Mini-Review

Reference Materials and Reference Measurement Procedures: An Overview from a National Metrology Institute

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Abstract
An outline of the processes involved in both certified clinical reference material production and clinical reference measurement procedure development at the National Institute of Standards and Technology (NIST), the national metrology institute of the United States, is presented. The role that NIST and other national metrology institutes play in the metrological traceability of certified reference material is discussed. Highlighted are the challenges associated with the development of reference measurement systems for complex clinical analytes, such as proteins, and examples of existing efforts in this area are given. Examples of recent international collaborations in developing certified reference materials for analytes such as cardiac troponin I, brain natriuretic peptide, and serum creatinine demonstrate the close cooperation that national metrology institutes must have with the clinical community to establish complete reference measurement systems.

Introduction

In medicine, the diagnosis, risk assessment, and treatment of patients depends on accurate and precise laboratory measurements of a wide range of clinical analytes. To establish and ensure the highest level of accuracy and precision in routine clinical measurements, a reference measurement system[1,2] must be established. The essential components of a reference measurement system are: the definition of the measurand, measurement procedures (including reference measurement procedures and value transfer protocols), and materials (including reference materials and calibrators). Once established, the reference measurement system provides metrological traceability to routine clinical analysis, linking the patient's laboratory measurement results to an established higher-order standard (ideally, an SI-unit[3] such as the mole or katal) through an unbroken chain of comparisons.[4] Linked through this metrological traceability chain are national metrology institutes, which provide primary and secondary reference materials and reference measurement procedures, accredited reference measurement laboratories, which provide secondary reference materials and measurement procedures, the manufacturers of clinical assays, which provide calibrators and utilise value transfer protocols, and clinical diagnostic laboratories.

As the national metrology institute of the United States, the National Institute of Standards and Technology (NIST) has been developing certified reference materials [also referred to by NIST as Standard Reference Materials[5] (SRMs)] for the clinical laboratory community since 1967 when SRM 911, a pure-substance reference material for cholesterol, was first introduced.[6] The list of clinical certified reference materials supplied by NIST and other national metrology institutes has increased substantially since then. This list includes both pure-substance or "primary" reference materials, to which a certified value of purity has been determined, and matrix-based or "secondary" reference materials, most often in the form of pooled human serum, plasma, or urine. The evaluation of the purity of a primary certified reference material typically involves the determination of the residual moisture of the material and the quantitative determination of known impurities, such as salts, metals, degradation products, and contaminants (from either chemical synthesis in the case of a synthetic material or isolation/extraction in the case of a natural product). For a small-molecule organic substance such as cholesterol, purity determination can be a relatively routine process. For a clinical analyte such as a protein hormone, purity assessment can be more challenging due to the heterogeneous nature of the molecule (including chemical heterogeneity and conformational heterogeneity) and the large number of potential contaminants. Because pure-substance certified reference materials often form the metrological foundation for the value assignment of the secondary, matrix-based materials, their importance should not be overlooked.

The availability and use of secondary or matrix-based certified reference materials is generally seen as a critical step to the measurement standardisation of any clinical analyte. Matrix-based certified reference materials are used by clinical assay manufacturers to access the trueness of their calibrators and quality control materials. Additionally, clinical laboratories often use matrix-based certified reference materials for routine quality control and more frequently as part of a periodic quality evaluation in an external quality assurance program. Because they are so important, the production of secondary certified reference materials needs to be done carefully and thoroughly; their production occurs through several research and development stages. These stages, discussed more thoroughly below, include: defining the measurand, development of a reference measurement procedure, acquiring the materials to produce the reference material, homogeneity and stability assessment, commutability evaluation, and the quality assurance involved in reference material production.

Defining the Measurand

The question "what is being measured?" would seem like an easy one to answer. For many molecules and elemental species of clinical interest, it is a relatively straightforward task to answer this question, to define the measurand. For example, the cholesterol found in human serum can be defined by a single molecular structure with a known molecular formula. Subsequently, the determination of cholesterol for clinical purposes can be measured in an SI-unit (i.e. mol/L) in a well-defined matrix (i.e. serum), leading to a relatively complete definition of the measurand. However, in far too many cases, the answer to the question "what is being measured?" is not readily known.

