Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine

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This document is an essential companion to the third iteration of the National Academy of Clinical Biochemistry [NACB,8 now the American Association for Clinical Chemistry (AACC) Academy] Laboratory Medicine Practice Guidelines (LMPG) on cardiac markers. The expert consensus recommendations were drafted in collaboration with the International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers (IFCC TF-CB).

We determined that there is sufficient clinical guidance on the use of cardiac troponin (cTn) testing from clinical practice groups. Thus, in this expert consensus document, we focused on clinical laboratory practice recommendations for high-sensitivity (hs)-cTn assays. This document utilized the expert opinion class of evidence to focus on the following 10 topics: (a) quality control (QC) utilization, (b) validation of the lower reportable analytical limits, (c) units to be used in reporting measurable concentrations for patients and QC materials, (d) 99th percentile sex-specific upper reference limits to define the reference interval; (e) criteria required to define hs-cTn assays, (f) communication with clinicians and the laboratory’s role in educating clinicians regarding the influence of preanalytic and analytic problems that can confound assay results, (g) studies on hs-cTn assays and how authors need to document preanalytical and analytical variables, (h) harmonizing and standardizing assay results and the role of commutable materials, (i) time to reporting of results from sample receipt and sample collection, and (j) changes in hs-cTn concentrations over time and the role of both analytical and biological variabilities in interpreting results of serial blood collections.

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Preamble

This companion article to the Laboratory Medicine Practice Guidelines (LMPG) on cardiac markers of the National Academy of Clinical Biochemistry (NACB), now the American Association for Clinical Chemistry (AACC) Academy, is based on expert opinion as there is insufficient evidence regarding the important issues addressed. This work was composed in conjunction with AACCAcademyLaboratoryMedicinePracticeGuidelinesCommitteeonCardiacMarkers: AlanH.B.Wu,Chair;FredS.Apple;RobertH.Christenson;Christopher deFilippis;Dina N. Greene;Allan S. Jaffe;Peter A. Kavsak;David M. Morrow;W.Franklin Peacock. IFCC Task Force on Clinical Applications of Cardiac Bio-Markers: Fred S. Apple, Chair. Members: Richard Body, Peter A. Kavsak, Su Ping Carolyn Lam, Guillaume Lefèvre, Torbjørn Omland, Kari Pulkkki, Amy Saenger. Consultants: Allan S. Jaffe, Paul Collinson, Jordi Ordonez-Llanos.
and endorsed by the International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers (IFCC TF-CB). The first LMPG on cardiac markers was published in 1999 (1), a few years after the commercial release of cardiac troponin (cTn) assays. Because this was the first set of published guidelines focused on cardiac markers, recommendations were made on both analytical specifications and the clinical applications of cTn results. Subsequently, the AACC Academy (NACB at that time) disseminated the second LMPG based on 5 manuscripts (2–6). In parallel, the Third Universal Definition of Myocardial Infarction (GTF-3rd MI) published their third revised recommendations for the role of cTn for the diagnosis of myocardial infarction and myocardial injury (7).

The third LMPG Committee determined that 2 types of recommendations were necessary: those based upon expert opinion and those that were evidence-based. Evidence-based recommendations that follow the procedures developed by the AACC Academy for LMPG promulgation are currently in preparation for a future document. However, there are numerous other primarily analytic testing issues that must be addressed to optimize the safety and effectiveness of cTn in clinical practice. Because there is limited and insufficient evidence to address many of these issues, an expert group was convened with the approval of the AACC Academy and in collaboration with the IFCC TF-CB that focused on laboratory medicine practice recommendations based on the opinions of these experts. Other expert groups have published recommendations that contain analytical considerations as well (8, 9).

Thus, while the third LMPG Committee determined that there is sufficient evidence-based clinical guidance on the use of cTn from clinical practice groups, for analytical considerations, there is no existing body of evidence from clinical trials; therefore, we determined that use of the expert opinion class of evidence was necessary and appropriate to focus on the analytical issues of cardiac marker testing, specifically high-sensitivity (hs) cTn assays, recognizing that the AACC Academy and the IFCC TF-CB had the most expertise. As future evidence evolves in this field, it will be incorporated into this guidance by appending addenda.

Several key recommendations from previous cardiac biomarkers LMPGs were discussed and retained. These include (a) elimination of creatine kinase-MB and myoglobin with the consensus statement that cTnI and cTnT are the biomarkers of choice for the diagnosis of acute MI and risk-outcomes stratification; (b) serum, plasma, and anticoagulated whole blood are acceptable specimens for the analysis of cTn, and that the choice of specimen must be based on sufficient evidence and the known characteristics of individual cTn assays (2, 3); and (c) different specimen types should not be used interchangeably for cTn or hs-cTn assays.

