and calibrators) in a calibration hierarchy may be modified by the parties implementing the calibration hierarchy, provided that the changes are validated and the metrologically highest elements of the hierarchy are retained (see Clause 5).

4.5 Selection and requirements for RMs and calibrators

4.5.1 General requirements

The calibrators (measurement standards) used at each step in the calibration hierarchy shall be documented to be fit for purpose by the party responsible for a given calibration step. The rationale for selection of each calibrator within the calibration hierarchy shall be included in the IVD MD manufacturer's documentation.

4.5.2 Characteristics to be documented

For each calibrator or RM applied in a defined calibration hierarchy for a particular IVD MD (excluding the end-user IVD MD calibrators), the following characteristics shall be identified and documented, and their consistency assured in replacement batches:

a) intended use of the material;

b) identity of the analyte (specifying as applicable atomic or molecular forms and/or chemical surrogate forms of the analyte);

c) origin of the material (e.g. synthetic, recombinant, microbial, human or animal);

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d) phase(s) (gas, liquid, solid);

e) state(s) of aggregation (solution, suspension, lyophilized);

f) matrix of the material (e.g. aqueous, other solvents, buffer, protein solution, human samples);

g) assigned values and their metrological traceability;

h) expanded measurement uncertainty, U(y), of RM assigned values;

NOTE 1 Expanded measurement uncertainty, U(y), divided by the coverage factor (reported on the RM certificate), is the standard measurement uncertainty, u(y), which is used in further calculation of combined measurement uncertainty.

NOTE 2 For non-certified *reference materials* (3.39) or calibrators, the standard uncertainty of the assigned value and corresponding coverage factor is sometimes expressed as a probability density distribution of the assigned value.

i) stability;

j) within-batch homogeneity;

k) commutability characteristics;

l) recognition if any (e.g. international, regional, national);

m) issuing authority if any (e.g. WHO, JISC, EU-JRC, NIST);

n) certificate status (certified, non-certified).

4.5.3 Higher order RMs that conform with ISO 15194

When higher order RMs are required for particular steps in a calibration hierarchy, those materials conforming to the requirements of ISO 15194 shall be used when suitable and available. Documentation of the ISO 15194 conformity status of any applicable RMs that comprise various stages in a calibration hierarchy for an IVD MD shall be included (or referenced) in the IVD MD manufacturer's technical file.

NOTE The Joint Committee for Traceability in Laboratory Medicine (JCTLM) lists[28] RMs that conform to requirements of ISO 15194.

4.5.4 RMs not conforming to ISO 15194

In cases where ISO 15194 conforming RMs are not available, or if available CRMs are not suitable for other reasons (for example, commutability not established or not satisfactory) other RMs not fulfilling all ISO 15194 requirements may be applied at the higher (highest) levels in a particular calibration hierarchy for an IVD MD, as long as the parties responsible for establishing the calibration hierarchy have demonstrated (with documentary evidence) the fitness for purpose and performance characteristics of such RMs. Documentation of such RMs as defined in the present clause shall address the material characteristics as specified in 4.5.2.

4.5.5 Commutability of RMs

Where applicable, the commutability of a RM relative to human samples shall be documented to be appropriate for its intended use at its position in the calibration hierarchy of an IVD MD.

NOTE MPs including those used to characterize and/or prepare primary (e.g. pure substance) RMs and primary calibrators (see Clause 5, Figs. 1 and 3, m.1 and m.2) usually cannot be applied to human samples as required when performing commutability assessments, hence commutability assessment is not required for such RMs at these levels (Clause 5, Figs. 1 and 3, m.1 and m.2) in a calibration hierarchy.

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4.5.6 Exception to commutability assessment requirements

When a RMP for the measurand (see Clause 5, Figs. 1, 2, and 3, p.3) is available, the first level where RM commutability can be assessed is at the level where a secondary (matrix) RM or other secondary calibrator (Clause 5, Figs. 1, 2 and 3, m.3), e.g. a CRM, is used in the calibration hierarchy, as in the case of a calibrator for the manufacturer's selected MP (Clause 5, Figs. 1, 2 and 3, p.4). For subsequent steps further down the calibration hierarchy, such as at the value transfer step employing a working calibrator (Clause 5, Figs. 1, 2 and 3, m.4) to calibrate the manufacturer's standing MP (Clause 5, Figs. 1, 2 and 3, p.5), commutability of the RM/working calibrator (Clause 5, Figs. 1, 2 and 3, m.4) shall be assessed to ensure appropriate value transfers and avoid bias.

4.5.7 Application of a non-commutable CRM

If a CRM (Clause 5, Figs. 1, 2 and 3, m.3) or international conventional calibrator intended to calibrate a manufacturer's selected MP (Clause 5, Figs. 1, 2 and 3, p.4) demonstrates commutability with human samples when measured by some but not all end-user IVD MDs intended for examination of a stated measurand, the CRM may still be used as a calibrator within the calibration hierarchy for a specified IVD MD for which the RM does not demonstrate commutability to the intended human samples, by application of a correction factor or function to the assigned value of the CRM. If applicable, details of the use and validation of such a correction to assigned values of the CRM or other RMs such as International conventional calibrators shall be disclosed in the documentation of the calibration hierarchy for the specified IVD MD, and the u_{cal} of values assigned to the end-user IVD MD calibrator(s) shall include any incremental uncertainty associated with the correction factor or function.

4.5.8 Alternative RMs

In the absence of commutable CRMs or international conventional calibrators, rationale shall be documented for selection of any alternative RMs (used as calibrators) at each applicable stage in the calibration hierarchy. Alternative RMs shall be documented to be fit for their intended purpose, shall each have an assigned value with a standard measurement uncertainty, and shall be demonstrated to be commutable with the intended human samples in each calibration transfer step in which they are deployed. Technical documentation for such alternative RMs shall include pertinent characteristics as outlined in 4.5.2.

NOTE 1 Alternative RMs include panels and/or pools of individual human samples, supplemented or "spiked" samples prepared in natural or artificial matrices, or other suitable materials.

NOTE 2 For guidance on appropriate selection of human sample panel members for use in a calibration hierarchy see CLSI EP09-A3, EP14-A3 and EP30-A.

NOTE 3 Human samples are assumed to be commutable when stored under conditions that have been validated not to alter the stability of the measurand or matrix.

NOTE 4 Validation of storage conditions for human samples, for a specified measurand, can be performed with a representative panel of individual human samples. Such validation of storage conditions for human sample panels can be used to support use of subsequent sample panels, obtained from persons with similar health/disease profiles, in sustaining the calibration hierarchy for the specified IVD MD, with no requirement for validation of commutability of stored sample panels.

4.5.9 Augmentation of alternative RMs

In cases where human sample panels are deployed as alternative RMs in a calibration hierarchy for a specified IVD MD, if the analyte in human samples (panels or pools) intended as RMs needs to be modified by augmentation or depletion to achieve appropriate quantity values, the commutability of the modified samples shall be validated. Where sample specific interferences or MP non-selectivity limitations are identified, individual human samples presenting with these limitations shall be excluded from human sample panels intended for use as calibrators in the calibration hierarchy.

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4.5.10 Non-commutable end-user IVD MD calibrators

When non-commutable materials are used as end-user calibrators (see Figures 1 to 6, m.5) for an IVD MD, commutable materials (for example a panel of human samples) shall be used in the calibration hierarchy to determine a correction factor or correction function to assign arbitrary values to the non-commutable end-user IVD MD calibrators to compensate for any bias due to non-commutability. If applicable, details of the use and validation of such a correction to assigned values of the non-commutable end-user IVD MD calibrators shall be disclosed in the documentation of the calibration hierarchy for the specified IVD MD, and the u_{cal} of values assigned to the end-user IVD MD calibrator(s) shall include any incremental uncertainty associated with the correction factor or function.

4.6 Selection and requirements for MPs

4.6.1 Rationale for selection of MPs and documentation responsibility

Each sequential value transfer step in a calibration hierarchy shall include a defined MP that is fit for purpose. The rationale for selection of MPs at each level of the established calibration hierarchy shall be included in the IVD MD manufacturer's documentation and shall be accompanied by supporting data demonstrating that the analytical performance characteristics of each MP meets performance requirements (i.e. is fit for purpose.) Elements of the documentation for a given MP in the calibration hierarchy may be obtained from a third party, for example from the developer of the applicable MP.

4.6.2 Metrological status of MPs

The MPs at each level of a defined calibration hierarchy shall be identified in terms of their metrological status. RMPs that comprise elements of a calibration hierarchy according to the models described in Clause 5 and that meet the requirements of ISO 15193 shall be considered to be MPs of higher metrological order. Different higher order RMPs may be deployed at different steps in the hierarchy. In the case that ISO 15193 conforming RMPs are not available, MPs that do not fulfil ISO 15193 requirements may still be applied in a hierarchy (for example a manufacturer's selected MP, or a manufacturer's standing MP), as long as the parties responsible for the calibration hierarchy have demonstrated (with documentary evidence) the fitness for purpose and performance characteristics of the relevant MPs.

EXAMPLE In the calibration hierarchy for a particular measurand, an SI-traceable higher order RMP, calibrated with a CRM, is deployed at the highest level in the calibration hierarchy. At subsequent (lower) levels in the calibration hierarchy, value transfer steps to assign values to commercial calibrators are introduced that deploy metrologically lower level MPs (e.g. international conventional RMPs, manufacturer's selected MPs and/or manufacturer's standing MPs), calibrated with secondary calibrators (with or without a certification.)

NOTE 1 Some MPs that are part of a calibration hierarchy, especially at the lower levels of a calibration hierarchy, are based on the same principle as the end-user IVD MD (e.g. a manufacturer's standing MP).

NOTE 2 Complete descriptions of higher order RMPs that establish traceability to SI units of measurement and conform with ISO 15193 are often published in the scientific literature.

4.6.3 Reference measurement laboratories

Reference measurement laboratories conforming with ISO 15195 may be selected by a manufacturer or other responsible party to provide reference measurement services in support of implementation of a metrologically traceable calibration hierarchy. The selected reference measurement laboratories, even if not conforming to ISO 15195, shall have demonstrated competence in providing best available measurements for the selected measurand in terms of the metrological traceability of values measured in human samples of the types intended and within the scope of the defined calibration hierarchy.

NOTE Conformance with ISO 15195 is independently demonstrated by achieving a listing of a reference measurement laboratory's reference measurement services in the JCTLM database[28].

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4.6.4 Impact of influence quantities

The description of a metrologically traceable calibration hierarchy for an IVD MD shall include results from investigation of the impact of influence quantities on the relevant MPs at each level of the calibration hierarchy.

4.6.5 Changes in the measured quantity within a calibration hierarchy

To ensure an unbroken chain of relationships and enable reporting of measured values that are traceable to the highest order available RMP (within medically acceptable limits), steps shall be taken at all levels of the calibration hierarchy to address and/or prevent problems associated with differences or changes in the measured quantity among the different MPs at the various levels in the calibration hierarchy. In this context, it is important to recognize and mitigate as necessary the differences between the measured quantity (or quantity being measured) and the measurand (quantity intended to be measured.)

NOTE 1 Multiple IVD MDs purporting to measure the same quantity but based on different chemical principles sometimes give different values for the same human sample or RM.

NOTE 2 Particular IVD MDs are sometimes influenced by measurement selectivity characteristics such as tertiary molecular structures, micro heterogeneity or chemical configurations of the target analyte.

EXAMPLE 1 Cases where there is variable micro heterogeneity of the analyte (isoforms, derivatives) in either or both the calibrator and/or the intended human samples (e.g. analyte classes such as enzymes, antibodies, glycoproteins, biomarkers from microorganisms, and other free or bound forms of analytes.)

NOTE 3 Metrological traceability problems often occur when the principle of the IVD MD is based on detection of a surrogate for the analyte of interest (e.g a peptide fragment of a large protein rather than the entire protein molecule) or when the IVD MD calibrator contains an analyte that is a surrogate for the analyte found in human samples.

EXAMPLE 2 Two or more IVD MD immunoassay MPs, all purport to measure the amount of substance concentration of a single protein hormone (e.g. thyroid stimulating hormone [TSH]). If different IVD MD immuno-MPs recognize and react to different extents with various epitopes of TSH, values for different although related quantities are generated by each IVD MD, possibly leading to lack of equivalence in the final measured values in certain human samples.

EXAMPLE 3 Non-equivalence of values among different IVD MDs may be observed among very selective (but different) measurement principles (e.g., a mass-spectrometric MP vs. an immunoassay procedure for a protein hormone in human serum). Each IVD MD is targeted toward detection of different isoforms or fragments of the same protein, but different values can be determined because different quantities are being measured with each IVD MD.

EXAMPLE 4 An end-user calibrator for an IVD MD intended to measure serum bilirubin may contain ditaurobilirubin (a synthetic surrogate analyte not found as a natural substance in human samples) in lieu of (or in addition to) naturally occurring unconjugated bilirubin and bilirubin glucuronide conjugates. Relative selectivity of the IVD MD for the surrogate analyte compared to the natural analyte found in human samples could change over the life of the IVD MD due to factors such as aging of one or more reagents, invalidating values assigned to the end-user IVD MD calibrators.

