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In vitro diagnostic medical devices — Requirements for international harmonisation protocols establishing metrological traceability of values assigned to calibrators and human samples

Dispositifs médicaux de diagnostic in vitro — Exigences relatives aux protocoles d'harmonisation internationaux établissant la traçabilité métrologique des valeurs affectées aux étalons et aux échantillons humains

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Foreword

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

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

Introduction

Results for a measurand in a human sample should be numerically equivalent, within clinically meaningful limits, among different laboratories using different in vitro diagnostic (IVD) medical devices (MDs). Clinical practice guidelines for diagnosis and treatment decisions that use fixed decision limits for interpreting laboratory results can only be appropriately applied when results are equivalent irrespective of the IVD MD used. Laboratory medicine has adopted the principle of metrological traceability of IVD MD calibration to higher order references as the basis to achieve equivalent results for the same measurand that are independent of the IVD MD, location or time the measurements were made.

ISO 17511:2020, describes 6 calibration hierarchies of reference measurement systems (referred to as cases in 5.2 to 5.7 of ISO 17511:2020) that fulfil the requirement for metrological traceability of a calibration to higher order references. Metrological traceability of calibrator assigned values for particular IVD MDs for measurands in cases 5.2, 5.3 and 5.4 are based on the availability of a reference measurement procedure. Case 5.5 includes measurands for which a certified reference material or an international conventional calibrator with a consensus-based protocol for value assignment is available but there is no reference measurement procedure. Cases 5.6 and 5.7 include measurands for which neither a reference measurement procedure nor a certified reference material or international conventional calibrator is available. Case 5.6 achieves standardization based on a consensus harmonisation protocol. The requirements for such a harmonisation protocol are described in this document. Case 5.7 includes measurands that are not addressed by traceability schemes in the preceding categories. For such measurands, metrological traceability is to the calibrator chosen by the manufacturer of an IVD MD but there is no traceability to a common reference. In case 6 the results from different IVD MDs can be different and not comparable to each other or to decision limits used in guidelines for making medical decisions.

Higher order references for measurands in case 5.6 have been technically difficult to develop thus requiring an approach for standardization based on a protocol for achieving equivalence of results among two or more IVD MDs. Research to develop suitable processes for harmonisation of case 5.6 measurands forms the basis for the requirements in this document[5][11]. Standardization of results based on a harmonisation protocol provides metrological traceability of particular IVD MD calibrators to that protocol. A harmonisation protocol is developed and administered by an international body to achieve equivalence among results for different IVD MDs thus meeting requirements for use of the results in medical decisions.

Annex A provides a worked example to illustrate the principles of a harmonisation protocol and one possible approach to implementing a harmonisation protocol. Other approaches are also possible and will likely be developed for particular measurands and IVD MDs.

In vitro diagnostic medical devices — Requirements for international harmonisation protocols establishing metrological traceability of values assigned to calibrators and human samples

1 Scope

This document specifies requirements for a protocol implemented by an international body to achieve equivalent results among two or more IVD MDs for the same measurand for cases where there are no reference measurement procedures and no fit-for-purpose certified reference materials or international conventional calibrators. In this case, the harmonisation protocol defines the highest level of metrological traceability for the stated measurand.

This document can be applied in cases when certified reference materials or international conventional calibrators exist but are not fit-for-purpose because, for example, they are not commutable with human samples.

NOTE This document addresses one case of traceability of assigned and measured values described in 5.6 in ISO 17511:2020.

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).

ISO 17511:2020, *in vitro diagnostic medical devices — Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples*

3 Terms and definitions

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

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

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

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

3.1
aliquot
known amount of a homogeneous material, assumed to be taken with negligible sampling error

[SOURCE: ISO 11074:2015]

3.2
calibration verification control
control provided by a manufacturer for use with a stated IVD MD to confirm that a satisfactory calibration was achieved using the end-user calibrator(s) intended for use with that IVD MD

3.3
harmonisation
harmonised
achievement of equivalent measured quantity values (within clinically meaningful limits) for human samples examined for a stated measurand among two or more IVD MDs by applying an international consensus protocol in their calibration hierarchies when fit-for-purpose higher order reference materials or reference measurement procedures are not available

Note 1 to entry: Harmonisation is one of the calibration hierarchy models described in ISO 17511:2020 to achieve metrologically traceable quantity values for human samples.

Note 2 to entry: Harmonisation is a special case of non-SI traceable standardization where the calibration of two or more IVD MDs is traceable to an international harmonisation protocol that defines the highest level of metrological traceability for the stated measurand, but with no traceability to SI.

Note 3 to entry: Harmonised is the condition in which harmonisation (equivalence among quantity values) is achieved among two or more IVD MDs.

3.4
harmonisation reference material
reference material used as a calibrator for an international *harmonisation* (3.3) protocol

Note 1 to entry: Specifications for these materials are included in the harmonisation protocol.

3.5
international harmonisation protocol
harmonisation protocol
standardization process implemented by an international body to achieve equivalence among measured quantity values for two or more IVD MDs intended for examination of the same measurand for cases where there are no higher order reference measurement procedures and no fit-for-purpose certified reference materials or international conventional calibrators

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

Note 2 to entry: A harmonisation protocol defines the highest level of metrological traceability for the stated measurand.

3.6
standardization
standardized
achievement of equivalent measured quantity values (within clinically meaningful limits) for human samples examined for a stated measurand among two or more IVD MDs, where each “standardized” IVD MD is calibrated according to a defined hierarchy of relationships to higher order references (materials and/or measurement procedures)

Note 1 to entry: Standardization of an IVD MD is achieved preferably by implementation of a calibration system that is traceable to higher order references, ideally with traceability to SI.

