Put Simply, Standardization of Cardiac Troponin I Is Complicated

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The analysis of heterogeneous protein analytes is very complicated to standardize, but these measurements must be viewed as fundamental to the practice of clinical chemistry. The clinical importance of selected protein measurements such as hemoglobin A1c (Hb A1c),5 thyroid-stimulating hormone, and cardiac troponin I (cTnI) is underscored by their routine use in the diagnosis, prognosis, monitoring, and management of disease, and their incorporation into professional guidelines. Standardization is difficult for proteins because there are few reference measurement procedures for these analytes, few primary (pure substance) reference materials (RMs) have been developed, and some of the RMs that are available can be used only for assay calibration with restrictions. In addition, few secondary (matrix-based) RMs with assigned values are available. In fact, the reference measurement system for the majority of clinically relevant proteins fall into the relatively weak Category 4, according to International Organization for Standardization (ISO) document 17511 (1). Furthermore, protein reference measurements sometimes appear to belong in a higher category but are in fact subject to analytical artifacts and prone to bias. By the criteria of the recently published “roadmap for harmonization” (2), standardization and harmonization of measurement would be regarded as high priority and mission critical for many proteins in laboratory medicine. cTnI is one such protein because its measurement represents the cornerstone for the diagnosis, prognosis, and management of patients with suspected and confirmed acute coronary syndromes (3, 4).

Standardization is based on the concept of metrological traceability, as described, for instance, in the ISO 17511 document (1). According to ISO 17511, standardization-of-measurement results for a substance require the metrological traceability chain outlined in Fig. 1A. This chain begins with a primary reference measurement procedure, which assigns quantity values to a primary RM. Primary RMs are used to assign values to a secondary RM. With this secondary RM (which typically has a matrix equivalent to that of patient samples), a strategy is executed to transfer values to working and product calibrators for the field methods that are designed for routinely quantifying the measurand in patient samples. As illustrated in Fig. 1A, this chain allows values reported for patient care to be traced to a fixed anchor point, i.e., the SI unit. In this way, metrological traceability supports long-term stability and comparability of routine measurement results.

Although complicated, standardization has been accomplished successfully for some important heterogeneous proteins. An example is Hb A1c, which is critical for the diagnosis, risk stratification, monitoring, and management of patients in the context of diabetes mellitus (5). The effort to standardize Hb A1c assays originated from the work of the National Glycohemoglobin Standardization Program (6) and others worldwide directed at harmonizing glycohemoglobin assays. To achieve standardization of Hb A1c measurements, however, required not only a reference measurement procedure but also defining the analyte in a more stringent and clinically meaningful way. Because the hemoglobin protein can react with glucose at multiple molecular locations and the glycohemoglobin measurand is heterogeneous, glycohemoglobin is not intrinsically a well-defined analyte. To pursue standardization of this heterogeneous protein analyte, the IFCC devel-
developed 2 reference measurement procedures that quantified with high specificity the concentration of only 1 predominant molecular species of glycohemoglobin: the hemoglobin molecules that have a specific hexapeptide in common, which is the stable adduct of glucose to the N-terminal valine of the hemoglobin β chain (βN-1-deoxyfructosyl-hemoglobin) (7). The IFCC approach was to redefine the analyte from something highly heterogeneous (i.e., glycohemoglobin) to a single, chemically discrete Hb A1c molecule. For this analyte, a primary RM was developed and used to calibrate the secondary reference measurement procedure, which in turn was used to assign values to whole-blood samples (the matrix for patient testing) used as secondary calibrators. These calibrators are used by manufacturers to calibrate their field methods and to ensure the traceability of patient results. A laboratory network has been established to implement and maintain the Hb A1c reference measurement system.
The challenges in standardizing the measurement of cTnI have similarities to the challenges faced in the Hb A1c standardization effort. Like glycohemoglobin, cTnI is a highly heterogeneous analyte. The cTnI complex undergoes substantial modification after release into the circulation. These modifications may include oxidation, reduction, and phosphorylation, as well as degradation by proteases (8, 9). Furthermore, the extent and nature of troponin modification, as well as the values obtained by immunoassay, are dependent on the time after myocardial infarction (10, 11). Like Hb A1c, standardization of cTnI measurements requires a reassessment of the analyte. Taking into consideration the molecular heterogeneity of cTnI, the IFCC Committee for the Standardization of Markers of Cardiac Damage recommended in 2001 that the antibodies used in routine cTnI assays preferably recognize epitopes “that are located in the stable part of the molecule and are not affected by complex formation (such as [the troponin] ICT [complex]) and other in vivo modifications” (12). By targeting the stable region of cTnI, routine assays ideally will measure the same chemical entity in the patient sample, regardless of the degree of cTnI heterogeneity. The stable region of cTnI also becomes the ideal target for the development of reference measurement procedures. Finally, like Hb A1c, standardization of cTnI measurement requires the development of RMs and reference measurement procedures. The proposed traceability chain developed by the IFCC Working Group on Standardization of Troponin I (13) incorporates all of these standardization elements (Fig. 1B). This IFCC working group is now focused on 2 tasks to establish the proposed traceability chain shown in Fig. 1B: development of a higher-order reference measurement procedure and establishment of a secondary RM (13).

