

## REVIEW

### Standardisation of cardiac troponin I measurement: past and present

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#### Summary

The laboratory measurement of cardiac troponin (cTn) concentration is a critical tool in the diagnosis of acute myocardial infarction (MI). Current cTnI assays produce different absolute troponin numbers and use different clinical cut-off values; hence cTnI values cannot be interchanged, with consequent confusion for clinicians. A recent Australian study compared patient results for seven cTnI assays and showed that between-method variation was approximately 2- to 5-fold.

A major reason for poor method agreement is the lack of a suitable common reference material for the calibration of cTnI assays by manufacturers. Purified complexed troponin material lacks adequate commutability for all assays; hence a serum-based secondary reference material is required for cTnI with value assignment by a higher order reference measurement procedure.

There is considerable debate about how best to achieve comparability of results for heterogeneous analytes such as cTnI, whether it should be via the harmonisation or the standardisation process. Whereas harmonisation depends upon consensus value assignment and uses those commercial methods which give the closest agreement at the time, standardisation comes closer to the true value through a reference measurement system that is based upon long-term calibration traceability.

The current paper describes standardisation efforts by the International Federation of Clinical Chemistry and Laboratory Medicine Working Group on Standardization of cTnI (IFCC WG-TNI) to establish a reference immunoassay measurement procedure for cTnI of a higher order than current commercial immunoassay methods and a commutable secondary reference material for cTnI to which companies can reference their calibration materials.

**Key words:** Cardiac troponin I, standardisation, measurement traceability, reference material, reference measurement procedure.

**Abbreviations:** AACC, American Association for Clinical Chemistry; ACS, acute coronary syndrome; C-SMCD, Committee on Standardization of

Markers of Cardiac Damage; cTn, cardiac troponin; CV, coefficient of variation; IFCC, International Federation of Clinical Chemistry; IRMM, Institute for Reference Materials and Measurements; LC, liquid chromatography; MAbs, monoclonal antibodies; MI, myocardial infarction; MS, mass spectrometry; NIST, National Institute of Standards and Technology; NSTEMACS, non-ST-elevation acute coronary syndrome; WG-TNI, IFCC Working Group on Standardization of cTnI.

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#### BACKGROUND

The measurement of cardiac troponin (cTn) concentrations is a critical tool in the diagnosis of acute myocardial infarction (MI). The almost 100% cardiospecificity of troponin is also of clinical value in these patients for use as prognostic indicators, as there is an excellent correlation between elevated troponin concentrations following MI and short-term morbidity and mortality.<sup>1</sup> Troponin is also useful for late MI diagnosis as concentrations remain elevated for 5–14 days and for the diagnosis of reinfarction when concentrations begin to rise again.

Troponin should correctly be interpreted as a continuous variable with only just detectable concentrations being informative for worsened prognosis in both high and low risk non-ST-elevation acute coronary syndrome (NSTEMACS) patients.<sup>2,3</sup> Using contemporary cTn assays (2nd generation cTnI and 4th generation cTnT), there appears to be no threshold below which troponin is not pathological.<sup>3–5</sup> By contrast, the pathophysiology of very low troponin concentrations which are detectable only with ultra-sensitive assays is uncertain. Using these assays, which have 10- to 100-fold increased analytical sensitivity, cTnI has been shown to be elevated in patients with unstable angina at zero and 2 hours post percutaneous coronary intervention,<sup>6</sup> in patients with stress-test-induced myocardial ischaemia,<sup>7</sup> and also in a majority of marathon runners whose median cTnT

increased more than 7-fold following completion of the marathon.<sup>8</sup>

The 2007 recommendations for diagnosis of MI and risk stratification of acute coronary syndrome (ACS) reiterate the central role of troponin measurement, recommending the detection of a rise and/or fall of cTn with at least one value above the 99<sup>th</sup> percentile limit of value distribution in a reference population, with a corresponding imprecision of ≤10% coefficient of variation (CV) at this cut-off.<sup>9,10</sup>