Note 2 to entry: Not all standardization approaches result in traceability of final measured values to SI but may be the best available means for achieving equivalent results for human samples among different IVD MDs. Such standardization approaches should be replaced when an approach becomes available that provides traceability to SI.

Note 3 to entry: Standardized is the condition in which standardization of results for human samples is achieved among two or more IVD MDs.

4 Abbreviated terms and symbols

CV coefficient of variation

IVD in vitro diagnostic

MD medical device

SI *Système international.*

5 Requirements for a harmonisation protocol

Figure 1 shows a flowchart for the main steps in a harmonisation protocol as described in this document. Subsequent subclauses provide the detailed requirements and considerations to implement a harmonisation protocol. Development and implementation of a harmonisation protocol is a collaboration among one or more harmonisation organizations, IVD MD manufacturers and regulatory bodies.

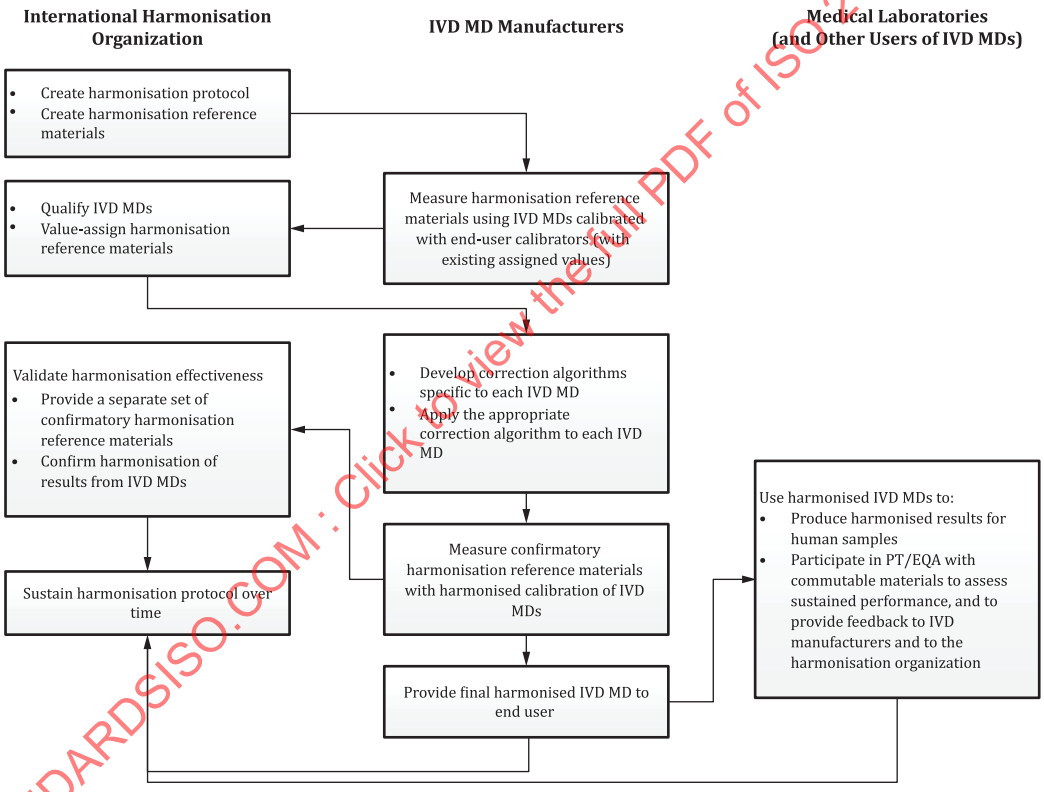


Figure 1 — Flowchart for steps in a harmonisation protocol

5.1 Description of the measurand

The measurand shall be defined as described in 4.2 of ISO 17511:2020.

5.2 Specifications for agreement among results from different IVD MDs

5.2.1 Specifications for agreement among results from different IVD MDs shall a priori be defined based on medical usefulness of decisions based on those results.

5.2.2 Specifications for agreement shall be defined at different amounts of the measurand when applicable.

NOTE The specifications set the criteria for a decision whether the harmonisation protocol achieves equivalent results[12][13].

5.3 Inclusion or exclusion of IVD MDs

5.3.1 Criteria for inclusion or exclusion of IVD MDs in the harmonisation protocol shall be stated.

5.3.2 Criteria shall specify the following performance characteristics: precision; proportional recovery of the measurand in a set of samples with known proportions of measurand present over the measuring interval; selectivity for the measurand, for example demonstrated as proportional and linear relationships for measured values from different IVD MDs for a panel of individual human samples that cover a substantial portion of the measuring interval; and other relevant performance characteristics as applicable.

5.3.2.1 Criteria should consider how results from an IVD MD influence a medical decision.

5.3.2.2 The decision to reject an IVD MD due to apparent poor selectivity should be carefully considered. An IVD MD that appears to generate outliers when compared to results from other IVD MDs purporting to measure the same measurand can have superior effectiveness in medical decisions. In such cases the definition of the measurand and the quantity actually measured should be re-considered.

5.3.2.3 The analytical performance of some IVD MDs may be inadequate and can require corrective action before inclusion in a harmonisation protocol. For example, the selectivity or imprecision of an IVD MD could need improvement before an IVD MD can be included in a harmonisation protocol.

5.4 Harmonisation reference materials required for a harmonisation protocol

5.4.1 Materials required for a harmonisation protocol and its sustainability shall be specified.

5.4.1.1 These materials can be a panel of human samples with limited shelf life and limited amount of material. The materials can be available only for a limited time period for performing the harmonisation protocol.