EXAMPLE 5 For the immunochemical measurement of ferritin amount of substance concentration in serum with analyte micro heterogeneity, where different isoforms of ferritin are recognized to different degrees by different monoclonal antibodies incorporated into different IVD MDs, leading to different reported values for various IVD MDs with certain human samples.

4.7 Estimating uncertainty of assigned values for end-user IVD MD calibrators

4.7.1 General requirements

The combined standard measurement uncertainty of the value assigned to an IVD MD calibrator (designated u_{cal} throughout this document) shall be estimated and made available to end-users by the

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manufacturer. The u_{cal} shall not exceed an acceptable fraction of the *Umax(y)* specification for the IVD MD taking into account a coverage factor *k*.

NOTE The development of an error budget allocation for u_{cal} is discussed elsewhere[33][34].

4.7.2 Documentation for method of estimating u_{cal}

The u_{cal} is estimated preferably according to the principles of the GUM. Regardless of whether the GUM method or a different method for estimation of the u_{cal} is followed, the method of statistical calculation of the u_{cal} shall be documented and maintained in the technical file of the IVD MD calibrator at least for the life of the product.

4.7.3 Statistical considerations and scope of u_{cal} estimates

For each IVD MD calibrator identified by a manufacturer for use in calibration of a specified IVD MD, the u_{cal} to be estimated and provided by the manufacturer of the IVD MD calibrator shall be determined by statistically combining the uncertainties associated with each of the sequential value assignment steps under the control of the manufacturer. In determining u_{cal}, the manufacturer shall also account for the known and foreseeable uncertainties contributed by all higher order value assignment steps in the defined calibration hierarchy, including steps not within the manufacturer's control such as (where applicable) the standard uncertainty of the value assigned to the highest order RM. Additional requirements in estimating u_{cal} include:

— Estimation of u_{cal} shall be based on at least one representative (single) lot or batch of reagent.

— Known and foreseeable variations and corresponding standard uncertainties in the specified IVD MD calibrators and reagents as well as in any intermediate RMs and measuring systems or MPs throughout the calibration hierarchy (due for example to factors such as but not limited to material heterogeneity and instability) shall be taken into account.

NOTE Estimated u_{cal} often varies among different lots of end-user IVD MD calibrators, especially in the case where different calibrator lots for the same IVD MD have substantially different assigned values.

4.7.4 Expression of u_{cal}

u_{cal} shall be expressed as a standard deviation (SD). When multiple component uncertainties are combined to estimate combined standard uncertainty, each component uncertainty (i.e. the $u(y)$ at each level in the calibration hierarchy) shall first be expressed as a variance, SD^2. The contributing variance components are then summed, and the square root of the sum of the variances is the combined standard uncertainty, $u(y)$; see EXAMPLE 1. u_{cal} can alternatively be calculated and expressed in terms of relative combined uncertainty, or percent relative combined uncertainty, $\%ru(y)$, i.e., relative uncertainty with respect to the mean or target value of the measurand in the calibrator; see EXAMPLE 2.

NOTE 1 The minimum information needed to estimate the uncertainty contribution of any MP within a calibration hierarchy is the standard deviation of the MP under repeatability conditions ($u_{Rw\text{-}p.x}$) as well as the uncertainty of the value assigned to any calibrators used for that MP.

NOTE 2 The calculations shown in EXAMPLES 1 and 2 are applicable only under circumstances where the input quantities are independent. If the input quantities are not independent, co-variances are appropriate.

EXAMPLE 1 Calculation of a combined standard uncertainty is performed according to generalized Formula (1):

$$u(y)=\sqrt{\left[u(y)_1^2+u(y)_2^2+u(y)_3^2+...+u(y)_n^2\right]} \quad (1)$$

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where

$u(\mathrm{y})$	is the combined standard uncertainty of the final measured value;
$u(\mathrm{y})_1, u(\mathrm{y})_2, u(\mathrm{y})_3, \ldots u(\mathrm{y})_\mathrm{n}$	are the standard uncertainties of the contributing variances from each step in the defined calibration hierarchy.

The expanded combined uncertainty, U, is calculated per Formula (2) as follows:

$$U = u(\mathrm{y}) \times k \tag{2}$$

where

$u(\mathrm{y})$	is the combined standard uncertainty determined according to Formula (1);
U	is the expanded combined uncertainty;
k	is the coverage factor (often 2, for a level of confidence of approximately 95 %).

EXAMPLE 2 Calculation of combined percent relative uncertainty of a measurement system is performed according to the generalized Formula (3):

$$\%\mathrm{r}u(\mathrm{y}) = \sqrt{\left[\%\mathrm{r}u(\mathrm{y})_1^2 + \%\mathrm{r}u(\mathrm{y})_2^2 + \%\mathrm{r}u(\mathrm{y})_3^2 + \ldots + \%\mathrm{r}u(\mathrm{y})_\mathrm{n}^2\right]} \tag{3}$$

where $\%\mathrm{r}u(\mathrm{y})$ is the combined percent relative standard uncertainty and where each component relative uncertainty is calculated according to Formula (4):

$$\%\mathrm{r}u(\mathrm{y})_\mathrm{n} = 100 \times u(\mathrm{y})_\mathrm{n} / \mathrm{m}(\mathrm{y})_\mathrm{n} \tag{4}$$

and where

$\%\mathrm{r}u(\mathrm{y})_\mathrm{n}$	is the percent relative standard uncertainty of the 'n'–th component uncertainty;
$u(\mathrm{y})_\mathrm{n}$	is the standard uncertainty of the 'n'–th component uncertainty;
$\mathrm{m}(\mathrm{y})_\mathrm{n}$	is the mean measured (or target) value of the measurand for the 'n'–th component measurement procedure.

The square of each component percent relative uncertainty, $\%\mathrm{r}u(\mathrm{y})^2$, i.e. the percent relative variance, is calculated per Formula (5):

$$\%\mathrm{r}u(\mathrm{y})_\mathrm{n}^2 = \left[100 \times u(\mathrm{y})_\mathrm{n} / \mathrm{m}(\mathrm{y})_\mathrm{n}\right]^2 \tag{5}$$

where $\%\mathrm{r}u(\mathrm{y})_\mathrm{n}^2$ is the percent relative variance of the 'n'–th component uncertainty.

The component percent relative variances are then summed, and the square root of the sum of the component percent relative variances is calculated according to Formula (3) to derive $\%\mathrm{r}u(\mathrm{y})$, the combined percent relative standard uncertainty for the measurement system.

4.7.5 Product modifications

When an IVD MD or a designated end-user calibrator for an IVD MD is modified by the manufacturer (either the original manufacturer or a different entity), the u_{cal} of assigned values for each relevant IVD MD calibrator shall be confirmed or re-estimated by the manufacturer, unless justification is provided for why the change does not affect u_{cal}.

NOTE In this clause, a manufacturer is any entity, including a medical laboratory, who modifies an IVD MD.

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4.7.6 Information to be provided to the end-user

For assigned values of IVD MD calibrators, the minimum information concerning the u_{cal} that shall be provided by the calibrator manufacturer to the end-user on request is: numerical value of y, u_{cal}(y), where y is the value assigned to the calibrator.

NOTE 1 Estimates for u_{cal} of IVD MD calibrators are sometimes presented as the expanded uncertainty (U_{cal}), where $U_{cal} = u_{cal}$ (y) × k, usually with the coverage factor k = 2, giving a level of confidence of approximately 95 %. Since the preferred information to be provided by the manufacturer is u_{cal} as a combined standard uncertainty only, the reporting of the expanded uncertainty (U_{cal}) of calibrator assigned values is discouraged.

NOTE 2 Dependent on local and regional requirements, medical laboratory end-users of IVD MDs often use the u_{cal} value provided by the manufacturer of the IVD MD calibrator to estimate the combined measurement uncertainty of the measured value for a human specimen as determined with the specified end-user IVD MD.

4.8 Validation of metrological traceability of values assigned to an IVD MD calibrator

4.8.1 General validation requirements

The IVD MD calibrator manufacturer shall validate a claim of metrological traceability of the value assigned to the IVD MD calibrator.

NOTE 1 As stated in 3.49, ISO 9000 defines validation as confirmation (supported by objective evidence) that the requirements for a specific intended use or application have been fulfilled. ISO 9000 further defines 'objective evidence' as data that supports the existence of something. Objective evidence is obtained by means of observation, measurement, testing or other means.

NOTE 2 Validation of metrological traceability of a calibration can be achieved using a continuum of tools and strategies. The most straightforward strategies for developing objective evidence of the validity of calibration traceability are for measurands with the most completely developed reference systems. The more complex validation strategies (and increased burden of responsibility for documentation) are required for calibration hierarchies supporting measurands with no existing higher order references or harmonisation protocols.

4.8.2 Validation strategies

Design of studies for the validation of a claim of traceability of assigned values for end-user IVD MD calibrators shall be documented by the manufacturer in the IVD MD technical file. The selection of a particular validation strategy for a given calibration hierarchy shall depend on the maturity and performance characteristics of the reference system for the measurand as well as the availability of materials (RMs) and MPs as needed to perform the types of studies listed below. For a given calibration hierarchy, several validation strategies may be applied, at the option of the party responsible (often the manufacturer) for defining the calibration hierarchy of the particular IVD MD. Study strategies applicable to validation of a calibration traceability claims for an IVD MD include but are not limited to:

a) Examination of commutable RMs (preferably, CRMs and/or trueness control materials; see 3.46).

b) Participation in EQA, proficiency testing (PT), or other inter-laboratory comparison schemes that utilize commutable test samples, with target values preferably assigned by a RMP (when available) or a harmonisation protocol.

c) Examination of banked human samples with values previously assigned by a RMP.

d) Method comparison studies on a set of human samples, comparing to a higher order RMP.

e) Method comparison studies on a set of human samples with another independent MP (that is not a RMP).

f) Higher order analytical controls embedded into the calibration hierarchy and value assignment MPs, focusing on use of carefully calibrated, SI traceable measurement tools and controls (for example, balances, volumetric glassware, spectrophotometers, thermometers, ambient environmental controls, reagents with highest available purity).

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NOTE 1 Among the validation possibilities described above, the availability of a RMP is the most critical factor.

NOTE 2 Among the generic validation strategies described above, bullets a) to e) are focused on the output (i.e. trueness of measured values) of the specified calibration hierarchy, while the strategies in bullet f) focus on the trueness and reproducibility of the value transfer process and procedures within the calibration hierarchy (i.e. critical steps such as volumetric and gravimetric measurement).

NOTE 3 For guidance on appropriate selection of human sample panel members for method comparison studies (bullets c), d) and e) above), refer to CLSI EP09-A3, EP14-A3, and EP30-A.

4.8.3 Test design considerations and acceptance criteria

For validation studies involving method comparisons with panels of human samples to support claims of metrological traceability of a value assigned to an IVD MD calibrator [see 4.8.2, c), d) and e)], known variables affecting human sample and/or calibrator measurements for both the test IVD MD being evaluated and the RMP (or other MP) against which results from the test IVD MD will be compared, shall be accounted for. Pre-determined acceptance criteria for validation shall be derived from and shall not exceed the *Umax(y)* specifications for the IVD MD as defined in the respective calibration hierarchy for the measurand (see 4.3). The number of replicates of each sample being measured using the test IVD MD shall be set such that the power to detect a bias as large as the validation criteria is reasonably high (e.g. >80 %), while the chance of incorrectly failing the validation criteria is low (e.g. <5 %).

NOTE Methods for derivation of *Umax(y)* specifications for IVD MDs are discussed in depth elsewhere [31]–[34].

4.8.4 Calibration hierarchies with an available RMP

For calibration hierarchies as described in 5.2, 5.3 and 5.4 (Figures 1, 2 and 3), with an available RMP for the measurand, traceability of values assigned to end-user calibrators and human samples shall be validated by comparison of measured values with sets of human samples of the type(s) intended for use with the IVD MD. These comparisons shall be made between values measured with the RMP (see Figures 1–3, [p.3]) and values measured with the calibrated end-user's IVD MD. Where physical limitations and costs make such comparisons impractical, in lieu of comparing the test IVD MD to the highest available RMP, a comparison with a secondary RMP (or other lower order RMP) that is part of the defined calibration hierarchy for the measurand (see Figures 1-3, [p.4]) shall be an acceptable alternative, with documented justification.