As the clinical information about the use of troponin for MI diagnosis and risk prognostication of adverse cardiovascular events has evolved, so has the analytical performance of the different generation cTnI and cTnT assays. Contemporary generations of cTnI and cTnT assays have improved sensitivity and corresponding analytical performance at low level concentrations compared with their predecessors. Of the 21 contemporary commercial cTn assays currently listed on the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) website by the Committee on Standardization of Markers of Cardiac Damage (C-SMCD), however, only five of these fulfill the guideline recommendations (Table 1).<sup>11</sup> Furthermore, these data relate to information given by the manufacturers themselves in the assay package inserts, which often portrays better performance than in clinical

practice.<sup>12</sup> Whether laboratories or clinicians realise it, the IFCC list, which is referenced in the MI guidelines document,<sup>9</sup> is a mixture of different generations of automated and point-of-care testing assays. cTnT results are less problematic, as assays are available from a single manufacturer and comparability of cTnT results for different generation assays is less of an issue. Standardisation of cTnT will not be discussed here. In contrast the various cTnI assays produce different absolute numbers and use different clinical cut-off values. Studies have shown there are differences in method concordance<sup>13,14</sup> and in clinical concordance<sup>15,16</sup> that are likely to be related to the less sensitive previous generation assays and to the different antibody configurations that exist between cTnI assays. Hence, cTnI assays are not equal in their clinical specificity and in their ability to identify patients who are at risk of future cardiovascular events.

With the ongoing improvement of troponin assays by manufacturers and development of assays that have increased analytical sensitivity and improved performance at low level troponin concentrations, it is likely that these newer generation assays will be more analytically accurate at the recommended MI cut-off levels (Table 1).<sup>11,17</sup> However, it is unknown if more sensitive assays will show an increased rate of false negative and/or false positive reactions despite the re-engineering of assays to reduce

**Table 1** Analytical characteristics of commercial and research high sensitivity cardiac troponin I and T assays determined by the manufacturer