5.4.1.2 Other types of materials can include: pools of human samples, human samples or pools supplemented with the measurand, or other preparations containing the measurand that do not fulfil the requirements for a certified reference material or an international conventional calibrator. When such materials are used, they should be as similar as possible (matrix-matched) to the types of samples intended to be measured by end-user IVD MDs. (See 5.4.9 regarding commutability requirements for harmonisation reference materials).

5.4.2 The number and quantity values of the harmonisation reference materials shall be appropriate for the measuring intervals of the IVD MDs as needed for implementation of the harmonisation protocol.

5.4.3 Preparation of the materials shall be described with sufficient detail that replacement batches with similar characteristics can be prepared.

5.4.4 When human samples or materials derived from human samples are used, the description shall provide characteristics and criteria used for selecting the human samples. Such characteristics and criteria shall consider the population from which the donors are selected, health or disease conditions and requirements for sample collection that the donors shall fulfil.

5.4.5 Procedures for collection, processing, storage and transportation of materials used in a harmonisation protocol shall be described.

5.4.6 The source and purity of any added components (e.g. measurand, substance similar to the measurand, stabilizers) shall be stated.

5.4.7 Stability characteristics shall be established and ensured over the intended use period for the materials. The influence of any stabilization and storage procedure(s) shall be validated to be suitable for the intended use.

NOTE 1 Freezing and thawing can alter the quantity or matrix from that in the human samples intended to be measured.

NOTE 2 Stability of harmonisation reference materials during transport and storage at the user location is also to be considered.

5.4.8 The procedures used to prepare the materials and their aliquots shall be designed to ensure a high probability of homogeneity. A statement regarding procedures to ensure homogeneity among aliquots of the materials shall be provided.

NOTE Homogeneity validation by sampling aliquots may not be practical for materials such as aliquots of individual human samples because of the limited amounts available. The procedures used to prepare the materials and their aliquots can be designed to ensure a high probability of homogeneity, for example by mixing a bulk quantity of serum during the aliquoting process.

5.4.9 A statement regarding the commutability of the materials with human samples shall be provided. Commutability assessment shall be performed when applicable[14].

5.4.9.1 Commutability validation may not be required when, for example, a panel of individual human samples is used. However, the potential influence of any stabilization procedure on commutability should be considered. The possibility of sample specific influences, for example from interfering substances, should be considered because such influences can affect the suitability of one or more of the individual human samples as harmonisation reference materials. Criteria should be included for exclusion of results from such individual human samples.

5.4.9.2 Commutability validation may be performed for a different batch of materials when limited quantities are available and commutability of a subsequent batch can be assumed to be acceptable. This assumption implies appropriate control of the production process to ensure consistency with specifications for the batch for which commutability was validated. Various characteristics of materials can be different for different batches or can become altered during material storage, thus representing conditions when reassessment of commutability can be applicable.

5.4.9.3 Commutability shall be validated when additives are used for stabilization or to supplement the quantity value (e.g. concentration) of the measurand or when a preparation process such as pooling human samples is used.

NOTE Additives or pooling can alter the matrix from that expected for human samples of the type intended to be measured.

5.4.9.4 Commutability shall be validated when harmonisation reference materials other than human samples are used.

5.5 Measuring the quantity values of harmonisation reference materials by participants in a harmonisation protocol

5.5.1 The sample handling procedure and measurement protocol for measuring the quantity values of the materials used in a harmonisation protocol shall be described.

5.5.1.1 The sample handling information shall be sufficiently detailed to ensure the same protocol is used by all participants. The information should consider sample receipt, storage and handling procedures, preparation for measurement, and disposal of materials.

5.5.2 A specification shall be set for either, or both, the standard deviation of multiple measurements and the uncertainty of the mean result from each IVD MD. Meeting the uncertainty specification can require sampling multiple variance components (e.g. IVD MDs, reagent lots, calibration events, days) during the measurement process. The inclusion of multiple measurements over a specific variance component can be required for the best estimate of the mean result. Multiple measurements can, however, increase the standard deviation seen in the results. Nevertheless, the inability of an IVD MD to meet either the uncertainty or the standard deviation specification could be grounds for exclusion from the harmonisation process.

5.6 Assigning a single quantity value to each harmonisation reference material used in a harmonisation protocol

5.6.1 Procedures for assigning quantity values to the materials used in a harmonisation protocol shall be described.

5.6.2 The scientific rationale for the value assignment process shall be described. The scientific rationale shall explain why the value assignment process is suitable for the intended use of the materials for achieving equivalence of results for human samples from two or more end-user IVD MDs.

5.6.3 The analytical and statistical processes and mathematical algorithms used for assigning quantity values (e.g. concentrations) to the materials used in a harmonisation protocol shall be described.

5.7 Modifying the calibration hierarchy for each IVD MD using the harmonisation reference materials to achieve harmonised results for human samples from different IVD MDs

5.7.1 Each IVD MD will already have a calibration hierarchy which shall be modified to accommodate harmonisation of results for human samples.

5.7.2 The harmonisation protocol shall describe the general approach for modifying the calibration hierarchy for an IVD MD that will be used to assign quantity values to human samples to make them equivalent to the quantity values from other IVD MDs in the harmonisation protocol.

5.7.3 The detailed procedure to develop and apply a harmonisation algorithm that modifies the calibration hierarchy shall be developed by each manufacturer as appropriate for their manufacturing process.

NOTE The detailed procedure to develop and apply a harmonisation algorithm can be different for different manufacturers.

