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EP32-R

Metrological Traceability and Its Implementation; A Report

This document provides guidance to manufacturers for establishing and reporting metrological traceability.

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Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087 USA
P: 610.688.0100
F: 610.688.0700
www.clsi.org
standard@clsi.org

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Metrological Traceability and Its Implementation; A Report

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Marc L. Salit, PhD
Dr. Thomas Ciesiolka
Neil Greenberg, PhD
Richard R. Miller, Jr.
W. Gregory Miller, PhD
Gary L. Myers, PhD
Mauro Panteghini, MD
Gerhard Schumann, PhD
Professor Dr. Lothar Siekmann
David Sogin, PhD

Abstract

Clinical and Laboratory Standards Institute document EP32-R—*Metrological Traceability and Its Implementation; A Report* provides guidance on establishing traceability of the chemical calibration step in clinical laboratory measurements, based on the traceability requirements for *in vitro* diagnostic (IVD) medical devices as given in ISO 17511[1] and ISO 15183,[2] and in accordance with the requirements for traceability as stated in the IVD Directive [i.e., Directive of the European Parliament on *In Vitro* Diagnostic Medical Devices (Directive 98/79/EC)[3]]. Though this report is aimed principally at manufacturers of IVD medical devices, the concepts and approaches recommended may be extended to apply to routine analysis conducted in the clinical laboratory either with commercially available or "home-brew" IVDs.

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Report

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Committee Membership

Working Group on Metrological Traceability

Marc I. Salit, PhD
Chairholder
National Institute of Standards and Technology
Gaithersburg, Maryland

Dr. Thomas Ciesiolka
Roche Diagnostics GmbH
Penzberg, Germany

Neil Greenberg, PhD
Ortho-Clinical Diagnostics, Inc.
Rochester, New York

Richard R. Miller, Jr.
Dade Behring Inc.
Newark, Delaware

W. Gregory Miller, PhD
Virginia Commonwealth University
Richmond, Virginia

Gary L. Myers, PhD
Centers for Disease Control and Prevention
Atlanta, Georgia

Professor Mauro Panteghini, MD
Azienda Ospedaliera "Spedali Civili"
Brescia, Italy

Privatdozent Dr. Gerhard Schumann, Ph.D.
Medizinische Hochschule Hannover
Hannover, Germany

Professor Dr. Lothar Siekmann
University of Bonn
Bonn, Germany

David Sogin, PhD
Abbott Laboratories
Abbott Park, Illinois

Staff

Clinical and Laboratory Standards Institute
Wayne, Pennsylvania

John J. Zlockie, MBA
Vice President, Standards

Jennifer K. McGeary, MT(ASCP), MSHA
Project Manager

Donna M. Wilhelm
Editor

Melissa A. Lewis
Assistant Editor

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Contents

Foreword

Clinical and Laboratory Standards Institute document EP32-R—*Metrological Traceability and Its Implementation; A Report* is intended to explain traceability, how it is established, and how it benefits the *in vitro* diagnostics (IVD) industry and the practice of clinical laboratory medicine.

Metrological traceability is one way to ensure comparability in laboratory test results between laboratories, regions, and countries. Much confusion exists on how to implement the traceability scheme on a practical level. For some measurands, there is a clearly established traceability pathway; for others, demonstrating traceability is more complex.

EP32-R explains the basics of traceability and defines a reference measurement system that includes reference materials, reference measurement procedures, and reference laboratories and laboratory networks.

EP32-R outlines what is required by manufacturers to demonstrate traceability, provides guidance on explaining the results of studies to the customers, and describes what laboratories must do to validate results based on traceability concepts. EP32-R has been developed as a companion to ISO 17511[1] and ISO 18153[2] standards on metrological traceability, and draws on discussions and outcomes of the Joint Committee on Traceability in Laboratory Medicine (JCTLM), which has developed criteria for acceptable reference materials and procedures and a provisional list of acceptable reference materials and procedures.

This report is intended for industry and clinical laboratorians.

The development of EP32-R—*Metrological Traceability and Its Implementation; A Report* is a joint responsibility of IFCC and CLSI. EP32-R has been developed by a working group composed of representatives from National Institute of Standards and Technology (NIST), International Bureau of Weights and Measures (BIPM), IFCC, and CLSI.

Key Words

Calibrator, certified reference material, commutability, metrological traceability, reference measurement procedure, uncertainty of measurement, validation, value assignment

Metrological Traceability and Its Implementation; A Report

1 Scope

EP32-R provides guidance on establishing traceability of the chemical calibration step in clinical laboratory measurements, based on the traceability requirements for *in vitro* diagnostic (IVD) medical devices as given in ISO 17511[1] and ISO 18153,[2] and in accordance with the requirements for traceability as stated in the IVD Directive (i.e., Directive of the European Parliament on *In Vitro* Diagnostic Medical Devices Directive 98/79/EC[3]). Though this report is aimed principally at manufacturers of IVD medical devices, the concepts and approaches recommended may be extended to apply to routine analysis conducted in the clinical laboratory either with commercially available or "home-brew" IVDs.

This report specifically addresses traceability of the chemical calibration of a routine measurement procedure to the highest order reference that is available for a measurand. A traceable result requires that traceability be established for all quantities that have significant influence on the magnitude of the results. Traceability is discussed in more complete scope in other references, most notably, the Eurachem/CITAC[a] Guide: Traceability in Chemical Measurements[4] (available at http://www.measurementuncertainty.org/), the principles of which are applied for laboratory medicine in this report.

The primary area of activity to which this report can be applied is the determination of "assigned" values for calibrators and trueness controls for IVD measurement devices that are intended for use in the quantitative measurement of defined substances in human body fluids. While the focus of this report is on establishing traceability of manufacturers' product calibrators, this is likely to be the key element in the traceability of results at the patient bedside performed on bodily fluids from patients.

This report discusses measurement uncertainty and method validation in relation to their respective roles in achieving traceability. Detailed descriptions of these processes are not provided, and may be found elsewhere (see the References section).

Throughout this report, it is assumed that laboratories or manufacturing facilities following the present guidance have in place effective quality assurance and control measures to ensure that all applicable measurement processes are stable and in control. These measures include, but are not limited to, appropriately qualified staff, continuous documented training of the technical staff, proper maintenance of equipment, correctly prepared reagents, and use of documented measurement procedures and control charts. ISO 17025[5] provides a detailed description of the expectations of a competent laboratory responsible for chemical calibration and testing in general. ISO 15189[6] builds on ISO 17025[5] and provides recommendations specific to medical laboratories. Also of interest is ISO 15195[7] which identifies specific aspects of calibration laboratories in the field of laboratory medicine.

2 Introduction

The primary goal of laboratory medicine is to provide information that is useful to assist medical decision-making and foster optimal health care. This information should be interpretable regardless of the laboratory or particular device employed to measure it. To achieve this, one must be able to obtain equivalent measurement results for the same measurand from a variety of measurement procedures and laboratories.

The ability to achieve equivalent results depends on *traceability* to common standards and is facilitated by expressing results in common units. A traceability network and common units lead to a harmonized

[a] CITAC is the Cooperation of International Traceability in Analytical Chemistry.

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measurement system, enabling comparability over space and time, and also enable quality systems to be put in place to enhance the reliability of laboratory results.

Results from a harmonized measurement system support the establishment of common reference intervals and decision limits, which allows for global application of clinical study findings.

To meet the goal of uniformly interpretable clinical information, results from test kits deployed in the field must be traceable to references of higher order as described in Table 1. While several comparisons may be required to establish traceability between field results and higher order standards, there is no requirement that these comparisons be based on identical, or even similar, protocols. The acceptability of more than one protocol permits the creation of a chain of traceable results with minimal disruption of systems already in place. As established in ISO 17511[1] and ISO 18153[2], a reference of higher order (hereafter often termed merely "reference") can be a certified reference material, a reference measurement procedure, or a network of reference laboratories.

Table 1. Certified Reference Materials and Reference Measurement Procedures of Higher Order

ISO 17511	Category A	Category B
	"SI-traceable"	"NON-SI traceable;" arbitrary units e.g., WHO IUs

JCTLM	List 1	List 2
Characteristics	Trueness	NO trueness
	Precision	Precision
		Consistency of performance only
	Results independent of method/procedure	Results dependent on method/procedure

2.1 Quality in Laboratory Medicine

Over time, measurement science has developed a robust set of concepts for the systematic establishment of measurement quality. That system is based on establishing traceability of the measurement result to recognized references, on establishing an uncertainty budget for the measurement result, and on establishing the validity of the measurement approach being applied to determine the result.

ISO 17511[1] and ISO 18153[2] are intended to establish a framework of "metrological traceability" for delivery of clinical measurement results of known and appropriate quality. These documents are intended to provide guidance to realize that framework. ISO 17511 begins its introduction with the following statement:

> "For measurements of quantities in laboratory medicine, it is essential that the quantity is adequately defined and that the results reported to the physicians or other health care personnel and patients are adequately accurate (true and precise) to allow correct medical interpretation and comparability over time and space." [1]

This statement refers to all three concepts that establish measurement quality: "*adequately defined and … adequately accurate (true and precise)…*" calls for a valid method with known uncertainty, and "*comparability over time and space*."

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2.1.1 Traceability

The concept of (metrological) traceability is defined in the International Vocabulary of Basic and General Terms in Metrology, (VIM), as:

> "property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties."[8]

When making practical measurements, results are typically determined by comparison against references (standards), often termed "calibration." Thus, traceability is usually just the property of a measurement result that connects it with the standard it was compared against in the calibration.

For laboratory medicine, the stated reference might be a value of a property established for a reference material; a reference measurement procedure that produces standard results; or a laboratory within a laboratory network whose results are considered as standards.

Linking a measurement result to the value of a reference provides a result that can then be compared against another result that was linked to the same reference. This comparison can be made in a different place, or at a different time, so long as the reference has been established to be stable.

This concept extends to a chain of references whose values can be compared, and a chain of results that can be compared, because all values were derived from comparison to references in the network.

For many interesting quantities, the chain of references derives from the International System of Units, the SI. For other quantities of interest in laboratory medicine, there are other chains of references, whose scope depends on the measured quantity or measurement procedure. Regardless, the concept of metrological traceability is consistent, and permits equivalency of results consistent with the scope of the chain of the references.

2.1.2 Uncertainty

VIM defines the term "Uncertainty of measurement" as follows:

> "parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand."[8]

Sound decisions based on a comparison of the result to another quantity require knowledge of the uncertainty of the two values (e.g., t-test to distinguish the significance of a difference).Values that may appear different may in fact be indistinguishable, depending on their uncertainties.

2.1.3 Validation

ISO 17511[1] calls for "adequate definition" and "accuracy" of measurement results. This is a call for method validation. That is, "for measurements of quantities in laboratory medicine, it is essential that the quantity is adequately defined and that the results reported to the physicians or other health care personnel and patients are adequately accurate (true and precise) to allow correct medical interpretation and comparability over time and space." In ISO 17025[5], method validation is formally defined as "confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled."

In the context of laboratory medicine, this definition can be interpreted as a call to make certain that what is measured and reported is what was intended to be measured and reported. Method validation

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establishes the suitability of the method to deliver adequate method performance according to established criteria. Consistent with the overall scope of this document, method validation includes validation of the comparisons associated with establishing traceability of measurement results.

Establishing commutability is a key aspect of validating a calibration chain for an *in vitro* diagnostic medical device. This is because, in laboratory medicine, different measurement procedures may yield different results on identical materials (i.e., two different answers from two approaches). These measurement procedures may be based on different physical principles, or may in fact be measuring different chemical entities, yet they are still measuring related biological phenomena. ISO 17511 defines a term, "commutability of a material," that addresses this issue:

> "closeness of agreement between the mathematical relationship of the measurement results obtained by two measurement procedures for a stated quantity in a given material, and the mathematical relationship obtained for the quantity in routine sample." [1]

The commutability of a material must be demonstrated to have a valid traceability chain.

2.1.4 Trueness and Traceability

The trueness of the values (closeness of agreement with "truth") assigned to reference materials of "higher order" is typically evaluated with more care and resources than applied to reference materials of "lower order." Teams of scientists from different organizations often evaluate such reference materials, sometimes collaborating across geographical borders. Sometimes these reference materials are in place over long periods of time, with a community of scientists accumulating great experience with them. This experience and community adds significantly to the reliability of the reference materials, through evaluation by application in numerous studies, with different techniques, and by different investigators.

In practice, establishing traceability to such a reference material offers the possibility for a routine, field clinical measurement result to "inherit" the trueness of the higher order reference materials. Where multiple comparisons are used, this concept applies to each, for example, when intermediate calibrators are employed in the manufacture of IVD test kits.

2.2 External Environment

In recent decades, a series of international standards establishing quality systems have been widely adopted and refined through experience, across numerous sectors and enterprises.

The key documentary standards that apply to quality in laboratory medicine are listed below in Table 2.

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Table 2. Key Standards That Apply to Quality in Laboratory Medicine

Standard	Scope
ISO 17511[1]	Traceability of Values Assigned to Calibrators and Controls
ISO 18153[2]	Traceability of Values for Catalytic Concentration of Enzymes
ISO 15189[6]	Quality Management in Medical Laboratories
ISO 15193[9]	Reference Measurement Procedures
ISO 15194[10]	Reference Materials for Biological Samples
ISO 15195[7]	Reference Measurement Laboratories
ISO 17025[5]	Quality Management in Testing and Calibration Laboratories

The IVD Directive describes specific requirements, which are derived from the ISO standards. As a rule of law, the IVD Directive has fostered numerous activities. The directive calls for clear statements describing how assays are calibrated, and on the identity of the reference to which results are traceable.