| Company/platform/assay           | LoD (µg/L) | 99 <sup>th</sup> % (µg/L) | %CV at 99 <sup>th</sup> % | 10% CV (µg/L) | Risk stratification | Epitopes recognised by antibodies    | Detection antibody tag      |
|----------------------------------|------------|---------------------------|---------------------------|---------------|---------------------|--------------------------------------|-----------------------------|
| Abbott AxSYM ADV                 | 0.02       | 0.04                      | 15.0                      | 0.16          | Yes                 | C: 87-91, 41-49; D: 24-40            | ALP                         |
| Abbott ARCHITECT                 | <0.01      | 0.028                     | 15.0                      | 0.032         | No                  | C: 87-91, 24-40; D: 41-49            | Acridinium                  |
| Abbott i-STAT                    | 0.02       | 0.08                      | 16.5                      | 0.10          | Yes                 | C: 41-49, 88-91; D: 28-39, 62-78     | ALP                         |
| Beckman Coulter Access Accu      | 0.01       | 0.04                      | 14.0                      | 0.06          | Yes                 | C: 41-49; D: 24-40.                  | ALP                         |
| bioMerieux Vidas Ultra           | 0.01       | 0.01                      | 27.7                      | 0.11          | No                  | C: 41-49, 22-29; D: 87-91, 7B9       | ALP                         |
| Inverness Biosite Triage         | 0.05       | <0.05                     | NA                        | NA            | No                  | C: NA; D: 27-40                      | Fluorophor                  |
| Inverness Biosite Triage (r)     | 0.01       | 0.056                     | 17.0                      | NA            | No                  | NA                                   | Fluorophor                  |
| Mitsubishi Chemical PATHFAST     | 0.008      | 0.029                     | 5.0                       | 0.014         | No                  | C: 41-49; D: 71-116, 163-209         | ALP                         |
| Ortho Vitros Eci ES              | 0.012      | 0.034                     | 10.0                      | 0.034         | Yes                 | C: 24-40, 41-49; D: 87-91            | HRP                         |
| Radiometer AQT90                 | 0.0095     | 0.023                     | 17.7                      | 0.039         | NA                  | C: 41-49, 190-196; D: 137-149        | Europium                    |
| Response Biomedical RAMP         | 0.03       | <0.1                      | 18.5                      | 0.21          | No                  | C: 85-92; D: 26-38                   | Fluorophor                  |
| Roche E170                       | 0.01       | <0.01                     | 18.0                      | 0.03          | Yes                 | C: 125-131; D: 136-147               | Ruthenium                   |
| Roche Elecsys 2010               | 0.01       | <0.01                     | 18.0                      | 0.030         | Yes                 | C: 125-131; D: 136-147               | Ruthenium                   |
| Roche Cardiac Reader             | <0.05      | <0.05                     | NA                        | NA            | No                  | C: 125-131; D:136-147                | Gold particles              |
| Siemens Centaur Ultra            | 0.006      | 0.04                      | 10.0                      | 0.03          | Yes                 | C: 41-49, 87-91; D: 27-40            | Acridinium                  |
| Siemens Dimension RxL            | 0.04       | 0.07                      | 20.0                      | 0.14          | Yes                 | C: 27-32; D: 41-56                   | ALP                         |
| Siemens Immulite 2500 STAT       | 0.1        | 0.2                       | NA                        | 0.42          | No                  | C: 87-91; D: 27-40                   | ALP                         |
| Siemens Immulite 1000 Turbo      | 0.15       | NA                        | NA                        | 0.64          | No                  | C: 87-91; D: 27-40                   | ALP                         |
| Siemens Stratus CS               | 0.03       | 0.07                      | 10.0                      | 0.06          | Yes                 | C: 27-32; D: 41-56                   | ALP                         |
| Siemens VISTA                    | 0.015      | 0.045                     | 10.0                      | 0.04          | Yes                 | C: 27-32; D: 41-56                   | Chemiluminescence           |
| Tosoh AIA II                     | 0.06       | <0.06                     | 8.5                       | 0.09          | No                  | C: 41-49; D: 87-91                   | ALP                         |
| Research high sensitivity assays |            |                           |                           |               |                     |                                      |                             |
| Beckman Coulter Access hs-cTnI   | 0.0020     | 0.0086                    | 10.0                      | 0.0086        | NA                  | C: 41-49; D: 24-40                   | ALP                         |
| Roche Elecsys hs-cTnT            | 0.001      | 0.013                     | 8.0                       | 0.012         | NA                  | C: 125-131; D: 136-147               | Ruthenium                   |
| Nanosphere hs-cTnI               | 0.0002     | 0.0028                    | 9.5                       | 0.0005        | NA                  | C: 136-147; D: 49-52, 70-73, 88, 169 | Gold-nanoparticles          |
| Singulex hs-cTnI                 | 0.00009    | 0.0101                    | 9.0                       | 0.00088       | NA                  | C: 41-49; D: 27-41                   | Capillary flow fluorescence |

Table reprinted with the permission of the International Federation of Clinical Chemistry and Laboratory Medicine.<sup>11</sup> LoD, limit of detection; 99<sup>th</sup>%, 99<sup>th</sup> percentile concentration; 10% CV, lowest concentration that has been shown to have a 10% CV (total imprecision); risk stratification claim per US Food and Drug Administration (FDA); epitopes (amino acid residues) recognised by antibodies were supplied by manufacturers; C, capture antibody(s); D, detection antibody(s); (r), revised assay submitted to FDA per Inverness; ALP, alkaline phosphatase; hs, high sensitivity designation per manufacturers; HRP, horseradish peroxidase.

interference due to haemolysis, heterophile antibodies and troponin autoantibodies.

Current cTnI assays are marketed by different companies and use various standard materials and antibodies with different epitope specificities. Hence, troponin I assays may give results that are unique to a certain method or instrument and different results may be obtained for the same patient sample depending on the assay and platform used. As a consequence, cTnI values obtained with different assays cannot be interchanged. This creates confusion for clinicians who may not know that values are not interchangeable, especially when patients are referred to different hospitals that may not use the same method as the referring hospital laboratory. Hence, reference values for cTnI and derived decision limits need to be determined separately for each assay and platform and must not be extrapolated to other assays until there is adequate cTnI standardisation.