4.8.5 Calibration hierarchies with no available RMP

In the case of calibration hierarchies for measurands with no available RMPs for the measurand, including calibration hierarchies supported by an international conventional calibrator or an international harmonisation protocol as described in 5.5 and 5.6, respectively (Figures 4 and 5), validation of metrological traceability of values assigned to calibrators for specified IVD MD's for these measurands shall be performed, using pre-determined acceptance criteria, by conducting method comparison studies using panels of human samples of the type(s) for which the IVD MD is intended. For IVD MDs for measurands standardized using an international conventional calibrator or a CRM with its value assigned by consensus of several qualified MPs (but not a reference MP) as described in 5.5 and Figure 4, at least one method comparison study shall be performed in comparison to a different and independent IVD MD intended for the same measurand that has been standardized with the same international conventional calibrator or CRM and which claims to be metrologically traceable to the specified international conventional calibrator or CRM. With IVD MDs for measurands standardized according to an international harmonisation protocol (see 5.6 and Figure 5) at least one method comparison study shall be performed in comparison to a different and independent IVD MD that has been harmonised according to the international harmonisation protocol.

4.8.6 Calibration hierarchies with no RMPs and no CRMs

In the case of measurands with no available RMPs or CRMs, and no international conventional calibrators or harmonisation protocols for the measurand (see 5.7 and Figure 6), internally developed and maintained calibration hierarchies defined by manufacturers of IVD MDs intended for these

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kinds of measurands shall be validated for metrological traceability of values assigned to calibrators, according to pre-determined acceptance criteria, by performing verification studies confirming that all known input quantities and influence quantities in the measurement formula are carefully controlled and reproducible. Key measurement variables and influence quantities contributing to the performance of the IVD MD shall be defined and characterized.

Normal variation of the known MP variables and influence quantities shall be assessed and quantified in terms of their contributions to the standard measurement uncertainty of the IVD MD, and their combined effects (when summed statistically) shall not exceed an appropriate fraction of the *Umax(y)* for the IVD MD.

4.8.7 Validation of design changes to an end-user IVD MD calibrator

In the case of design changes to an IVD MD calibrator, and as mandated per the results of appropriate risk assessments, the manufacturer shall perform re-validation of the metrological traceability of values assigned to the IVD MD calibrator or shall justify in the manufacturer's technical documentation (e.g. design history file) rationale as to why re-validation of metrological traceability is not required. In the course of implementation of any design changes, end-users shall be informed if any new information becomes available regarding the performance expectations for the calibrator and its intended IVD MD.

NOTE Design changes include (but are not limited to) changes in specifications of raw materials, changes in sources of raw materials (e.g. changing from one tissue source to another tissue source for an enzyme), manufacturing process or vendor changes, amount of measurand specification changes, value-assignment protocol changes.

4.9 Additional calibration hierarchy documentation responsibilities

4.9.1 Obligation to end-users

The manufacturer of end-user IVD MD calibrator(s) shall provide to end-users on request the assigned target value, the associated metrological traceability and u_{cal} for each level of calibrator provided for use with a specified IVD MD.

4.9.2 Maintaining documentation

Documentation of procedures and data supporting a calibration hierarchy of an IVD MD for measurement of a particular measurand(s) in human samples, including the manufacturing specifications, estimated standard measurement uncertainties, materials, verification and validation studies, and operating procedures, shall be maintained in the manufacturer's technical file at least for the life of the IVD MD.

4.9.3 Third party manufacturers of IVD MD calibrators

In some cases, manufacturers of IVD MDs specify end-user IVD MD calibrators manufactured by a different (second or independent) manufacturer. Such independent (third party) manufacturers of IVD MD calibrators shall maintain the technical file supporting claims of metrological traceability of assigned values for each measurand claimed in the intended use statement for such applicable IVD MD calibrator(s). Similarly, any manufacturer of an IVD MD calibrator who sells a calibrator intended for use with "other" (third party) IVD MDs (with or without collaboration with the manufacturer of the IVD MD measuring system) is responsible for fulfilment of all documentation requirements defined in this document.

4.9.4 Modifications introduced by independent entities

If modifications to an IVD MD are defined and implemented by a medical laboratory or other independent entity, third party or person who is not the original manufacturer of the IVD MD, full description and re-validation of the calibration hierarchy underlying the reported values for human samples when examined with the modified IVD MD shall be the responsibility of the entity(s) that specified and implemented the modifications.

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4.9.5 Calibration hierarchies supporting IVD MDs developed by a single entity for its own use

In the case of an IVD MD developed by a single entity for its own use, the developing and/or implementing entity shall be responsible for validating and describing the full calibration hierarchy down to and including the results for human samples.

4.9.6 RMs other than end-user IVD MD calibrators

For RMs other than end-user IVD MD calibrators (e.g. IVD MD trueness control materials, 3.46), the RM manufacturer shall be responsible for validating and describing the calibration hierarchy that is the basis for any measurand values assigned to such RMs and for documenting the status of the material's commutability with human samples (if applicable) when used with any intended MPs, including any IVD MDs. Combined standard measurement uncertainty of assigned values for these kinds of RMs for IVD MDs (that are not IVD MD calibrators) shall be estimated by the manufacturer and provided to end-users on request.

4.9.7 EQA and PT materials with claims of metrologically traceable target values

The manufacturer of a commutable trueness-based (see 3.46) EQA and/or PT material with an assigned value(s) claimed to be metrologically traceable to higher order references (for one or more measurands), shall define, describe and validate the relevant calibration hierarchy supporting the assigned values for each stated measurand. Where claimed by the producer, commutability of such EQA or PT materials shall be demonstrated according to published recommendations (see CLSI EP30-A and [35]–[37]) for representative IVD MDs widely used by end-user medical laboratories. The assigned values for each measurand and the estimated u_{cal} values shall be determined and provided to end-users upon request.

5 Model calibration hierarchies for metrological traceability

5.1 Elements of the description of a calibration hierarchy

Calibration hierarchies for IVD MDs shall be described in the manufacturer's technical documentation. A description of a calibration hierarchy shall include the following elements:

a) a definition of the measurand.

b) a description of the sequence of calibration and measurement steps, each of which consists of a MP and a calibrator, where the "unknown" sample(s) being measured at each step in the hierarchy (except for the final step) functions in turn as the calibrator(s) for the next/subsequent step (a MP).

c) an estimate of uncertainty of assigned values of the measurand in RMs (IVD MD calibrators) deployed at the lowest level in the calibration hierarchy (typically with an IVD MD), so as to enable end-user estimation of the combined standard uncertainty of reported values in the intended samples (e.g., human samples, EQA materials, or other calibrators).

NOTE 1 The six generic model calibration hierarchies described (5.2 to 5.7) are hierarchies that can be implemented by IVD manufacturers to support metrologically traceable calibrations for various measurands. In these models, "trueness" from the first (highest order) calibration material and/or MP is carried through to the very last material being measured (human samples) at the final measurement step of the sequence (usually an IVD MD).

NOTE 2 The model calibration hierarchies described are representative of current state of the art and widely available technologies, and are applicable to particular classes of measurands, depending on availability of higher order references. The models presented are not intended to be inclusive of all possibilities and do not exclude other possibilities; additional models can be described to support particular measurands and/or new technologies.

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NOTE 3 The number of levels (i.e. pairs of MPs and calibrators) applied in a calibration hierarchy for a given measurand is the responsibility of the parties implementing the calibration hierarchy, provided that the highest order available elements (e.g., RMs and/or RMPs) remain embedded in the final hierarchy. The final choice of the particular metrological levels to be included in a given calibration hierarchy depends on chemical characteristics of the measurand, target uncertainty of measurement for the end result, and availability of MPs, calibrators, and other relevant technology (e.g. information technology).

5.2 Cases with RMPs and primary RMs

5.2.1 General considerations

A model calibration hierarchy for measurands supported with available RMPs and primary RMs, with full metrological traceability to the SI is described in Figure 1. The characteristics to be addressed in the description of these types of calibration hierarchies are elaborated in 5.2.2 to 5.2.13.

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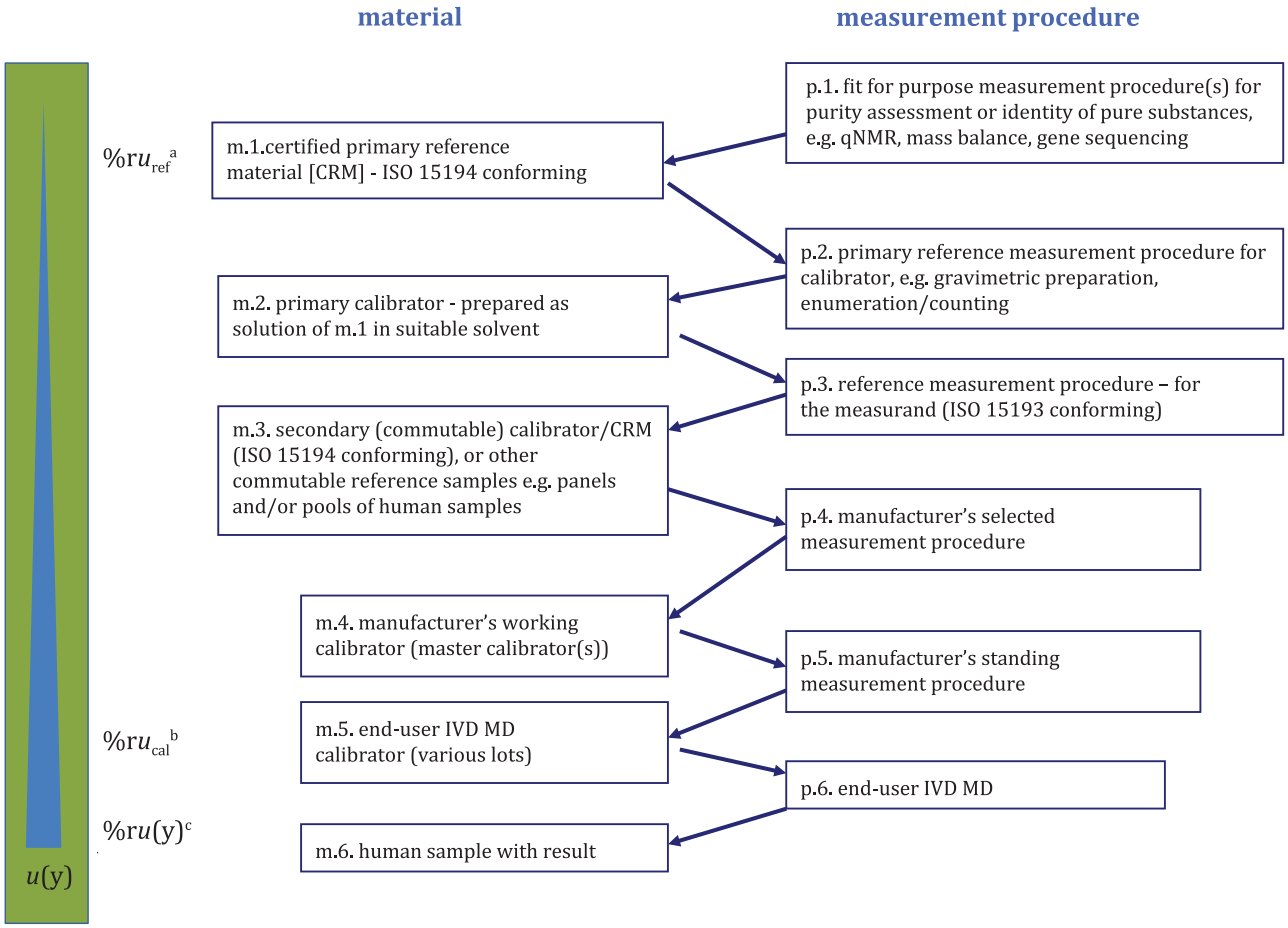

a Relative percent standard uncertainty of value assigned to the primary RM [m.1].

b IVD MD calibrator [m.5] value assignment relative percent combined uncertainty, according to the following formula:

$$\%ru_{cal} = \sqrt{(\%ru^2_{ref} + \%ru^2_{Rw\text{-}p.2} + \%ru^2_{Rw\text{-}p.3} + \%ru^2_{Rw\text{-}p.4} + \%ru^2_{Rw\text{-}p.5})}$$

where $\%ru_{Rw\text{-}p.2}$, $\%ru_{Rw\text{-}p.3}$, etc., represent the percent relative standard uncertainties for each applicable MP in the calibration hierarchy.

c Relative percent combined standard measurement uncertainty for reported values of the measurand with the end-user IVD MD, calculated per the following equation:

$$\%ru(y) = \sqrt{(\%ru^2_{cal} + \%ru^2_{Rw\text{-}p.6})}$$

where $\%ru^2_{Rw\text{-}p.6}$ is the relative percent standard uncertainty of the IVD MD based on long-term precision (repeatability conditions of measurement).

Figure 1 — Calibration hierarchy — Full metrological traceability to SI

5.2.2 Definition of the measurand

Definition of the measurand shall include the SI unit of measurement, whether base or derived quantity, to which metrological traceability shall refer.

EXAMPLE 1

1) base quantities: mole, kilogram;

2) derived quantities: mole per cubic metre (= millimole per litre), gram per kilogram.

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NOTE 1 The measured quantity at different steps in the calibration hierarchy is subject to change as the material being measured changes. Changes to the measured quantity often lead to different SI reporting units.