This document was initially developed and revised by the American, Canadian, and Spanish authors, and was then discussed and revised separately by the entire IFCC Task Force membership who voted unanimously to be partners in the shaping of this document, including participation via teleconference and in person as part of the vigorous discussion of the recommendations. However, we acknowledge that the recommendations herein are particularly relevant for the US audience because hs-cTn assays are only now becoming available there. On the other hand, hs-cTn assays have been in use in Europe, Canada, and parts of Asia for >5 years. It is important to note, however, that the current experts’ opinions are relevant globally. Although others have reflected on these issues, we performed a needs assessment and found no published guidelines from these other countries that list in a single source all the recommendations contained in this document. All the information presented herein falls into the expert opinion class, as there are no clinical trials from which to generate rigorous scientific evidence on these topics.

### Quality Control Characteristics

The Third Universal Definition of MI includes a decision point for all cTn assays at the 99th percentile upper reference limit (URL) from the distribution of a reference population (7). Metrics for implementing and monitoring cTn assays over time have typically been performed using QC materials with concentrations higher than the 99th percentile. Hence, there has been a quality gap because users do not know the performance of cTn assay at the 99th percentile.

**Recommendation 1:** For hs-cTn assays, laboratories should measure at least 3 different concentrations of QC materials at least once per day. For contemporary cTn assays, at least 2 different concentrations of QC materials must be assessed at least once per day. Before patient testing can be initiated, the values for acceptable imprecision must be at a minimum, consistent with those specified by the manufacturer.

A. QC concentrations for hs-cTn assays (ng/L units with 1 decimal place):

1. **Concentration 1:** a concentration between the limit of detection (LoD) and the lowest sex-specific 99th percentile.
2. **Concentration 2:** a concentration that is higher than but close (within 20%) to the highest sex-specific 99th percentile URL.
3. **Concentration 3:** a concentration that challenges the upper analytical range of reportable cTn results (e.g., multiples above the 99th percentile concentration).
B. QC concentrations for contemporary cTn assays (ng/mL or μg/L units) with 3 decimal places:

1. Concentration 1: a concentration at or close to (within 20%) the overall 99th percentile.
2. Concentration 2: a concentration that challenges the upper analytical range of reportable cTn results (e.g., multiples from the 99th percentile concentration).

Note that the QC concentration that challenges the upper analytical range is dependent on the patient population served by the laboratory. High cutoffs (such as 10 × 99th percentile for patients undergoing coronary artery bypass grafting) (7), may be an appropriate level to monitor in hospitals that provide this procedure.

For hs-cTn assays, the IFCC TF-CB has established the analytical requirement that an assay has a %CV at the 99th percentile of ≤ 10% (10). For contemporary cTn assays, ≤ 20% CV at the 99th percentile is acceptable (7, 10). There are no published guidelines regarding what concentrations of QC material should be used to monitor performance of cTn testing. For contemporary cTn assays, the analytical sensitivity is not sufficient to reliably measure below the 99th percentile URL. For hs-cTn assays, it is necessary to monitor the assays’ performances within the reference interval and slightly above the 99th percentile URL, as previously recommended from the pre-hs-cTn era (11). Monitoring cTn testing at higher concentrations may also be useful for laboratory professionals developing or discussing serial change criteria, which are important for ruling in and ruling out acute cardiac injury, including MI, with clinicians (7, 10).

We recommend that QC manufacturers produce materials that meet these recommendations for all cTn assays. Until they are available, laboratories should obtain QC materials that comply as closely as possible to the recommended concentrations.

For hs-cTn assays, we acknowledge that there are no recommendations on allowable imprecision and bias at concentrations below the 99th percentile. Monitoring assay performance at or below the 99th percentile will provide laboratories with assay stability information near the clinical decision limits. Quantitative shifts at these low concentrations have previously been reported, and the analytical variation within the low concentration range may exceed some of the deltas reported in clinical studies (12, 13). According to the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) guidelines on models for deriving analytical performance specifications, the outcome-based model should be preferred for cardiac troponin (14, 15). Lyon et al. (13) have applied a simulation model for estimating the misclassification rate of patients with suspected acute myocardial infarction (AMI) when an hs-cTn assay is employed in conjunction with its 99th percentile limit. For example, a false-positive rate of approximately 1% was obtained when both bias and imprecision of measurements were kept around 10% (13). However, for laboratories whose physicians use the early rule-out algorithms as proposed by the European Society of Cardiology guidelines in management of patients without persistent ST-segment elevation (12), an error goal of < 1 ng/L for concentrations < 10 ng/L may be preferred to avoid patient misclassification (13). We recently proposed that a total analytic absolute error of < 3.5 ng/L for both hs-cTnT and hs-cTnL concentrations ≤ 10 ng/L be used as performance specification, which is based on long-term imprecision and bias estimates obtained with hs-cTn assays (16). This criterion, which we endorse, would fit the EFLM’s model 3 (state-of-the-art) and would represent < 35% of the total error, which is essentially identical to the recommendations made by the Australasian Association of Clinical Biochemists, who recommended a total error goal of ≤ 34% at 99th URL (17). These limits could be lowered with the development of cTn assays with even higher analytical sensitivity. Point-of-case tests should adhere to the same proposed QC concentrations for contemporary and hs-cTn assays.

