Assigning Traceable Values to Commercial IVD Calibrators

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Calibration Traceability … *Basic Concepts*

**National & International Metrology Infrastructure Responsibilities**

- International or National Reference Materials
- Accredited Calibration Labs

**Manufacturer's Responsibilities**

- Manufacturer's Calibration Center: In-house reference materials & methods
- In-house calibration lab: Working or Factory standards and Test Equipment

*Adapted from: ILAC-G2:1994 Traceability of Measurements (ILAC 1996)*
Calibration Traceability... *ISO Standards*

- ISO/DIS 17511 Metrological traceability of values assigned to calibrators and control materials
- ISO/DIS 18153 Metrological traceability ... for catalytic concentration of enzymes in calibrators ...
- ISO 15193 Presentation of reference measurement procedures
- ISO 15194 Description of reference materials
- ISO/DIS 15195 Requirements for reference measurement laboratories in laboratory medicine
Calibration Traceability - ISO/DIS 17511 Overview

- Main Objective: Ensure TRUENESS of test results based on AVAILABLE internationally recognized reference materials or procedures, when using a routine (lower metrological order) commercial method

- Specifies process documentation needed when establishing traceable assigned values for analytes in a product calibrator

- Requires definition of an UNBROKEN series of linked steps, starting at higher order (available) reference methods or materials, moving stepwise downward to assigned values for product calibrators
Calibration Traceability is...

- A tool to ensure accurate results
- A process that relates measurement values to a reference standard
- A property of the test result
- Maintained through monitoring and correction over time
Calibration Traceability Requires...

- Definition of the measurand
  \[ \Rightarrow \text{(Analyte)} + \text{(type of quantity)} + \text{(sample)} + \text{(units)} \]

- Higher order Reference Systems
Calibration Traceability - *Scope of ISO17511*

- Specifies how to assure metrological traceability of values assigned to calibrators and control materials intended to establish or verify trueness of a method.
- Applies to calibrators and some control materials sold by manufacturers.
- 4.1.7 “The responsibility of the manufacturer for describing the traceability chain ... *start(s) at the value of the manufacturer's product calibrator and end(s) at the metrologically highest reference* used by the manufacturer.”
The traceability chain for a product calibrator must be described, from the highest metrological level down to the assigned value for the calibrator.

Each subsequent/lower level in a traceability hierarchy alternates between a measurement procedure and a measurement standard (i.e., a reference material)...

A given measurement standard (material) with its assigned value at one level calibrates the measurement standard (material) at the next lower level, by applying a measurement procedure...specified in a transfer protocol.
Calibration Traceability

*Complete Hierarchy - Tracing to SI*  
[Courtesy - E. Voelkert, Roche Diagnostics, Gmbh; modified from ISO/CD 17511, Figure 1]

- **primary calibrator**
- **secondary calibrator**
- **manufacturer’s working calibrator**
- **manufacturer’s product calibrator**
- **routine sample**

- **primary reference measurement procedure**
- **secondary reference measurement procedure**
- **manufacturer’s selected measurement procedure**
- **manufacturer’s standing measurement procedure**
- **user's routine measurement procedure**

Diagram shows the hierarchy of calibrators and their measurement procedures, tracing back to primary reference.
Calibration Traceability...Key Definitions

Primary Measurement Standard

- Standard (material) that is ... widely acknowledged as having the highest metrological qualities and whose value is accepted without reference to other standards (*ISO 15194, 3.1*)

- Value is assigned either directly with a primary reference method, or indirectly by analysis of composition and impurities
Secondary Measurement Standard

- Standard (material) whose value is assigned by comparison to a primary standard

*(ISO 15194, 3.2)*
Reference Measurement Procedure

• Thoroughly investigated measurement procedure shown to yield values having an uncertainty...commensurate with the intended use, especially in assessing the trueness of other ... procedures ... and in characterizing reference materials.

*(ISO 15193, 3.7)*
Calibration Traceability…Key Definitions

**Primary Reference Measurement Procedure**
- Has the highest metrological qualities
- Detailed procedure and performance is completely described and understood
- Proved to be analytically specific
- Low uncertainty, with complete uncertainty statement available (in terms of SI units)
- Results accepted without reference to a measurement standard for the same quantity.