A large number of important clinical analytes are proteins, some with a relatively simple molecular structure such as insulin[7] or with very complex molecular structures such as carcinoembryonic antigen.[8] For most of these proteins, sequencing of the human genome has provided information on their amino acid sequence, at least until the point in time that biochemical translation from genetic code into protein has occurred. After translation, biochemical modifications to the protein can occur that alter the sequence of amino acids, chemically modify the amino acids with the addition of small chemical groups like phosphate or large molecular structure like branched glycoforms, or join different proteins together, either covalently or through non-covalent interactions, to form protein complexes. The consequence of all these protein post-translational modifications is that most human proteins cannot be readily represented by one known molecular formula and cannot be assigned a single molecular mass. In fact, for many proteins of clinical importance, little is known about the extent of actual post-translational modification or the chemical nature of the modification even when their amino acid sequence is confidently known. For these proteins, SI-traceability is not possible without further molecular characterisation. However, for these analytes, standardisation can be possible with traceability to an "artifact standard". Many examples of this form of standardisation exist in clinical chemistry,[9] the most prevalent being those clinical measurement systems traced to World Health Organization reference preparations[10] with concentrations given in International Units (IUs). Building a robust reference measurement system for these types of analytes remains a significant challenge.

An example of the standardisation of an important clinical analyte for which complete molecular structural information is not known is human cardiac troponin I, a cardiac marker. In 2004, NIST released SRM 2921 (Human Cardiac Troponin Complex)[11] which was developed with the critical assistance of the joint Cardiac Troponin I Standardization Committee of the International Federation of Clinical Chemistry (IFCC) and the American Association for Clinical Chemistry (AACC). The path to the release of SRM 2921 was a long one because very little direct information existed on the molecular structure of troponin I present in blood after myocardial damage. Because of this, a number of candidate reference materials, composed of potential molecular forms of troponin I, were first evaluated by the Committee through two rounds of interlaboratory studies involving commercial troponin I assay manufacturers.[12,13] The assay performance of candidate reference materials was compared to that of patient samples and this comparison was used to choose the ideal form of troponin I for the standard. In the end, the human troponin complex (composed of the troponin T, troponin I, and troponin C subunits) which had been extracted from human hearts under non-denaturing conditions, was found to have an assay response most similar to the form found in patient samples, as compared to the other candidate reference materials evaluated. Even today, there is still little direct structural information available about the molecular form of troponin I released into the bloodstream after myocardial damage. Therefore, a robust, SI-traceable reference measurement system for cardiac troponin I has not been achieved.

Recently, NIST has been collaborating with the IFCC's Committee for the Standardization of Markers of Cardiac Damage to develop reference standards and reference measurement procedures for human brain natriuretic peptide (BNP) and the N-terminal fragment of the BNP precursor (NT-proBNP), two markers of heart failure. Initially, this project appeared to be more straightforward than that of troponin I standardisation because both BNP and NT-proBNP were thought to be small polypeptides with very simple and homogeneous chemical structures, leading to the belief that

the measurand could be readily defined. However, recent research indicates that neither BNP nor NT-proBNP circulating in the blood of patients with heart failure possess a simple, homogeneous molecular structure. There is growing evidence that both molecules are glycosylated[14] and/or multimeric.[15] Because of this, it appears that the path towards developing a complete reference measurement system for both BNP and NT-proBNP might not be a short one. Defining the measurand for both of these heart failure makers is the first critical step and the focus of ongoing research.