## CAUSES OF NON-HARMONISED CTNI RESULTS

### Differences in assay calibration

One main reason for poor agreement of values between methods is the lack of a suitable common reference material for the calibration of cTnI assays by manufacturers. Typically, research assays used independent sources of calibrators that were usually purified from heart muscle tissue with a total protein concentration determined. Variations in results of between 20- and 40-fold and as much as a 100-fold were reported among the initial first generation cTnI assays.<sup>12,18,19</sup> More recently, in 2006 an Australian study compared patient results for seven cTnI assays and showed the variation to be reduced to approximately 2- to 5-fold.<sup>14</sup> Troponin I survey data for samples from the 2009 Royal College of Pathologists of Australasia Quality Assurance Program (RCPA QAP) for General Chemistry do not allow information about between-method comparability to be assessed due to the possible matrix effect of the materials used. In this program cTnI values are divided into two groups of methods according to median cTnI sample values, and within-method inter-laboratory imprecision ranges from 5.3% to 15.9%. End-of-cycle low and high concentrations range between 0.01 and 0.30 µg/L and 1.18 and 2.74 µg/L, respectively, for cTnI measured by group A methods, and between 0.37 and 0.87 µg/L and 1.65 and 4.40 µg/L by group B methods. Such method differences in cTnI results, if replicated in patient samples, highlight a lack of comparability preventing use of a common decision cut-off value for cTnI assays.

The major prerequisite for guaranteeing comparability of results among different assays is availability of commutable reference materials for calibration. Katrukha *et al.*<sup>20</sup> showed for six first-generation cTnI assays that use of a material containing equimolar concentrations of human cTns I, T, and C as common calibrator may significantly reduce the interassay variability of cTnI values for a positive serum sample. However, a collaborative study of the American Association for Clinical Chemistry (AACC) and the National Institute of Standards and Technology (NIST) using a similar purified ITC-troponin complex (NIST SRM 2921), has shown that this material does not

behave in the same way as individual patient samples with elevated cTnI concentrations and failed to harmonise cTnI values between methods. This indicated a potential commutability issue in more than half of 15 tested cTnI assays, whereby the matrix containing the purified cTnITC material affected the measured result independent of the cTnI analyte.<sup>19</sup> Differences in method harmonisation between the two studies may be due to a different matrix used to spike the NIST standard into, instability of the diluted standard, or study specimen differences, i.e., pooled patient serum versus individual patient serum. The same AACC study showed that use of matrix-matched serum pooled from cTnI positive patients with matrices and concentrations more similar to patient samples is more likely to harmonise methods than manufactured materials which can suffer from matrix effects.

### Antibody specificity

Apart from calibration differences, cTnI assays vary from each other by differences in assay format, by the types of antibody used, which may be combinations of mouse monoclonal and/or polyclonal anti-cTnI antibodies, by the epitopes to which they bind, and by the type of indicator molecule that is used.<sup>21</sup> Immunoassays for cTnI may be influenced by the antibody species and the specificity of the polyclonal and/or monoclonal anti-cTnI antibodies used in the immunometric sandwich assays and these specificity differences are likely to contribute to assay differences for both contemporary and high sensitive assays.