First, a mission-critical requirement is not only a clearly defined analyte but also that the defined analyte (epitope) represent the clinically relevant isoforms. A key link in the traceability chain is the development by the IFCC working group of a noncommercial immunoassay (14) to be used as the higher-order reference measurement procedure for this cTnI target (Fig. 1B). This secondary reference method is currently under development (14), and its specificity focuses on key cTnI epitopes (i.e., amino acid residues 41–49 and 83–93), which are in the stable portion of the molecule common to all clinically relevant cTnI forms present in blood, i.e., cTnITC (complex of cardiac troponins I, T, and C), cTnIC (complex of cardiac troponins I and C), cTnI, and so forth.

The secondary reference measurement procedure must show the same response for the different isoforms to guarantee consistency in results across samples. Once developed and validated, this procedure will be used to assign traceable values to the secondary RMs (Fig. 1B), which will be the native cTnI analyte in a natural serum (i.e., commutable) matrix. In this way, the value assigned to working and product calibrators for each assay will be traceable to the value carried by the primary RM. With regard to the primary RM, a purified intact troponin ITC complex from human heart was selected after consideration of several candidates (15). The purity and protein concentration of this RM were evaluated with reversed-phase liquid chromatography–mass spectrometry and with amino acid analysis (16). This pure cTnI material is designated NIST SRM 2921. Because of the molecular heterogeneity of cTnI in patient samples, it is very likely that only a small fraction of the molecules detected in the measurement process (i.e., the particular quantity of cTnI subject to measurement in the biological matrix) may exist in the intact ternary troponin form represented by SRM 2921. Consequently, after dilution with an appropriate diluent or human serum, SRM 2921 was found not to be commutable with the majority of cTnI commercial methods (17). It is important to note that the noncommutability of SRM 2921 is metrologically acceptable, and traceability to it is appropriate if one considers the intended use of SRM 2921, which is primarily for calibrating a secondary reference measurement procedure, not for directly assigning values to the working calibrators for the field assays. With use of SRM 2921 to calibrate the secondary reference method (higher-order immunologic procedure), there is traceability to the SI for value assignment of the serum-based RM.

It is noteworthy that many characteristics of available commercial cTnI assays, including the key epitopes targeted, have been recently collated and updated on the IFCC Web site (see http://www.ifcc.org/index.asp?cat=Scientific_Activities&scat=Troponin_Assay_Analytical_Characteristics&rif=4&dove=1).

Complicated work remains for the standardization of cTnI measurements. Notable challenges include completing the development of a robust higher-order reference method that can be transferred to a number of reference laboratories and characterizing a stable native matrix RM that displays commutability in appropriate experiments (a panel of 3 cTnI-positive serum pools is presently planned) (13).

cTnI standardization is certainly complicated; however, the substantial effort required is richly justified, given the critical clinical role of this analyte in patient management (3, 4). Therefore, cTnI must unquestionably be considered a high-priority clinical parameter, and there is powerful motivation for clinical, governmental, and manufacturing organizations to assist in bringing this activity to full fruition. Rather than
throwing up our collective hands when facing the acknowledged challenges involved, we believe that the end point of cTnI standardization is worthy of the efforts necessary to overcome these challenges along the path to standardization.

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