While the requirements for device labeling are straightforward, assertion of the traceability to higher order references is flexible.

Other factors contributing to new documentary and procedural requirements include:

- Laboratories conform with (and are audited against) either ISO 15189[6] or ISO 17025.[5] To comply with these standards, laboratories must document the traceability of their results. To do this they require more complete information from the providers of their goods and services.

- The call for "higher order" references affects the entire diagnostic community, and is being addressed through an international effort, the Joint Committee for Traceability in Laboratory Medicine (JCTLM), described below.

2.2.1 Joint Committee for Traceability in Laboratory Medicine

In response to the need to establish lists of available higher order references in laboratory medicine, an ad-hoc committee has been formed. Its Executive, made up of representatives from the Bureau Internationale de Poids et Measures (BIPM), International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and International Laboratory Accreditation Cooperation (ILAC), oversees the Joint Committee. With the creation of the JCTLM, a framework has been established which can be used for the identification of such reference materials, procedures, and laboratory networks. Members and Observers include clinical laboratory professionals, reference material providers, scientists from National Metrology Institutes worldwide (NMIs), organizers of proficiency testing (PT) and external quality assessment (EQA) schemes, and IVD manufacturing representatives. The work of JCTLM is ongoing and can be expected to be a resource for the identification of reference materials and reference procedures. Note that a reference material may or may not be commutable with patient samples for a particular comparison; the user must validate commutability unless suitable information is included in the certificate of analysis. It will also be a forum where the various stakeholders can exchange views on the best way to provide consistent patient results across the world. Current information on the committee's progress is posted and can be viewed on the Internet at http://www.bipm.org.

3 Definitions

analyte – component represented in the name of a measurable quantity (ISO 17511)[1]; **NOTE:** This is the chemical entity/substance that is actually intended to be measured.

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calibration transfer protocol – detailed description for assigning a value of a quantity to a reference material using a specified sequence of measurement procedures calibrated by higher-order reference materials for the same type of quantity. (ISO 17511)[1]

certified reference material (CRM) – reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes metrological traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. (VIM93) [8]

commutability (of a material) – closeness of agreement between the mathematical relationship of the measurement results obtained by two measurement procedures for a stated quantity in a given material, and the mathematical relationship obtained for the quantity in routine samples; **NOTE:** For reference materials used to calibrate measurement procedures intended for use by medical laboratories, the routine samples shall include samples from healthy and relevantly diseased individuals.

fitness for purpose – a term used to indicate that a method or service fits the analyst's defined purpose for that measurand.[11]

influence quantity – quantity that is not the measurand but that affects the result of the measurement. (VIM93)[8]

international conventional calibrator – calibrator whose value of a quantity is not metrologically traceable to the SI but is assigned by international agreement; **NOTE:** The quantity is defined with respect to the intended clinical application. (ISO 17511)[1]

international measurement standard – standard recognized by an international agreement to serve internationally as the basis for assigning values to other standards of the quantity concerned. (VIM93)[8]

manufacturer's product calibrator – calibration material provided to the customer for use with a routine clinical measurement procedure.

manufacturer's selected measurement procedure – highest level measurement procedure within the manufacturer's operation unless the manufacturer maintains his/her own reference laboratory; **NOTE 1:** Generally used to transfer a value to the "Manufacturer's Working Calibrator"; **NOTE 2:** The calibration may make use of a Primary Calibrator or a Secondary Calibrator.

manufacturer's standing measurement procedure – measurement procedure that is calibrated by one or more of the manufacturer's working calibrators or higher types of calibrator and validated for its intended use (ISO 15197[12]; ISO/DIS 17593[13]); **NOTE:** Testing measurement procedure used to assess the product calibrator and is calibrated with the "Manufacturer's Working Calibrator."

manufacturer's working calibrator – standard that is used routinely to calibrate or check material measures, measuring instruments or reference material (ISO/DIS 17593[13]); **NOTE:** Material used to calibrate the "Manufacturer's Standing Measurement Procedure."

matrix effect – influence of a property of the sample, other than the measurand, on the measurement of the measurand according to a specified measurement procedure and thereby on its measured value (ISO 17511)[1]; **NOTE 1:** A specified cause of a matrix effect is an influence quantity (ISO 17511); **NOTE 2:** The term 'matrix effect' is sometimes erroneously used for the lack of commutability due to a denatured analyte or an added nongenuine component ('surrogate analyte') meant to simulate the analyte. (ISO 17511)

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measurand – particular quantity subject to measurement (VIM93)[8]; **NOTE:** Generally includes the "*analyte*" as measured with respect to specific conditions. Examples include: *glucose* in plasma, *protein* in 24 hour urine, serum *cholesterol*.

primary reference material//primary calibrator – reference material having the highest metrological qualities and whose value is determined by means of a primary reference measurement procedure, directly to the SI or indirectly by determining the impurities of the material by appropriate analytical methods (adapted from ISO 17511).[1]

primary reference measurement procedure – reference measurement procedure having the highest metrological qualities, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units, and where results are, therefore, accepted without reference to a measurement standard of the quantity being measured. (ISO 17511)[1]

reference measurement procedure – thoroughly investigated measurement procedure shown to have an uncertainty of measurement commensurate with the intended use, especially in assessing the trueness of other measurement procedures for the same quantity and in characterizing reference materials. (ISO 15193)[9]

reference measurement laboratory – laboratory that performs a reference measurement procedure and provides results with stated uncertainties; **NOTE:** ISO/IEC 17025 uses the term "calibration laboratories."

secondary calibrator – a reference material whose value is assigned using a reference (secondary or primary) procedure calibrated with a Primary Calibrator.

secondary reference measurement procedure – a procedure usually calibrated with a Primary Calibrator; **NOTE:** Often these procedures are appropriate for a patient's sample.

trueness – closeness of agreement between the average value obtained from a large series of test results and an accepted reference value (ISO 3534-1)[14]; **NOTE 1:** The measure of trueness is usually expressed in terms of bias (ISO 3534-1); **NOTE 2:** Trueness has been referred to as "accuracy of the mean." This usage is not recommended (ISO 3534-1).

uncertainty of measurement – parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand. (VIM93; GUM 1993)[8,15]

validation – confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. (ISO 9000)[16]

4 Traceability

In the context of this document, traceability means metrological traceability. Traceability of clinical laboratory measurements to agreed-upon references is a prerequisite for achieving the needed comparability and reliability of laboratory results for patient care. Traceability will play a critical role in achieving harmonized laboratory results.

Eurachem and CITAC have recently published a document, "Traceability in Chemical Measurement: A guide to achieving comparable results in chemical measurement[4]," that discusses the concepts of traceability and provides detailed guidance for its establishment. The Eurachem/CITAC guide[4] was created through the effort of an international working group, and is freely available over the Internet. This report will not attempt to reproduce the Eurachem/CITAC guide, but encourages the adoption of its

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principles and methods, and provides implementation information for its application in laboratory medicine.

From the Preface to the Eurachem/CITAC guide:

> To achieve comparability of results over space and time, it is essential to link all the individual measurement results to some common, stable reference or measurement standard. Results can be compared through their relationship to that reference. This strategy of linking results to a reference is termed "traceability."[4]

The Eurachem/CITAC guide, in its introduction (paragraph 2.3), identifies the role of a measurement equation to define the measurand, and a validation process to demonstrate the equation's completeness.[4] The measurement equation provides a means to calculate the value of the measurand in terms of other measured quantities. Traceability of the measurand is established by ensuring that these other measured quantities are themselves traceable.

Returning to the VIM definition of traceability, it becomes clear that two elements are needed to achieve traceability: a reference to compare to, and a comparison procedure. The definition goes on to suggest that the uncertainty of the comparison be propagated to the uncertainty of the result. This concept of the propagation of variability underlies the generally accepted manner of calculating uncertainties in measurement, and has been adopted by the *Guide to the Expression of Uncertainty in Measurement* (GUM)[15], a joint publication of BIPM, IEC, IFCC, ISO, IUPAC, IUPAP, and OIML[b].

Numerous methods of comparison are applied in laboratory medicine, where an unknown sample is compared to a "calibrator." This comparison is often achieved indirectly, through calibration of an instrumental measurement. In calibration, the scale of the instrument is transformed to reportable units by the measurement of one or more "calibrator" samples, for instance providing a conversion factor between millivolts and units of chemical concentration. When unknown samples are presented to the instrument at some later time, a signal is obtained (in the measuring units of the instrument) and the result is converted to the reportable units, relying on the conversion factor established at the time of calibration. The conversion to and from the natural units of the instrument (typically volts) and those of the "calibrator" sample(s) reduces the calibration to a comparison between the calibrators and the unknown. A comparison procedure will often be an analytical method.

Expressing the elements of traceability as this comparison between "calibrator" and unknown samples reveals that traceability is already ubiquitous in laboratory medicine. These "calibrators" are the *references* called for in the VIM definition.[8] Where great opportunity exists is in establishing the pedigree of these references, as that pedigree determines the scope of traceability. The scope of traceability extends so far as a reference is shared. When a reference is shared only within a given laboratory, the scope is limited to that laboratory. When a reference is shared internationally, the scope is international, and comparability of results traceable to that reference can be supported. For instance, references that are restricted to a single IVD device manufacturer's laboratory, and that are not themselves traceable to outside references, may prevent the comparison of results from other devices calibrated with different references.

[b] BIPM International Bureau of Weights and Measures
IEC International Electrotechnical Commission
IFCC International Federation of Clinical Chemistry and Laboratory Medicine
ISO International Organization for Standardization
IUPAC International Union of Pure and Applied Chemistry
IUPAP International Union of Pure and Applied Physics
OIML International Organization of Legal Metrology

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4.1 Overview of the Process for Establishing Traceability

The process for establishing traceability is described in Section 6 of the Eurachem/CITAC Guide.[4] It identifies the following key elements in establishing traceability. These elements apply to each stage in the transfer process, where values are transferred by comparison from one material or procedure to another.

- specification of the measurand, the scope of the measurements, and the required uncertainty;
- selection of a suitable method of estimating the value of the measurand;
- demonstration, through validation, that all significant influence quantities appear in the measurement equation and the specified conditions;
- identification of the relative importance of each input quantity;
- selection of appropriate reference materials or procedures; and
- estimation of the uncertainty of the measurement result, or of the value assigned to a standard (e.g., a "product" calibrator, as defined in ISO 17511).

4.2 Process to Establish Traceability

4.2.1 Specifying the Measurand and Required Uncertainty

4.2.1.1 Identity of the Analyte

Measurements in laboratory medicine require care in specification of the chemical form of, and matrix containing, the analyte. This must include a description of the specific condition of the entity being measured, the units used to report the measurement result, and any special descriptors that may be needed.

Example 1: The concentration of glucose is often measured using glucose oxidase/peroxidase and a colorimetric reaction that is spectrophotometrically proportional to the actual content of D-Glucose. The samples are often serum, plasma, urine, or cerebral spinal fluid (CSF). Definition of the measurand, glucose, under those conditions is substance concentration of D-Glucose in serum, plasma, urine, or CSF in mmol/L.

Example 2: The manufacturer of a test kit intended for measuring human chorionic gonadotropin (hCG) as a tumor marker (to detect molar pregnancy, choriocarcinoma, or testicular tumors) or as an aid to the detection of Trisomy 21 (Down's syndrome) would not want to establish the values assigned to the kit calibrators by calibration with the WHO International Standard for hCG, which is prepared from urine of pregnant women. This is because the various forms of hCG present in tumors are qualitatively different in their carbohydrate ligand content and branching compared to those forms of hCG present in women in normal pregnancy. Definition of the measurand, hCG, under those conditions would be human chorionic gonadotropin (hCG) in serum from nonpregnant individuals.

4.2.1.2 Implied Measurement Conditions

Normally, the implied measurement conditions may be a portion of the description of the analyte. If not, the measurement conditions must be described; this may take the form of specific descriptors such as pH, temperature, or other such variables that may have an effect on the measurement results. An example is ionized (free) calcium activity in plasma at 37 °C, at the sample pH in mmol/L.

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4.2.1.3 Method-defined Measurand

When a procedure defines the measurand, the specification is through enumeration of the procedure. Measuring alanine aminotransferase (ALT) by the IFCC procedure, as described below, is an example of this.

Example 3: IFCC has established a reference measurement procedure that defines a catalytic measure of alanine aminotranferase (ALT) in human serum. The specifications for the procedure include attributes such as incubation temperature, buffer type and strength, substrate concentration, coenzyme concentration, etc. Due to substantial differences in the conditions of measurement, assay of this "analyte" according to an alternative methodological principle should not be considered to be measurements of the same entity.

4.2.2 Choosing a Suitable Method for Comparison

The transfer of a value from a reference material to a test sample (perhaps another reference material, to be used in a different process) is a comparison between the reference material and the test sample. The specific needs of the transfer will dictate the method of comparison to be used.