cTnI exhibits considerable chemical and structural heterogeneity in blood, unlike simpler chemical molecules such as glucose or creatinine. Following myocardial damage, cTns are released rapidly from the myocyte and appear in blood as a heterogeneous mixture of different sized molecular species including intact free cTnI and a range of modified and complexed products. cTnI is sensitive to proteolysis and can undergo proteolytic degradation both in necrotic cardiomyocytes<sup>22</sup> and the bloodstream or in collected blood. In particular, the N-(amino) and C-(carboxyl) terminal parts of molecules are less stable compared with the mid-molecule region of cTnI at amino acid residues 30–110, which is more stable possibly due to protection by TnC. Labugger *et al.* showed there were up to eight degradation products of cTnI present in sera from MI patients, three of these being of a higher molecular mass than the native troponin I.<sup>23</sup> Apart from degradation, different plasma forms may also undergo chemical modifications such as oxidation, reduction, phosphorylation, dephosphorylation and N-terminal acetylation. The phosphorylation state of cTnI in serum may reflect the functional status of viable heart, while degraded products of cTnI may be specific for certain disease states.<sup>24</sup> This progressive modification of cTnI found in the blood of MI patients after tissue release results in a heterogeneous mixture of plasma forms including predominantly the binary IC complex with smaller amounts of the ternary ITC complex, binary IT complex and free cTnI.<sup>25</sup> The percentage of free versus complexed cTnI and the extent of fragmentation may vary over the MI time course as well as between individuals with MI. In addition, polymorphisms of cTnI that may contribute to the development of certain cardiomyopathies have the potential to modify the

troponin molecule's immunogenicity and to result in false negative results.<sup>26</sup>

Ideally, cTnI assays should recognise both complexed and free cTnI equally, and their modified forms, to allow monitoring of total cTnI concentrations present in samples from the same subject over the MI course. However, variable antibody immunoreactivity to different isoforms has been demonstrated for various cTnI assays. An earlier study performed on nine commercially available cTnI assays showed that although assays recognised both the complexed and free forms, some did not give equal relative responses to the various forms of cTnI, which may result in over- or under-estimation of the true cTnI concentration in patient samples.<sup>27</sup> Unfortunately, there are limited data available on the relative immunoreactive response by contemporary assays to the various cTnI forms present in plasma.

## STANDARDISING CTNI MEASUREMENTS

In addressing standardisation of cTnI, the following major factors need to be considered: (1) heterogeneity of cTnI and the requirement for a suitable definition of the analyte intended to be measured by an assay, i.e., the measurand; (2) differences in antibody specificity between assays and targeting of the clinically relevant analyte; (3) the availability of a suitable commutable reference material for cTnI measurements and calibration traceability of commercial assays to this material; and (4) value assignment of the reference material by a higher order reference measurement procedure with validated performance characteristics.<sup>28</sup>

### Definition of the clinically relevant measurand

A way to address the definition of cTnI that exists in blood in multiple forms is to define the measurand as a unique, invariant part of the molecule that is common to all cTnI forms present in blood (e.g., a specific amino acid sequence) and is the clinically relevant form(s).<sup>29</sup> Commercial immunoassay methods should recognise this particular sequence of amino acids common to all cTnI molecules present in blood, resulting in an increased comparability of assay reactivity. In so doing, it is implied that all plasma cTnI forms are measured by current assays and that assays measure the forms either equally or the difference in reactivity is not clinically relevant. As previously alluded to, this may not be the case<sup>27</sup> and there is the need for all manufacturers of cTnI assays to test for recovery of different cTnI forms.

### Antibody assay characteristics

Standardisation of cTnI assays requires that differences in antibody specificities among the assays be minimised. Due to proteolytic susceptibility of N- and C-terminal parts of cTnI, the IFCC C-SMCD has recommended that diagnostic manufacturers use cTnI antibodies directed to the more stable mid-molecule epitopes.<sup>30</sup> Therefore, antibodies used for the development of cTnI assays should selectively recognise epitopes that are located in the stable part of the molecule and not be affected by IC or ITC complex formation and other 'in vivo' modifications.<sup>30,31</sup> The possibility of the presence in blood of anti-cTnI autoantibodies

should also be considered, as these may cause under-estimation of cTnI.<sup>32,33</sup> All but two of the currently available cTnI assays use antibodies that are directed to the mid-molecule epitopes, i.e., amino acids 30–110, which is the more stable part of the molecule.<sup>34</sup>

