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Reference Measurement Procedures of Higher Metrological Order

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**MATERIAL
MEASUREMENT
LABORATORY**

VIM Definition of a Reference Measurement Procedure:

“Measurement procedure accepted as providing measurement results **fit for their intended use** in assessing **measurement trueness** of measured quantity values obtained from other measurement procedures for **quantities of the same kind**, in calibration, or in characterizing reference materials”

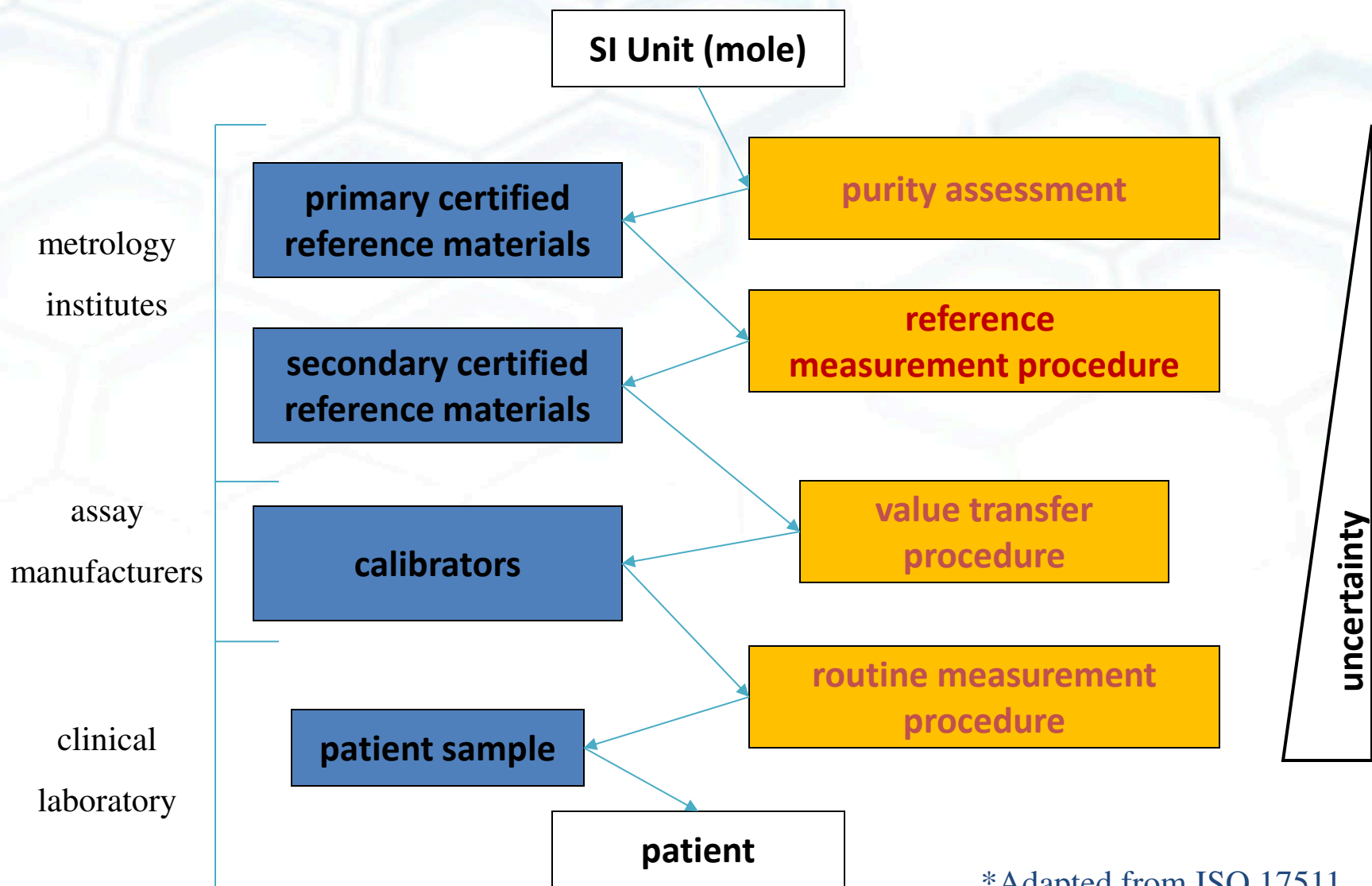
In simpler terms, a RMP is a measurement procedures which:

- has been validated to measure what it is intended to measure
- provides measurements which have been thoroughly assessed for bias
- provides the results that we need

Uses of Reference Measurement Procedures:

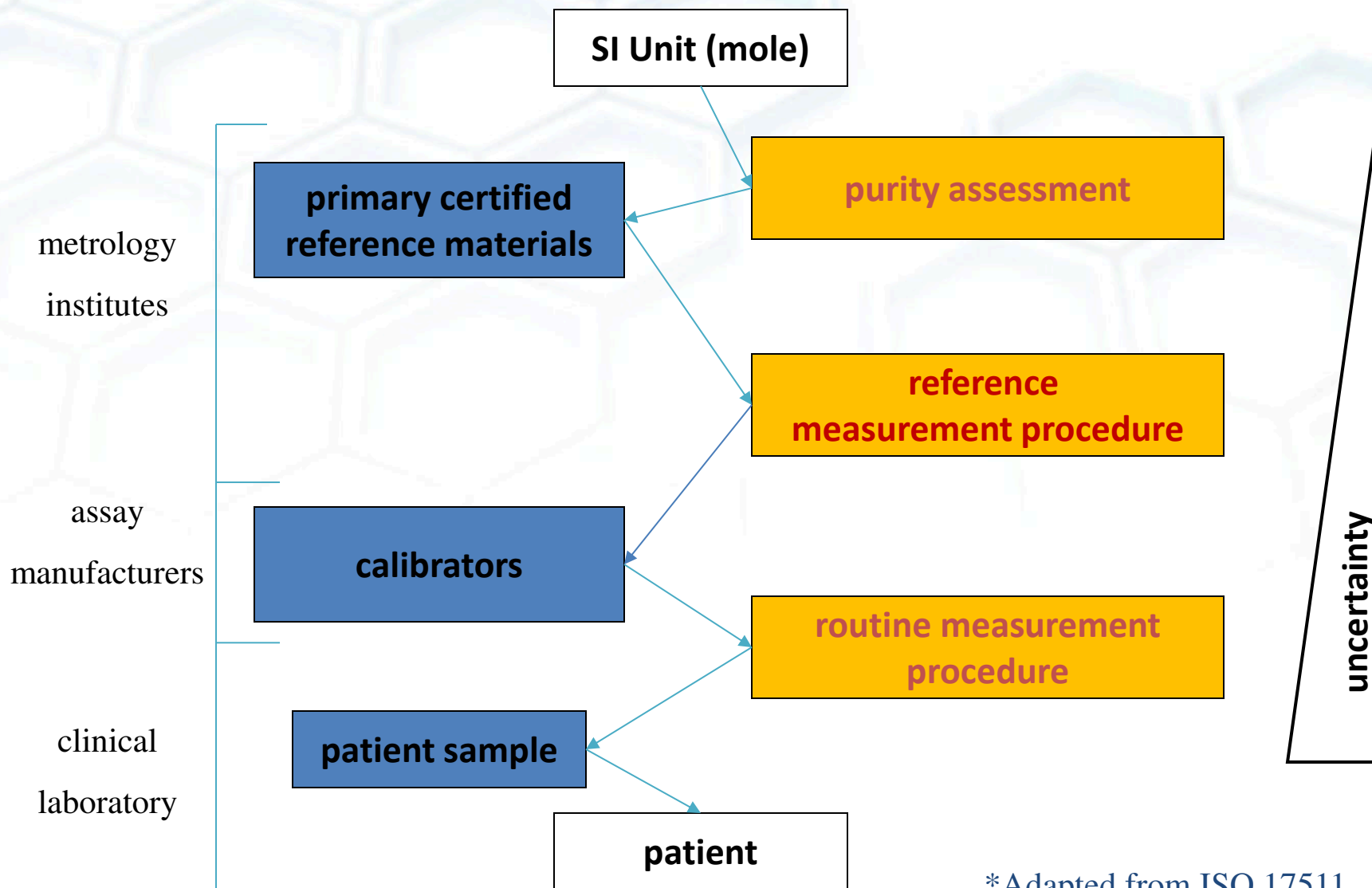
- value-assignment of certified reference materials and higher-order calibration solutions
- Assessing the performance characteristics of routine assay systems (instrumentation and reagents)
- Comparison of routine assays
- Detection of analytical influence quantities in routine samples