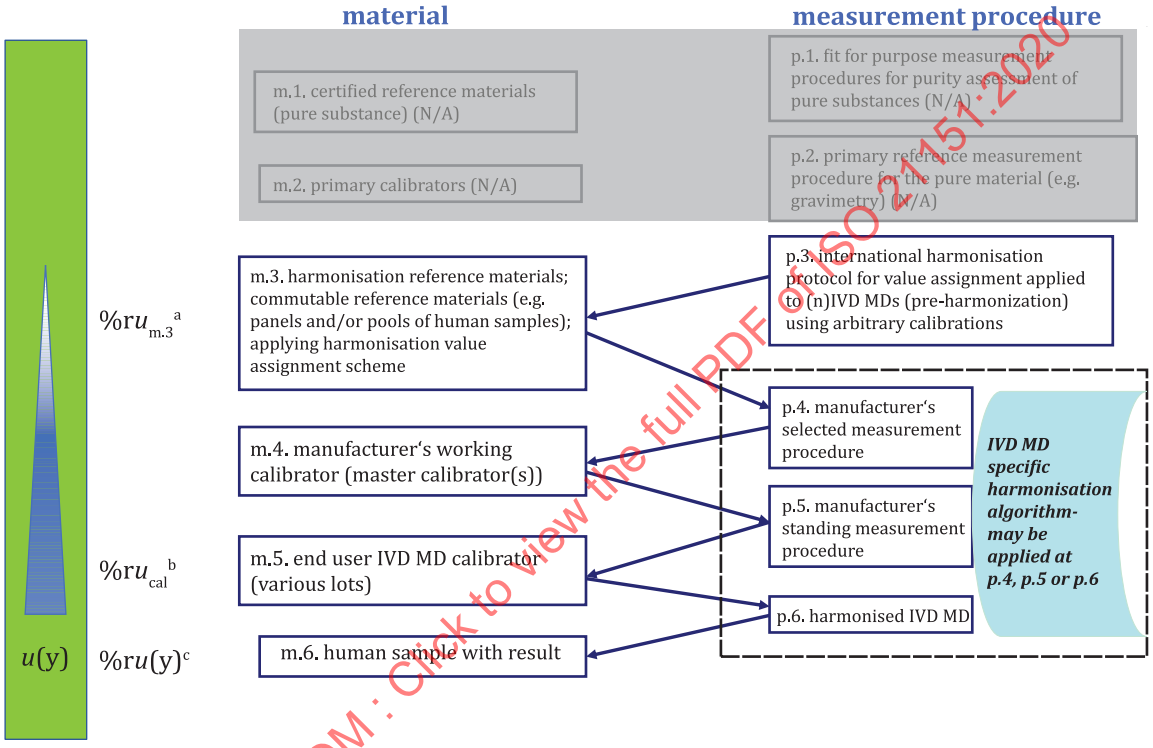
The following approaches can be considered to apply the harmonisation algorithm for assigning results to human samples to achieve harmonised results (see Figure 2):

— A calibration correction based on the harmonisation algorithm can be applied to the results as currently measured by an IVD MD with no change to the values assigned to the existing end-

user (product) calibrators. This correction will add an additional step in the calibration hierarchy between the end-user (product) calibrator and the value assigned to the human sample.

— A manufacturer can reassign the value(s) of their end-user (product) calibrator(s) according to the harmonisation algorithm. This reassignment will add an additional step in the calibration hierarchy between the standing measurement procedure and the end-user calibrator(s).

— A manufacturer can reassign the value(s) of their working calibrator(s) according to the calibration algorithm that will then be propagated to new values assigned to the end-user (product) calibrators. This reassignment adds an additional step in the calibration hierarchy between the selected measurement procedure and the working calibrator(s).



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 2 — 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)

[SOURCE: ISO 17511:2020, 5.6, Figure 5]

5.7.4 The approach used for assigning quantity values to human samples shall be transparent to the end-user and be an automated component of the end-user's calibration process for the IVD MD.

5.7.5 The manufacturer shall describe in end-user documentation the approach taken to achieve harmonisation of human sample results.

5.7.6 The uncertainty of the step added by the harmonisation process shall be included when determining the combined standard uncertainty of the end-user calibrators. The uncertainty results shall meet the requirements as specified in 5.2.

NOTE The uncertainty of this additional step is a measure of the degree of alignment achieved after the calibration algorithm has been applied. This uncertainty for each IVD MD is determined as the standard error of the mean bias over the human sample panel used to demonstrate effectiveness of the harmonisation process (see 5.9).

5.8 Assigning quantity values to calibration verification controls

5.8.1 A manufacturer shall document the process used to assign quantity values and standard uncertainties to calibration verification controls when such controls are provided.

NOTE A manufacturer can include calibration verification controls as well as working calibrators and end-user calibrators in the process to implement a harmonisation protocol. In this case, the process to assign values to end-user calibrators can also be used to assign values to calibration verification controls.

5.9 Effectiveness of the harmonisation protocol

5.9.1 The effectiveness of the harmonisation protocol to achieve equivalent results among different IVD MDs shall be validated based on results from individual human samples or other commutable samples.

NOTE The criteria used to validate that equivalent results were achieved are specified in 5.2.

Samples used to validate that harmonisation was achieved shall conform to the requirements given in 5.4.

5.9.2 Samples used for validation that equivalent results were achieved among different IVD MDs shall be different than those used as harmonisation reference materials.

5.10 Sustainability of the harmonisation protocol over time

5.10.1 The process for sustaining harmonisation over time shall be described with adequate detail that any materials and other resources needed for the process can be developed and implemented by a competent organization.

5.10.1.1 A commitment should be obtained from at least one organization for sustaining the resources needed for the harmonisation protocol.

5.10.2 Preparation and qualification of replacement batches of materials used for harmonisation shall be described.

NOTE The type of samples used for sustainability can be different than the type of samples used for the original harmonisation process.

5.10.2.1 The metrological traceability to the original harmonisation protocol shall be specified.

5.10.2.2 Guidance for estimating the uncertainty of values assigned to replacement batches of harmonisation materials shall be provided and shall consider the combined uncertainty related to the process for ensuring consistency from batch to batch.

