

## Standardisation of cardiac troponin I measurements – the way forward

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### ABSTRACT

Cardiac troponin I (cTnI) assays produce different results. To achieve closer comparability of cTnI values among assays, the use of a suitable reference material is proposed for use to calibrate commercial assays. To assign true cTnI concentrations to this material, establishment of a reference procedure for cTnI is also required.

### BACKGROUND

The measurement of cardiac troponin I (cTnI) is critical in the diagnosis of acute myocardial infarction (AMI), in treatment management of acute coronary syndromes (ACS) and as a prognostic indicator of future cardiovascular disease risk. The international guidelines for diagnosis of AMI and risk stratification of ACS reiterate the central role of troponin measurement (1, 2). Particularly, for AMI diagnosis a rise and/or fall of troponin concentrations in blood is requested with at least one value above the 99<sup>th</sup> percentile limit of the value distribution in a reference population, when an appropriate clinical and/or electrocardiographic/imaging situation is present (1).

Currently, however, various cTnI assays available in the market produce different results and, consequently, use different clinical cut-off values. In other words, cTnI values obtained with different assays cannot be interchanged nor can the clinical cut-offs. This creates problems for clinicians when interpreting patient results, especially when patients are referred to different hospitals that may not use the same assay as the referring hospital laboratory. Standardisation of cTnI assays would give the same measurement results independent of the method used or the laboratory doing the cTnI testing, so that the same decision cut-off can be used.

### CAUSES OF NON-HARMONISED TROPONIN I MEASUREMENTS

Currently there is at least a 5-fold difference in cTnI concentrations between commercially available assays (3). One main cause of non-harmonised cTnI assays is

the lack of a suitable reference material for calibration of cTnI assays by manufacturers. Commercially available cTnI assays marketed by different companies use various standard materials. Apart from calibration differences, cTnI assays use different assay formats, antibody combinations with different specificity to epitopes on the cTnI molecule, and different indicator molecules which can affect sensitivity and specificity of assays (4, 5). cTnI exhibits considerable structural heterogeneity in blood unlike simpler molecules like glucose and creatinine. Following myocardial injury, troponins are released from the myocyte into the blood as a heterogeneous mixture of different-sized molecular species, including intact ternary cTnI-T-C complex, binary cTnI-C complex, which is the main cTnI form in the blood after an AMI, cTnI-T complex, and free cTnI. Proteolytic degradation and chemical modifications of cTnI, such as oxidation, reduction, phosphorylation, dephosphorylation and N-terminal acetylation, occur that produce post-translational forms of the molecule. About two-thirds of contemporary cTnI assays use antibodies that are directed to the more stable, central part of the cTnI molecule located between amino acid residues 30 and 110 (5, 6). Ideally, the assays should recognise equally both complexed and free cTnI, and their modified forms, in equimolar amounts to allow monitoring of total cTnI concentrations present in samples from the same subject over the course of an AMI. There are, however, limited data available on the relative immunoreactivity of assays to the various clinically relevant cTnI forms present in plasma.

Differences in analytical performance between cTnI assays will also impede efforts to harmonise measurement results. Particularly, imprecision and assay detection limit may markedly be different (7).

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## STANDARDISING THE TROPONIN I MEASUREMENT

### Requirements

Standardization approaches are based on the concept of metrological traceability as described in the ISO 17511 standard and requires the availability of a complete metrological traceability chain (8). This chain begins with the definition of the measured analyte (the "measurand") and the preparation of a primary reference material (RM). Then, the primary RM and a high-order reference measurement procedure are used to assign values to a secondary RM, which typically has a matrix equivalent to that of biological samples. In turn, a strategy is carried out to transfer values to manufacturers' working ("master") and product calibrators for routine assays to be used for quantification of the "measurand" in patient samples. This chain allows traceability of values reported for patient care to a fixed point in time and space, i.e. the International System (SI) unit, allowing long term stability and comparability of routine measurement results independent of the assay and the laboratory performing it.

### Difficulties

Standardization is difficult for heterogeneous molecules, like cTnI, because they are typically quantified

by measuring the amount of substance of a certain part of all isoforms or of particular isoforms (epitopes for immunoassays, peptides for mass spectrometry methods). Hence, multimeric heterogeneity may affect different measurement procedures to a different extent and it can be difficult to implement adequate reference measurement procedures and characterize secondary (matrix-based) RMs suitable for direct calibration of commercial assays (9).