Development of Reference Measurement Procedures

For clinical analytes in which the measurand has been defined, the next step towards the goal of the production of a higher-order reference material and, ultimately, a reference measurement system, is the development of a reference measurement procedure. At NIST, the ideal approach to value assignment of reference materials incorporates a primary reference measurement procedure, although other modes of certification are used and are discussed below. A primary reference measurement procedure is defined by the Consultative Committee for the Amount of Substance (CCQM)[16] of the International Bureau of Weights and Measures (BIPM) as "a method having the highest metrological properties, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units". For many clinical analytes, isotope dilution mass spectrometry (IDMS)[17] is the analytical method used to achieve a primary reference measurement procedure. IDMS requires that a purified form of the analyte, labelled with a stable isotope (most commonly, deuterium, ^{13}C, ^{14}N, or ^{18}O for organic analytes), be available for use as an internal standard. Additionally, the analyte must be amenable to analysis by mass spectrometry, either directly or through chemical modification. The success of IDMS in a primary reference measurement procedure lies in the use of the isotopically-labelled internal standard. The isotopically-labelled internal standard, when added to a sample at the start of the analysis and properly equilibrated, allows the analyte to be isolated from the sample matrix without introducing quantitative bias due to incomplete isolation/extraction. The isotopically-labelled version of the analyte, because it is chemically-equivalent to the analyte itself, is an ideal internal standard. When tandem mass spectrometry is used to measure the analyte-to-internal standard ratio, the measurement can be sensitive, selective, and a direct measure of the concentration of the analyte.

Most current NIST clinical reference materials have been value-assigned using a primary reference measurement procedure involving IDMS. This has been possible for small molecule analytes such as cholesterol,[18] creatinine,[18] glucose,[18] cortisol,[19] homocysteine,[20] and steroid hormones[21] because they are well-characterised analytes, defined measurands, and therefore SI-traceable. Incorporating the measurement approach of IDMS for the quantification of more molecular complex analytes such as proteins has not been routinely accomplished but may potentially be feasible. IDMS quantification of serum insulin,[22] serum prostate specific antigen,[23] and urinary albumin[24] has been demonstrated.

Once a reference measurement procedure has been developed, there are a number of potential approaches to assigning a value to the concentration of an analyte in a certified reference material. At NIST,[25] the value-assignment methods commonly used are:

- a single primary reference measurement procedure with confirmation by other method(s)
- two independent and critically-evaluated reference measurement procedures
- one reference measurement procedure at NIST and different methods by outside collaborating laboratories
- interlaboratory studies in collaboration with NIST
- a method-specific protocol

As stated above, reference measurement procedures based on IDMS have predominantly been developed and used at NIST. However, for analytes such as enzymes[26] and others whose measurement is not amenable by IDMS, certification by a method-specific protocol is often the only means possible.

Obtaining Materials for Reference Material Production

Generally, NIST does not make or fabricate the materials used to produce certified reference materials. To produce certified secondary reference materials for clinical applications, large pools, typically from 1 to 50 litres, of human serum, plasma, or urine are needed to produce the quantity needed. While there have been panels of individual patient samples produced as reference materials,[27,28] the small volume of an individual patient sample provides only a limited lot size of a certified reference material. Considering the substantial effort that goes into the production, certification, and distribution of clinical certified reference materials, a lot size large enough to meet the needs of the clinical community for 5 to 10 years necessitates the use of pools of patient samples rather than individual samples.

Because NIST, like other national metrology institutes, does not have sufficient medical and clinical expertise to decide on the ideal specifications of a human patient pool for reference material production, the specifications are developed in close collaboration with medical experts, often through a

committee of a professional organisation such as the College of American Pathologists, the AACC, or the IFCC. Decisions that typically have to be made on the pool specifications are the desired concentration(s) of analyte(s), demographics of the patient samples that will make up the pool, and the pre-analytical variables and processing that will be used.[29] Once pool specifications have been decided, NIST often relies again on professional societies to organise the collection of the individual patient samples from hospital clinical centres. Alternatively, NIST has made arrangements with commercial blood collection centres to collect and pool patient samples.