5.10.3 Recommendations for surveillance that harmonisation has been maintained over time shall be provided.

NOTE 1 A surveillance or certification program can be made available by the organization that developed the harmonisation protocol or by a collaborating organization.

NOTE 2 A commitment is desirable from one or more organisations to provide surveillance of harmonisation for a measurand to which this document applies.

5.10.3.1 Samples used for surveillance shall be human samples or commutable with human samples[15][16].

5.10.3.2 Recommendations regarding materials suitable for surveillance shall be provided to include: preparation instructions, target quantity values (e.g. concentrations), assignment of quantity values, criteria to evaluate the surveillance results to determine that harmonisation has been sustained, and instructions for notification of providers of IVD MDs that do not meet the evaluation criteria.

5.10.3.3 The criteria for harmonisation should be the same as specified in 5.2.

5.10.4 Recommendations shall be provided for the frequency to reassess that harmonisation continues to be sustained.

5.11 Harmonisation of IVD MDs not included in the original group

5.11.1 A process shall be described for harmonisation of IVD MDs not included in the group that participated in the original development and validation of a harmonisation protocol.

NOTE 1 It is desirable to include as many IVD MDs as possible when implementing a harmonisation protocol.

NOTE 2 IVD MDs that did not meet the performance requirements for inclusion (see 5.3) need a process for harmonisation when their performance is improved to meet the requirements for inclusion.

NOTE 3 New IVD MDs can be introduced that did not exist at the time a harmonisation protocol was originally implemented.

NOTE 4 A new IVD MD that has different clinical performance from others already in use for a measurand could not qualify for inclusion in an existing harmonisation protocol. Results from such a new IVD MD could have superior performance in medical decisions than other devices already in use.

6 Information on metrological traceability to be provided in instructions for use

Requirements of ISO 18113 *in vitro diagnostic IVD medical devices — Information supplied by the manufacturer (labelling) — Part 2: in vitro diagnostic reagents for professional use*, 7.5, shall apply[1]. The information should indicate that a harmonisation protocol was used in the calibration hierarchy and identify the organization responsible for the harmonisation protocol.

EXAMPLE The calibration hierarchy of [insert name of measurand] followed the international harmonisation protocol developed by the [insert name of professional organization].

Annex A
(informative)

Worked Example of a Harmonisation Protocol

A.1 Introduction

The following example illustrates the principles of a harmonisation protocol and represents one possible approach to implementing a harmonisation protocol. Other approaches are also possible and will likely be developed for particular measurands and IVD MDs. This example should not be taken as a recommended approach.

The steps described in this Annex refer to example calculations provided in a spreadsheet available from the ISO website at https://standards.iso.org/iso/21151/ed-1/en/.

A.2 Description of the measurand

The measurand is peptide-R (a fictitious measurand used for the example) measured in human serum as arbitrary units per litre (U/L). Peptide-R has genetic variants consisting of a 68-98 amino acid peptide that exists in blood plasma and serum as a complex with at least one other peptide each of which has a variable number of glycosylation sites. Peptide-R is present in plasma at low concentrations and becomes elevated in cancers of the pancreas. Peptide-R is stable for at least 24 h in blood stored at 4-8 °C and at least 1 year in serum stored at −70 °C (a citation would be provided for a real measurand).

A.3 IVD MD performance specifications

The recommended approaches for defining analytical performance specifications should preferentially be based on the effect of measurement performance on clinical outcome or on the biological variation of the measurand[12][13].

The within-individual and within-group biological variation of peptide-R have been estimated to be 10 % and 30 % respectively. Accordingly, the biological variation model suggests desirable IVD MD performance specifications of:

— total imprecision as coefficient of variation (CV) ≤5 %,

— mean bias within ±8 % of the all IVD MDs assigned values for patient samples.

The performance specifications defined in this example are intended for illustration purposes only, in order to present a simplified example of how a harmonisation protocol can be developed and implemented. The performance specifications to be selected when designing a harmonisation protocol for a given measurand should be appropriate for that particular measurand, and defined according to guidance from published sources[12][13].

A.4 Qualification of IVD MDs included in the harmonisation protocol

IVD MDs included in this harmonisation protocol (for the measurand amount of substance concentration of peptide-R in serum) shall have the following performance characteristics:

— within-laboratory total imprecision (following Clinical and Laboratory Standards Institute guideline EP05[3]) as %CV of 5 % or less near the concentration of the upper reference interval limit,

— for a panel of human samples that cover a substantial portion of the measuring interval, each IVD MD has a proportional and linear relationship for measured values when compared to results from other IVD MDs being considered for inclusion in the harmonisation protocol. The criterion for an acceptable relationship is a maximum 10 % deviation for an individual result from the weighted Deming regression line for all results from two IVD MDs, and an intercept whose 95 % confidence interval includes zero.

Results for samples with outlier values may be excluded if those samples are less than 10 % of the total number of human samples in the comparison.

The four IVD MDs included in Table A.1 meet the performance requirements and are included in the harmonisation protocol example.

Table A.1 — Example IVD MDs for measurement of peptide-R in human serum

	A	B	C	D
Measuring interval	10–500 U/L	2–225 U/L	10–500 U/L	20–700 U/L
Upper reference interval limit for positive result	50 U/L	20 U/L	45 U/L	70 U/L
Within-laboratory imprecision at upper reference interval limit (as CV)	3,7 %	3,9 %	3,4 %	3,6 %

A.5 Harmonisation reference materials

A.5.1 A panel of 120 individual human serum samples is prepared for use as harmonisation reference materials for this protocol. Eighty (80) samples are used for the harmonisation experimental design. The remaining 40 samples are used to validate the effectiveness of the harmonisation protocol.