EXAMPLE 2 In a mass balance measurement for purity assessment of a certified primary RM for cortisol, the mass fraction of impurities (the measured quantity in this case) is determined rather than the mass fraction of cortisol, and the purity of the material is stated as a mass fraction, using the unit g/kg. For other RMs used at lower levels in the calibration hierarchy of an IVD MD for cortisol, such as secondary RMs (e.g. CRMs) or manufacturer's working calibrators, the amount-of-substance concentration of cortisol (in serum or other body fluid) is measured and measurement results are expressed with the appropriate SI units (µmol/L).

EXAMPLE 3 For complex measurands such as specific proteins in human blood serum (e.g. albumin), the measured quantity in a calibration hierarchy at the highest level is often the purity of the intact protein (e.g. mass fraction, mg/g). At other lower levels in the calibration hierarchy, the measurand is often the amount-of-substance concentration of specific epitopes or peptides derived from the protein of interest. In such cases the measured quantity is different at different levels in the hierarchy, and the assigned values for various RMs across the hierarchy will be expressed in different SI units.

NOTE 2 Some measureable quantities cannot be expressed in terms of the seven base quantities of the SI, but have the nature of a count[38]. Examples are a number (i.e. a count) of specified molecules, a number of specified cellular or biomolecular entities (e.g. number of copies of a particular nucleic acid sequence or number of specified lipoprotein particles). A full description of the quantity being counted is essential.

EXAMPLE 4 Number of CD4 cells per unit volume[40].

EXAMPLE 5 Number of copies of a defined KRAS nucleic acid sequence per unit volume[41].

NOTE 3 Formal traceability to the SI for counts is established through appropriate, validated counting MPs (see ISO 20391, ISO 20395 and [38], [42]).

5.2.3 Selecting RMPs

Primary RMPs and other fit for purpose MPs (see Figure 1, p.1, p.2) shall be based on principles of measurement demonstrated to have fit for purpose performance, providing metrological traceability to an SI unit of measurement with the smallest achievable measurement uncertainty. More than one primary RMP can exist at a given time for assigning values for quantities of a given kind to primary calibrators. The values obtained by two or more primary RMPs for a given measurand shall not be significantly different within a stated uncertainty at a certain level of confidence.

NOTE Counting (enumeration-based) MPs can form the basis of a primary RMP subject to a detailed description of the measurand, establishment of selectivity and completeness of count and a statement of measurement uncertainty

EXAMPLE 1 Two counting MPs for DNA copy number concentration that do not require a calibration standard are flow cytometric counting (FCM) and digital polymerase chain reaction (dPCR)[41][43].

EXAMPLE 2 Two counting MPs for cell number concentration not requiring a calibration standard are microscopy and FCM[40][42].

5.2.4 Primary RMPs

A selected primary RM (see Figure 1, m.1) shall be the best available realization (embodiment) of the unit of measurement with the smallest achievable relative standard measurement uncertainty (denoted by the abbreviation $\%ru_{\mathrm{ref}}$ in Figure 1.) The primary RM shall have its value assigned either directly by a primary RMP or by a fit for purpose MP for identity and/or purity assessment of pure substances, e.g. qNMR, mass balance, gene sequencing[38][39]. The value assignment and documentation for a primary RM shall conform to ISO 15194.

NOTE The primary RM (see Figure 1, m.1) usually is highly purified, containing a physico-chemically well-defined analyte, evaluated for stability, compositional integrity, and accompanied by a certificate (i.e. a CRM).

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EXAMPLE 1 β-D-Glucose as SRM 917b[1)] from NIST; the mass fraction of β-D-Glucose in the material is 997,0 mg/g, with an expanded measurement uncertainty of 0,2 mg/g. (The measurand is the mass fraction of **β**-D-glucose in crystalline glucose material expressed in mg/g).

EXAMPLE 2 Cholesterol as SRM 911b[1)] from NIST; mass fraction 0,998 ± 0,001 where the purity and estimated uncertainty is based upon scientific judgment and evaluation of numerous analytical tests applied to this CRM in the certification process. The uncertainty given approximates two standard deviations about the certified value. (The expanded uncertainty is 0,001 with a coverage factor $k = 2$, giving a level of confidence of approximately 0,95).

5.2.5 Primary calibrators

A primary calibrator (see Figure 1, m.2) shall be prepared from a primary reference material [m.1] and value-assigned using a primary RMP (see Figure 1, p.2).

NOTE Frequently the primary RMP is gravimetry, with the dissolution of a measured mass of the primary RM in a measured mass of an appropriate solvent.

EXAMPLE A primary calibrator for uric acid can be prepared by gravimetric dissolution into a solvent of a CRM of pure uric acid, e.g. SRM 913b[2)], value assigned by NIST, with a certified value of the mass fraction of uric acid in the pure material of 0,998 kg/kg, with an expanded uncertainty (level of confidence 95 %, $k = 2$) of 0,002 kg/kg.

5.2.6 Assigning a value to a secondary RM or calibrator

An appropriate RMP (see Figure 1, p.3) for the measurand shall be used to assign a value to a secondary calibrator or secondary RM (see Figure 1, m.3) with a complex matrix. For the documentation of the RMP (see Figure 1, p.3) for the measurand, the requirements of ISO 15193 shall apply.

NOTE 2 In cases where there is more than one RMP, or multiple reference laboratories capable of performing the same MP for the measurand, EQA programs such as IFCC External Quality Assessment Scheme for Reference (calibration) Laboratories in Laboratory Medicine[44] can provide helpful information regarding equivalence among different RMPs and different reference laboratories.

5.2.7 Commutability of secondary RMs

The secondary calibrators or secondary RM (see Figure 1, m.3) shall be commutable with human samples as determined in commutability assessment studies.

NOTE See CLSI EP30-A and other published recommendations[36]–[38] for conducting commutability studies.

EXAMPLE NIST SRM 967a[1)] creatinine in frozen human serum, two separate vials with certified values of 0,074 9 mmol/L and 0,342 7 mmol/L, is an example of a commutable reference material appropriate for use as a secondary calibrator (see Figure 1, m.3). The measurand is the amount of substance concentration of creatinine in frozen human serum expressed in mmol/L. The certified concentration values for each level of this material are based on isotope dilution liquid chromatography/mass spectrometry (ID LC/MS).

5.2.8 Manufacturer’s Selected MP

The manufacturer's selected MP (see Figure 1, p.4) shall comprise a measuring system that is calibrated by one or more (commutable) calibrators or RMs (see Figure 1, m.3), when available.

EXAMPLE For the concentration of cortisol in blood plasma, isotope dilution-gas chromatography-mass spectrometry (ID-GC/MS) can be a selected MP.

5.2.9 Working calibrators

The manufacturer's working calibrator (see Figure 1, m.4) shall have its value assigned according to the manufacturer's selected MP (see Figure 1, p.4), or (depending on commutability characteristics of the working calibrator), according to the RMP (see Figure 1, p.3). The secondary (working) calibrators (see

1) This RM is an example of a suitable product available commercially. This information is given solely for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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Figure 1, m.4) shall be commutable with human samples as determined in commutability assessment studies (see 4.5.5) comparing the manufacturer's selected MP (see Figure 1, p.4) and the manufacturer's standing MP (see Figure 1, p.5), or comparing the RMP (see Figure 1, p.3) and the manufacturer's standing MP (see Figure 1, p.5) if steps (see Figure 1, m.3) and (see Figure 1, p.4) are omitted from the calibration hierarchy.

NOTE 1 The manufacturer's working calibrator (see Figure 1, m.4) is sometimes called "manufacturer's master calibrator", "in-house calibrator" or "master calibrator lot."

NOTE 2 A manufacturer's working calibrator is usually a material with a matrix resembling that of the human samples intended to be measured by the end-users' IVD MD.

NOTE 3 Manufacturers often use panels of clinical samples or a series of pools of human clinical samples for working calibrators.

5.2.10 Manufacturer's standing MP

The manufacturer's standing MP (see Figure 1, p.5) shall define a MP that is calibrated by one or more of the manufacturer's working calibrators or other commutable matrix calibrators and is validated for analytical selectivity.

5.2.11 Manufacturer's end-user calibrator

The manufacturer's end-user calibrator (see Figure 1, m.5.) shall have its value assigned according to the manufacturer's standing MP (see Figure 1, p.5) or the manufacturer's selected MP (see Figure 1, p.4) and is intended for calibration of the end-user's IVD MD (see Figure 1, p.6).

5.2.12 u_{cal} of the assigned value of the end-user calibrator

The u_{cal} of the assigned value of the end-user calibrator (see Figure 1, m.5) shall be estimated by the manufacturer (see 4.7), incorporating all appropriate higher order uncertainties such as the uncertainty (u_{Ref}) of the assigned value of the primary RM (see Figure 1, m.1) in addition to the uncertainties of each of the subsequent MPs in the calibration hierarchy down to and including the manufacturer's standing MP (see Figure 1, p.5).

5.2.13 End-user IVD MD

The end-user IVD MD (see Figure 1, p.6) shall describe a measuring system calibrated by one or more end-user calibrators. This MP, the final MP in the calibration hierarchy for the defined measurand, is used to examine human samples and generate final measured values for the measurand, with combined standard measurement uncertainties of the reported values to be estimated by the end-user, taking into account all known measurement uncertainties accrued at each higher step in the defined calibration hierarchy.

5.3 Cases with a primary RMP that defines the measurand

5.3.1 General Considerations

A model calibration hierarchy for measurands with a primary RMP that defines the measurand, (with metrological traceability to SI) is described in Figure 2. For these types of measurands, there are no certified primary RMs available. In such cases, as exemplified by calibration hierarchies for some measureable quantities for catalytic activity concentration of enzymes measured in human serum (or other body fluids), metrological traceability to SI is based on well-defined and internationally agreed RMPs. The characteristics to be addressed in the description of these calibration hierarchies are elaborated in 5.3.2 to 5.3.11.

NOTE Certain blood coagulation factors are also examined by measurement of their catalytic activity concentration in blood or blood plasma, e.g. Factor VIII[45].

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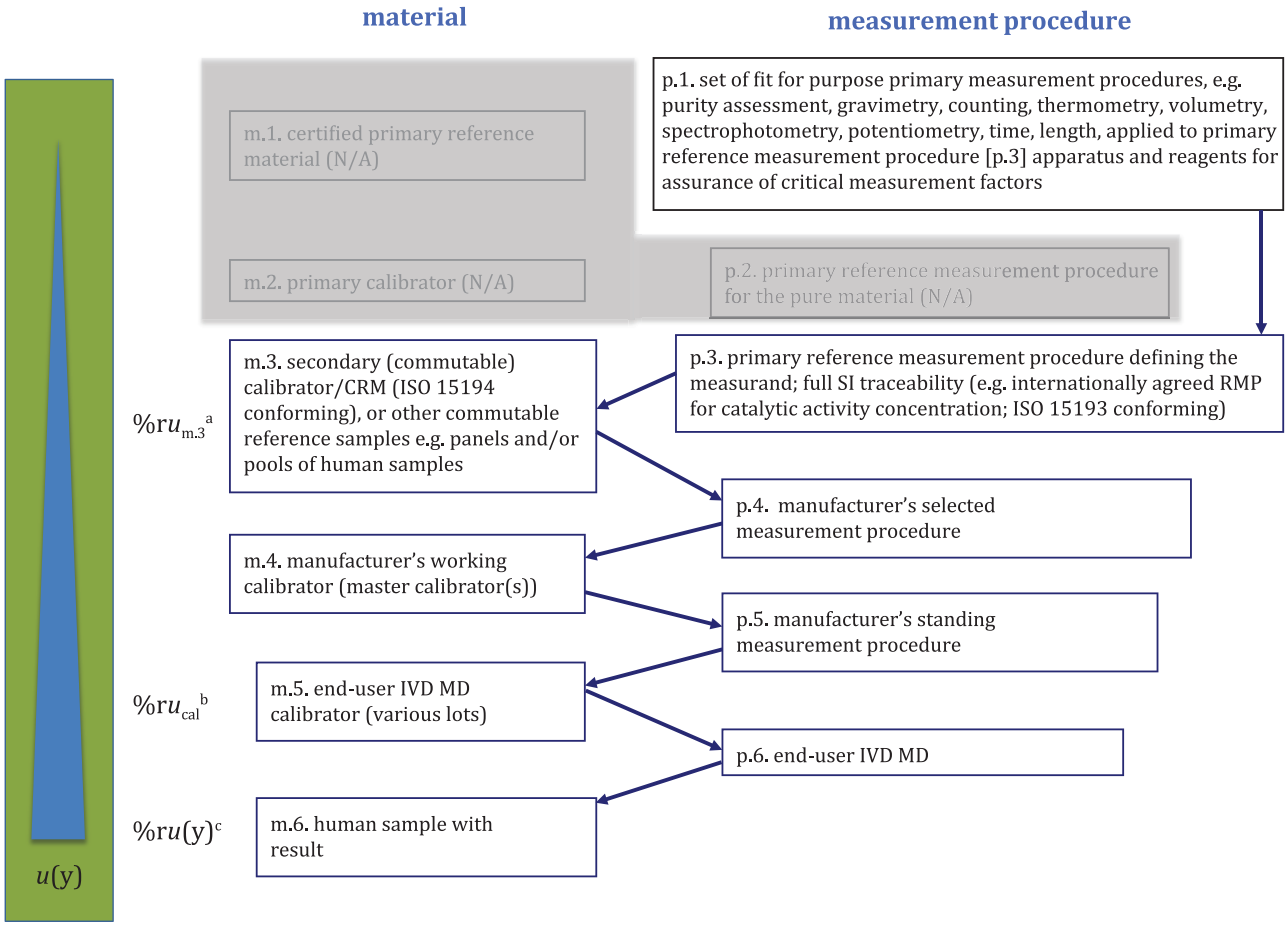

a Relative percent combined value assignment uncertainty of the [m.3] reference material, calculated according to the following formula:

$$\%ru_{m.3} = \sqrt{(\%ru_{c\,p.1}^2 + \%ru^2_{Rw\text{-}p.3})}$$

where

$\%ru_{c\,p.1}^2$ is the relative percent combined standard measurement uncertainty for the [p.1] higher order MPs with relation to e.g. thermometry, volumetry, spectrophotometry, pH, time, length, etc.;