**Assay Limits**

The IFCC TF-CB has recommended the LoD as the lowest reportable limit for determining an hs-assay designation (10). According to the Clinical and Laboratory Standards Institute, it is the lowest amount of analyte in a sample that can be detected with stated acceptable probability, although not quantified as an exact value (18). For laboratories not using this limit, this document recommends that the laboratory ideally convert to this approach, but at a minimum it should discuss and collaborate with its clinical users when a lower reportable limit that has a higher concentration, such as the limit of quantification (LoQ), is being used to define important assay metrics. Currently the US Food and Drug Administration (FDA) only allows reporting of cTn assays to the LoQ (typically the 20% CV concentration) (19). This definition of LoQ based on assay imprecision differs from the Clinical and Laboratory Standards Institute standard (18).

hs-cTn assays concentrations below the 99th percentile and above the LoD may have clinical utility (12). Accordingly, if clinicians are incorporating both detectable and nondetectable hs-cTn concentrations into their clinical decision-making process, then clinical laboratories should assess and monitor the lower reportable limit. If possible, this includes validating the limit of blank (LoB), LoD, and the LoQ of the hs-cTn assay before implementation to document the baseline performance within individual instrument(s) and across different reagent lots. If this is not possible, relying on peer-reviewed literature information is acceptable. Once established,
this parameter(s) should be used as a troubleshooting metric and/or as a quality indicator for bias and imprecision.

Recommendation 2: During initiation of hs-cTn testing, clinical laboratories should validate the LoB, LoD outside the US, or LoQ as applicable per FDA regulations in the US. These analytical parameters should be validated minimally on an annual basis or more frequently as deemed necessary.

There is evidence that drift over time at the 99th percentile medical decision concentration of hs-cTn assays is associated with reagent lot changes, calibrator lot changes, regularly scheduled instrument maintenance, and instrument to instrument variability, as well as instrument decline and malfunctioning (13, 16, 20–23). Concentration changes owing to these variables may go unnoticed and could contribute to analytical shifts in reportable cTn concentrations in the region of the 99th percentile URL, leading to clinical misdiagnosis or mismanagement (13, 21). These analytical deviations may be difficult to detect because common assessment tools like QC and many proficiency testing materials are not manufactured to challenge hs-cTn assays at such low concentrations. One exception is the UK National External Quality Assessment Service that regularly challenges low cTn concentrations. The current document endorses the IFCC TF-CB–recommended LoD, to be defined as the lower analytical reportable limit (10), or at the very least that the LoD be communicated to the clinical users, especially if a higher concentration such as the LoQ is being utilized for the lower reporting limit. With our current document, and subsequent studies demonstrating clinical value for very low cTn test results, we hope that the FDA will allow in vitro diagnostics companies and, in turn, clinical laboratories to report down to the LoD. Until then, the LoQ, which is almost always greater than the LoD, is the lowest value that can be reported in the US. There may be some labs in the US that use hs-cTn assays for risk stratification for primary care after proper validation. In this situation, measured hs-cTn values may be lower that the LoQ but ≥LoD, and therefore validation of the assay’s LoB and LoD is appropriate as a laboratory-developed test.

After determining and validating the lower reportable limit, it is important for laboratories to subsequently verify this metric and continually educate clinical users regarding what this value indicates. The understanding by clinicians is that different lower reportable limits may be possible for the same hs-cTn assay and may influence rule-out decisions based solely on detectable versus nondetectable concentrations. The LoB, LoD, and LoQ are determined by use of Clinical and Laboratory Standards Institute guidelines (24, 25). The clinical information provided by hs-cTn assays, especially at very low concentrations, portends a need for accuracy of results within a given assay. The validation of the lower analytical limit and subsequent monitoring will aid laboratories in ensuring that detectable hs-cTn concentrations can be consistently quantified over time (26).