The Role of RMPs in Measurement Traceability:



*Adapted from ISO 17511

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Requirements of Reference Measurement Procedures

(according to ISO Guide 15193)

ISO 15193:2009

In vitro diagnostic medical devices -- Measurement of quantities in samples of biological origin -- Requirements for content and presentation of reference measurement procedures

Mandatory Descriptive Elements:

- Title
- Warning and safety precautions
- Scope :
 - type of materials to which the RMP will be applied
 - objective of the measurement
 - limits for values
 - interferences
- Forward
- Dates of authorization and revision
- References

Requirements of Reference Measurement Procedures

(according to ISO Guide 15193)

Mandatory Descriptive Elements:

- Principle and method of measurement
- Sampling and Samples:
 - preanalytical factors that influence measurement
 - sample storage
 - sample preparation
- Reagents (description and how used)
- Apparatus (description, preparation and use)
- Data processing
- Analytical performance
- Inter-laboratory validation

What's Missing from ISO Guide 15193?

- Assessment of bias
 - how to assess?
 - how much to assess?
- For the analytical performance, how good is good enough?

Examples of Reference Measurement Procedures

— *Quantitation of Creatinine in Human Serum by Isotope Dilution Mass Spectrometry*

Elements of the Measurement Procedure*:

- Isotopically-labeled internal standard ($^{13}\text{C}_2$ - creatinine) added to sample immediately and equilibrated thoroughly
 - the completeness of equilibration experimentally validated*
 - isotopically-labeled internal standard minimizes measurement bias*
- Creatinine separated from interfering substance (creatine and other matrix components) by ion-exchange chromatography
 - the ion-exchange chromatography was experimentally validated using pure substance standards of analyte and interferences*
- Isolated creatinine and $^{13}\text{C}_2$ -creatinine derivatized as the ethyl ester of N-(4,6-dimethyl-2-pyrimidiny)-N-methylglycine
 - absence of isotope effects on derivitization reaction experimentally validated*

* Anal. Chem., 1986, 58(8), 1681-1685

Examples of Reference Measurement Procedures

— *Quantitation of Creatinine in Human Serum by Isotope Dilution Mass Spectrometry*

Elements of the Measurement Procedure:

- Quantitative determination of creatinine performed by isotope dilution gas chromatography-mass spectrometry
-absence of interferences in the GC separation and MS analysis experimentally validated
- Procedure validated through multi-laboratory inter-comparison study
-thorough evaluation of both the repeatability and reproducibility of the procedure
- Procedure validated against an LC-MS reference measurement procedure
-validation using “orthogonal” methods for the same measurand

Examples of Reference Measurement Procedures

— *Quantitation of HbA1c in Human Hemolysate by LC-MS*

Elements of the Measurement Procedure*:

- Hemoglobin in human hemolysate samples enzymatically digested with endoproteinase Glu-C
 - conditions for repeatable and reproducible digests thoroughly evaluated*
- LC-MS quantification of the concentration ratios of the glycosylated and non-glycosylated β -N-terminal hexapeptides of hemoglobin
 - LC separation and MS analysis evaluated for interferences*
 - analysis method optimized for repeatability*
- Calibrants are defined mixtures of HbA0 and HbA1c, which undergo all sample preparation steps as hemolysate samples
 - treating calibrants and samples identically minimizes bias*
- Procedure validated through multi-laboratory inter-comparison study
 - thorough evaluation of both the repeatability and reproducibility of the procedure*