Producing a human sample pool with elevated concentrations of a clinical analyte can often be accomplished by spiking of the pure analyte. This approach was taken to produce NIST SRM 967 (Creatinine in Frozen Human Serum), in which pure creatinine was added to create an elevated level of creatinine from a normal serum pool.[30] Spiking can only be used in situations where the analyte is both simple and well-characterised. As new certified reference materials are developed for complex clinical analytes, such as human troponin I in serum, spiking of the purified analyte into normal serum might not produce a viable reference material. Studies performed using human pooled serum spiked with NIST SRM 2921 (Human Cardiac Troponin Complex) have shown that this spiked material does not behave the same way as an individual patient sample with an elevated troponin I concentration.[13] These studies indicated a potential commutability issue (see discussion below) of pooled serum spiked with purified troponin I, a problem that will probably be encountered with other clinical protein analytes. To produce a large pool of human serum with a troponin I concentration elevated above normal levels, it is likely that hundreds to thousands of "leftover" diagnostic test samples from patients who have experienced a substantial cardiac event will have to be collected from many hospital laboratories. Or course, this is a challenge by itself, but with the additional requirement of obtaining informed consent from patients and only including in the pool patient samples that have tested negative for known contagions, it could be one of the most difficult serum pools yet collected.

Homogeneity and Stability Testing

The goal of any standardisation effort is to provide measurements that are stable and consistent across time and space. In order to achieve this, the certified reference materials used must be both homogeneous and stable. The homogeneity of a reference material refers to both the homogeneity of the material within one bottle and the homogeneity between bottles. Because most clinical certified reference materials are composed of either frozen liquids (i.e. serum, plasma, or urine) or liquids that have been lyophilised after aliquotting, within-bottle inhomogeneity is rarely observed. However, when the aliquotting of these large pools of reference materials into thousands of bottles takes several hours, changes in the pool can occur during this time that produce measurable differences in analyte concentration between bottles. Therefore, evaluating the between-bottle homogeneity of the concentration of the certified analyte(s) is critically important. Between-bottle homogeneity assessment is typically done by assaying the concentration of the certified analyte(s) from multiple bottles within the entire lot of reference materials produced. The number of bottles sampled for homogeneity evaluation will depend on the lot size and the precision of the measurement method used to assay analyte concentration.[31]

The stability of a certified reference material must also be evaluated, including both short-term and long-term stabilities. The short-term stability evaluation of NIST certified reference materials aims to determine if the value assigned to the analyte(s) changes in the time and under the conditions of shipment from NIST to the end-user. This is accomplished by subjecting the reference material to the temperature extremes expected in the duration of a shipment worldwide and then measuring the concentration of the analyte(s) relative to a control sample maintained at long-term storage conditions. Long-term stability evaluation aims to determine if the certified values of the analyte(s) remain valid during the 5 to 10 year lifetime of the certified reference material. There are a number of specific approaches to stability testing,[31-33] the choice of which often depends on the anticipated stability of the analyte. Because most of the reference measurement procedures used to certify reference materials are developed and performed at NIST, substantial information and experience is gained about the stability of the analyte(s) during these efforts. It is this experience that influences decisions about the frequency of sampling for long-term stability assessment of NIST certified reference materials.

Evaluation of Commutability

The commutability of a reference material is defined in ISO 15194 as the "ability of a material to yield the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships obtained when the same procedures are applied to other relevant types of material".[34] This is a complicated way of saying that a reference material must functionally behave the same as a patient sample during measurement by a routine procedure. Because most clinical certified reference materials are pooled samples that have undergone some type of processing, changes in the matrix or in the analyte itself can create differences in the way an assay behaves relative to an actual patient sample. Commutability issues may not

surface during certification of the reference material because most reference measurement procedures involve an isolation/extraction of the analyte from the matrix, making them fairly matrix-independent measurement procedures. Therefore, it is usually after a reference material is produced and certified that commutability issues are encountered.

When a reference material proves to not be commutable with one, some, or all routine measurement procedures, it has limited utility in developing a complete reference measurement system.[35] Commutability testing of a new certified reference material against all routine measurement procedures in which it is expected to be used is essential. However, most national metrology institutes do not have direct access to routine measurement procedures. Therefore, commutability testing is another area in which NIST and other national metrology institutes rely heavily on the assistance of professional societies to arrange studies with commercial clinical assay manufacturers worldwide.