$\%ru_{Rw\text{-}p.3}$ is the relative percent standard deviation (CV%) for MP [p.3] under repeatability conditions.

b Relative percent combined value assignment uncertainty of the IVD MD calibrator [m.5] calculated according to the following formula:

$$\%ru_{cal} = \sqrt{(\%ru^2_{m.3} + \%ru^2_{Rw\text{-}p.4} + \%ru^2_{Rw\text{-}p.5})}$$

where $\%ru_{Rw\text{-}p.4}$, $\%ru_{Rw\text{-}p.5}$, represent the percent relative standard uncertainties for each applicable MP in the calibration hierarchy.

c Relative percent combined standard measurement uncertainty for reported values of the measurand with the end-user IVD MD, calculated per the following formula:

$$\%ru(y) = \sqrt{(\%ru^2_{cal} + \%ru^2_{Rw\text{-}p.6})}$$

where $\%ru^2_{Rw\text{-}p.6}$ is the relative percent standard uncertainty of the IVD MD based on long-term precision (repeatability conditions of measurement).

Figure 2 — Calibration hierarchy — Measurand defined by a RMP, but no primary RM for the quantity; traceable to SI. Materials [m.1] and [m.2], and MP [p.2] are not applicable (N/A)

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5.3.2 Definition of the measurand

Definition of the measurand shall include the SI unit of measurement, whether base or derived unit, to which metrological traceability shall refer.

EXAMPLE For catalytic concentration of enzymes, the relevant derived quantities include: mole per second per cubic metre (= mol s^{-1} m^{-3}), katal per litre (= kat L^{-1}).

NOTE 1 The kind–of-quantity 'catalytic concentration' is catalytic activity of component in katal (or mole per second) divided by volume of (original) system sampled in cubic metres.

NOTE 2 In laboratory medicine, the denominator can be chosen to be "litre", giving the non-coherent derived unit "katal per litre", symbolized = kat L^{-1} = kat/L = mol s^{-1} L^{-1} = (mol/s)/L.

NOTE 3 Another, non-coherent unit used is based on the unit for catalytic activity "enzyme unit" (or "international unit"), symbolized U, with the conversion formula, 1 U = 1 μmol min^{-1} = 16,667 × 10^{-9} kat. Consequently, 1 U/L = 16,667 × 10^{-9} kat/L. The unit of measurement is independent of the MP.

5.3.3 Higher order RMP that defines the measurand

The higher order (primary) RMP that defines the measurand (see Figure 2, p.3) shall be performed with a measuring system(s) calibrated according to various fit for purpose primary RMPs (see Figure 2, p.1), such as gravimetry, thermometry, volumetry, spectrophotometry, potentiometry, time, length, as applicable.

NOTE As defined by the Consultative Committee for Amount of Substance (CCQM), cases with no primary calibrator require a set of primary methods of measurement (see Figure 2, p.1) to be directly applied to the measuring system, to enable SI-traceable standardization of the primary RMP Figure 2, [p.3].

5.3.4 The primary RMP and definition of the measurand

For a measurand that is the catalytic concentration of an enzyme, the primary RMP (see Figure 2, p.3) is an integral part of the definition of the measurand. Accordingly, the primary RMP (see Figure 2, p.3) shall be specified in sufficient detail regarding equipment, reagents, reaction conditions and calculation from the measured signal so that the RMP can be reproduced in any qualified laboratory that intends to perform the measurement.

NOTE Results of catalytic concentration measurements are only comparable among different laboratories if the enzyme activities are measured under the same conditions. Therefore, an enzyme measurand cannot be described only by kind-of-quantity (e.g. catalytic concentration), name of enzyme and of system, but also requires the specified MP, especially the indicator component of the measured reaction. At the top of the calibration hierarchy, the primary RMP is internationally agreed.

EXAMPLE 'Creatine kinase measured by the conversion rate of $NADP^+$ according to the IFCC RMP'[46].

5.3.5 Documentation of the primary RMP

The documentation for a primary RMP deployed in a calibration hierarchy as described in Figure 2, p.3 (for example, for the catalytic concentration of an enzyme in a body fluid) shall meet the requirements of ISO 15193. In addition, the description of the primary RMP for the measurand (see Figure 2, p.3) shall include the following information (if applicable):

a) kind of substrate and its concentration;

b) activators or inhibitors and their concentrations;

c) direction of catalysed reaction;

d) indicator reaction;

e) buffer system and pH;

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f) volume fraction of sample;

g) volume fraction of start reagent solution;

h) measurement temperature;

i) incubation time;

j) reagent blank;

k) material used for starting the reaction;

l) delay time;

m) measurement interval;

n) measurement wavelength;

o) optical bandwidth;

p) optical path length;

q) kind of regression line for analysis of data points.

5.3.6 Assignment of values to secondary RMs

The primary RMP for the measurand (see Figure 2, p.3) shall be used to assign a value to a secondary calibrator or secondary RM (see Figure 2, m.3) with a complex matrix.

NOTE Such secondary RMs or calibrators (see Figure 2, m.3) often have a matrix resembling the human samples intended to be measured by the end-users' routine MPs, to improve the likelihood that these materials will be commutable with human samples when used in lower order MPs in the calibration hierarchy. Panels or pools of human samples are a type of secondary RM applicable in this context (see Figure 2, m.3), depending on biochemical characteristics (e.g. stability) of the particular enzyme measurand.

EXAMPLE ERM-AD457/IFCC[2], from the European Commission Joint Research Centre Directorate F — Health, Consumers and RMs, is certified for catalytic activity concentration of aspartate aminotransferase and listed with JCTLM[28]. Commutability studies[47] demonstrated that this material performed equivalent to human serum samples in 5 of 11 comparisons with the RMP using available IVD MDs.

5.3.7 Manufacturer’s selected MP

A manufacturer's selected MP (see Figure 2, p.4) shall define a MP that is calibrated by one or more secondary RMs or secondary calibrators (see Figure 2, m.3), and is used to assign values to the manufacturer’s working calibrator(s) (see Figure 2, m.4).

NOTE The secondary RMs (see Figure 2, m.3) have certified values with associated uncertainties, and are value-assigned by a calibration laboratory using a fit-for-purpose primary RMP.

5.3.8 Manufacturer's working calibrator

The manufacturer's working calibrator (see Figure 2, m.4) shall have values assigned according to the manufacturer's selected MP (see Figure 2, p.4) or (depending on the commutability characteristics of the working calibrator) according to a primary RMP (see Figure 2, p.3) for the measurand. The secondary (working) calibrators (see Figure 2, m.4) shall be commutable with human samples as determined in commutability assessment studies (see 4.5.5) comparing the manufacturer’s selected MP (see Figure 2, p.4) and the manufacturer’s standing MP (see Figure 2, p.5), or comparing the RMP (see Figure 2, p.3) and the manufacturer’s standing MP [see Figure 2, p.5) if steps (see Figure 2, m.3) and (see Figure 2, p.4) are omitted from the calibration hierarchy.

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5.3.9 Manufacturer’s standing MP

The manufacturer’s standing MP (see Figure 2, p.5) shall define a MP calibrated by one or more of the manufacturer’s working calibrators (see Figure 2, m.4) or higher types of calibrators and is validated for analytical selectivity.

5.3.10 Manufacturer's end-user calibrator

The manufacturer's end-user calibrator (see Figure 2, m.5) shall have its value assigned according to the manufacturer's standing MP (see Figure 2, p.5) and is intended for calibration of the end-user's IVD MD. The u_{cal} of the assigned value of the end-user calibrator (see Figure 2, m.5) shall be estimated by the manufacturer (see 4.7), incorporating all appropriate higher order uncertainties in addition to the uncertainties of each of the subsequent MPs in the calibration hierarchy down to and including the manufacturer’s standing MP (see Figure 2, p.5).

5.3.11 End-user IVD MD

The end-user IVD MD (see Figure 2, p.6) shall describe a measuring system calibrated by one or more end-user calibrators. This MP, the final MP in the calibration hierarchy for the defined measurand, is used to examine human samples and generate final measured values for the measurand, with combined standard measurement uncertainties of the reported values to be estimated by the end-user, taking into account all known measurement uncertainties accrued at each higher step in the defined calibration hierarchy.

5.4 Cases for measurands defined by a RMP calibrated with a particular primary calibrator

5.4.1 General considerations

Calibration hierarchies for measurands that are defined by a RMP that is calibrated with a particular primary calibrator (with traceability to SI) are described in Figure 3. In such cases, the RMP detects a quantity that is a component of the measurand (e.g. a peptide fragment or an epitope), rather than the entire molecular structure of the quantity intended to be measured. The characteristics to be addressed in the description of these calibration hierarchies are elaborated in 5.4.2 to 5.4.10.

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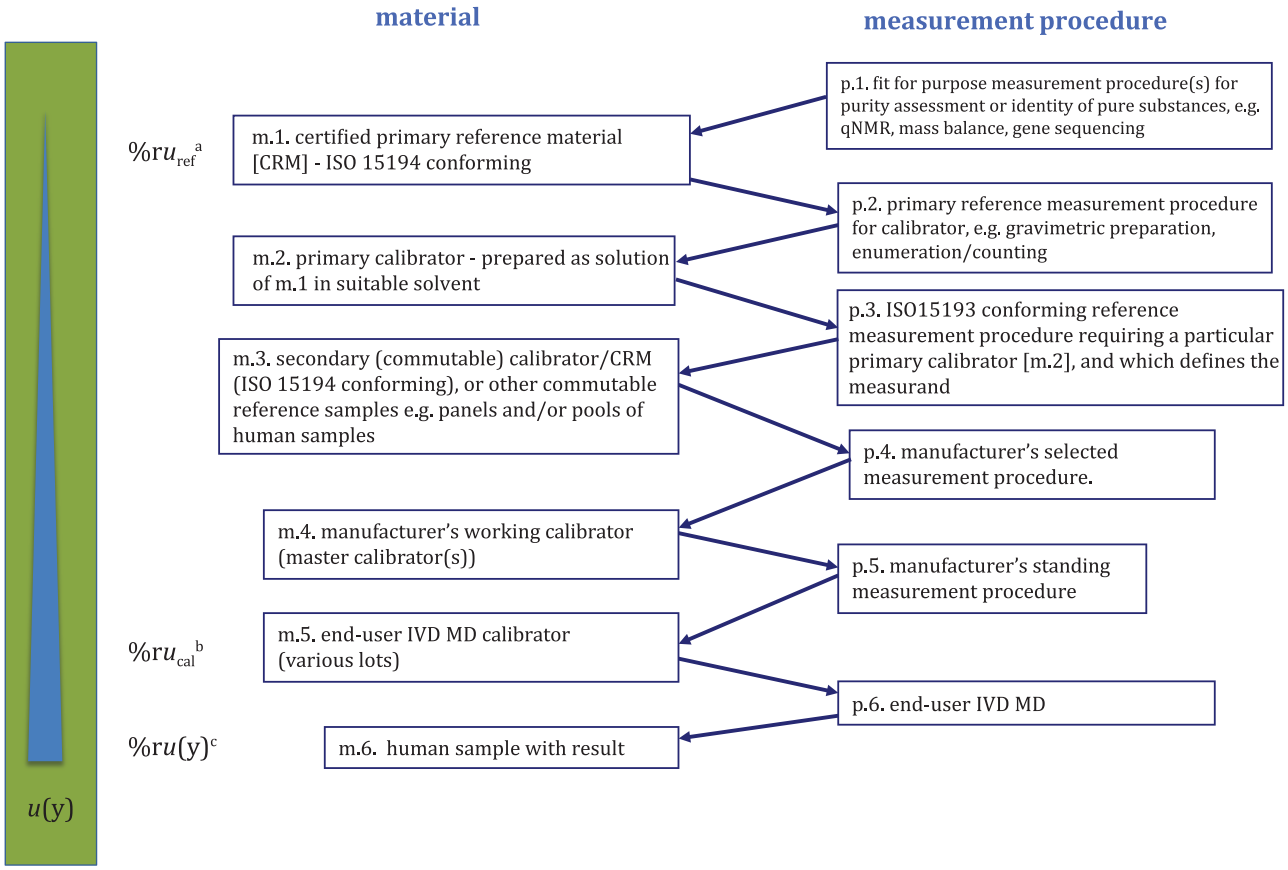

a Relative percent standard uncertainty of value assigned to the primary RM [m.1].

b IVD MD calibrator [m.5] value assignment relative percent combined uncertainty, according to the following formula:

$$\%ru_{cal} = \sqrt{(\%ru^2_{ref} + \%ru^2_{Rw\text{-}p.2} + \%ru^2_{Rw\text{-}p.3} + \%ru^2_{Rw\text{-}p.4} + \%ru^2_{Rw\text{-}p.5})}$$

where $\%ru_{Rw\text{-}p.2}$, $\%ru_{Rw\text{-}p.3}$, etc., represent the percent relative standard uncertainties for each applicable MP in the calibration hierarchy.

c Relative percent combined standard measurement uncertainty for reported values of the measurand with the end-user IVD MD, calculated per the following formula:

$$\%ru(y) = \sqrt{(\%ru^2_{cal} + \%ru^2_{Rw\text{-}p.6})}$$

where $\%ru^2_{Rw\text{-}p.6}$ is the relative percent standard uncertainty of the IVD MD based on long-term precision (repeatability conditions of measurement).