* Clin Chem Lab Med, 2002, 40(1), 78-89

Examples of Reference Measurement Procedures

— *Quantitation of HbA1c in Human Hemolysate by LC-MS*

Elements of the Measurement Procedure:

- Procedure validated against an CE-UV reference measurement procedure
-validation using “orthogonal” methods for the same measurand

Examples of Reference Measurement Procedures

— *Quantitation of Catalytic Concentration of Alkaline Phosphatase in Human Serum*

Elements of the Measurement Procedure*:

- Measurement of the catalytic and chemical conversion of 4-nitrophenyl phosphate to 4-nitrophenoxide ion
 - substrate used is a defining characteristic of the RMP*
- All enzymatic reaction conditions are thoroughly defined with stated allowable tolerances
 - reaction conditions are defining characteristics of the RMP*
 - reaction conditions optimized to maximize repeatability*
- Procedure validated through multi-laboratory inter-comparison study
 - thorough evaluation of both the repeatability and reproducibility of the procedure*
- Procedure cannot be validated using another method
 - RMPs for catalytic concentrations are procedurally-defined*

* Clin Chem Lab Med, 2011, 49(9), 1439-1446

Examples of Reference Measurement Procedures

— *Quantitation of Cystatin C in Human Serum using a Consensus Process*

Elements of the Measurement Procedure*:

- Routine assays (single radial immunodiffusion, immuno-turbidimetry, immuno-nephelometry) used for the measurement
 - method/laboratory performance evaluated in a pilot study prior to certification measurements*
 - QC procedures in place during the certification measurements*
 - different reagents and/or different instruments for each assay type were used to reduce bias*
- Common calibrants used for all routine assay measurements
 - common calibrants provide traceability*
 - common calibrants establish accuracy of routine methods*
 - calibrant matrix evaluated for bias*

* Certification Report, IRMM CRM ERM-DA471/IFCC

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Examples of Reference Measurement Procedures

— *Quantitation of C-reactive Protein in Human Serum using IDMS*

Elements of the Measurement Procedure*:

- Isotopically-labeled internal standard (¹⁵N-labeled recombinant human CRP) added to the sample at the start of the measurement process
 - the completeness of equilibration experimentally validated*
 - isotopically-labeled internal standard minimizes measurement bias*
- Immuno-purification of CRP from serum accomplished with immobilized monoclonal antibodies
 - appropriateness of internal standard experimentally validated*
 - immuno-purification validated through comparison with different monoclonal antibodies and immobilized phosphatidyl choline*
- Enzymatic digestion of CRP with trypsin used to produce measurable peptide
 - appropriateness of internal standard experimentally validated*

* Anal. Chem., 2009, 81(20), 8610-8616

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Examples of Reference Measurement Procedures

— *Quantitation of C-reactive Protein in Human Serum using IDMS*

Elements of the Measurement Procedure:

- Quantitation of multiple(5) CRP peptides achieved using LC-MS/MS
-measurement of multiple peptides reduces the potential of measurement bias
- Calibrants for the LC-MS/MS measurements are prepared from recombinant CRP and are carried through the immuno-purification and trypsin digestion
-treating calibrants and samples identically minimizes bias

The Joint Committee for Traceability in Laboratory Medicine (JCTLM)

- JCTLM WG 1 reviews nominations of clinical reference measurement procedures and certified reference materials
- Currently there are 158 reference measurements procedures for 76 unique measurands listed in the JCTLM database
- JCTLM review teams review the content of RMP nominations based on ISO Guide 15193
- JCTLM review teams review the fitness for intended use of the RMP nominations based on their expertise
- Reference measurement laboratories listed by JCTLM WG2 must use RMP listed in the JCTLM database



A scenic landscape photograph featuring a town with red-tiled roofs and a prominent church steeple in the foreground. The town is situated on a hillside overlooking a large, calm blue lake. In the background, a range of mountains with snow-capped peaks stretches across the horizon under a clear blue sky. The text "Thank You" is overlaid in a large, bold, black font across the center of the image.

Thank You