Quality Assurance of Reference Materials

With the goal of establishing a complete reference measurement system, NIST efforts do not stop at supplying certified reference materials and developing reference measurement procedures. As with any other products or services, providing reference material with quality assurance is critical to their successful use world-wide. NIST certified reference materials are produced under a quality system[36] based on ISO 17025[37] and ISO Guide 34.[38] In addition to this quality system, NIST also contributes to providing the clinical chemistry community with high quality reference materials by participating in international comparison studies and through the Joint Committee for Traceability in Laboratory Medicine (JCTLM) in the review of clinical certified reference materials.

In order to provide high quality certified reference materials, NIST and other national metrology institutes routinely participate in international comparison studies of measurement capabilities, through the CCQM of the BIPM.[39] The CCQM organises international comparison studies to establish the technical basis for the mutual recognition of measurement capabilities among national metrology institutes in the field of chemical measurement. Many of these comparisons have focused on important clinical analytes such as serum creatinine,[40] glucose,[41] or calcium.[42] By participating in these comparison studies, national metrology institutes can validate that their measurement capabilities and the certified reference materials produced using these reference materials have comparability worldwide.

NIST also plays an active role in the BIPM's JCTLM which was established to **"**provide a worldwide platform to promote and give guidance on internationally recognised and accepted equivalence of measurements in laboratory medicine and traceability to appropriate measurement standards".[43] All of NIST's certified reference materials for clinical applications have been submitted for inclusion or are listed in the JCTLM database of higher order reference materials. Therefore, from the very inception of a new clinical certified reference material, all JCTLM requirements (which generally mirror ISO requirements) are taken into consideration. NIST staff also have active roles on the many review teams that make up the JCTLM, both for the working groups that evaluate reference materials and reference measurement laboratories.

Summary

The journey to a viable clinical reference material can be a long one. However, given the importance of standardised laboratory medicine for current clinical analytes and future ones, this journey is a necessary one. Thankfully, national metrology institutes such as NIST have excellent support from clinical professional societies such as the IFCC and AACC, who give guidance and direction along the path to a clinical reference material. A recent example of this partnership resulted in NIST SRM 967 (Creatinine in Frozen Human Serum), which was released in January 2007.

The development of SRM 967 started with a goal of the Laboratory Working Group of the U.S. National Kidney Disease Education Program (NKDEP) to improve estimates of glomerular filtration rate (GFR) based on serum creatinine measurements.[30,44] The Laboratory Working Group contacted NIST in 2005 to begin development of a serum creatinine certified reference material to support their goal through the standardisation of serum creatinine measurements. NIST obtained critical guidance and support from the NKDEP in the design of a reference material with creatinine levels that would most benefit clinical measurements, to validate the existing IDMS reference measurement procedure, and to validate the commutability of SRM 967.

SRM 967 is now available to support improved estimated GFR calculations through standardised serum creatinine measurement. SRM 967 can be used by clinical laboratories for internal QC purposes, which can provide useful evidence to accrediting authorities that the laboratory is genuinely interested in the accuracy of its results. Accrediting authorities can use SRM 967 to support their quality assurance programs. Another value of SRM 967 is to the manufacturers of clinical creatinine assays, who can utilise this reference material in the value assignment of their routine calibrators. Wider use of reference materials by the clinical diagnostic industry has the potential to reduce inconsistencies in results that are evident and confusing to many clinical laboratories and the medical

professionals they support.[45] National metrology institutes provide the certified reference materials and the reference measurement procedures that underpin the development of complete reference measurement systems such as the one that is now reaching fruition for clinical creatinine measurements.