For example, when a high-purity reference material is available, an appropriate method of establishing traceability is to prepare calibrators from that material. In this case, the comparison is made with a balance and calibrated volumetric apparatus. Traceability to the high-purity material is established from the purity of the material and the use of calibrated equipment.

4.2.3 Validation

Validation is defined as confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled (ISO 9000:2000). A value transfer process is validated by demonstrating that the process consistently meets specified requirements. The principles of traceability assume that all comparisons made in the unbroken chain are valid.

Responsibility for validation lies with the party responsible for reporting the results. Validation relies upon effective quality management and good manufacturing and laboratory practices at all stages. When the calibration traceability strategy is altered, validation must be reestablished.

The performance of the measurement procedures is what must be validated, against a specified set of expectations. These expectations establish the degree to which the measured results reflect what was intended to be measured, and that the uncertainty associated with the results is adequate and consistent with predictions. When performance is confirmed, a value-transfer process can be employed to establish traceability.

Section 6.4 in the Eurachem/CITAC guide on traceability suggests a number of experiments useful in validation.[4] In addition to these experiments, it is essential to establish the commutability of any reference materials used in establishing traceability in laboratory medicine. Commutability assures that results obtained during a value transfer are reasonably free from bias (sometimes termed "matrix effects") potentially introduced by the matrix of the reference materials.

For a traceability chain to be completely effective, the reference material used to calibrate the next procedure must be demonstrated to be commutable with the samples used in that procedure. For example, if a high-purity material is used to calibrate a reference procedure used to assign values to a secondary reference material, the commutability of the secondary reference material for that particular segment of the traceability chain must be validated. This continues analogously for each link of the traceability chain. Please refer to Appendix B for an example of a traceability chain.

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One will see SRM 917 is the first reference material in the "chain." It is assigned using primary methods, and the producer (NIST) is responsible for assuring the validation of that value assignment process.

The second set of reference materials in the chain are gravimetrically prepared aqueous standards that are used as working calibrators by the manufacturer. The commutability of these materials will be the responsibility of the IVD manufacturer assigning values to the product calibrator.

The third set of reference materials in the chain are the product calibrators. The commutability of these materials must be demonstrated by the producer of the product calibrator for the uses that the producer claims the calibrators are to be used.

NOTE: In some cases, the product calibrator may be made and sold by a different manufacturer than the one selling and providing the analytical system. In that case, the user of the analytical system should assure that the producer of the product calibrator has adequately validated the commutability of the calibrator for that specific use.

4.2.4 Establishing Calibrator Commutability

A practical approach to evaluating and demonstrating commutability is described in the following example procedure:

(1) Select candidate materials to be validated for commutability.

(2) Collect samples of each clinically relevant sample type appropriate to the material to be validated (i.e., serum, plasma, urine, or others).

- Single donor samples are preferred.
- Spiking samples should be avoided and only allowed if the resulting sample mimics natural samples.

(3) Select two measurement procedures for which commutability of a material is to be validated (one may be a reference procedure of higher order).

(4) Obtain results on all of the above samples using each measurement procedure (i.e., verify that each measurement procedure is being performed correctly and using the appropriate calibration for each procedure; then assay clinically relevant samples and candidate materials to be validated for commutability on each measurement procedure).

(5) Compare the results. Statistically demonstrate that the results obtained for the candidate reference materials have essentially the same numeric relationship between the two measurement procedures as the results obtained for the clinically relevant samples.

The intent of a commutability study is to assure there are no significant matrix effects, or altered analyte effects, in the materials used for calibration. One mechanism for doing the above validation is the use of CLSI document EP14—*Evaluation of Matrix Effects*.

An example, shown in Figure 1, uses the EP14 evaluation protocol to validate commutability of calibrators between two procedures. In this example, the two manufacturers' working calibrators were commutable with the native human serum samples between the two measurement procedures. If a result for a manufacturer's working calibrator had exceeded the 95% prediction interval, that material would have been considered noncommutable between the two measurement procedures.

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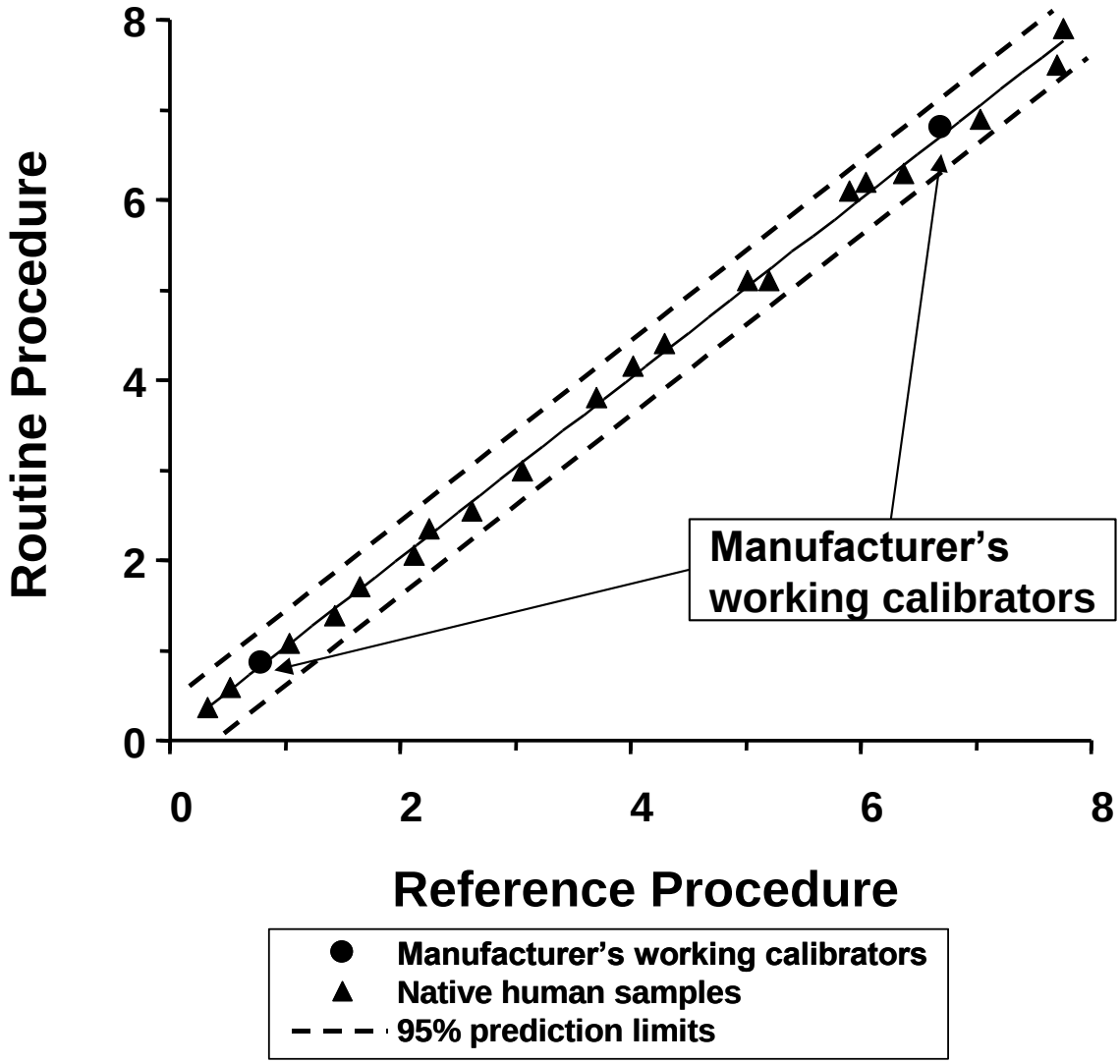

Figure 1. Validation of Manufacturer's Working Calibrator

One caution should be noted, however, when multiple working calibrators are used. As many as ten working calibrators can be used in a value assignment process. CLSI document EP14 examines the matrix effect of each calibrator independent of the others to a 95% confidence. If multiple calibrators are used, the probability of a false rejection increases by 0.95^{n}, where n = the number of calibrators to be studied. In these cases, alternative statistical analysis may be useful, such as a chi square analysis of the deviations of the working calibrators compared to the $S_{y.x}$ deviations of the patient samples.

Alternate approaches to validate the equivalence of numeric relationships between results for the two measurement procedures have been described by Baadenhuijsen et al.,[17] Franzini,[18] Rej,[19] and Bretaudiere[20] for materials intended for use as trueness controls or reference materials. CLSI/NCCLS document C37—*Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures* used a specialized material preparation design to demonstrate commutability of a reference material by comparing the measured numeric value for the pooled serum material to the weighted mean value predicted from individual measurements on each donor serum used to prepare the pool.

Section 7.2 of ISO 17511[1] describes validation of commutability of a working calibrator in the following manner: The working calibrator and human samples are measured using both the reference measurement procedure and the routine measurement procedure. If the mathematical relationship between the results of

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the reference measurement procedure and the results of the routine measurement procedure for the human samples is not significantly different from that found for the manufacturer's working calibrator(s), then commutability of the working calibrator has been demonstrated.

Section 7.3 of ISO 17511[1] addresses commutability of a product calibrator in the following manner. The results for a set of human samples are determined using a reference measurement procedure, calibrated with an appropriate calibrator, and the routine measurement procedure calibrated with the manufacturer's product calibrator. For metrological traceability to be achieved, the results for the human samples measured with the routine procedure shall be equivalent to those of the reference procedure. Comparisons may be by linear regression, with suitable slopes and intercepts, or other techniques that demonstrate the equivalency of the numeric results. Note that it is not required that the product calibrator be directly measured using the reference measurement procedure; the acceptance criteria for traceability are based on verification that results for human samples measured with the routine procedure are equivalent to the results for those same human samples measured using the reference measurement procedure.

Establishing the commutability of calibration materials used in more than one measurement procedure is an essential component to obtaining results for human samples that can be compared between two, or more, procedures. When the methods are valid, including demonstration that the calibration materials are commutable, two or more methods, calibrated by the same calibrators, will yield equivalent results for the measurand in the clinically relevant samples. Consequently, the results of those procedures can be traceable to the calibrator, and can be compared. In practice, equivalence is determined in part by the fit-for-purpose uncertainty of the method results.

If a manufacturer's product calibrator is intended for use only with a specific routine measurement procedure, commutability may not be necessary to have results for native patient samples that are traceable to a higher order reference. An example that illustrates this case is given in Appendix C. In this example, the manufacturer uses panels of human samples as working calibrators. This is accomplished by measuring the quantity of glucose in each member of the panel using a reference measurement procedure for glucose, the CDC hexokinase procedure. The panels of human samples can then be used (with their reference method-determined values) as calibrators in subsequent steps of the traceability chain, applying the commercially available method as a Selected Measurement Procedure and/or Standing Measurement Procedure (e.g., Glucose System X) to assign values to Working Calibrators (manufacturer's master lot of the product calibrator), or directly to product calibrator lots. Under these circumstances, validation of the values assigned to product calibrators (i.e., validation of calibration traceability according to the requirements of Clause 7.4 of ISO 17511[1], and the recommendations in Section 4.2.3 above) will demonstrate that any matrix-related bias applicable to the particular design of the product calibrators has already been accounted for; i.e., the values assigned to the product calibrators incorporate any correction needed to eliminate systematic biases. Under these circumstances, the commutability of the additional Working Calibrator or the Product Calibrators will have been established only with respect to the particular commercial product system, i.e., Glucose System X. In the product labeling, the manufacturer should indicate that the commutability of the product calibrator has been established only with respect to Glucose System X, and that commutability with respect to any other glucose measurement procedures is unknown. Similarly, a manufacturer's product calibrator that is value assigned using a reference material other than a panel of native samples, and is intended only for a specific routine measurement system, may not be required to be commutable as long as the results for native patient samples assayed using that routine measurement system are traceable to a higher order reference system.

4.2.5 Importance of Different Input and Influence Quantities

As noted in Section 6.5 of the Eurachem/CITAC guide,[4] it is crucial to establish the degree of care that must be taken to control or calibrate the different factors that influence the measurement result. Those

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factors that have large quantitative influence must be addressed with care and attention, while those with secondary influence can be addressed less rigorously.

Specificity of a measurement procedure for the measurand in relevant clinical samples is an influence quantity. Interfering substances, such as protein with creatinine in serum when measured with an alkaline picrate reagent, can influence the validation of commutability and the demonstration of traceability. It is beyond the scope of this document to address interfering substances, except to note that nonspecificity of a measurement procedure may be a factor to consider in disqualifying that procedure as a component of a traceability comparison.

The equation used to calculate the measurement result, and a comprehensive uncertainty budget, are the tools used to assess the degree of influence of the different contributors to the result. As noted in the Eurachem/CITAC guide,[4] it is likely that the chemical effects will require more attention and care than the physical effects (time, mass, temperature) in a chemical measurement. Using the proper reference material for calibrating the IVD device, by assuring the commutability of the standards used to establish its traceability, is likely to be a critical influence quantity.

4.2.6 Considerations When Selecting a Reference

Annex I, paragraph A3, In Vitro *Diagnostic Directive 98/79/EC* states:

> "The traceability of values assigned to calibrators and/or control materials must be assured through available reference measurement procedures and/or available reference materials of a higher order."[3]

NOTE: Higher order references are being identified by the JCTLM, which publishes lists of approved reference materials, reference measurement procedures, and reference laboratories (see Section 2.2 for additional details).