Figure 3 — Calibration hierarchy — Measurand defined by a RMP calibrated with a particular primary calibrator; traceable to SI

5.4.2 Definition of the measurand

Due to its selectivity for a particular epitope or molecular structure that is part of the measurand, a higher order RMP (see Figure 3, p.3) that is calibrated with a particular primary calibrator (see Figure 3, m.2) shall define the measurand.

EXAMPLE In the IFCC reference measurement system for hemoglobin A1c (HbA1c), the measurand is defined as the molar fraction of beta chains of haemoglobin A1 with glycation at the N-terminal valine or epsilon-amino acid residues (HbA1c) relative to the non-glycated fraction of beta chain haemoglobin A (HbA0), in whole blood. The analyte is defined as hemoglobin (Hb) that is irreversibly glycated at one or both N-terminal valines and epsilon-amino acids of the beta chains.

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5.4.3 Value assignment of the primary RM

The primary RM (see Figure 3, m.1) shall be value assigned by one or more MPs (for confirmation of identity and determination of purity of the pure substances) (see Figure 3, p.1). The MPs selected shall be ones with performance characteristics to help ensure the smallest relative achievable standard measurement uncertainty (denoted by the abbreviation %ru_{ref} in Figure 3) for the assigned value of the primary RM.

5.4.4 Value assignment of the primary calibrator

The primary calibrator (see Figure 3, m.2) shall be value assigned by one or more primary RMPs (e.g. gravimetry) (see Figure 3, p.2). The choice of primary RM (see Figure 3, m.1), in addition to preparation and value assignment of the primary calibrator (see Figure 3, m.2) are critical to the definition of the measurand, in conjunction with the RMP (see Figure 3, p.3).

EXAMPLE 1 In the IFCC reference measurement system for hemoglobin A1c (HbA1c), for value assignment of the primary calibrator (see Figure 3, m.2) for use in calibration of the RMP (see Figure 3, p.3), mixtures are made of pure HbA1c and pure HbA0, which have been isolated using cation exchange and affinity chromatography, and characterized using capillary isoelectric focusing and electrospray ionization mass spectrometry[48].

EXAMPLE 2 Quantitative measurement of C-reactive protein (CRP) by homogenous immunoassay is dependent on the oligomeric state of the analyte[51]. A conventional reference method based on the detection of CRP-derived monopeptide would be blind regarding the oligomeric state of CRP. To rule out bias as a function of differences in the oligomeric state of the protein in the RM compared to the intended human samples, the fraction of monomer in the primary RM and the calibrator are independently determined.

5.4.5 Selection and intended use of the RMP in the calibration hierarchy

The RMP (see Figure 3, p.3) (which, when calibrated with the primary calibrator [see Figure 3, m.2] defines the measurand) shall be used to assign a value to a secondary calibrator or secondary RM with a complex matrix (see Figure 3, m.3). The secondary calibrator or secondary RM (see Figure 3, m.3) shall be commutable with human samples in both the initial MP (see Figure 3, p.3) used to assign its value as well as in the subsequent MP (see Figure 3, p.4), where it is to be used as a calibrator for purposes of assigning values to the manufacturer’s working calibrator(s) (see Figure 3, m.4).

EXAMPLE In the IFCC reference measurement procedure for haemoglobin A1c (HbA1c), there are two RMPs available (see Figure 3, p.3) that selectively measure the glycated n-terminal residue of the haemoglobin (Hb) beta-chain. Hb is then cleaved into peptides by a proteolytic enzyme. The specific glycated and non-glycated N-terminal peptides of the Hb beta-chain are measured by HPLC separation followed by either mass spectrometry or capillary electrophoresis[49][50].

5.4.6 Manufacturer's selected MP

The manufacturer's selected MP (see Figure 3, p.4) shall define a measuring system that is calibrated by one or more secondary calibrators or secondary RM (see Figure 3, m.3). Its main purpose is to transfer trueness to the manufacturer’s working calibrator (see Figure 3, m.4). As such, this MP shall be selected in part because the calibrators (see Figure 3, m.3 and/or m.4) are commutable with human samples.

NOTE Such secondary RMs or secondary calibrators will generally have a matrix resembling the human samples intended to be measured by the end-user IVD MD, to improve the likelihood that these RM(s) will be commutable with human samples, helping to ensure their suitability for use with the MPs that they are intended to calibrate (i.e. [Figure 3, p.4 and/or p.5].)

5.4.7 Manufacturer’s working calibrator

The manufacturer's working calibrator (see Figure 3, m.4) shall have its value assigned according to the manufacturer's selected MP (see Figure 3, p.4). The calibration material (see Figure 3, m.4) shall have demonstrated commutability with the intended human samples, to help ensure its suitability for

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use with the manufacturer's selected MP (see Figure 3, p.4) and the procedure to be calibrated, i.e. the manufacturer's standing MP (see Figure 3, p.5).

NOTE A manufacturer's working calibrator is often a material with a matrix resembling the intended human samples to be used with the end-user IVD MD, such as a panel or a series of pools of human samples.

5.4.8 Manufacturer's standing MP

The manufacturer's standing MP (see Figure 3, p.5) shall define a MP calibrated by one or more of the manufacturer's working calibrators (see Figure 3, m.4) or higher types of calibrator and is validated for analytical selectivity.

5.4.9 End-user IVD MD calibrator

The manufacturer's end-user IVD MD calibrator (see Figure 3, m.5) shall have its value assigned according to the manufacturer's standing MP (see Figure 3, p.5) and is intended for calibration of the end-user IVD MD. The u_{cal} of the assigned value of the end-user IVD MD calibrator (see Figure 3, m.5) shall be estimated by the manufacturer (see 4.7), incorporating all appropriate higher order uncertainties in addition to the uncertainties of each of the subsequent MPs in the calibration hierarchy down to and including the manufacturer's standing MP Figure 3, [p.5].

5.4.10 End-user IVD MD

The end-user IVD MD [Fig. 3, p.6] shall describe a measuring system calibrated by one or more end-user calibrators. This MP, the final MP in the calibration hierarchy for the defined measurand, is used to examine human samples and generate final measured values for the measurand, with combined standard measurement uncertainties of the reported values to be estimated by the end-user, taking into account all known measurement uncertainties accrued at each higher step in the defined calibration hierarchy.

5.5 Cases with an international conventional calibrator that defines the measurand

5.5.1 General considerations

The calibration hierarchy described in Figure 4 shall apply to cases where there is an international conventional calibrator that defines the measurand (see Figure 4, m.3), and which conforms with the requirements of ISO 15194. For these kinds of measureable quantities, there are no RMPs (see Figure 4, p.2), no primary RMs (see Figure 4, m.1) or primary calibrators (see Figure 4, m.2), and no traceability to SI. The value assigned to the international conventional calibrator (see Figure 4, m.3) has an arbitrary value for the measurand that is assigned by an internationally agreed value assignment protocol (see Figure 4, p.3), which comprises the highest level of metrological traceability for the specified measurand. The characteristics to be addressed in the description of these types of calibration hierarchies are elaborated in 5.5.2 to 5.5.9.

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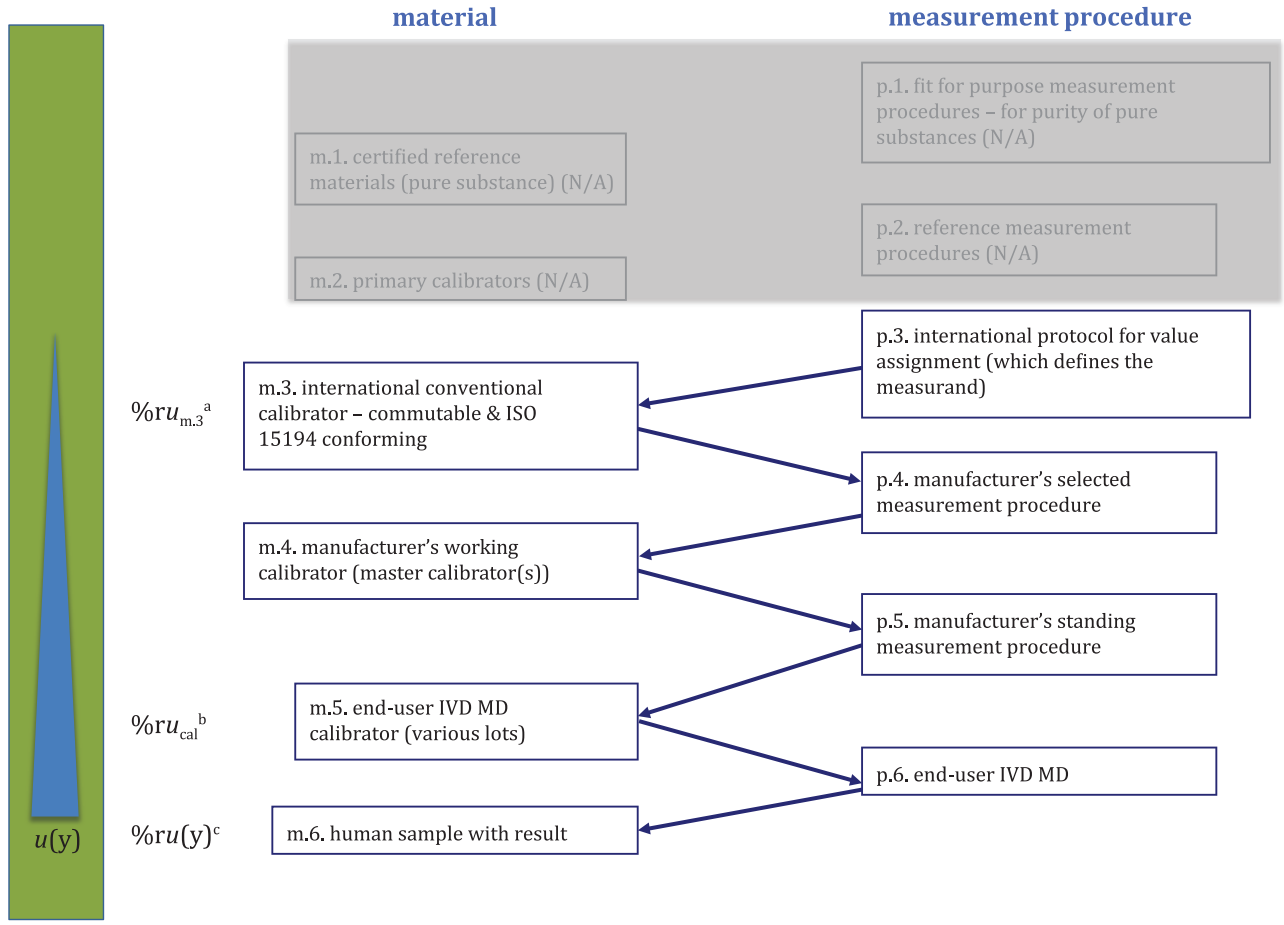

a Relative percent combined value assignment uncertainty of the [m.3] reference material, calculated according to the following formula:

$$\%ru_{m.3} = \%ru_{Rw\text{-}p.3}$$

where $\%ru_{Rw\text{-}p.3}$ is the relative percent standard deviation (CV%) for MP [p.3] under repeatability conditions, i.e. the uncertainty of the international protocol for value assignment of the international conventional calibrator [m.3].

b Relative percent combined value assignment uncertainty of the IVD MD calibrator [m.5], calculated according to the following formula:

$$\%ru_{cal} = \sqrt{(\%ru^2_{m.3} + \%ru^2_{Rw\text{-}p.4} + \%ru^2_{Rw\text{-}p.5})}$$

where $\%ru_{Rw\text{-}p.4}$ and $\%ru_{Rw\text{-}p.5}$ represent the percent relative standard uncertainties for each applicable MP in the calibration hierarchy.

c Relative percent combined standard measurement uncertainty for reported values of the measurand with the end-user IVD MD, calculated per the following formula:

$$\%ru(y) = \sqrt{(\%ru^2_{cal} + \%ru^2_{Rw\text{-}p.6})}$$

where $\%ru^2_{Rw\text{-}p.6}$ is the relative percent standard uncertainty of the end-user IVD MD based on long-term precision (repeatability conditions of measurement).