*(adapted from ISO 17511, 3.25)*
Calibration Traceability...Key Definitions

**Secondary Reference Measurement Procedure**

- Any Reference Measurement Procedures that are NOT primary, e.g.
  - Abell Kendall Cholesterol Method
  - GC or HPLC for many therapeutic drugs
  - CDC Hexokinase Glucose Method
  - CDC Urease Method for UREA
  - Atomic Absorption Spectrometry for various metals (Na, K, Ca, Mg, Mn, Fe)
Calibration Traceability…Key Definitions

**Selected Measurement Procedure**

- A measurement procedure that is calibrated by one or more primary or secondary calibrators (*ISO 17511, 4.2.2*)

- Can be a secondary reference method
Calibration Traceability…Key Definitions

Manufacturer’s Working Calibrator

• A reference material that has its value assigned via measurement with a selected measurement procedure (ISO 17511, 4.2.2)

• Commonly known as “Master Calibrator Lot”
Calibration Traceability...Key Definitions

Manufacturer’s Standing Measurement Procedure

• Is calibrated by Manufacturer’s Working Calibrator (Master Lot)

• Can be based on same principle/method as the routine commercial method, but with lower uncertainty due to robust implementation (ie., replications; strict quality control measures) (ISO 17511, 4.2.2)
Assigning Traceable Calibrator Values…

Field Serum Cholesterol Method with no Matrix Effects

Primary Measurement Standard
SRM 911a (NIST) - gravimetry

Manufacturer’s Working Calibrator (master lot)
Value-assigned Master Calibrator Lot

Manufacturer’s Product Calibrator
-New Lot with assigned values supporting new and existing reagent lots

Reference Measurement Procedure
Abell-Kendall Method

Manufacturer’s Standing Measurement Procedure/Product Calibrator Value Assignment Protocol
Master Reagent Lot plus multiple product reagent new lots & multiple analyzers (with new product calibrator lots as UNKNOWNs); multiple days and reps

Suitable for systems without calibrator matrix effects, and demonstrated commutability of Product Calibrator vs. Patients.
Assigning Traceable Calibrator Values...

Example 1: *Commercial* Cholesterol Method (with matrix effects)

- **Primary Measurement Standard**
  - SRM 911a (NIST) - gravimetry

- **Secondary Calibrator(s)**
  - Human Sample Panels (up to n=200 samples spanning range)

- **Manufacturer’s Working Calibrator**
  - (master lot)
  - Value-assigned Master Calibrator Lot

- **Manufacturer’s Product Calibrator**
  - New Lot with assigned values supporting new and existing reagent lots

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**Reference Measurement Procedure**
- Abell-Kendall Method

**Manufacturer’s Selected Measurement Procedure/Working Calibrator Value Assignment Protocol**
- Multiple analyzers (n>3) & one Master Reagent Lot; Master Calibrator Lot as UNKNOWN; multiple days and reps

**Manufacturer’s Standing Measurement Procedure/Product Calibrator Value Assignment Protocol**
- Master Reagent Lot plus multiple product reagent new lots & multiple analyzers (with new product calibrator lots as UNKNOWNs); multiple days and reps
Assigning Traceable Calibrator Values...

Calibration Traceability is...

• Maintained through monitoring and correction over time
Maintaining Traceability Over Time

- To verify assigned-value stability, Calibrator Master Lot values are re-checked periodically with human sample panels assayed with Reference Method.
- Master Calibrator Lot Assigned-values are adjusted as needed.

Accuracy Not “zero”-bias

Accuracy “zero”-bias after calibration adjustment
Assigning Traceable Calibrator Values…

Maintaining Traceability Over Time

- Primary Measurement Standard
  SRM 911a (NIST) - gravimetry

- Secondary Calibrator(s)
  Human Sample Panels (up to n=200 samples spanning range)

- Manufacturer’s Working Calibrator (master lot)
  Value-assigned Master Calibrator Lot

- Manufacturer’s Product Calibrator - New Lot with assigned values supporting new and existing reagent lots

Reference Measurement Procedure
Abell-Kendall Method

Manufacturer’s Selected Measurement Procedure/Working Calibrator Value Assignment Protocol
Multiple analyzers (n>3) & one Master Reagent Lot; Master Calibrator Lot as UNKNOWN; multiple days and reps