Four situations are possible:

- both reference materials and reference measurement procedures are available (e.g., glucose, cholesterol, creatinine, hemoglobin);
- a reference material, but no reference measurement procedure, is available (e.g., specific plasma proteins);
- a reference measurement procedure, but no reference material, is available (e.g., some coagulation factors, some enzymes);
- neither a reference measurement procedure nor a reference material is available (e.g., cancer markers, CK-MB, new measurands).

4.2.6.1 Considerations When Reference Materials and a Reference Measurement Procedure of Higher Order are Available

The choice of a reference material and a measurement procedure when results can be traced to the SI unit system is relatively straightforward. In these instances there are often several choices. The manufacturer best determines the criterion for selection as it relates to the production of the product calibrator. Clause 4.3 of ISO 17511[1] provides good guidance in this area. Among the considerations are:

- Define the analyte being measured in the human samples.
- Consider if the analyte is heterogeneous and the impact of heterogeneity on the measurement.

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- Recognize potential differences when different measurement procedures are used (often the case for immunochemistry assays where the epitopes being detected may vary between assays.)
- When calibrating with a common purified material, the native clinical sample being run may not generate a signal with the same relative magnitude as the purified material (e.g., protein determination of serum by a biuret reaction calibrated with an albumin solution).
- When using a surrogate analyte in the calibrator, studies should be included to establish the validity of the surrogates used.
- Allowances should be made for any modifications to the analyte during the measurement procedure that affect the calibrator and human samples differently.
- Commutability between the reference material and relevant clinical samples must be validated for each comparison step.

4.2.6.2 Considerations When Reference Material(s) but No Reference Measurement Procedure of Higher Order is Available

When no reference measurement procedure of higher order exists (e.g., specific plasma proteins), the choice of the reference material is critical. The considerations in the previous section apply to this circumstance.

4.2.6.3 Considerations When a Reference Measurement Procedure but No Reference Material of Higher Order is Available

In the absence of reference materials, the manufacturer is limited to establishing performance through the validation of each significant input and influence quantity in the procedure. It is also necessary to demonstrate that the reference and routine measurement procedures produce equivalent results for patient samples, within the limits of fit for purpose.

4.2.6.4 Considerations When No Appropriate Reference Materials or Procedures of Higher Order Are Available

When no reference material or reference measurement procedure is recognized as "higher order," the reference must be established by the manufacturer. ISO 15194 provides guidance that can be applied to the establishment of a reference material. While all the concerns listed above are relevant, the manufacturer should take into account those issues normally considered when reference materials are developed. For example, establishing the stability of the material is essential to maintaining a standard and providing for its eventual replacement.

For some diagnostic assays, a procedure itself may define the concentration of the calibrators used for field methods. ISO 15193[9] provides guidance in this case. Such procedures should be stable and reproducible over space and time, reporting consistent results when the physical components of the procedure are changed. Such components could include column packing materials for column separation procedures or proprietary substrates for a unique enzymatic assay system.

The consistency of product calibrator from lot to lot will depend upon the consistency of the manufacturing process and the system used to assign a value to that material. Maintaining the reference includes the maintenance of materials, instruments, and the capability of the individuals performing the test. ISO 15193,[9] ISO 15194,[10] and ISO 15195[7] provide guidance in these areas. Following these recommendations should result in a stable reference system.

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4.2.7 Uncertainty Estimation

The measurement of uncertainty estimates the expected dispersion of the result. All measured quantities have variability—the intent of determining this variability is to guide the decision maker in distinguishing between measurement variability and significant clinical effects.

Uncertainty is not error. Error is the difference between an individual measurement result and the true value of the measurand.[8] Where error can be estimated, for instance when an interference that can be estimated is present, a correction should be made. Uncertainty is not doubt about the validity of the result; in fact, a quantitative estimate of uncertainty brings more confidence in a result by providing information that the true value lies within a certain range.

Multiple sources typically contribute to uncertainty. Effects of these sources are combined and used in aggregate as an estimate of the uncertainty of a result. The adoption of the 1995 ISO document *Guide to the Expression of Uncertainty in Measurement*[15] provides a consistent approach to calculate and report quantitative uncertainty estimates. The GUM outlines statistical and mathematical procedures that can be applied to quantifying uncertainties in any field of measurement.

Due to the wide range of measurement technologies covered in the GUM,[15] a number of interpretations have been written that are applicable to specific fields. For example, the Eurachem/CITAC guide, *Quantifying Uncertainty in Analytical Measurement* (QUAM),[21] is useful for clinical diagnostics. This key resource for application of the principles of the GUM[15] in chemical analysis is applicable to laboratory medicine as well. This guidance document is rich in examples, and establishes a four-step procedure for establishing an uncertainty budget for a laboratory measurement. The steps focus on 1) specification of the measurand; 2) identification of the sources of uncertainty; 3) quantification of these sources (uncertainty components), often in groups; and 4) combination of the quantified components. It is important to recognize that the calculation is an estimate that depends upon technical judgment with respect to what components of uncertainty are included. Sources of uncertainty that are relevant for atomic absorption calibrators (for example, natural isotope distribution for lithium) may not be significant sources of uncertainty for calibrators for glucose.

Since its introduction in 2000, the QUAM document[7] has been widely adopted by both the laboratory analyst and the accreditation community, and is readily available on the Internet at: http://www.measurementuncertainty.org. (This site offers the QUAM guide and also provides collected examples, a glossary, a discussion forum, relevant announcements, and useful links.)

The examples in the appendixes include estimations of uncertainty for several traceability protocols.

While the motivation for the clinical laboratory to provide calculations of uncertainty for reported results may be viewed as providing full disclosure of the ability of the laboratory to provide accurate values, there is an unstated benefit for both the laboratory and the manufacturer. The exercise of calculating uncertainty forces careful consideration of each manufacturing and testing step. As estimates are made, often process improvements that would lower the uncertainty are identified, as well as areas where a less stringent process could improve workflow with no impact on the final result. For the manufacturer, uncertainty calculations can provide a tool for setting and meeting product specifications. To provide the greatest utility, uncertainty calculations may be best presented by breaking out the components as either internal or external.

By convention, the uncertainty of the zero calibrator is zero unless there is data to suggest otherwise. In some instances there may be endogenous analyte present in the "zero" calibrator that is below the sensitivity of available assay technology and therefore cannot be estimated. In most instances the uncertainty of the level of this material will have little impact on the overall uncertainty of the calibration curve. The calibration algorithm will assume a "zero" intercept in some applications. If there is a potential

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impact on the measurement curve, and data is available, information on the uncertainty of the zero calibrator should be provided.

4.3 Reporting Traceability in Product Literature

According to ISO 17511,[1] the following information is the minimum to be included by the manufacturer in the product instructions or labeling (**NOTE:** The examples below are only one of the means of reporting traceability. They may not be the only example for each measurand, or describe the best method(s) to use in establishing traceability of a measurand):

- The measurand and applicable body fluids in concise terms. For example: Glucose in serum, plasma, or urine measured in mmol/L. (A useful reference is the Logical Observation Identifiers Names and Codes (LOINC) database accessible at http://www.regenstrief.org/.)

- A statement describing what references, i.e., procedures and materials, were used to establish the traceability of the calibrators. An example of such a statement may be:

  The values assigned for Glucose to this calibrator (or set of calibrators) are traceable to the SI Unit by utilizing SRM 917b and the ID-GCMS or Glucose Hexokinase reference procedure for Glucose.

- If procedures and/or materials of a JCTLM-approved higher order are not available, the manufacturer should state what is used to assign the values. For example:

  The values assigned for xyz analyte are not traceable to the SI unit. They are assigned using purified analyte in a human serum matrix and an internal analytical procedure using the xyz method (measurement procedure).

Additional information, such as uncertainty estimates, the results of commutability experiments, details of the procedures used, and statistical approaches to analyzing the calibrator validation data may be included at the manufacturer's option. The uncertainty estimates and demonstration of the commutability of the calibrators need not be in the labeling but must be on file and available to customers upon request. All statements made must be supported by data on file as part of the validation process for the calibrators.

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Appendix A. Traceability and Calculating Uncertainty of Calibrator Levels for hCG

The example in Figure A1 below follows the guidance outlined in Section 4.1, Overview of the Process for Establishing Traceability. A more detailed description of the manufacturing process can be found in the figure at the end of this example.

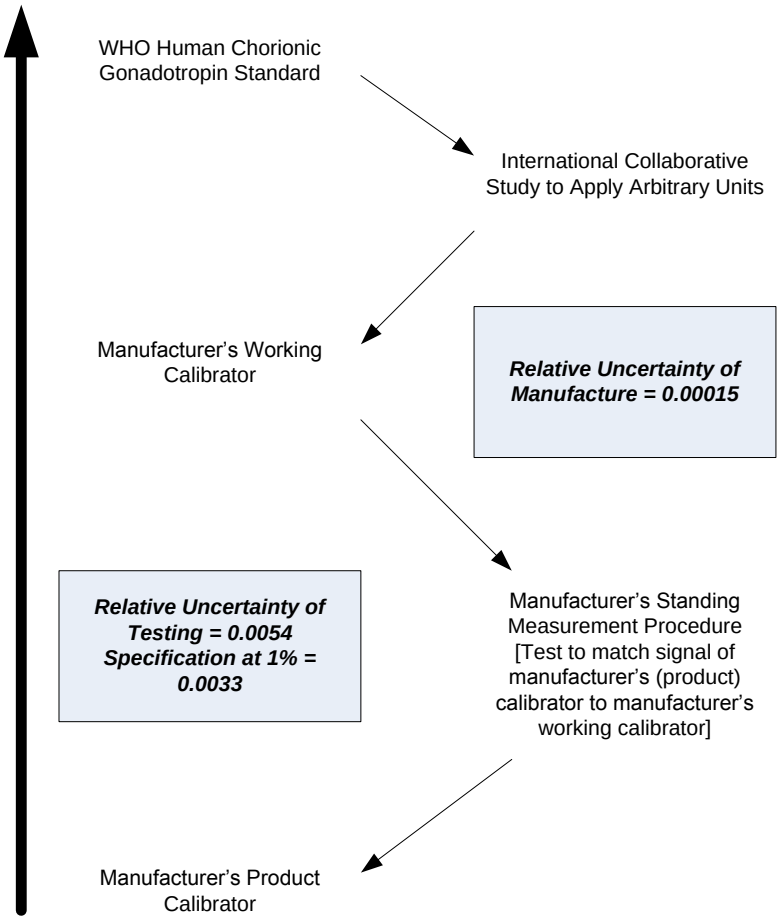

Figure A1. Traceability for Generic hCG Immunoassay

A1. Specification of the Measurand, the Scope of the Measurements, and the Required Uncertainty

Human chorionic gonadotropin (hCG) is a protein whose presence is used for the detection of a number of diseases. Assays can be directed toward particular epitopes that are appropriate for the intended use of the assay. The choice of the standard is dictated by the intended purpose of the assay and the matrix (e.g., serum or urine). For example, for the diagnosis of pregnancy the use of WHO 75/589 is a reasonable choice for the measurement in serum. There are several other standards available which for other intended uses may be more appropriate.

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Appendix A. (Continued)

A2. Selection of a Suitable Method of Estimating the Value of the Measurand

There are no recognized reference measurement procedures for hCG, and consequently standardization makes use of an internationally recognized WHO standard reference material for most commercial assays. A manufacturer's standing test method is required for this value transfer, and in the absence of a recognized reference method, the choice is based on the technical judgment of the manufacturer.

A3. Demonstration, Through Validation, That All Significant Input Quantities Appear in the Measurement Equation and the Specified Conditions

In choosing the manufacturer's standing method, all relative influences on that method such as matrix effects should be considered, as well as the analytical capability of the method with respect to analytical specificity and precision. The number of replicates run for value transfer is a key decision that is governed by the precision of the assay and the allowable uncertainty of a particular calibrator concentration. These considerations should be part of the method validation. For immunoassays, signals are frequently matched between the Manufacturer's Working Calibrator and Manufacturer's Product Calibrator using the components of the assay sold to the customer. The value assigned is nominal and does not vary lot to lot for calibrators (see Figure A2), and the uncertainty of the value is controlled by the test method and underlying internal manufacturing specifications for matching the Manufacturer's Working Calibrator to the Manufacturer's Product Calibrator (provided with test kits to customers). The degree to which the analyte reacts differently in the calibrator matrix as in the matrix of the intended sample should be characterized. Since there is no higher order reference procedure for hCG that can be used with patient samples, the commutability of the calibrator cannot be established. The comparison of the assay performance against other marketed assays provides valuable information. Ideally all assays should return results that are similar with respect to the numerical values. Claims with respect to analytical range and other performance characteristics (e.g., interferences) can be expected to vary from one manufacturer to another.