### Reference materials for cTnI

#### *Purified reference material for cTnI*

Ideally, cTnI assays should be calibrated against the material representing the natural and major forms of the molecule present in blood after tissue release.<sup>25,30</sup> Some years ago, NIST released a purified reference material (Standard Reference Material 2921, human cTn complex composed of cTnT, cTnI, and TnC subunits), which was extracted from human heart. Structural characterisation of SRM 2921 was performed by liquid chromatography (LC) coupled to mass spectrometry (MS) and tryptic digestion followed by matrix-assisted laser desorption/ionisation (MALDI)-MS. The concentration of cTnI in SRM 2921 was determined through a combination of reversed-phase (RP)-LC and amino acid analysis.<sup>35</sup> However, studies performed using SRM 2921 have shown that this material does not behave in the same way as individual patient samples with elevated cTnI concentrations, and after assay recalibration using the purified ITC material approximately 50% of methods were still non-harmonised.<sup>19</sup> The study conclusion was that the proportion of measurement procedures demonstrating commutability for the NIST cTnI material was too low to use this material as a common calibrator by manufacturers.

#### *Secondary reference material for cTnI*

Closer agreement of values between methods has been shown after realignment to serum-based cTnI pools, suggesting that use of matrix-matched secondary reference material will likely better harmonise methods. Several studies have shown that cTnI values are more comparable between routine measurement systems if one or more serum samples are used as the common calibrator.<sup>19,20,36</sup> Tate *et al.* assessed harmonisation by determining the among-systems CV after correction for calibration differences among seven assays by reference to two different materials used as common calibrator, a purified ITC material, similar to SRM 2921, and a serum sample collected from a patient with MI.<sup>36</sup> Generally, test values were better harmonised among systems using the serum sample rather than the processed material as calibrator. In the AACC cTnI standardisation study, similar findings were observed after the realignment of values against the median cTnI concentrations of six pools and the variability of results among cTnI assays decreased from CVs of ~90% to CVs of 7–28%.<sup>19</sup>

### Standardisation and metrological traceability

Comparability of patient values is dependent upon calibration traceability, which is the property of a measurement tracing its value and measurement uncertainty to a higher order reference.<sup>37–39</sup> There is considerable debate about how best to achieve comparability of measurements of heterogeneous analytes such as cTnI, whether it should be via the harmonisation or the



standardisation process. Harmonisation of cTnI measurement aims to establish consensus values for cTnI in 'real' patient specimens by a normalisation or realignment process to minimise between-method variability. However, it is generally biased in terms of trueness and is possible only in a method-dependent manner with no long-term anchor of trueness available. Harmonisation depends upon consensus value assignment, generally using those commercial methods which give the closest agreement at the time. In contrast, standardisation comes closer to the true value through a reference measurement system that is based upon long-term calibration traceability by referencing the values of different batches of calibration materials to the same reference measurement procedure and the same well-characterised primary reference material.

#### Reference measurement system

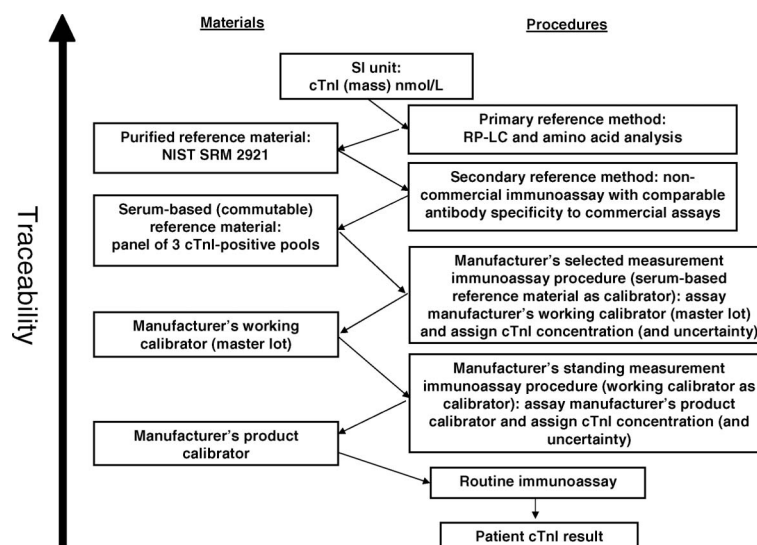
The foundation of a metrologically correct approach for the standardisation and traceability of cTnI measurements is the availability of a reference measurement system (Fig. 1).<sup>40</sup> This comprises: (1) a purified reference material with values assigned by mass determination/calculation, (2) a higher order reference procedure for the value assignment of secondary reference materials, and (3) matrix-based (commutable) reference materials, represented by a panel of appropriately selected and characterised human pooled serum samples. The reference measurement system links higher order reference methods and reference materials to routine calibrators and procedures used in clinical laboratories through an unbroken, metrologically-based traceability chain.