Figure 4 — Calibration hierarchy — Measurand defined by value assignment protocol for international conventional calibrator (conforming with ISO 15194; no SI traceability). Materials [m.1], [m.2], and MPs [p.1] and [p.2] are not applicable (N/A)

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5.5.2 The international conventional calibrator — Material description

An international conventional calibrator (see Figure 4, m.3) shall be a preparation containing the measurand (see 3.17). Where appropriate, the calibrator prepared from the material provided shall have matrices resembling those of the human samples intended to be measured by IVD MDs, to make it more likely that the RM(s) (see Figure 4, m.3) will be commutable with human samples in the MPs (e.g. [Fig. 4, p.3, p.4 and/or p.5]) that they are intended to calibrate in the defined calibration hierarchy.

NOTE 1 The measurand is usually present in a well-characterized form and is typically contained in a matrix resembling that of the samples of human origin to be measured by end-user IVD MD.

EXAMPLE An international conventional calibrator provided in a matrix closely resembling the samples of human origin as intended to be measured by a specified IVD MD (e.g. pooled serum, pooled urine.)

NOTE 2 The international conventional calibrator is sometimes not available in a form suitable for direct use as a calibrator in a MP. Some international conventional calibrators are provided as a powder or lyophilisate, requiring dilution with a suitable diluent prior to use. In some cases, the provider of a calibrator includes specific instructions for preparation and dilution in the instructions for use.

NOTE 3 An international conventional calibrator can be a single material, or multiple materials, e.g. a panel of samples or a series of sample pools, each with different amounts of the measurand.

5.5.3 Value assignment of an international conventional calibrator

An internationally agreed protocol (see Figure 4, p.3) shall be used to assign the quantity value(s) to the international conventional calibrator(s) (see Figure 4, m.3).

NOTE 1 An example of a protocol is a scheme in which the mean value (after outlier removal) of the measurand in the international conventional calibrator is determined among a group of MPs that have been qualified to have suitable performance characteristics. Determination of suitability of performance characteristics is usually based on results measured for a panel of human samples and includes, in particular, consideration of: imprecision, selectivity, correlation, reduction of differences among procedures when the candidate international conventional calibrator is used to recalibrate the procedures, along with other key influence quantities.

NOTE 2 The WHO Expert Committee on Biological Standardization (ECBS) establishes international biological RMs called "International Standards (IS)" (and previously "International Reference Preparations (IRP)") for use with bioprocedures and immunoprocedures. For the first batch of these materials, an "international unit" is defined as an arbitrarily specified amount of the material and characterized by its specified biological activity. Subsequent batches are calibrated by interlaboratory collaborative measurements against the previous material. The batches in a series are specified by "1st IS", "2nd IS", etc. The assigned value(s) of such a RM, even when it is highly purified, are related to a dedicated biological MP (or other internationally agreed protocol) without metrological traceability to SI units. Such materials, therefore, cannot be called primary RMs. These international conventional RMs can only be used as calibrator(s) for IVD MDs if the material is developed on the basis of a clear definition of the quantity related to the intended medical application and if the assigned value(s) of the material have uncertainty that is acceptable for calibration of IVD MDs.

5.5.4 Commutability of an international conventional calibrator

The commutability of the international conventional calibrator with human samples shall be validated for a representative number of different IVD MDs, consistent with the intended use of the calibrator. The provider of the international conventional calibrator, prior to first release of the material for its intended use, shall conduct the commutability assessment. A manufacturer responsible for defining the calibration hierarchy for a particular IVD MD is responsible for any additional commutability assessments as needed to ensure that the selected international conventional calibrator is suitable for use with the specified IVD MD, if applicable.

NOTE 1 Use of a human sample-like matrix in an international conventional calibrator (or any other calibrator) is no guarantee that the resulting calibrator is actually a commutable material.

NOTE 2 Protocols for performing commutability assessment are available in CLSI EP30-A and other published recommendations[35]–[37].

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NOTE 3 Selection of MPs for participation in a commutability study takes into account factors such as market share as well as differences in technology among available IVD MDs for the measurand of interest.

5.5.5 Calibration and selection of the manufacturer's selected MP

An international conventional calibrator (see Figure 4, m.3) shall be used to calibrate the manufacturer's selected MP (see Figure 4, p.4). The manufacturer's selected MP (see Figure 4, p.4) shall define a measuring system that is calibrated by one or more international conventional calibrators. The selected MP, when calibrated appropriately, shall be used to determine assigned values for the manufacturer's working calibrator (see Figure 4, m.4).

5.5.6 Characteristics and value assignment of the manufacturer's working calibrator

The manufacturer's working calibrator (see Figure 4, m.4) shall have its value assigned according to the manufacturer's selected MP (see Figure 4, p.4). The manufacturer's working calibrator (see Figure 4, m.4) shall have demonstrated commutability with the intended human samples as regards the manufacturer's selected MP (see Figure 4, p.4) and the manufacturer's standing MP (see Figure 4, p.5).

NOTE The manufacturer's working calibrators are often a material with a matrix resembling the human samples intended to be measured by the end-user IVD MD. The manufacturers working calibrators in some instances consist of a panel or a series of pools of human samples.

5.5.7 Manufacturer's standing MP

The manufacturer's standing MP (see Figure 4, p.5) shall define a MP calibrated by one or more of the manufacturer's working calibrators (see Figure 4, m.4) or higher types of calibrator such as the international conventional calibrator (see Figure 4, m.3) and is validated for analytical selectivity.

5.5.8 End-user IVD MD calibrator

The end-user IVD MD calibrator (see Figure 4, m.5) shall have its value assigned according to the manufacturer's standing MP, and is intended for calibration of the end-user IVD MD (see Figure 4, p.6). The u_{cal} of the assigned value of the end-user IVD MD calibrator (see Figure 4, m.5) shall be estimated by the manufacturer (see 4.7), incorporating all appropriate higher order uncertainties in addition to the uncertainties of each of the subsequent MPs in the calibration hierarchy down to and including the manufacturer's standing MP (see Figure 4, p.5).

5.5.9 End-user IVD MD

The end-user IVD MD (see Figure 4, p.6) shall describe a measuring system calibrated by one or more end-user calibrators. This MP, the final MP in the calibration hierarchy for the defined measurand, is used to examine human samples and generate final measured values for the measurand, with combined standard measurement uncertainties of the reported values to be estimated by the end-user, taking into account all known measurement uncertainties accrued at each higher step in the defined calibration hierarchy.

5.6 Cases with metrological traceability supported by an international harmonisation protocol

5.6.1 General Considerations

The calibration hierarchy described in Figure 5 shall apply to cases in which the measurand in human samples is defined by an international harmonisation protocol; but there are no internationally agreed RMPs, no primary RMs, no conventional RMPs or RMs, and no metrological traceability to the SI. The characteristics to be addressed in the description of these types of calibration hierarchies are elaborated in 5.6.2 to 5.6.5.

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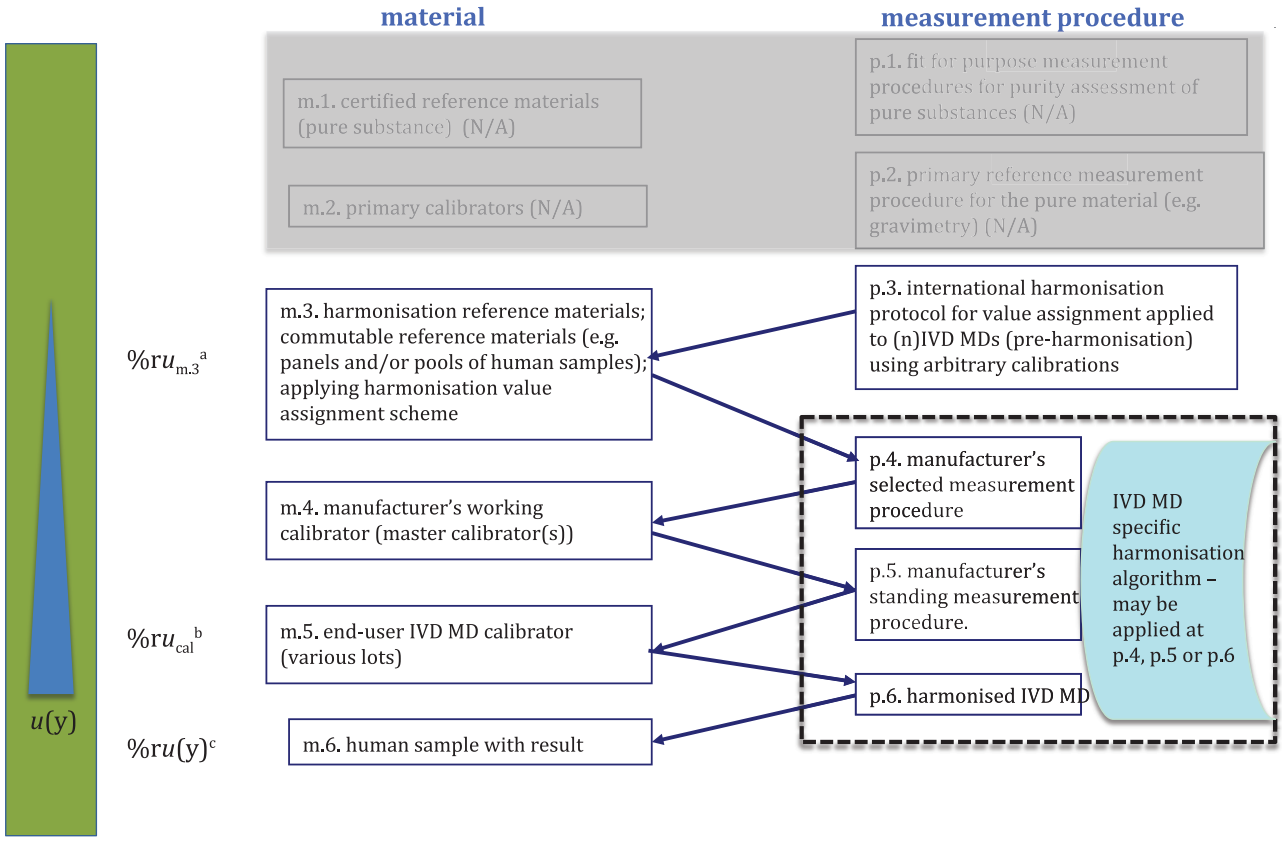

a Relative percent combined value assignment uncertainty of the [m.3] reference material, calculated according to the following formula:

$$\%ru_{m.3} = \%ru_{Rw\text{-}p.3}$$

where $\%ru_{Rw\text{-}p.3}$ is the relative percent standard deviation (CV%) for MP [p.3] under repeatability conditions, i.e. the uncertainty of the protocol for value assignment of the harmonisation reference material(s) [m.3].

b Relative percent combined value assignment uncertainty of the IVD MD calibrator [m.5], calculated according to the following formula:

$$\%ru_{cal} = \sqrt{(\%ru^2_{m.3} + \%ru^2_{Rw\text{-}p.4} + \%ru^2_{Rw\text{-}p.5})}$$

where $\%ru_{Rw\text{-}p.4}$ and $\%ru_{Rw\text{-}p.5}$ represent the percent relative standard uncertainties for each applicable MP in the calibration hierarchy.

c Relative percent combined standard measurement uncertainty for reported values of the measurand with the end-user IVD MD, calculated per the following formula:

$$\%ru(y) = \sqrt{(\%ru^2_{cal} + \%ru^2_{Rw\text{-}p.6})}$$

where $\%ru^2_{Rw\text{-}p.6}$ is the relative percent standard uncertainty of the end-user IVD MD based on long-term precision (repeatability conditions of measurement).

Figure 5 — Calibration Hierarchy — Measurand defined by international harmonisation protocol (No CRM; not traceable to SI). Materials [m.1], [m.2], and MPs [p.1] and [p.2] are not applicable (N/A)

5.6.2 International harmonisation protocol

The prescribed international harmonisation protocol for the measurand (see Figure 5, p.3) shall define the highest metrological level in the calibration hierarchy, and is intended to achieve equivalence of

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reported results for the intended human samples (see Figure 5, m.6) among available harmonised end-user IVD MDs (see Figure 5, p.6) for the measurand.