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References

1. Dybkaer R. Metrology in laboratory medicine – Reference measurement systems. Accred Qual Assur 2001:6:16-9.
2. Thienpont LM, Van Uytfanghe K, De Leenheer AP. Reference measurement systems in clinical chemistry. Clin Chim Acta 2002;323:73-87.
3. Bureau International des Poids et Mesures. The International System of Units (SI). http://www.bipm.org/en/si/ (Accessed 24 May 2007).
4. International Organization for Standardization. International Vocabulary of Basic and General Terms in Metrology. 2nd ed. Geneva, Switzerland: ISO; 1993.
5. National Institute of Standard and Technology. Standard Reference Materials. http://ts.nist.gov/MeasurementServices/ReferenceMaterials/232.cfm (Accessed 24 May 2007).
6. Schaffer R, Bowers GN Jr, Melville RS. History of NIST's contributions to development of standard reference materials and reference and definitive methods for clinical chemistry. Clin Chem 1995;41:1306-12.
7. Brange J, Langkjoer L. Insulin structure and stability. Pharm Biotechnol 1993;5:315-50.
8. Thompson JA, Grunert F, Zimmermann W. Carcinoembryonic antigen gene family: molecular biology and clinical perspectives. J Clin Lab Anal 1991;5:344-66.
9. Kallner A. International standards in laboratory medicine. Clin Chim Acta 2001;307:181-6.
10. World Health Organization. International reference materials. http://www.who.int/bloodproducts/ref_materials/en/ (Accessed 24 May 2007).
11. Bunk DM, Welch MJ. Characterization of a new certified reference material for human cardiac troponin I. Clin Chem 2006;52:212-9.
12. Christenson RH, Duh SH, Apple FS, Bodor GS, Bunk DM, Dalluge J, et al. Standardization of cardiac troponin I assays: Round robin of ten candidate reference materials. Clin Chem 2001;47:431-7.
13. Christenson RH, Duh SH, Apple FS, Bodor GS, Bunk DM, Panteghini M, et al. Toward standardization of cardiac troponin I measurements Part II: Assessing commutability of candidate reference materials and harmonization of cardiac troponin I assays. Clin Chem 2006;52:1685-92.
14. Schellenberger U, O'Rear J, Guzzetta A, Jue RA, Protter AA, Pollitt NS. The precursor to B-type natriuretic peptide is an O-linked glycoprotein. Arch Biochem Biophys 2006;451:160-6.
15. Seidler T, Pemberton C, Yandle T, Espiner E, Nicholls G, Richards M. The amino terminal regions of proBNP and proANP oligomerise through leucine zipper-like coiled-coil motifs. Biochem Biophys Res Commun 1999;255:495-501.
16. Minutes from the Fifth Meeting (February 1999) of the Consultative Committee on the Quantity of Material (CCQM) of the Bureau International des Poids et Mesures (BIPM), Sevres, France.
17. De Leenheer AP, Thienpont LM. Applications of isotope dilution-mass spectrometry in clinical chemistry, pharmacokinetics, and toxicology. Mass Spectrom Rev 1992;11:249-307.
18. Phinney CS, Murphy KE, Welch MJ, Ellerbe PM, Long SE, Pratt KW, et al. Definitive method certification of clinical analytes in lyophilized human serum: NIST Standard Reference Material (SRM) 909b. Fresenius J Anal Chem 1998;361:71-80.
19. Tai SS, Welch MJ. Development and evaluation of a candidate reference method for the determination of total cortisol in human serum using isotope dilution liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. Anal Chem 2004;76:1008-14.
20. Nelson BC, Satterfield MB, Sniegoski LT, Welch MJ. Simultaneous quantification of homocysteine and folate in human serum or plasma using liquid chromatography/tandem mass spectrometry. Anal Chem 2005;77:3586-93.
21. Tai SS, Welch MJ. Development and evaluation of a reference measurement procedure for the determination of estradiol-17beta in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Chem 2005;77:6359-63.
22. Van Uytfanghe K, Rodriguez-Cabaleiro D, Stockl D, Thienpont L. New liquid chromatography/electrospray ionisation tandem mass spectrometry measurement procedure for quantitative analysis of human insulin in serum. Rapid Commun Mass Spectrom 2007;21:819-21.
23. Barnidge DR, Goodmanson MK, Klee GG, Muddiman DC. Absolute quantification of the model biomarker prostate-specific antigen in serum by LC-MS/MS using

protein cleavage and isotope dilution mass spectrometry. J Proteome Res 2004;3:644-52.