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Appendix A. (Continued)

Table A1. Identification of the Relative Importance of Each Input and Influence Quantity

Identification of Relative Importance of Each Input and Influence Quantity			Selection of Appropriate Reference Materials and Procedures
Input/Influence Quantity	**Relative Importance**	**Control Strategy**	**Selected Reference**
Reaction Temperature	+++	Instrument controls temperature	NIST Traceable Thermometers
Precision	+++	Number of Replicates chosen to statistically provide necessary resolution	Statistics Reference Texts
Volumetric Flask	+++	Class A Volumetric	Traceable to NIST Standard
Endogenous hCG	+++	Use plasma from males assayed for HCG	
Calibration	+++	Use Calibrated Equipment	Calibrate using certified standards
Balance Error	+/-	Choose Self-calibrating Balance with appropriate specifications	NIST Traceable Calibration Weights
Instrument	++	Single Instrument	Maintenance current
Interferences in Matrix	+/-	Measure	Characterize sources and methods to measure
Reagent Lot	+/-	Use Single Lot	
Matrix Variability	+/-	Characterize Potential Differences	
Mixing	+/-	Use validated mixing methods	
Barometric Pressure	-	None	
Room Temperature	-	None	
Humidity	-	None	

A4. Selection of Appropriate Reference Materials or Procedures

The standard is a lyophilized protein in a vial with a set amount of arbitrary International Units. Since the units are arbitrary, there is no uncertainty associated with the assigned value. Although the amount of protein is indicated on the vial, the usually reported value is in units. The contents of the vial can be used to prepare a number of dilutions (Manufacturer's Working Calibrators) under controlled conditions that introduce a relatively low uncertainty to the resulting concentrations. These dilutions can then be used to transfer their values to Manufacturer's Product Calibrators.

A5. Estimation of the Uncertainty of the Value Assigned to a Standard (e.g., a "product" calibrator, as defined in ISO 17511)

To determine the uncertainty of the Manufacturer's Product Calibrator concentrations, each step of the manufacturing process is examined for its contribution. As discussed above, the process is initiated by the preparation of a stock solution prepared from a reconstituted recognized standard (e.g., WHO). Each manufacturing step will contribute an uncertainty based on the methodology chosen. Volumetric dilutions tend to introduce more uncertainty as compared to appropriately chosen gravimetric dilutions. The final result is a series of Manufacturer's Working Calibrators (A through F) that are equivalent in concentration to the series of Manufacturer's Product Calibrators. Each of the Manufacturer's Working Calibrators will have included the relative uncertainty of the preparation of the highest concentration of hCG (Calibrator F

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Appendix A. (Continued)

at 100 mIU/mL). The relative uncertainty for the Calibrator F working calibrator includes the relative uncertainty for the volumetric flask, and the two weighing operations as follows:

$$\text{Uncertainty Working Calibrator F} = \sqrt{U_{100\text{ mL vol Flask}}^{2} + U_{\text{wt of hCG stock}}^{2} + U_{\text{wt of serum diluent}}^{2}}$$

$$= \sqrt{2.13333\text{E}-11 + 3.14804\text{E}-08 + 2.7052\text{E}-09} = 0.00018$$

Each of the other working calibrators will have both the relative uncertainty of the Calibrator F and the two weights for diluting to the lower concentration.

$$\text{Relative Uncertainty Working Calibrator} = \sqrt{U_{\text{CalibratorF}}^{2} + U_{\text{wt of } \textit{CalibratorF}}^{2} + U_{\text{wt of serum diluent}}^{2}}$$

For example, for Working Calibrator C (50 mIU/mL Serum)

$$= \sqrt{3.421\text{E}-08 + 1.133\text{E}-11 + 9.234\text{E}-09} = 0.0002084$$

The range of relative uncertainties based on the similar calculations is from 0.00021 to 0.00023, where most of the contribution is from the preparation of the stock solution. Had these solutions been prepared using volumetric tools, the uncertainties would be more than an order of magnitude greater.

In the next step for manufacture of the product calibrators is the value transfer from the working calibrator to the in-process Manufacturer's Product Calibrator, which is accomplished by matching the signal output of the two calibrators by the Manufacturer's Standing Measurement Procedure. In this example the procedure is identical to that which is sold to the customer. The Manufacturer's Product Calibrator is adjusted with a commercially available hCG (or material produced internally) until the signal matches the Manufacturer's Working Calibrator. There are two contributions to the uncertainty of the calibrator values from this procedure: the first is the specification that describes the degree that these two signals from each pair of calibrators tested will be matched (typically +/- 1 to 4%, depending upon the assay requirement); and the second is the test method performance, including the number of replicates and the variance of the assay. The contribution to the uncertainty can be represented by the comparison $\mathbf{S_{pecification}}$ and by $\mathbf{U_{Test\ method}}$ for the test method contribution. (An alternative to this approach to matching has been described.[1])

The total relative uncertainty for the Manufacturer's Product Calibrator at a single concentration can then be represented as:

$$\text{Relative Uncertainty for (k=1)} = \sqrt{U_{\text{Manufacturing}} + S_{\text{pecification}} + U_{\text{Test method}}^{2}}$$

Typical values may result in the following:

For the manufacture of the Manufacturer's Working Calibrator

$M_{\text{anufacturing}} = 0.00022$

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Appendix A. (Continued)

Assuming a matching specification within 1% and a Gaussian distribution so the specification is divided by $\sqrt{9}$ to give the contribution to the standard error:

$$S_{pecification} = 0.0033$$

For testing, the CV of the assay is 3% and the number of replicates is 96 each for both the Manufacturers Working Calibrator and the Product Calibrator being tested. The test method contribution to uncertainty is:

$$SEM = \sqrt{2 \text{ x } \frac{Var_{REPLICATE}}{n_{REPLICATE}}}$$

$$U_{Test\ method} = 0.004330$$

The total relative uncertainty is then equal to:

$$\sqrt{0.00022^2 + 0.0033^2 + 0.0004330^2} = 0.005469$$

Assuming a concentration of 250 mIU/mL, the uncertainty for the 95% confidence interval (k = 2) would then be:

250.0 mIU/mL • 0.005469 • 2 = ± 2.734 mIU/mL

Note that most all the uncertainty is derived from the testing process as controlled by the matching specification. Inclusion of the uncertainty of the working calibrators has almost no impact on the uncertainty estimate. Including its contribution increases the 95% uncertainty range by only 0.002 mIU/mL.

For Calibrator “A” (0 mIU hCG/mL serum) has by convention an uncertainty of zero for its concentration. The serum diluent used to prepare the calibrators is in this case the same as the “A” calibrator. While it is relatively simple to obtain plasma samples lacking hCG, this does not always hold for all analytes. Care should be taken that zero is a correct value assignment when required.

In the absence of any data to the contrary, the uncertainty of the zero concentration calibrator is taken at zero by convention. In some instances there may be endogenous analyte present in the "zero" calibrator that is below the sensitivity of available assay technology and therefore cannot be estimated. In most instances the uncertainty of the concentration of this material will have little impact on the overall uncertainty of the calibration curve. The calibration algorithm will assume a "zero" intercept in some calibration algorithms. If there is a potential impact on the measurement curve, and data is available, information on the uncertainty of the zero calibrator should be provided.

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Appendix A. (Continued)

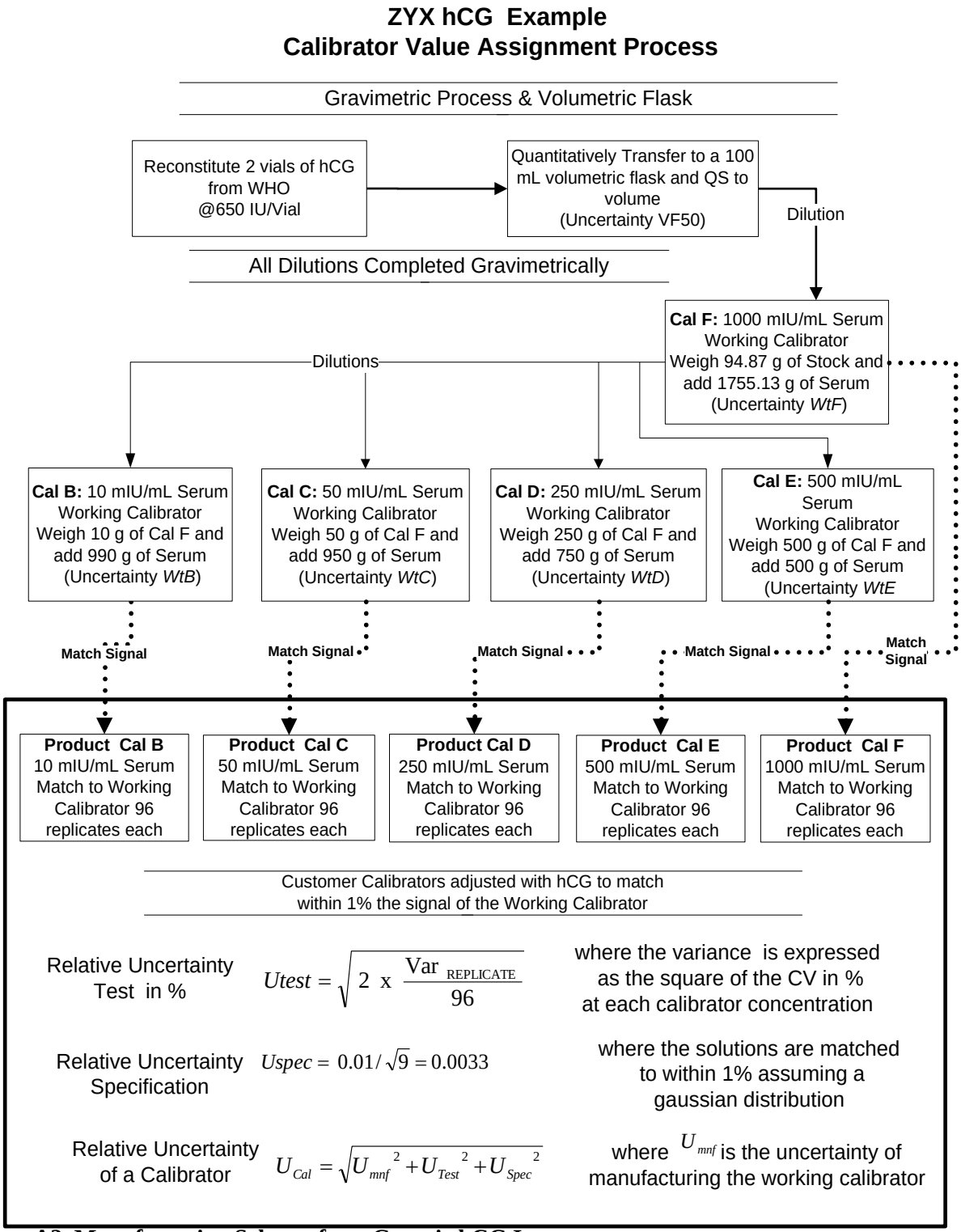

Figure A2. Manufacturing Scheme for a Generic hCG Immunoassay

Reference for Appendix A

[1] Schlain B. A stochastic approximation method for assigning values to calibrators. *Clin Chem*. 1998;44(4):839-848.

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Appendix B. XYZ Glucose Analytical System Glucose Calibrator Traceability Summary

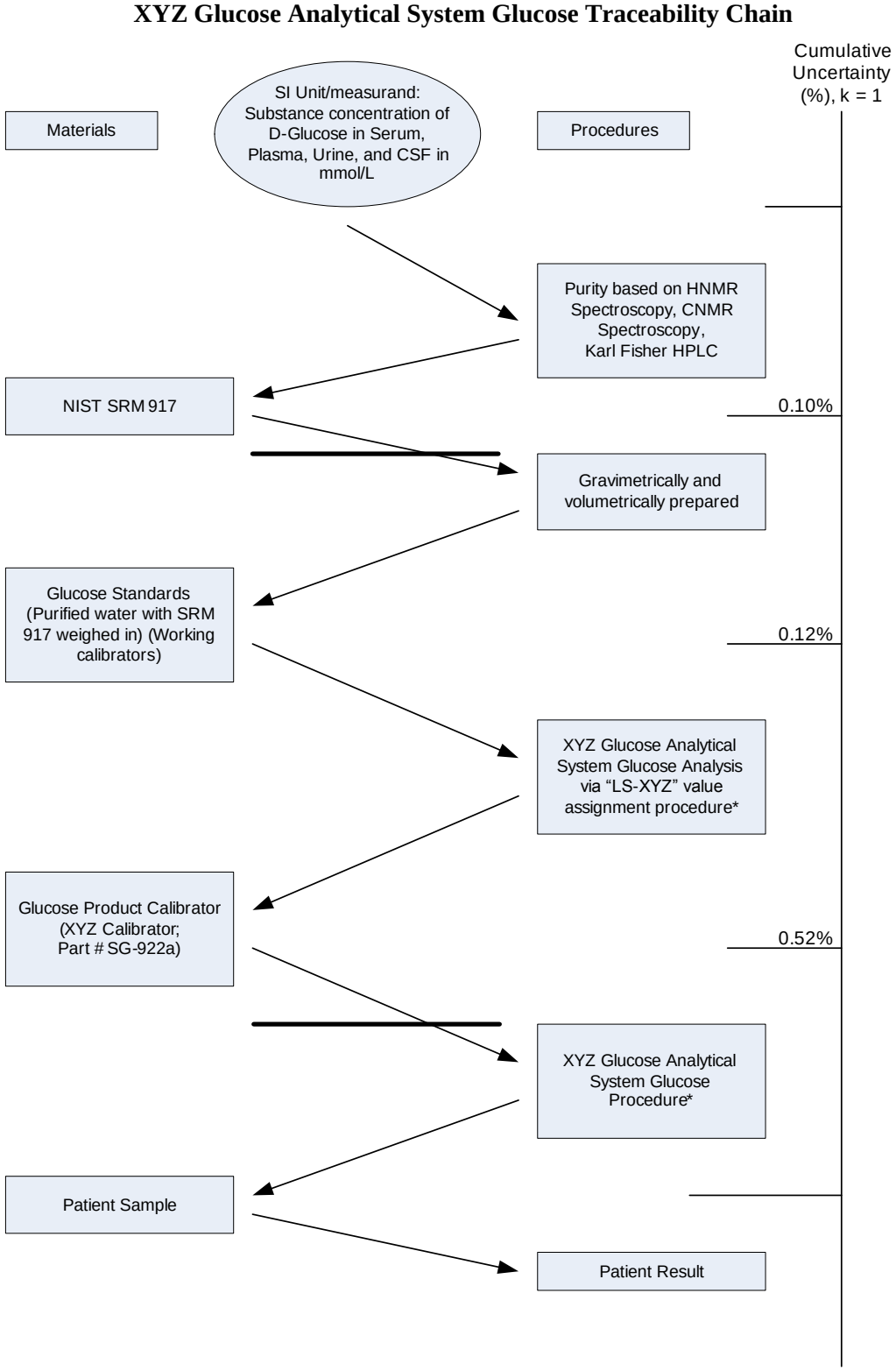

Figure B1. XYZ Glucose Analytical Systems Glucose Traceability Chain

*See example in Section B3.1 for commutability studies.