A.5.2 Donors are identified as follows (note the specifications for human samples are arbitrary and intended only to provide an example):

— 25 % have no known chronic diseases and screening values within the reference intervals for peptide-R, pancreatic amylase, lipase, alanine aminotransferase, alkaline phosphatase, γ-glutamyltransferase, total bilirubin and haemoglobin,

— 75 % have peptide-R values distributed evenly in 5 bins representing 20 % intervals between the upper reference interval limit and the upper limit of the measuring interval using one of the IVD MDs to be included in the harmonisation protocol.

A.5.3 The human serum is collected and processed according to Clinical and Laboratory Standards Institute guideline C37[2] without pooling or filtration steps. Donor serum identified as haemolyzed, icteric, turbid or positive for infectious disease markers is discarded. Each donor unit is maintained at 4-8 °C and stirred during aliquoting to ensure homogeneity. Aliquots of 0.5 ml are prepared at 4-8 °C and frozen in polypropylene cryovials with silicone o-rings at or below −70 °C within 24 h of blood collection. Approximately 360 aliquots are available for each human sample. Aliquots are shipped on frozen CO_2 to participants in the harmonisation protocol.

A.5.4 The individual human sera stored at or below −70 °C are assumed to be commutable with freshly collected samples. This assumption is based on using the Clinical and Laboratory Standards Institute C37[2] collection and processing procedure with precautions to handle the blood and serum similarly to how human serum from a clinical sample is handled. A literature report has verified that a single freeze thaw cycle does not alter recovery of peptide-R and that aliquots frozen at −70 °C were stable for 1 year measured with 5 commercially available IVD MDs (a citation would be provided for a real measurand).

A.5.5 Stability of the stored samples beyond 1 year will be validated by biannual circulation of 4 representative aliquots from each of the 6 combinations of donor type and concentration bins for

measurement by the IVD MDs in the original protocol. Samples will be considered stable if the measured values are within ±5 % of those measured at the time of initial preparation (see example in A.10).

A.6 Assigning quantity values to the harmonisation reference materials

A.6.1 Arbitrary quantity values for peptide-R are assigned to each serum sample as the mean value of results from all IVD MDs that qualify for inclusion in the harmonisation protocol. The choice of how to assign the value will depend on the number of IVD MDs and the distribution of the data. The experimental design for value assignment follows.

A.6.1.1 Aliquots of each sample are measured in triplicate on each of 3 different days with a new calibration of each IVD MD performed on each day. Manufacturer's internal quality control and other quality parameters are verified to meet specifications prior to performing measurements on the aliquots. The same IVD MD, reagent lot and calibrator lot are used on each day that measurements are performed. Outliers may be removed due to blunders or clearly different values from other results for the same sample. A single outlier may be removed from a set of triplicate results, or all results may be removed for a sample if all results are clearly different from those from other measurement days with a given IVD MD. The mean value for all remaining results for a sample will be used as the result for a given IVD MD (MD in the spreadsheet). In the worked example spreadsheet file tab "Data," there were no outlier values among replicates, so all data were used in the subsequent analysis.

NOTE In this example study it was determined that day-to-day changes should be sampled to reduce the effects of this variance component on the accuracy of the mean estimate. Different IVD MDs, reagent lots and/or calibration events could be sampled in other study designs.

A.6.1.2 Refer to the worked example spreadsheet file tab "Analysis 1." An initial all IVD MDs mean (column AV) is calculated for each sample. A plot of mean value for each sample vs. the initial all IVD MD mean is prepared for each IVD MD. The plot is inspected visually to identify samples with outlier values that are clearly separated from the cluster of other results. A statistical test for outliers could be used to confirm visual observations if desired. For this example, only visual outlier detection was used. In the example, IVD MD B had two outlier values and IVD MD C had one outlier value. Samples with results that are clearly outliers for at least one IVD MD are removed from the data for all IVD MDs. Such outliers are likely due to sample specific influences with a given IVD MD that reflect inadequate selectivity for the measurand. It is necessary to remove all results for a sample identified as an outlier for one IVD MD to avoid a biased calculation of the all IVD MDs mean when data for a given IVD MD are removed. An IVD MD with >10 % samples identified as outlier values will be excluded from the harmonisation protocol and the outlier results re-evaluated for the remaining IVD MDs. In the example, results for three samples (ID 42, 70 and 71 with outliers highlighted in yellow) are removed from the data and all IVD MDs are included in the next step in the harmonisation protocol.

A.6.1.3 Refer to the worked example spreadsheet file tab "Analysis 2." All results for samples identified as having outlier values in the preceding step are removed from the data. The standard deviations (SDs) of the replicates increase over 10-fold from the low to high concentrations of the samples (columns BT to BW). Consequently, a weighted Deming regression that assumes the variability (SD) being proportional to the concentration (as described in Clinical and Laboratory Standards Institute guideline EP09[4]) is applied for the mean of each remaining sample result for each IVD MD (columns AQ-AT) vs. the outliers removed all IVD MDs mean (column AU). The regression lines are examined and any IVD MD whose results deviate more than 10 % from a linear response over the interval of concentration values would be excluded from the harmonisation protocol. In this example, all results for each IVD MD have a linear response over the interval of the data and are retained in the harmonisation protocol. In addition, the CV for each sample is calculated from the 9 replicate measurements (see columns BE to BH). A pooled CV for the values from all samples (columns BO to BR) is <5 % for each IVD MD. Examination of CVs for individual samples near the decision limits (columns BE to BH; sample IDs 13 to 24) confirms the CV is <5 % for each IVD MD at those concentrations as specified in the criteria for inclusion in the harmonisation protocol. All remaining data for all IVD MDs are acceptable and retained. The outlier-

removed all IVD MDs mean (column AU) becomes the concentration value assigned to each sample that is used as a harmonisation reference material.