Manufacturer’s Standing Measurement Procedure/Product Calibrator Value Assignment Protocol
Master Reagent Lot plus multiple product reagent new lots & multiple analyzers (with new product calibrator lots as UNKNOWNS); multiple days and reps
Calibration Traceability

ISO/CD 17511 - Specific Requirements

• The value assigned to a standard (material) at a given level ... (has an) uncertainty ...
  • includes inherited contributions from measurement standards and procedures at all higher levels of the calibration hierarchy.
  • additive and cumulative
  • Includes all measurement factors (e.g. pipetting, weighing)
  • Should be estimated (preferably) according to the GUM (International Guide to Uncertainty in Measurement)
  • Uncertainty information must be available, if requested
Assigning Traceable Calibrator Values…

**Example 2: Commercial Cortisol Method**

- **Primary Measurement Standard**
  - Definition of SI unit by CGPM

- **Secondary Calibrators**
  - ID/GCMS value-assigned patient sample pools

- **Manufacturers Working Calibrator Reference Calibrator set** (n=7 levels) forms the master curve

- **Manufacturers Product Calibrator**
  - New calibrator lot with values for a specific reagent master lot

- **Primary Reference Measurement Procedure**
  - Isotope dilution-gas chromatography - mass spectrometry (ID/GCMS)

- **Manufacturers Selected Measurement Procedure**
  - Immunoassay measurement of a set of seven working calibrators using multiple reagents lots and instruments

- **Manufacturers Standing Measurement Procedure**
  - Immunoassay measurement of Product Calibrators and generation of lot specific Master Calibration Curve
Calibration Traceability

- *Is Traceability to SI Required?*

- ISO 17511 Distinguishes between SI-traceable and non-SI-traceable quantities or measurands (analytes)
  - **SI-traceable**: physico-chemically well-defined substances - Type ‘A’ Quantities
    - Applies to ~25 analytes which are well defined, e.g. some electrolytes, metabolites, and hormones.
  - **Non-SI-traceable**: complex mixtures of different molecular entities, measured together because of a clinically relevant characteristic (e.g. lipoproteins, tumor markers) - Type ‘B’ Quantities
    - Applies to 100’s of quantities/analytes
Calibration Traceability…Key Definitions

**International Conventional Calibrator**

- Calibrator with a value not metrologically traceable to SI;
- Assigned value is based on international agreement

**International Conventional Reference Measurement Procedure**

- Yields values not metrologically traceable to SI, but the values obtained are agreed as reference values by international agreement
Calibration Traceability - *Some Limitations*

- For a large number of analytes, there may not be traceability above the level of manufacturer's “selected measurement procedure” or a manufacturer's “working calibrator,” absent universally agreed reference methods and materials. - *The Standard recognizes this.*

- In such cases, TRUENESS might be defined only in terms of a given manufacturer’s in-house calibration hierarchy (working methods and/or materials) until a universally agreed reference measurement procedure and/or calibrator becomes available.
Assigning Traceable Calibrator Values…

Example 3: Commercial Troponin I Method

Traceable?

- **Secondary Calibrators**
  - Human sample panel (n=200)

- **Manufacturers Working Calibrator**
  - In-House Reference Calibrator set forms the master curve

- **Manufacturers Product Calibrator**
  - New product calibrator lot with reagent-lot specific values and cal curves

- **Alternate Commercial Method/Market Leader**
  - Measure (n=200) human samples

- **Manufacturers Selected Measurement Procedure**
  - In-house immunoassay, including multiple reagent lots and instruments

- **Manufacturers Standing Measurement Procedure**
  - Immunoassay based on product principle; value assignment of Product Calibrators; generation of reagent-lot specific Master Cal Curves
Example 3: Commercial Troponin I Method

What is the calibrator traceable to?