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Appendix B. (Continued)

B1. Scope and Specification of the Measurand and of the Measurements

Glucose is the predominant monosaccharide utilized by the body as an energy source. Its diagnostic uses are multiple and beyond the scope of this report. Glucose may be reported in mmol/L or mg/dL. The measurands are, therefore, substance concentration of glucose (mmol/L) or mass concentration (mg/dL) in serum, plasma, CSF, or urine.

B2. Selection of Suitable Procedures for Estimating the Value of the Measurand

The recognized reference measurement procedures for glucose are Isotope Dilution, Mass Spectrometry employing gas chromatography (ID/GC-MS), and the "CDC" Glucose Hexokinase procedure, which measures glucose in a protein-free filtrate of the sample. Both procedures use SRM 917 as their primary calibrator.

The selected measurement procedure is a direct glucose hexokinase procedure performed directly on the patient sample with no purification step adapted to the XYZ Glucose analytical system. The above traceability chain describes the analytical processes for each value transfer. Aqueous working calibrators are prepared gravimetrically and volumetrically from SRM 917. The working calibrators are used to calibrate the XYZ Glucose Analyzer. Values are assigned to the product calibrator using multiple XYZ analyzers, reagent lots, runs, and replicates.

B3. Demonstration Through Validation That All Significant Input and Influence Quantities Appear in the Measurement Equation and Specified Conditions

To minimize uncertainty, gravimetrically and volumetrically prepared solutions of SRM 917 have been used as working calibrators. To demonstrate the commutability of these calibrators, ID/GC-MS was used. In order to minimize the uncertainty of the manufacturer's standing measurement procedure, a test design, which uses multiple instruments, reagents, runs, and replicates to assign values to the product calibrators, was defined. Report XYZ describes the validation of the value transfer processes from SRM 917 to the product calibrator and the commutability of the calibrators.

B3.1 Validation of Commutability

In Figure B1, the commutability of the product calibrators has been demonstrated by correlation of the XYZ system, calibrated with product calibrators, with ID/GC-MS. Commutability was demonstrated by verifying that results for clinical samples were the same when measured by either the routine or reference measurement procedures. The simple linear regression analyses gave the following results:

For serum and plasma, a correlation was performed on 20 patient samples each of plasma and serum. The correlation equation is Y (XYZ) = 0.977 X (ID/GC-MS) + 0.11 mmol/L. The samples ranged from 1.33 to 29 mmol/L, and the results of the Y (XYZ) procedure had an average bias of 0.75% vs. the X (ID/GC-MS) procedure with a standard deviation of the individual sample biases of 1.26%.

For CSF, the correlation equation is Y (XYZ) = 0.995 X (ID/GC-MS) – 0.1 mmol/L. The samples ranged from 1.17 to 8.78 mmol/L. The XYZ procedure had an average bias of 0.12 mmol/L with a standard deviation of 0.02 mmol/L.

For urine, the correlation equation is Y (XYZ) = 0.991 X (ID/GC-MS) + 0.34 mmol/L. The samples ranged from 0.16 to 164 mmol/L. The average bias for the XYZ procedure for values greater than 1.2 mmol/L was 1.14% with a standard deviation of 4.2%.

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Appendix B. (Continued)

Commutability of the working calibrators has been demonstrated by utilization of CLSI document EP14—*Evaluation of Matrix Effects* and all working calibrators fell within the 95% prediction interval obtained from patient samples in the above mentioned correlations. Additionally, a chi-squared analysis was performed on the differences between the ID/GC-MS glucose values and the glucose values for the calibrators as measured with the XYZ procedure. When the populations of deviations from the line of regression for patient samples and for calibrators were compared, the deviations were not significantly different. The p-values ranged from 0.37 for spinal fluid to 0.99 for serum and plasma.

NOTE: In this case the aqueous working calibrators are commutable with patient samples when the XYZ glucose method is used. If this were not the case, several options would still be available to the manufacturer. These options could include appropriate patient pools whose values were measured by the reference method for a reference material (perhaps lyophilized or frozen serum materials) for which commutability has been demonstrated. If these other options are used, the uncertainty of the values assigned to the calibrators will be greater, because the uncertainty of the value assignment of those materials will contribute variability to the value assignment process.

B4. Identification of Relative Importance of Each Input and Influence Quantity

The input and influence quantities for the working calibrators are the uncertainty of the purity of NIST SRM 917, the uncertainty of the gravimetric procedures for the preparation of the high calibrator, and the relative uncertainty of each volumetric dilution for the remaining calibrators. Uncertainties for the gravimetric measurements are based on a triangular distribution of the calibration tolerances of the balances at the appropriate measurement ranges. The uncertainty of the volumetric measurements is based on a triangular distribution of the tolerances of the appropriate Class A volumetric glassware according to Federal Specification DD-V-581.[1] DD-V-581 is consistent with ASTM E969[2] for Class A pipettes and ASTM E288[3] for volumetric flasks. This will be quantitatively assessed in the uncertainty estimates below.

The input and influence quantities for the analytical steps for determination of the assigned values of the product calibrators are the within-run variation of the analytical run and the variability attributed to calibrations, instruments, and reagents. The overall variation of the process was determined by reviewing the value assignment of six lots of product calibrator, each repeated twice. These 12 exercises resulted in nine mean values, each of which included the above mentioned influence quantities. These, too will be quantitatively assessed in the uncertainty estimates below.

B5. Selection of Appropriate Reference Materials and Procedures

Both SRM 917 and the ID/GC-MS procedure are listed in the JCTLM list of reference materials and reference procedures of higher order. The reference procedure has been validated for all four sample types by a reference measurement laboratory. The laboratory's quality system meets the requirements of ISO 15195. The use of SRM 917 and the gravimetric and volumetric preparations of working calibrators for the XYZ system are validated by the commutability studies listed above.

B6. Estimation of the Uncertainty of the Values Assigned to the Working and Product Calibrators

The table below illustrates the influence quantities considered when the uncertainties of the calibrators were estimated.

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Appendix B. (Continued)

Identification of Relative Importance of Each Influence Quantity			Selection of Appropriate Reference Materials and Procedures
Influence Quantity	Relative Importance	Control Strategy	Selected Reference
Quantities for Working Calibrator Process			
Purity of the glucose weighed into the working calibrators	+	Use NIST-certified pure glucose	Use NIST SRM 917
Moisture content of SRM 917	-	Dry material under vacuum at 60 °C for 24 h before use	Controlled descriptions in the two rows below
Time glucose is dried in vacuum oven	—-	Dry for 24-30 hours	Calibrated timers not required
Drying temperature of vacuum oven	—-	Measure 60 °C using a calibrated thermometer	Calibrated to NIST thermometer
Tolerance limits of the weighing procedure for dried glucose	++	Controlled by procedure	None needed
Weighing accuracy of the balance used to weigh the dried glucose	++	Use of calibrated balances tolerances set by SOP	Calibrated to NIST weights
Volume of water added to NIST glucose	+++	Use of ASTM Class A volumetric flasks	Certificate or declaration from flask manufacturer
Temperature of water, flask, and dried glucose	—	All materials are at ambient temperature (18-25 °C)	Room temperature controls and area validation; no further control needed
Volume of diluent used for dilutions of stock to prepare working calibrators	+++	Use of ASTM Class A volumetric flasks	Certificate or declaration from flask manufacturer
Volume of stock used for dilution to prepare working calibrators	+++	Use of ASTM Class A volumetric pipettes	Certificate or declaration from pipette manufacturer
Temperature of water, flask, and pipettes when dilutions are made	—-	All materials are at ambient temperature (18-25 °C)	Room temperature controls and area validation; no further control needed

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Appendix B. (Continued)

Identification of Relative Importance of Each Influence Quantity Quantities for Product Calibrator Uncertainties			
Influence Quantity	Relative Importance	Control Strategy	Selected Reference
Accuracy of calibration of the XYZ instruments	++	Use of primary calibrators prepared with NIST SRM 917	Influences listed above
Reaction temperature for XYZ reaction	-	Controlled by temperature Controlled to within acceptable tolerances Confirm acceptable temperatures during maintenance	Per instrument maintenance procedures
Pipetting device in XYZ instrument	-	Controlled by XYZ Instrument confirmed during maintenance	Per instrument maintenance procedures
Lot – Lot variation of reagents	+	Reagents must meet acceptance testing before use. Use of multiple reagent lots to minimize variation	Acceptance testing procedure and use of controls to demonstrate acceptable consistency
Calibration error for xyz instrument	+	Instrument must demonstrate acceptable calibration curve based on slope, intercept, and residuals of each of six calibrator levels. Use of multiple calibrations to minimize variation	Acceptance criteria based on SOP
Instrument – Instrument variation from other sources	+	Instruments must demonstrate acceptable performance on control samples. Use of multiple instruments to minimize variation	Acceptance criteria for control samples, and acceptance criteria for between-instrument variation based on SOP

Working calibrator uncertainties—As stated before, the influence quantities for the working calibrators include the uncertainties for:

- the impurity of SRM 917;
- the balances used to prepare the high calibrator; and
- the volumetric equipment used for the remaining calibrators.

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Appendix B. (Continued)

In these calculations, the distributions of volumetric flasks and balances are all assumed to be triangular in nature. The standard deviation is derived from the tolerance divided by √6. All of the uncertainties are converted to relative uncertainties. The calculation of the uncertainty of the high calibrator is:

Relative Uncertainty (Ru) = $\sqrt{(\text{Ru SRM } 917)^2 + (\text{Ru bal } 1)^2 + (\text{Ru bal } 2)^2}$

where Ru (component) = uncertainty ÷ measured value.

To express as percent (%), multiply Ru by 100.

For the high working calibrator Ru (in percent) = $\sqrt{(0.1)^2 + (0.0352)^2 + (0.01225)^2}$ or 0.1068%

For the remaining working calibrators the estimate is:

Relative Uncertainty (Ru) = $\sqrt{(\text{high calibrator})^2 + (\text{Ru pipette})^2 + (\text{Ru vol flask})^2}$

For the lowest working calibrator the Ru (in percent) = $\sqrt{(0.1068)^2 + (\text{Ru } 0.061)^2 + (\text{Ru } 0.1095)^2}$ or 0.1238%.

The Ru (in percent) for the remaining five calibrators was estimated as approximately 0.11%. Since the six calibrators are used as a set to calibrate the XYZ system, the worst-case estimate of 0.12% is the relative uncertainty assigned to the working calibrators.

B6.1 Uncertainty of the Product Calibrators

An example of the process of calculating the uncertainty of a nonzero concentration is as follows. The value assignment to a Product Calibrator is based on the mean of nine runs using multiple reagent lots and instruments. To estimate the uncertainty, the standard deviations from the value assignment of 12 consecutive lots of Product Calibrator were examined. The root mean square of those 12 standard deviations was used to estimate the variation of the process. The uncertainty of the value assignment portion of the process then becomes the root mean square of the standard deviations divided by the square root of the number of runs in an exercise (i.e., nine). The relative uncertainty for each level is, therefore,

Relative Uncertainty (Ru) = $\sqrt{(\text{Ru standards})^2 + (\text{Ru value assignment})^2}$,

where Ru (component) = uncertainty ÷ measured value; and

Ru value assignment = SD of the value assignment ÷ $\sqrt{9}$.

For example, the uncertainty of a 15.28 mmol/L calibrator is:

The Ru value assignment (in percent) = 100 x (0.23 mmol/L ÷ $\sqrt{9}$)/15.28 mmol/L = 0.509%

Relative Uncertainty of the Product Calibrator is:

Ru (in percent) = $\sqrt{(0.12)^2 + (0.509)^2}$ = 0.523%.

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Appendix B. (Continued)

The expanded uncertainty, with a coverage factor of 2, is 1.05%.