A.7 Developing an IVD MD specific harmonisation algorithm

The IVD MDs included in a harmonisation protocol are representative of each manufacturer's measurement procedure. The relationship between the assigned values of the harmonisation reference materials and the values measured by each IVD MD is used to derive an IVD MD specific harmonisation algorithm to be applied to each respective manufacturer's calibration hierarchy such that harmonisation of the results for human samples among all of the IVD MDs is achieved. Details of how an algorithm is derived and how it is used to assign values to human samples are the responsibility of each manufacturer and may include proprietary information. Consequently, the harmonisation protocol specifies that such a process occur but does not provide detailed mathematical or procedural instructions how the process is implemented by manufacturers. This example uses one possible approach to developing and applying a calibration correction based on a harmonisation algorithm for illustrating the concept, but is not intended to represent a recommended approach.

NOTE The number of IVD MDs, number of lots of calibrator and reagents, measurement replication and other experimental design details are determined by each manufacturer based on performance characteristics and the manufacturer's internal processes for calibration of IVD MDs produced as implementations of that measurement procedure.

A.7.1 Refer to the example file spreadsheet tab "Analysis 3." A relative difference plot (as difference in ln(value) in columns J to M) between the values assigned to the harmonisation reference materials and the mean measured values by a given IVD MD is examined to determine if the scatter is approximately constant over the measuring interval. In this example, the scatter is consistent for each IVD MD over the interval of measured values which supports that a proportional relationship exists among results from the IVD MDs and that the values for human samples can be harmonised among the IVD MDs.

NOTE In this example study, some relative differences (as percent) between IVD MDs are large at numerically small quantity values so a plot of the data as percent differences would present a skewed picture of their performance. To prevent this inappropriate skewing, differences of ln values are used.

A.7.2 Refer to the example file spreadsheet tab "Analysis 4." The relationship between the assigned values of the harmonisation reference materials and the values measured by each IVD MD are established from a weighted Deming linear regression as performed in spreadsheet tab "Analysis 2". The linear regression parameters are shown under column B and are used as the harmonisation algorithm to assign values to end user calibrators. When the intercept is significantly different from zero as in MD-B and MD-D, a manufacturer can choose to partition the data into lower and higher values and develop two harmonisation algorithms over the two different concentration intervals. In this example, manufacturer B chose not to partition the data and manufacturer D chose to partition the data into two concentrations represented by sample IDs 1-25 and IDs 26-80. A different approach to partitioning the data or to fitting a relationship could have been used but the chosen example illustrates that different manufacturers can use different approaches for a harmonisation algorithm.

A.7.3 In the example file spreadsheet tab "Analysis 4," each manufacturer has assigned a value (column H) to their end user calibrator(s) using their standing measurement procedure calibrated using the existing working calibrator(s) according to each manufacturer's standard operating procedure. In this example, each manufacturer uses the regression-derived parameters for the IVD MDs from spreadsheet tab "Analysis 2" shown in spreadsheet tab "Analysis 4" as the harmonisation algorithm (column I) to assign a new value to each end user calibrator (column J) that will achieve harmonisation of results among the IVD MDs.

A.7.4 Figure A.1 shows how this process changes the calibration hierarchy. The end user calibrator(s) with new harmonisation algorithm derived assigned values will be used to calibrate the end user IVD

MDs based on each of the measurement procedures. The uncertainty of this harmonisation step is determined using the harmonisation protocol effectiveness validation described in A.9.5 below.