Once appropriate reference materials are available, these materials and the manufacturer's testing procedures can be used by industry to assign values to working and product calibrators through a value transfer process. Through this calibration process clinical laboratories, using routine procedures with validated calibrators to measure patient samples, will obtain standardised and traceable values, with

no calibration bias among the commercial procedures. However, it is important to note that the practical implementation of the reference system concept cannot compensate for poor analytical performance and lack of analytical sensitivity and specificity of commercial assays. Neither standardisation nor harmonisation can improve comparability of patient cTnI results for assays that are not optimised for imprecision, suffer from pre-analytical and analytical interference, lack robust and reproducible calibration for different reagent lots, or do not give equal immunoreactivity to the clinically relevant cTnI forms released in acute MI. Method comparison studies of patient samples collected over the time course of a MI are required to identify less robust assays that are affected by cTnI form bias and may hamper the comparability of cTnI measurements.

#### Commutable reference materials for cTnI

Future efforts to standardise results of cTnI assays will require a suitable secondary reference material, which must be commutable among all of the measurement procedures for which it is intended to be used including the selected secondary reference measurement procedure.<sup>40</sup> A secondary reference material for cTnI is intended to establish the traceability of cTnI results obtained by a routine method to the reference system and to produce results for clinical samples that are equivalent for all methods. A reference material for cTnI would be considered commutable when a measurement procedure produces the same result for the material as it does for an authentic patient sample that contains the same cTnI concentration.<sup>41</sup> It is the matrix in which cTnI is contained that creates non-commutability in assays. As noted previously, the use of the purified cTnITC complex when spiked into troponin negative serum was unsuitable in over half of the commercial cTnI assays. Similarly, use of spiked cTnIC as calibrator did not achieve satisfactory cTnI assay harmonisation.<sup>19</sup> An alternative approach is to use a small number of human serum pools



**Fig. 1** Suggested approach for the standardisation of cardiac troponin I (cTnI) measurements through traceability implementation to the reference measurement system consisting of a hierarchy of analytical measurement procedures and different kinds of reference materials (modified from Panteghini *et al.*<sup>40</sup>). SI, International System; RP-LC, reversed-phase liquid chromatography; NIST, National Institute of Standards and Technology; SRM, Standard Reference Material; Conversion factor (SI in nmol/L to mass in  $\mu\text{g/L}$ ) is 23.9184.<sup>46</sup>

(e.g., three pools from cTnI positive patients and containing cTnI around the clinically relevant concentrations) that are obtained from hospitalised individuals with myocardial injury (i.e., MI/ACS) as a secondary reference material for cTnI measurement. In this way serum pools will consist of a blend of the clinically relevant cTnI forms and act as a surrogate reference material rather than reflecting the cTnI composition of each individual clinical sample, which can change over the time after MI.<sup>42</sup> In contrast to a well-defined simple analyte such as creatinine, serum-based reference materials for cTnI may only represent an 'average' condition. Provided commutability can be confirmed using this 'pools' approach which comes closest to resembling cTnI in blood, then larger volumes of each pool would be collected. Following their preparation, the next step in standardisation would be value assignment of the secondary reference materials by a reference method.