### The reference measurement system for cardiac troponin I

The Figure 1 shows the suggested approach for the standardisation of cTnI measurements through traceability implementation to the reference measurement system consisting of a hierarchy of analytical measurement procedures and different kinds of RMs (3, 10). These include:

- 1) a purified RM with values assigned by mass determination/calculation [NIST Standard Reference Material (SRM) 2921, which is a purified human cTn complex composed of cTnT, cTnI, and TnC subunits] (11);
- 2) a higher-order reference method for the value assignment of secondary RM, i.e. an ELISA-based procedure currently being evaluated (12); and
- 3) matrix-based (commutable) RMs, represented by a panel of appropriately selected and characterised

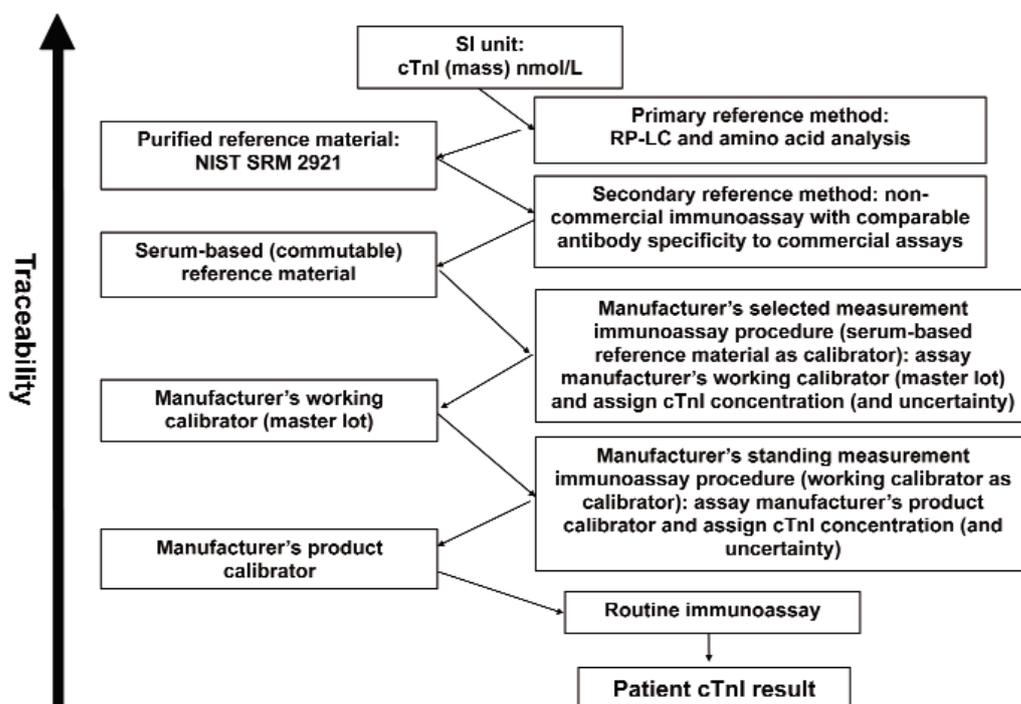


Figure 1

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 refs. 3 and 10).

SI, International System; RP-LC, reversed-phase liquid chromatography; NIST, National Institute of Standards and Technology; SRM, Standard Reference Material.

human pooled serum samples from AMI patients.

A RM for cTnI would be considered commutable when a measurement procedure produces the same result for the material as it does for a patient sample that contains the same cTnI concentration (13). Serum pools will therefore consist of a blend of the clinically relevant cTnI forms and act as a surrogate RM rather than reflecting the cTnI composition of each individual clinical sample, which can change over the time after AMI.

At present, higher-order mass spectrometry methods, commonly used as reference measurement procedures for many analytes, lack the analytical sensitivity to measure the low microgram concentrations of cTnI present in serum. As an alternative, the development of an immunochemical reference method based on the availability of monoclonal antibodies with well-defined epitope specificity has been proposed and currently pursued (10, 12).

#### The IFCC WG-TNI standardization project

The IFCC WG-TNI has undertaken a project to address the establishment of a secondary reference immunoassay measurement procedure for cTnI of a higher metrological order than current commercial immunoassay methods, and the development of a serum-based commutable RM for cTnI to which companies can reference their calibration process (3, 10). A pilot study has been planned to compare the candidate reference procedure for cTnI with commercial assays and to investigate the feasibility of preparing a commutable and stable secondary RM for cTnI by use of serum pools that are obtained from hospitalised individuals with acute myocardial injury (i.e. AMI/ACS) (14). The pilot study has started early this year and involves the National Metrology Institutes of United States, U.K., and the European Union, the University of Maryland School of Medicine, and the majority of diagnostic companies in the field. Method comparability and sample commutability and stability from the evaluation of individual patient samples and pools by 23 commercial assays and by candidate reference procedure are planned. Particularly, commutability of the pools as candidate RM will be tested between routine assays and candidate reference procedure utilizing sets of individual patient samples at different cTnI concentrations. At the same time, the short term stability of cTnI in the pool materials will be evaluated.

#### CONCLUSIONS

It is imperative that troponin measurement, on which important clinical decisions are based, is made with standardised methods to achieve comparable results regardless of the assay system or laboratory where the measurement is performed. The success of the cTnI standardisation project will enable pooling of

clinical results to generate a larger information database permitting the establishment of common reference limits and/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 consensus recommendations for AMI diagnosis. In addition, the availability of higher-order references will permit a more accurate evaluation of laboratory performance of cTnI measurements in EQAS programs with suitable information about uncertainty of cTnI measurement and analytical performance of assays in clinical laboratories (15, 16).

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