24. Singh R, Crow FW, Babic N, Lutz WH, Lieske JC, Larson TS, et al. A liquid chromatography-mass spectrometry method for the quantification of urinary albumin using a novel 15N-isotopically labeled albumin internal standard. Clin Chem 2007;53:540-2.
25. May W, Parris R, Beck C, Fassett J, Greenberg R, Guenther F, et al. Definitions of terms and modes used at NIST for value-assignment of reference materials for chemical measurements. NIST Special Publication 260-136. Washington: U.S. Government Printing Office; 2000.
26. Schumann G, Bonora R, Ceriotti F, Ferard G, Ferrero CA, Franck PF, et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clin Chem Lab Med 2002;40:725-33.
27. Thienpont LM, De Leenheer AP. Efforts by industry toward standardization of serum estradiol-17beta measurements. Clin Chem 1998;44:671-4.
28. Thienpont LM, Van Uytfanghe K, Marriott J, Stokes P, Siekmann L, Kessler A, et al. Feasibility study of the use of frozen human sera in split-sample comparison of immunoassays with candidate reference measurement procedures for total thyroxine and total triiodothyronine measurements. Clin Chem 2005;51:2303-11.
29. Bienvenu J, Later R, Pontet F. [Reference material (CRM 470) for serum proteins: preparation, characteristics and conditions of use.] [French]. Ann Biol Clin (Paris) 1995;53:499-505.
30. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum creatinine measurement: A report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem 2006;52:5-18.
31. International Organization for Standardization. Certification of reference materials – General and statistical principles. ISO Guide 35. 3rd ed. Geneva, Switzerland: ISO; 2006.
32. Pauwels J, Lamberty A, Schimmel H. Quantification of the expected shelf-life of certified reference materials. Fresenius J Anal Chem 1998;361:395-9.
33. Linsinger TP, Pauwels J, Lamberty A, Schimmel HG, van der Veen AM, Siekmann L. Estimating the uncertainty of stability of matrix CRMs. Fresenius J Anal Chem 2001; 370:183-8.
34. ISO 15194:2002. In vitro diagnostic medical devices – Measurement of quantities in samples of biological origin – Description of reference materials. Geneva, Switzerland: ISO; 2002.
35. Miller WG, Myers GL, Rej R. Why commutability matters. Clin Chem 2006;52:553-4.
36. NIST Quality System. http://ts.nist.gov/qualitysystem/ (Accessed 24 May 2007).
37. ISO 17025:2005. General requirements for the competence of testing and calibration laboratories. 2nd ed. Geneva, Switzerland: ISO; 2005.
38. ISO Guide 34. General requirements for the competence of reference material producers. 2nd ed. Geneva, Switzerland: ISO; 2000.
39. Wielgosz RI. CCQM primary methods symposium: how far does the light shine? Accred Qual Assur 2001;6: B329-31.
40. Welch MJ, Phinney CP, Parris RM, May WE, Heo GS, Henrion A, et al. CCQM–K12: The determination of creatinine in serum. Metrologia 2003;40:08005.
41. Welch MJ, Sniegoski LT, Parris RM, May WE, Heo GS, Henrion A. CCQM–K11: The determination of glucose in serum. Metrologia 2003;40:08003.
42. Van Nevel L, Aregbe Y, Taylor PDP. CCQM-K14: Calcium amount content in serum. Metrologia 2004;41:08003.
43. JCTLM: Joint Committee for Traceability in Laboratory Medicine. http://www.bipm.org/en/committees/jc/jctlm/ (Accessed 24 May 2007).
44. Peake M, Whiting M. Measurement of serum creatinine - Current status and future goals. Clin Biochem Rev 2006;27:173-84.
45. Bais R. What information should manufacturers provide on their procedures? Clin Chem 2006;52:1624-5.