#### *Proposed secondary reference measurement procedure for cTnI*

At present, there is no reference method and no preferred methods for cTnI able to value assign a serum-based secondary reference material. Analytical principles commonly used in reference measurement procedures, e.g., MS, currently lack the measurement sensitivity to measure the low microgram concentrations of cTnI present in serum. As an alternative, the development of immunochemical reference methods based on the availability of monoclonal antibodies (MAbs) with well-defined epitope specificity has been proposed.<sup>43</sup>

Initial research work at NIST has involved characterisation of commercial MAbs to human cTnI by epitope mapping and affinity binding studies to determine the optimal combination of antibodies. MAbs were selected based on the manufacturer's recommendation for appropriate capture and detection pairs for a sandwich immunoassay and according to the IFCC recommendations to target the central, more stable part of the cTnI molecule for serum cTnI measurements.<sup>30</sup> Developmental work at the National Physical Laboratory (NPL) is underway to establish the candidate reference procedure for cTnI, based on ELISA technology, to validate the method using clinical samples and to compare its immunoreactivity with commercial assays. Analytical requirements of the reference measurement procedure include: (1) comparable cTnI specificity to commercial assays; (2) acceptable assay precision; (3) calibration against the purified reference material for cTnI (NIST SRM 2921); (4) ability of the assay to be unaffected by interferences; and (5) technical validation and transferability in a laboratory network.<sup>40,44</sup>

#### *IFCC WG-TNI Standardization Project*

The IFCC Working Group on Standardization of cTnI (WG-TNI), working in collaboration with the metrology institutes [NIST, NPL, and the Institute for Reference Materials and Measurements (IRMM)] and industry, has undertaken a project to address: (1) establishment of a secondary reference immunoassay measurement procedure for cTnI of a higher order than current commercial immunoassay methods; and (2) development of a serum-based commutable reference material for cTnI to which companies can reference their calibration materials (Table 2).

**Table 2** IFCC Working Group on Standardization of Cardiac Troponin I (cTnI) project aims

1. Development of a candidate secondary reference immunoassay measurement procedure for cTnI;
2. Selection, characterisation (including commutability) and value (and measurement uncertainty) assignment of a secondary reference material for cTnI by use of the above reference measurement procedure;
3. Validation of cTnI standardisation through a round robin after a value transfer to commercial assays using the secondary reference material as common calibrator;
4. Depending on the success of the above tasks, to seek acceptance with the Joint Committee for Traceability in Laboratory Medicine (JCTLM) of the reference measurement procedure and commutable reference materials for cTnI.

As a part of this work a pilot study is currently underway involving NIST, NPL and diagnostic industry to compare a candidate secondary immunoassay reference measurement procedure for cTnI with commercial assays and to investigate the feasibility of preparing a commutable, stable secondary reference material for cTnI by use of serum pools. The cTnI pools will be prepared in different ways including the addition of individual cTnI positive native patient samples to produce low, medium and high cTnI concentration pools, and blending of a high cTnI concentration pool with low and medium concentration pools or with a normal pool to ensure a range of different clinically relevant cTnI forms are represented in the material. The commutability and stability of the reference material will also be tested in routine methods and in the candidate cTnI reference method to ensure that it behaves the same as a patient sample during measurement by a routine procedure and that the material is stable for up to 5 years when stored frozen at  $\leq -70^{\circ}\text{C}$ . Although this is a research project and there is no guarantee of success, such experimental work is needed if there is to be progress in the standardisation of cTnI and, in general, of heterogeneous clinical analytes measured by immunoassays.

The successful standardisation of cTnI assays has benefits for clinical laboratories, clinicians and patients. Laboratories will be confident that results reported to clinicians and patients are adequately accurate (true and precise) to allow for correct medical interpretation and comparability over time and space. Traceable cTnI results will enable pooling of clinical results to generate a larger database of clinical information that can enable the definitive determination of the diagnostic specificity and sensitivity of cTnI assays, and the establishment of common reference limits or decision cut-points for medical intervention. Use of the same decision cut-off across cTnI assays of the same generation will be beneficial to clinicians and enable a wider uptake of the MI guideline recommendations. In addition, in a traceability context the comparison of laboratory performance of cTnI measurements in external quality assessment programs will provide better information about uncertainty of cTnI measurement and analytical performance of assays in clinical laboratories. If specifications for optimal performance of troponin measurements are clearly defined,<sup>45</sup> this will permit laboratories to identify and abandon poorer performing cTnI assays.

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