- Alternate Commercial Method - Market Leader?
- Selected Measurement Procedure?
- Working Calibrator?
- Something Else?
Example 3: *Commercial* Troponin I Method

**Beware and Avoid Lateral Traceability!**

- **Secondary Calibrators**
  - Human sample panel (n=200)

- **Alternate Commercial Method/Market Leader**
  - Measure (n=200) human samples

- **Manufacturers Selected Measurement Procedure**
  - In-house immunoassay, including multiple reagent lots and instruments
Example 3: *Commercial* Troponin I Method

**Beware and Avoid Lateral Traceability!**

- **Secondary Calibrators**
  - Human sample panel (n=200)

- **Alternate Commercial Method/Market Leader**
  - Measure (n=200) human samples
  
- \( U = ? \)

**Points to Consider & Questions to Ask…**

- Frequency of method comparison between Alt Commercial Method & in-house Immunoassay (Selected Method)?
  - Once? More often?
- Is variation of Alternate Commercial Method controlled and known?
  - If NO, Alt Com Method is at SAME METROLOGICAL LEVEL as Manufacturer’s Method in routine customer use
Example 3: *Commercial* Troponin I Method

**Beware and Avoid Lateral Traceability!**

- **Alternate Commercial Method/Market Leader**
  Measure (n=200) human samples

- **Selected Measurement Procedure**
  In-house immunoassay

**Points to Consider/Questions to Ask…**

- Alternate Commercial Method - NOT a Higher Order method
- Selected Measurement Procedures are calibrated with a Secondary Calibrator or higher, or International Conventional Calibrator.
- Is the Human Sample panel (n=200) with values measured by the Alt Commercial Method actually a Secondary Calibrator set?
  ⇒ NO! Secondary Calibrators can only be value-assigned against methods calibrated either with Primary Calibrators or Conventional International Calibrators.
Example 3: *Commercial* Troponin I Method

**Beware and Avoid Lateral Traceability!**

**Secondary Calibrators**
- Human sample panel (n=200)

**Manufacturers Working Calibrator**
- In-House Reference Calibrator set forms the master curve

**Alternate Commercial Method/Market Leader**
- Measure (n=200) human samples

**Selected Measurement Procedure**
- In-house immunoassay

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**What is the calibrator traceable to?**
- *Selected Measurement Procedure*, if fully validated and variation in assigning values to working calibrators is known, or...
- *Working Calibrator*, if retained for longterm use, & stability monitored and controlled; no longterm reference back to Selected MP or higher.
Calibration Traceability Requirements - Recommendations for Manufacturer Documentation

Calibration Traceability tech report - include in Calibrator Product Technical File - for each analyte/method/system combination supported. Document...

- Calibrator Value Assignment process map
  • Follow format in ISO/CD 17511, e.g. Fig. 1
- Descriptions of measurement procedures (including protocols) & reference materials applied at each level
- Reference to process/test method validation reports - each measurement step; each value transfer step
- Estimates of uncertainties at each level
  • Calculations of combined uncertainty
- References to documentation/literature publications describing relevant reference materials and methods
- Certificates from higher order reference materials
Calibration Traceability Requirements - Recommendations for Manufacturer Documentation

Other items to include in calibrator technical file…

– Procedures for replacement of intermediate elements of traceability chain (e.g. working calibrators or master calibrator lots) when supplies/inventories are depleted or expired

– Methods/procedures and certificates of analysis from 3rd party labs and/or suppliers of reference materials.
  • Include providers of assayed patient sample panels used as secondary or working calibrators.

– In-house or external Reference Labs certification of conformity to ISO/CD 15195, if available
Calibration Traceability Requirements - Recommendations for Manufacturer Documentation

- Information to be Provided on Product Labeling
  - Provide brief, high level summary of Calibrator Value Assignment process chain map
  - Most important to identify highest order available reference methods and materials applied
  - If applicable, define and explain known biases vs. reference methods
  - Define commutability of the calibrator
    - Define which methods the assigned values apply to
    - Important to limit commutability claims only to methods for which the calibrator is validated for use
Calibration Traceability Requirements - Recommendations for Manufacturers __Key Learnings

• Use ISO language correctly
• Traceability requires re-verification over time
  – Unbroken series of linked steps
  – Avoid claiming traceability to metrological levels not supported by ongoing process to re-verify
• Always trace to higher order (materials & methods)
  – Beware Lateral Traceability
• Uncertainty is additive; fewer steps is best
• When standards don’t exist, traceability only to manufacturer’s in-house reference system is OK
• Validate each step in the chain
Calibration Traceability - *Selected References*

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Thank You!

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