NOTE Additional detail concerning use and application of a harmonisation protocol in a metrologically traceable calibration hierarchy can be found in ISO 21151.

5.6.3 Assignment of values to harmonisation RMs

The international harmonisation protocol (see Figure 5, p.3) shall specify the process used to assign harmonised values (arbitrary units, not traceable to SI) to the harmonisation RMs (see Figure 5, m.3). The international harmonisation protocol (see Figure 5, p.3) shall specify how the harmonisation RMs are used to estimate relationships between the results for the harmonisation RMs (see Figure 5, m.3) among the IVD MDs (see Figure 5, p.6) participating in the harmonisation protocol. The measurement uncertainties, $u_{\mathrm{m.3}}$, associated with the assigned quantity values for the harmonisation RMs shall be estimated and accounted for in the estimate of u_{cal} for the specified IVD MD end-user calibrators.

5.6.4 Application of harmonisation RMs

Each IVD MD manufacturer shall determine and document an IVD MD-specific algorithm that when applied to (a) their selected MP (see Figure 5, p.4) for assignment of values to their working calibrator or calibrators (see Figure 5, m.4), or (b) to their standing MP (see Figure 5, p.5) for assignment of values to their end-user IVD MD calibrator or calibrators (see Figure 5, m.5), or (c) to their harmonised IVD MD (see Figure 5, p.6) for assignment of values to human samples (see Figure 5, m.6), will enable achievement of equivalent results for human samples with their specified IVD MD, when compared to other IVD MDs participating in the harmonisation protocol. The measurement uncertainty introduced by the IVD MD-specific harmonisation algorithm defined by each IVD MD manufacturer shall also be accounted for and included in the estimate of the incremental measurement uncertainty component at the applicable position in the calibration hierarchy, as well as in the final combined measurement uncertainty of the end-user calibrator(s), u_{cal}, for each harmonised IVD MD.

5.6.5 End-user IVD MD

The end-user IVD MD (see Figure 5, p.6) shall describe a measuring system calibrated by one or more specified end-user IVD MD calibrators (see Figure 5, m.5). This MP, the final MP in the calibration hierarchy for the defined measurand, is used to examine human samples and generate final measured values for the measurand, with combined standard measurement uncertainties of the reported values to be estimated by the end-user, taking into account all known measurement uncertainties accrued at each higher step in the defined calibration hierarchy.

5.7 Cases for measurands with metrological traceability only to manufacturer's internal arbitrarily defined RM(s)

5.7.1 General considerations

The calibration hierarchy described in Figure 6 shall apply to measurands with metrological traceability only to a manufacturer's internal, arbitrarily defined RMs (see Figure 6, m.3), for certain measurands with no certified pure RMs (see Figure 6, m.1), no primary calibrators (see Figure 6, m.2), no RMPs (see Figure 6, p.3), no harmonisation protocol, and no traceability to SI. Characteristics to be addressed in the description of these types of calibration hierarchies are elaborated in 5.7.2 to 5.7.7.

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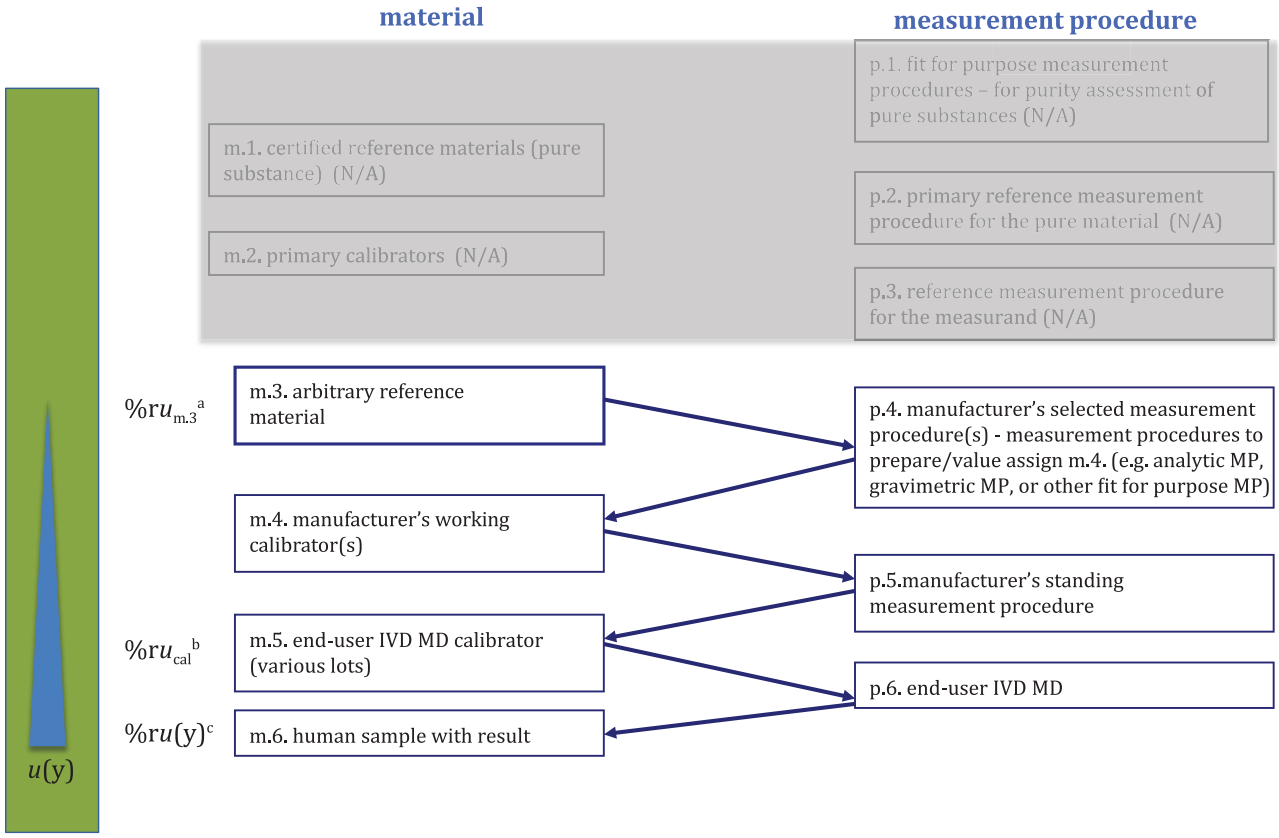

a Relative percent combined value assignment uncertainty of the [m.3] arbitrary reference material.

b Relative percent combined value assignment uncertainty of the IVD MD calibrator [m.5], calculated according to the following formula:

$$\%ru_{cal} = \sqrt{(\%ru^2_{m.3} + \%ru^2_{Rw\text{-}p.4} + \%ru^2_{Rw\text{-}p.5})}$$

where $\%ru_{Rw\text{-}p.4}$ and $\%ru_{Rw\text{-}p.5}$ represent the percent relative standard uncertainties for each applicable MP in the calibration hierarchy.

c Relative percent combined standard measurement uncertainty for reported values of the measurand with the end-user IVD MD, calculated per the following formula:

$$\%ru(y) = \sqrt{(\%ru^2_{cal} + \%ru^2_{Rw\text{-}p.6})}$$

where $\%ru^2_{Rw\text{-}p.6}$ is the relative percent standard uncertainty of the end-user IVD MD based on long-term precision (repeatability conditions of measurement).

Figure 6 — Calibration Hierarchy — Measurand defined by manufacturer's internal arbitrarily defined RMs (no primary RMs or CRMs; no RMP; no harmonisation protocol; not traceable to SI). Materials [m.1] and [m.2], and MPs [p.1], [p.2], and [p.3] are not applicable (N/A)

5.7.2 Selection of RMs

Since higher order RMs and MPs (see Figure 6, m.1, m.2, p.1, p.2, p.3) do not exist for these types of measurands, arbitrary RMs (see Figure 6, m.3) shall be established, if applicable, as the metrologically highest level in the defined calibration hierarchy for the measurand.

NOTE 1 To help ensure consistency in the calibration of the specified end-user IVD MDs of this type, manufacturers sometimes establish arbitrary MPs (see Figure 6, p.4) and/or arbitrary RMs (see Figure 6, m.3) as the highest level of these calibration hierarchies,

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NOTE 2 Arbitrary RMs (see Figure 6, m.3) are at times prepared from, e.g., purified biomarkers, then measured with a selected MP (see Figure 6, p.4), enabling further calibration and value assignment to a working calibrator(see Figure 6, m.4). Such working calibrators are often prepared in a human sample matrix material or other appropriate matrix, or alternatively are comprised of, e.g., panels (or pools) of human samples or "spiked" human samples.

5.7.3 Manufacturer's Selected MP

In cases where a manufacturer establishes an independent calibration hierarchy using a selected MP (see Figure 6, p.4) where there is no availability of an RM for the measurand (e.g. MPs dependent on other properties such as UV absorptivity; procedures based on counting; etc.), the selected MP shall be the metrologically highest level in the specified calibration hierarchy for the defined measurand.

5.7.4 Manufacturer's Standing MP

The manufacturer's standing MP (see Figure 6, p.5) shall define a measuring system that is calibrated with the manufacturer's working calibrator (see Figure 6, m.4), which may be comprised of arbitrary RMs including panels of human samples or sample pools. The standing MP (see Figure 6, p.5) shall be used to determine assigned values for the manufacturer's end-user IVD MD calibrator(s) (see Figure 6, m.5).

5.7.5 End-user IVD MD calibrators

The manufacturer's end-user IVD MD calibrators (see Figure 6, m.5) shall have values assigned according to the manufacturer's standing MP (see Figure 6, p.5), and are intended for use in the calibration of the end-user's IVD MD (see Figure 6, p.6). The u_{cal} of the assigned value of the end-user IVD MD calibrator (see Figure 6, m.5) shall be estimated by the manufacturer (see 4.7), incorporating all appropriate higher order uncertainties in addition to the uncertainties of each of the subsequent MPs in the calibration hierarchy down to and including the manufacturer's standing MP (see Figure 6, p.5).

5.7.6 End-user IVD MD

The end-user IVD MD (see Figure 6, p.6) shall describe a measuring system calibrated by one or more end-user IVD MD calibrators. This MP (see Figure 6, p.6), the final MP in the calibration hierarchy for the defined measurand, is used to examine human samples and generate final measured values for the measurand (see Figure 6, m.6), with combined standard measurement uncertainties in the reported values to be estimated by the end-user, taking into account all known measurement uncertainties accrued at each higher step in the defined calibration hierarchy.

5.7.7 Documentation of the calibration hierarchy

Documentation of the calibration hierarchy to be included in the manufacturer's technical file for the specified IVD MD for a measurand with metrological traceability according to the scheme in Figure 6 shall include (as a result of a risk assessment) specifications and validation documentation for elements critical to the performance and reproducibility of the calibration hierarchy, including but not limited to:

a) specification of raw materials to be prepared, purchased, processed or otherwise acquired for preparation of any arbitrary RMs (see Figure 6, m.3, m.4) or reagents and other components of the MPs (see Figure 6, p.4, p.5) including specifications for any human samples (or pools) or other types of samples intended to serve as RMs at various levels (see Figure 6, m.3, m.4) within the calibration hierarchy.

b) specification of the measurand of interest and any associated measured quantities and/or influence quantities appropriate to each clinical intended use of the specified IVD MD in sufficient detail to enable reproducible human sample selection and/or pool preparation for subsequent batches of RMs (see Figure 6, m.3, m.4) as applicable.

NOTE 1 The measurand present in each human sample selected as a calibration panel member (see Figure 6, m.3 and/or m.4) is assumed to represent the measurand of interest for each stated intended use of the IVD MD.

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c) for steps in the calibration hierarchy that are under control of the manufacturer, procedures and work instructions for assignment of quantity values to human samples (or pools) or other arbitrary materials intended to serve as RMs (see Figure 6, m.3, m.4), including descriptions of measures to be taken to ensure consistency of the value assignment process for replacement batches of calibration panels or other arbitrary RMs.

NOTE 2 Internal RMs (see Figure 6, m.3, m.4) are assigned values by the manufacturer using protocols including, for example (a) arbitrary units of measurement, (b) standard addition of weighed or volumetrically dispensed volumes of a concentrate, (c) direct measurements using the manufacturer's selected MP (see Figure 6, p.4) (e.g. an available commercial MP), or (d) other scientifically valid methods as appropriate to the measurement technology and type of analyte.

EXAMPLE A sub-panel of human samples from the first calibration panel (see Figure 6, m.3) is often used to transfer assigned values to a subsequent calibration panel.

6 Labelling information to be provided to end-users by the manufacturer

The requirements given in ISO 18113-2, 7.5, shall apply.

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