This could also be expressed as the assigned value ± the uncertainty. For example:

Glucose = 15.28 +/- 0.16 mmol/L. ($\kappa = 2$)

The uncertainty of a zero calibrator is "0" by definition.

NOTE: To accurately calculate the uncertainty, one must carry the estimates of the impact of each influence quantity out to as many significant figures as are obtained. Once the final uncertainty is obtained, however, the final result should be reported to the number of significant figures indicated by the uncertainty estimate and the uncertainty reported to one additional significant figure. In this example, the uncertainty of 0.16 mmol/L indicated the reported glucose content should be reported to the nearest 0.1 mmol/L, and the uncertainty is rounded to two decimal places.

References for Appendix B

[1] Processing KODAK Motion Picture Films, Module 3, Analytical Procedures. H24.03. The Selection, Care, and Use of Volumetric Glassware and Weighing Equipment. ULM-0005/1. Available at: http://www.kodak.com/US/plugins/acrobat/en/motion/support/processing/h243/ulm0005-1.pdf. Accessed January 13, 2006.

[2] ASTM. *Standard Specification for Glass Volumetric (Transfer) Pipets*. E969-95. West Conshohocken, PA: ASTM; 1995.

[3] ASTM. *Standard Specification for Laboratory Glass Volumetric Flasks*. E288-03. West Conshohocken, PA: ASTM; 2003.

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Appendix C. System X Glucose Calibrator Traceability Example

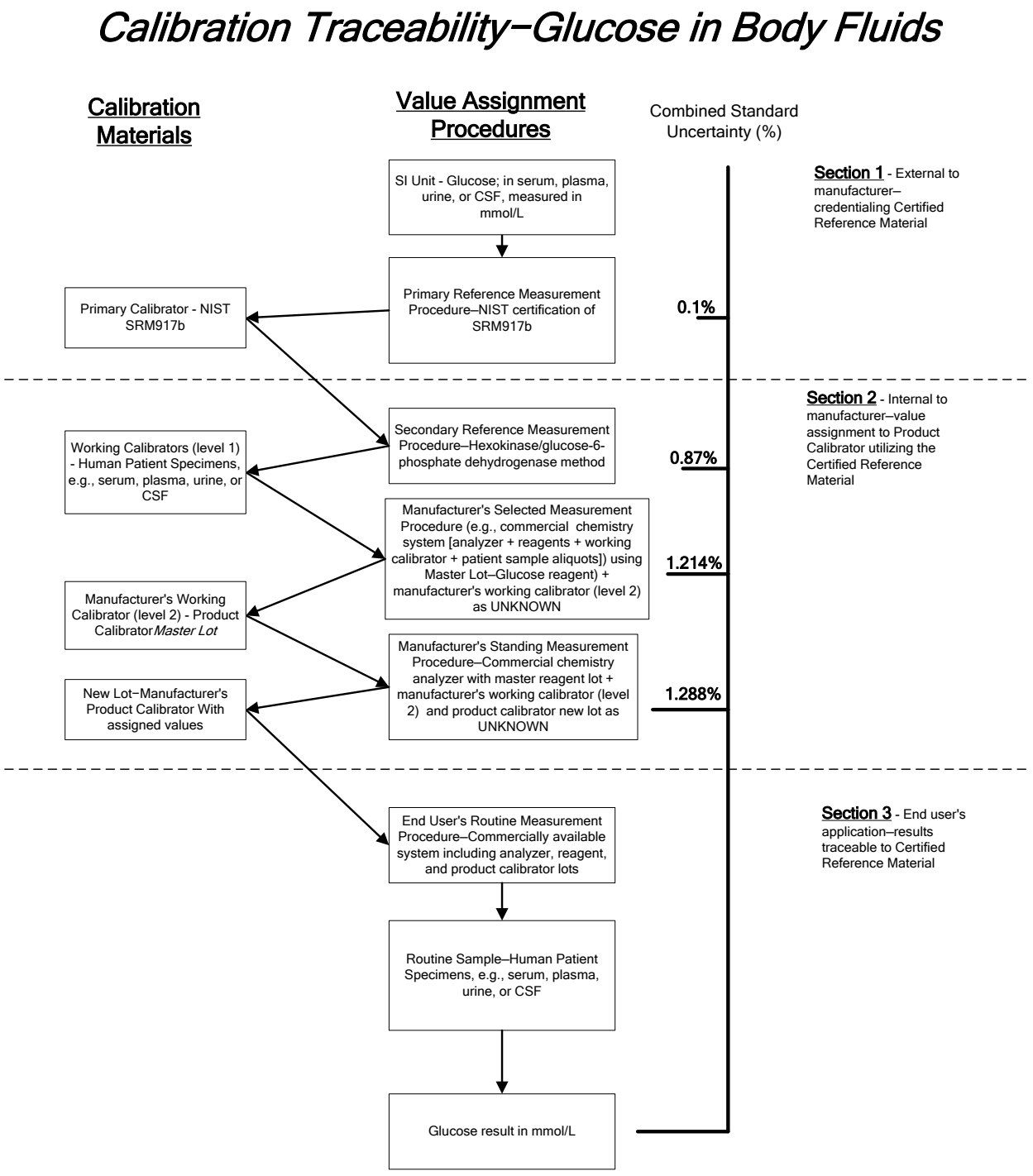

Figure C1. Traceability Chain for Values Assigned to Commercial System X Calibrator for Glucose in Serum, Plasma, Urine, and CSF

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Appendix C. (Continued)

C1. Scope and Specification of the Measurand and the Measurements

Glucose is the predominant monosaccharide utilized by the body as an energy source. Its diagnostic uses are multiple and beyond the scope of this report. Some examples of the clinical applications of glucose measurements are: 1) in serum and plasma, glucose is predominantly used for the diagnosis of diabetes mellitus and for monitoring the maintenance and control of diabetic patients; 2) in cerebral spinal fluid (CSF), glucose levels decrease substantially in bacterial infection; and 3) in urine, multiple conditions can cause altered glucose output including septicemia, pheochromocytoma, pregnancy, and many other conditions.

Glucose quantity may be reported in mmol/L or mg/dL of the respective body fluid. The measurand is defined as amount of glucose (mmol/L or mg/dL) in serum, plasma, CSF, or urine.

C2. Selection of Suitable Procedures for Estimating Values of the Measurand

According to JCTLM List 1 (http://www.bipm.org/en/committees/jc/jctlm/jctlm-db/) and Table C1, the recognized reference measurement procedures for glucose in human serum include isotope dilution/gas chromatography mass spectrometry (ID/GC-MS) and the "CDC" glucose hexokinase spectrophotometric procedure for glucose in a protein-free filtrate prepared from the original body fluid sample. Both procedures use NIST SRM 917b as a primary reference material.

Table C1. Excerpt from JCTLM List 1, Reference Measurement Procedures

Procedure Name	Applicable Matrices	Measurement Principle
NIST definitive method for serum glucose	human serum; lyophilized, fresh, or frozen	ID/GC/MS[1]
U. of Ghent reference method for glucose	human serum; lyophilized, fresh, or frozen	ID/GC/MS[2-4]
CDC Hexokinase reference method for glucose	human serum	Spectrophotometry[5]

The *reference measurement procedure* for serum glucose in this system is the CDC hexokinase procedure (Neese, et al) as performed on a protein-free filtrate. The traceability chain described in Figure C1 defines the analytical processes for each value transfer step.

To calibrate the CDC hexokinase reference measurement procedure, aqueous calibrators are prepared gravimetrically and volumetrically from SRM 917b. The CDC hexokinase reference measurement procedure is used to determine glucose reference values for a panel of human samples (*Manufacturer's Working Calibrators—Level 1*). The panel of human samples is in turn used to calibrate the *Manufacturer's Selected Measurement Procedure*. The *Manufacturer's Selected Measurement Procedure* utilizes the commercial Glucose System X instrument, along with a Master Lot of Glucose System X commercial reagent, to assign values to the *Manufacturer's Working Calibrators—Level 2* (master lot of System X glucose calibrator). The *Manufacturer's Standing Measurement Procedure*, calibrated with the *Manufacturer's Working Calibrator—Level 2,* is used to assign values to each production lot of the *Manufacturer's Product Calibrator*, according to a statistically defined protocol that includes a series of measurement replications across multiple System X glucose reagent lots, runs, and System X instruments.

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Appendix C. (Continued)

C3. Demonstration Through Validation That All Significant Influence Quantities Appear in the Measurement Equation and Specified Conditions

C3.1 Validation of Commutability of Product Calibrators

Serum Validation:

An internal method comparison study of 145 human serum samples with glucose values ranging from 24 to 620 mg/dL was performed according to CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples*. Values obtained with the System X Glucose procedure, calibrated with product calibrators, were compared to values obtained with the CDC hexokinase glucose reference measurement procedure. Least squares linear regression analysis demonstrated the following relationship:

$$Y = 0.99x + 1.64 \text{ mg/dL},$$

where y = glucose result using System X Glucose procedure; and
x = glucose result using CDC hexokinase glucose reference measurement procedure.

The standard error of the estimate ($s_{y.x}$) was 5.12 and the correlation coefficient, r, was 1.000.

Urine Validation:

An internal method comparison study of 145 human urine samples with glucose values ranging from 21 to 621 mg/dL was performed according to CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples*. Values obtained with the System X Glucose procedure, calibrated with product calibrators, were compared to values obtained with the CDC hexokinase glucose procedure. Least squares linear regression analysis demonstrated the following relationship:

$$Y = 1.00x - 0.18 \text{ mg/dL},$$

where y = glucose result using System X Glucose procedure; and
x = glucose result using CDC hexokinase glucose procedure.

The standard error of the estimate ($s_{y.x}$) was 5.81 and the correlation coefficient, r, was 1.000.

CSF Validation:

An internal method comparison study of 143 human cerebrospinal fluid samples with glucose values ranging from 21 to 625 mg/dL was performed according to CLSI/NCCLS document EP9—*Method Comparison and Bias Estimation Using Patient Samples*. Values obtained with the System X Glucose procedure, calibrated with product calibrators, were compared to values obtained with the CDC hexokinase glucose procedure. Least squares linear regression analysis demonstrated the following relationship:

$$Y = 1.00x + 0.32 \text{ mg/dL},$$

where y = glucose result using System X Glucose procedure; and
x = glucose result using CDC hexokinase procedure.

The standard error of the estimate ($s_{y.x}$) was 4.27 and the correlation coefficient, r, was 1.000.

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Appendix C. (Continued)

Commutability of the product calibrator was demonstrated by verifying that results for clinical samples were the same when measured by either the routine or reference measurement procedures.

C3.2 Validation of Commutability of Working Calibrators

Since the *Manufacturer's Working Calibrators (Level 1)* for this traceable calibration process (see Figure C1) are comprised of panels of individual human samples for each of the claimed body fluid matrices, demonstration of commutability of these working calibrators is not required, since they are identical with the samples intended for measurement with the System X Glucose procedure. The *Manufacturer's Working Calibrator (Level 2)* is a master lot of the Product Calibrator. As such, demonstration of commutability for this material is inferred by demonstration of commutability of the product calibrator, as discussed in Section C3.1.

C4. Identification of Relative Importance of Each Influence Quantity

The listing of influence quantities and applicable methods of control underlying the value assignment process for product calibrators supporting the System X Glucose procedure is provided in Table C2.

The assigned values for the *Manufacturer's Product Calibrators* for the System X Glucose procedure include the uncertainty of the purity of NIST SRM 917b, and the uncertainties of the gravimetric and volumetric procedures for the preparation of the multilevel aqueous calibrator series used to calibrate the CDC hexokinase reference measurement procedure. Uncertainties for the gravimetric measurements of the reference material are based on a triangular distribution of the calibration tolerances of the balances at the appropriate levels. The uncertainty of the volumetric measurements is based on a triangular distribution of the tolerances of the appropriate Class A volumetric glassware according to U.S. Federal Specification DD-V-581.[6] DD-V-581 is consistent with ASTM E969[7] for Class A pipettes and ASTM E288[8] for volumetric flasks. These influence quantities are quantitatively assessed in the uncertainty estimates below.

The influence quantities for the analytical steps for determination of the assigned values of the *Manufacturer's Product Calibrators* also include the run-to-run within-laboratory variation of the analytical runs for the CDC hexokinase procedure, the *Manufacturer's Selected Measurement Procedure*, and the *Manufacturer's Standing Measurement Procedure,* including the variability attributed to multiple test days, calibrations, instruments, and reagent lots.

The overall variation of the complete value assignment process was determined by reviewing value assignment data across six lots of product calibrator, with each calibrator lot value assignment study repeated twice. These additional influence quantities are accounted for in the expanded uncertainty estimate discussed below.