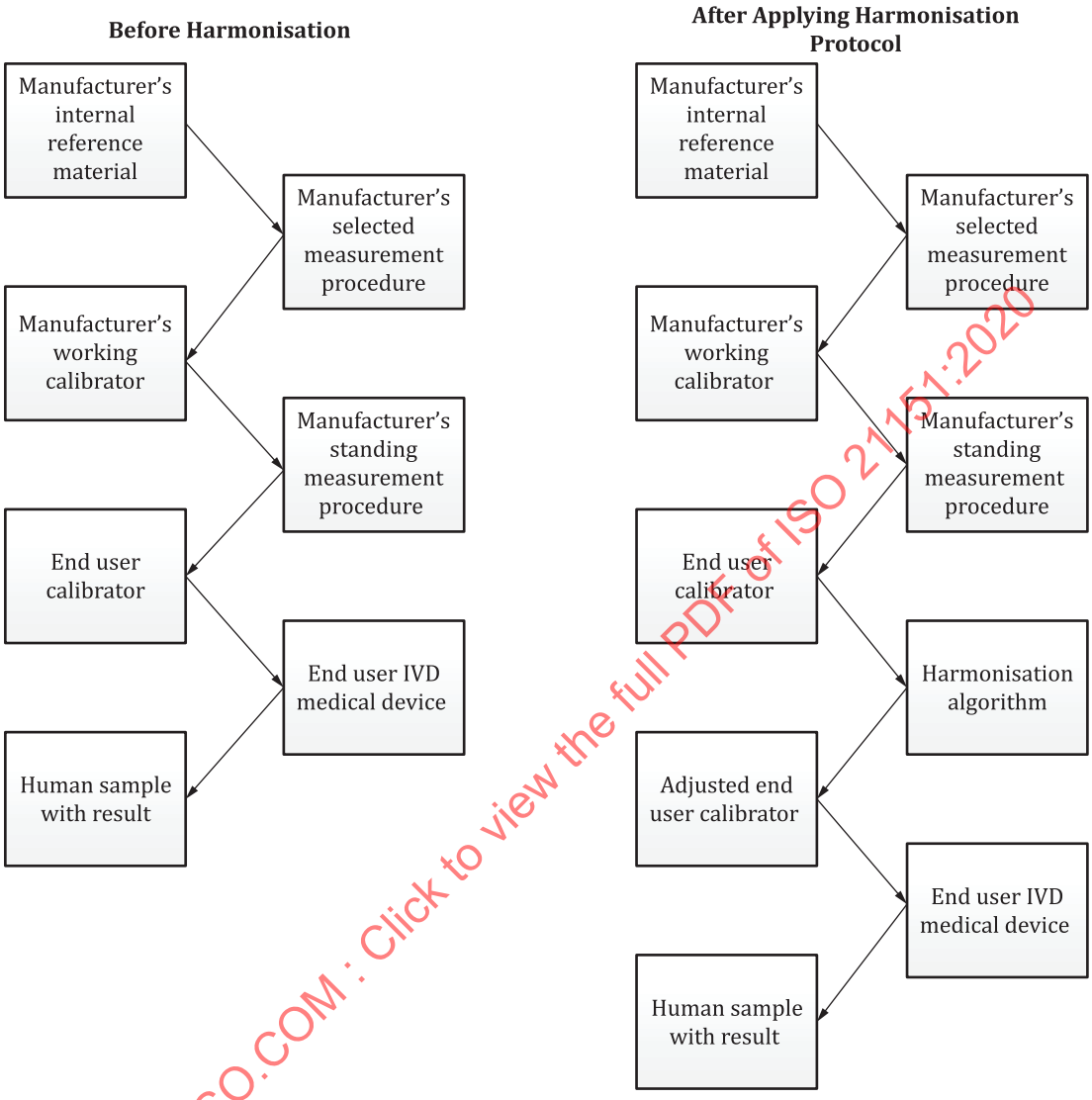


Figure A.1 — Calibration hierarchy before and after applying the harmonisation algorithm to the end user calibrator

NOTE In this example, the harmonisation algorithm is applied at the step of the end-user calibrator. As stated in 5.7.3, a harmonisation algorithm could have been developed and applied at the manufacturer's internal reference material or at the manufacturer's working calibrator steps.

A.8 Assigning values to calibration verification controls

If a manufacturer provides end user calibration verification controls intended for use with that manufacturer's IVD MD to verify that a correct calibration was performed, then such controls are value assigned according to the manufacturer's procedure analogous to that used for end user calibrators described in example A.7. No example is provided for this situation.

A.9 Validating the effectiveness of the harmonisation protocol

A.9.1 The effectiveness of the harmonisation protocol is validated in this example by measuring the validation set of 40 individual human samples using each of the IVD MDs in the harmonisation protocol

that are now calibrated using the respective end user calibrator(s) with new adjusted value(s) assigned based on the harmonisation protocol. Note that the validation set of human samples was prepared along with those used as harmonisation reference materials in the harmonisation protocol, but were not used in the harmonisation protocol to assign new values for the end user calibrators.

A.9.2 According to the criteria set at the beginning of the harmonisation activity, the mean bias vs the all IVD MDs assigned values should be within ±8% of the all IVD MDs assigned values for patient samples.

A.9.3 An experimental design like that described in example A.6 can be used for validation of effectiveness of the harmonisation protocol. Note that this validation experiment is a new set of measurements with each IVD MD calibrated using the end user calibrator(s) with new adjusted value(s) assigned according to the harmonisation algorithm determined in example A.7.

A.9.4 Refer to the example file spreadsheet tab "Analysis 5." The reserve set of human samples ID 81-120 were initially measured by each IVD MD at the same time as the measurements for samples ID 1-80. The value assigned to each of the reserve samples is the all IVD MDs mean value (column C) using the same criteria to examine and remove samples with outlier values that was used for the 80 harmonisation reference materials. The complete data set for these 40 samples is not shown in the example spreadsheet.

The reserve set of human samples are now re-measured with each of the IVD MDs after the harmonisation algorithm is applied and the mean of the triplicate measurements is shown (columns D to G). The slopes, intercepts and small scatter of results for weighted Deming regression vs. the assigned values for the reserve set of human samples are now uniform (regression plots under column K). The mean biases shown in the difference plots (under column W) meet the criteria for harmonisation. For comparison, difference plots are also shown for the original data (under column AD) before developing the harmonisation algorithms by each IVD MD manufacturer. This data for the reserve samples verifies that the harmonisation protocol was effective in developing harmonisation algorithms and achieved the desired equivalence among results from the four IVD MDs.

A.9.5 Refer again to the example spreadsheet file tab "Analysis 5." Calculation of the uncertainty of the additional harmonisation step in the calibration hierarchy is computed using this reserve set of samples. This reserve set can be considered a validation set of samples as opposed to the initial learning set that was used to develop the harmonisation algorithms. This validation set, because it is an independent set of measurements and because the sample size is smaller, provides a more conservative estimate of uncertainty. The underlying assumptions are that:

— the variability is proportional to concentration and thus the relative uncertainty is constant over the measuring interval of each IVD MD,

— the harmonisation process effectively provides a proportional adjustment resulting in a zero intercept, and

— any remaining bias after adjustment plays no role in the estimation of uncertainty as long as the remaining bias meets the harmonisation protocol requirements.

Under these assumptions, the standard error of the bias for an IVD MD uses the distribution of sample % difference results (mean of three replicates per sample) to determine the standard uncertainty of the harmonisation step for that IVD MD as shown in the example spreadsheet file tab "Analysis 5" columns P through S at row 47 (yellow highlight). The standard uncertainty of the harmonisation step is combined with the uncertainties from other steps in the calibration hierarchy to estimate the combined standard uncertainty assigned to the end-user calibrator. If the above assumptions cannot be made, an alternative approach for estimating uncertainty should be used.