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Appendix C. (Continued)

Table C2. Influence Quantity Analysis

Identification of Relative Importance of Each Influence Quantity			**Selection of Appropriate Reference Materials and Procedures**
Influence Quantities for Secondary Calibrator (panel of patient samples) Value Assignment Process — System X Glucose			
Influence Quantity	Relative Importance	Mitigations and Control Strategies	Selected Reference
Purity of the glucose weighed into the working calibrators	+	Use NIST-certified pure glucose	Use NIST SRM 917
Moisture content of SRM 917	-	Dry material under vacuum at 60 °C for 24 h before use	Controlled descriptions in the two rows below
Time glucose is dried in vacuum oven	—-	Dry for 24 – 30 hours	Calibrated timers not required
Drying temperature of vacuum oven	—-	Measure 60 °C using a calibrated thermometer	Certificate from thermometer manufacturer
Tolerance limits of the weighing procedure for dried glucose	++	Controlled by procedure	None needed
Weighing accuracy of the balance used to weigh the dried glucose	++	Use of calibrated balances tolerances set by SOP	Certificate from balance manufacturer; annual recertification from third-party auditor
Volume of water added to NIST glucose	+++	Use of ASTM Class A volumetric flasks	Certificate or declaration from flask manufacturer
Temperature of water, flask, and dried glucose	—	All materials are at ambient temperature (18-25 °C)	Room temperature controls and area validation; no further control needed
Volume of diluent used for dilutions of stock to prepare working calibrators	+++	Use of ASTM Class A volumetric flasks	Certificate or declaration from flask manufacture
Volume of stock used for dilution to prepare working calibrators	+++	Use of ASTM Class A volumetric pipettes	Certificate or declaration from pipette manufacturer
Temperature of water, flask, and pipettes when dilutions are made	—-	All materials are at ambient temperature (18-25 °C)	Room temperature controls and area validation; no further control needed
Accuracy of calibration of the instruments for CDC hexokinase procedure	++	Use of primary calibrators prepared with NIST SRM 917	Influences listed above
Reaction temperature for CDC hexokinase reaction	-	Controlled by precision analytical water bath and temperature controller to within acceptable tolerances. Confirm temperatures to spec during maintenance.	Per instrument maintenance procedures
Pipetting device for samples and reagents for manual CDC hexokinase procedure	-	Controlled by use of high precision and accuracy of automated analytical pipetting device; confirmed during maintenance and periodic calibration checks	Per pipetting device specifications and maintenance procedures
Lot – Lot variation of reagents—CDC hexokinase procedure	+	Reagents prepared in-house; must meet acceptance criteria before use.	Acceptance testing procedure and use of controls to demonstrate acceptable consistency
Calibration error for photometric instrument	+	Instrument must demonstrate acceptable calibration curve based on slope, intercept, and residuals of each of six calibrator levels. Use of multiple calibrations to minimize variation	Certificate from spectrophotometer manufacturer; periodic photometric calibration check/verification with primary photometric standards. Acceptance criteria based on SOP

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Appendix C. (Continued)

Table C2. (Continued)

Identification of Relative Importance of Each Influence Quantity			**Selection of Appropriate Reference Materials and Procedures**
Influence Quantities for Secondary Calibrator (panel of patient samples) Value Assignment Process — System X Glucose			
Influence Quantity	Relative importance	Mitigations and Control Strategies	Selected Reference
Calibration error and other sources of measurement error for System X analyzer – Manufacturer's Selected Measurement Procedure	++	• Use of multiple (at least 2) carefully maintained instruments (System X analyzers) • Use of redundant QC checks, including use of Master Calibrator lot as a quality control sample series. • Use of well-characterized reagent master lots and quality control samples • Use of at least a statistically significant minimum number and range of aliquots of assayed (CDC hexokinase) patient samples as Working Calibrators	• Internal company SOPs for System X maintenance and setup • Procedures for establishment and maintenance of reagent and calibrator Master Lots • Staff training • ISO 13485 Quality System Registration
Calibration error and other sources of error for System X Standing Measurement Procedure	+	Same as for Selected Measurement Procedure, except calibration of System X glucose assays performed with Calibrator Master Lot (multiple vials and replicates, statistically validated per SOPs) instead of aliquots of patient samples	• Internal company procedures, policies, and release specifications for value assignment and acceptance of product calibrator assigned values • Staff training • ISO 13485 Quality System Registration

C5. Selection of Appropriate Reference Materials and Procedures

Both NIST SRM 917b (crystalline high-purity glucose) and the CDC hexokinase reference measurement procedure for glucose in serum are listed in the JCTLM list of reference materials and reference measurement procedures of higher order. The CDC hexokinase reference measurement procedure has also been validated for all sample types by the reference laboratory contracted to support these value assignment studies. The reference laboratory's quality system has been accredited against the requirements of ISO 17025 and is also consistent with the requirements of ISO 15195, based on internal audit. The use of NIST SRM 917b reference material and the gravimetric and volumetric preparations of secondary calibrators for the CDC hexokinase reference measurement procedure for glucose have been validated by routine examination of serum trueness control materials, including SRM 909b and CAP survey validated reference materials (with target values assigned by the NIST definitive method for glucose, isotope dilution mass spectrometry).

C6. Estimation of the Uncertainty of the Values Assigned to the Working and Product Calibrators

C6.1 Defining the Uncertainty Model

Calibrator assigned-value **combined standard uncertainty** may be estimated as a standard deviation calculated from the elements of uncertainty comprising each of the process stages described in Figure C1.

The error model is:

$$\sigma \text{Total Uncertainty} = \sqrt{\sigma_{SM}^2 + \sigma_{\text{Ref/SMP}}^2 + \sigma_{SMP\,/\,Feature}^2}$$

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Appendix C. (Continued)

where:

σ_{SM} is the estimate of the standard uncertainty of the assigned value of the highest order calibration material, in addition to the processes associated with preparation of working solutions of the reference material using gravimetric and volumetric procedures.

$\sigma_{Ref/SMP}$ is the estimate of the combined standard uncertainty associated with the process of applying the *Secondary Reference Measurement Procedure* (CDC hexokinase procedure) to assign values to the *Manufacturer's Working Calibrator—Level 1* (panel of human samples), followed by use of the Level 1 Working Calibrator in the Manufacturer's Selected Measurement Procedure to assign values to the System X Glucose calibrator master lots (Working Calibrator—Level 2).

$\sigma_{SMP/Feature}$ is the estimate of the combined standard uncertainty associated with use of the *Manufacturer's Standing Measurement Procedure* in assigning values to subsequent production lots ("feature" lots) of product calibrators.

C6.2 Estimating σ_{SM}

The higher order calibrators used for calibration of the CDC hexokinase reference measurement procedure for glucose are prepared from the purest available reference material, NIST SRM 917b. The certificate of analysis from the material producer provides information concerning the uncertainty of the material assay value and a confidence interval. The estimate of σ_{SM} is therefore calculated as:

$$\sigma_{SM} = \sqrt{\left(\frac{[C]*(U_{ref})}{k}\right)^2 + \left(U_{bal}\right)^2 + \left(U_{pipette}\right)^2}$$

where **[C]** is the calibrator concentration, **(U_{ref})** is the uncertainty stated by the supplier of the reference material, and ***k*** is the Z value from a standard normal distribution associated with the confidence statement provided by the reference material supplier. If the uncertainty statement is based on two standard deviations, then ***k* = 2**. U_{bal} and $U_{pipette}$ are the respective uncertainties of the gravimetric balance and the volumetric pipetting device.

C6.3 Estimating $\sigma_{Ref/SMP}$

The error model for the application of the reference measurement procedure (CDC hexokinase method) to the panel of patient samples (Level 1 working calibrators) includes the components of variability associated with the measured glucose value for each member of the panel of human samples. The square root of the sum of the relevant variance components equates to the combined standard uncertainty for the values assigned to the Level 1 working calibrators. The major sources of variability associated with this measurement system are:

- calibration error for photometric instrument;
- optical cell uniformity of measuring instrument;
- reaction temperature for CDC hexokinase reaction; and
- pipetting device for samples and reagents for CDC hexokinase procedure.

These sources of measurement variability in the reference measurement procedure can be characterized and quantified indirectly by analysis of variance of long-term quality control data for multiple levels of quality control material. Analysis of variance (ANOVA) with a fully nested design yields estimates for components of variation, including replicate-to-replicate and day-to-day variation (which includes reagent lot-to-lot and multiple batches of calibration material).

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Appendix C. (Continued)

In addition to the error components associated with determination of glucose values for the panel of patient specimens using the CDC hexokinase method, additional variation in the process is encountered in use of the assayed patient sample aliquots to calibrate and run the Manufacturer's Selected Measurement Procedure, and to assign values to the Level 2 working calibrators (Master Lot of Glucose System X commercial calibrators). These additional sources of variation include System X Glucose analyzer-to-analyzer, day-to-day, replicate-to-replicate, and random bias unique to each patient sample. As noted above (see Section C4), the overall variation of the complete value assignment process was determined by reviewing value assignment data across six lots of product calibrator, with each calibrator lot value assignment study repeated twice.

The overall error model includes the following components:

- Glucose System X analyzer-to-analyzer, σ_{Eana};
- Glucose System X procedure day-to-day, σ_{Eday} (confounded with reagent pack-to-pack);
- Hexokinase reference glucose procedure day-to-day, σ_{Rday};
- Glucose System X procedure rep-to-rep, σ_{Erep};
- Hexokinase reference glucose procedure rep-to-rep, σ_{Rrep}; and
- Glucose System X procedure random bias, σ_{Erb}, associated with the selection of human specimen panel members.

NOTE: An estimate of this error component may be based on the standard error of the estimate ($S_{y.x}$) from the linear regression analysis of the method comparison study for values obtained with the commercial field method (Glucose System X – "Y" axis) vs. values obtained with the reference method ("X" axis).

Using the sources of variability described above and assuming a fully nested design, the error model is defined as:

$$\sigma^2_{\Delta_{rw}} = \frac{\sigma^2_{Eana}}{A} + \frac{\sigma^2_{Eday}}{A*D} + \frac{\sigma^2_{Erb}}{A*D*P} + \frac{\sigma^2_{Erep}}{A*D*P*E} + \frac{\sigma^2_{Rday}}{A*D} + \frac{\sigma^2_{Rrep}}{A*D*P*R}$$

where:
A is the number of System X analyzers in the test;
D is the number of days in the test;
P is the number of human samples tested per day;
E is the number of replicates per sample on System X; and
R is the number of replicates per sample on the CDC hexokinase glucose procedure.

The estimate of $\sigma_{\text{Ref/SMP}}$ is calculated as:

$$\sigma_{\text{Ref}/SMP} = \sqrt{\sigma^2_{\Delta rw}}$$

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Appendix C. (Continued)

C6.4 Estimating $\sigma_{SMP/Feature}$

This standard uncertainty associated with $\sigma_{SMP/Feature}$ is calculated at each concentration level of glucose System X calibrators, from n = 6 replicate independent studies of the assigned value for a new product calibrator lot, using identical test materials (Glucose System X reagent lots, System X analyzers, and Glucose System X calibrator master lots).

Therefore,

$$\sigma_{SMP/Feature} = \sigma_{Test-test},$$

or the run-to-run variation in the estimate of the assigned values for each of three calibrator levels in the commercial System X Glucose calibrator set.

C6.5 Calculation of the Expanded Uncertainty of a Given Calibrator Assigned Value

An example calculation of the expanded uncertainty for values assigned to Glucose System X calibrators is provided in Table C3. The example provides the calculations for estimation of expanded uncertainty for the highest concentration calibrator only. Similar calculations will be applied to derive the expanded uncertainties associated with each of the lower level calibrators in the complete product calibrator kit.

Table C3. Example Calculation of Expanded Uncertainty—Glucose System X Calibrator at 600 mg/dL

Influence Name	Nominal Calibrator Assigned Value (Glucose)	Type of Uncertainty	Distribution	Divisor	Quotient (%)	Square
SRM 917 impurity	600 mg/dL	A	normal	1	0.1	0.010000000
Gravimetric device		B	triangular	6	0.352	0.020650667
Volumetric device		B	triangular	6	0.1225	0.002501042
Value assign test—Selected Measurement Procedure ($\sigma_{\text{Ref}/SMP}$)	600 mg/dL	A	normal	1	1.2	1.440000000
Value assign test—Standing Measurement Procedure ($\sigma_{SMP/Feature}$)	600 mg/dL	A	normal	1	0.43	0.1849
Sum of Squares						1.658051709
Combined standard uncertainty						1.28765%
Expanded uncertainty (K = 2)						2.5753%
Conclusion: Expanded uncertainty of the assigned value for Glucose System X calibrator at 600 mg/dL is 2.58%.						

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Related CLSI/NCCLS Publications*

C24-A2 **Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline—Second Edition (1999).** This guideline provides definitions of analytical intervals, plans for quality control procedures, and guidance for quality control applications.

C37-A **Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures; Approved Guideline (1999).** This guideline details procedures for the manufacture and evaluation of human serum pools for cholesterol measurement.

EP5-A2 **Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline—Second Edition (2004).** This document provides guidance for designing an experiment to evaluate the precision performance of quantitative measurement methods; recommendations on comparing the resulting precision estimates with manufacturer's precision performance claims and determining when such comparisons are valid; as well as manufacturer's guidelines for establishing claims.

EP6-A **Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (2003).** This document provides guidance for characterizing the linearity of a method during a method evaluation; for checking linearity as part of routine quality assurance; and for determining and stating a manufacturer's claim for linear range.

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EP21-A **Estimation of Total Analytical Error for Clinical Laboratory Methods; Approved Guideline (2003).** This document provides manufacturers and end users with a means to estimate total analytical error for an assay. A data collection protocol and an analysis method which can be used to judge the clinical acceptability of new methods using patient specimens are included. These tools can also monitor an assay's total analytical error by using quality control samples.

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