Reporting Units

Since the release of cTn assays in the late 1990s, there has been a reduction in the 99th percentile URL due to improved analytical sensitivity, improved assay precision, use of better antibodies, increased number of antibodies, and other factors. With the redefinition of MI (the predecessor of the Third Universal Definition of MI) in 2000, the European Society of Cardiology and the American College of Cardiology recommended the use of the 99th percentile URL of a healthy population (7). This further reduced the cutoff concentration for AMI detection. With the emergence of hs-cTn testing and the analytical ability to reliably measure cTn below the 99th percentile, further changes are needed to highlight and aid clinicians in interpreting these lower cTn concentrations.

Recommendation 3: Report hs-cTn in whole numbers, using ng/L without decimal points. For reporting QC values, we recommend 1 decimal point. For contemporary cTn assays, units are reported in μg/L to 2 significant figures, with QC values reported to 3 significant figures.

For hs-cTn assays, the reporting of concentration units as nanograms per liter, as originally recommended by the IFCC TF-CB (10, 24), is endorsed, including the reporting of results as whole numbers; e.g., a contemporary cTn assay result of 0.014 ng/mL (μg/L) will be 14 ng/L for an hs-cTn assay (10, 24). Because the unit of nanograms per milliliter does not conform to conventions established by the Système international d’unités, SI units (8), we have recommended conversion to nanograms per liter, a concentration unit that has also been endorsed by the Third Universal Definition of MI (7). For analysis of QC data, use of an additional significant figure is needed to avoid round-off error, which can artificially increase the observed CV (22, 23). This is in contrast to contemporary cTn assays in which the QC material near the 99th percentile should be reported to 3 decimal places (ng/mL or μg/L), with patient results reported to 2 decimal places. The Committee recognizes that a conversion from nanograms per milliliter (micrograms per liter) to nanograms per liter for hs-cTn assays will require education and may initially cause confusion among clinicians; nevertheless, such conversion avoids ambiguity with the use of results that contain several zeros after the decimal point. The clinical laboratory plays an important role in educating the clinical staff well in advance of this change (10, 27).
99th Percentile Sex-Specific URLs

As we progress globally into the era of hs-cTn assays, reference interval studies using both hs-cTnI and hs-cTnT assays have demonstrated that the 99th percentile URL for men is substantially higher than that for women. The higher hs-cTn URL concentration for men is justified, as in the case of creatine kinase-MB, by the larger heart mass of men over that of women (28).

Recommendation 4: Use a defined reference population to report 99th percentile concentrations according to sex-specific cutoffs for hs-cTn assays. This recommendation is not relevant for contemporary cTn assays.

The IFCC TF-CB (10, 24) and the Third Universal Definition of MI (7) have endorsed sex-specific 99th percentiles recognizing that URLs for women are less than those of men (24). At least 300 men and 300 women are needed to appropriately define a 99th percentile URL for each sex. The approaches for determining the 99th percentile URL vary tremendously (29–33). Multiple studies have shown that the more rigorous one is in eliminating potential comorbidities, the lower the URL value becomes (29, 31, 32). When only a health questionnaire screening form is utilized, the 99th percentile URL will be higher than when reference individuals are excluded on the basis of cardiac medication use, including statins, and screening by abnormalities of specific surrogate biomarkers to identify silent pathologies: amino-terminal pro B-type natriuretic peptide (NT-proBNP) or BNP (hemodynamic stress), hemoglobin A1c (diabetes), and eGFR (renal insufficiency). Presently, there are no specific cutoff concentrations established to exclude individuals on the basis of the surrogate biomarker concentrations. We recommend the following cutoffs: NT-proBNP of 125 ng/L for reference individuals <75 years of age and 450 ng/L for individuals ≥75 years of age, or BNP at 35 ng/L; hemoglobin A1c of 6.5%; and an eGFR of either 60 or 90 mL/min/1.73 m² (according to the Chronic Kidney Disease Epidemiology Collaboration equation) (34), recognizing that a cutoff of 90 mL/min/1.73 m² not only excludes cases of mild renal insufficiency but also limits the number of individuals who could have been included, making recruitment for reference interval studies more difficult to achieve particularly for healthy elderly individuals (often the target group for acute coronary syndrome studies). It is not known if an exclusion threshold of 90 mL/min/1.73 m² will affect the hs-cTn values; for now, therefore, the committee favors the 60 mL/min/1.73 m² cutoff. Likewise, an HbA1c cutoff of <5.7% would exclude prediabetes, although this condition has not been shown to influence cTn results (35). Age-specific cutoffs for the natriuretic peptides could also be included. Ultimately, the use of imaging studies should be included as a routine part of reference-participant vetting, recognizing that their use is financially burdensome and challenging for clinical laboratories and in vitro diagnostic companies.

Ideally each study should strive to have sex-group reference individuals that are representative of the patient population observed in their geographic area for patients that present with symptoms suggestive of myocardial injury, including MI. Since age >60 years has been shown to bias the 99th percentile URL to higher concentrations (30–32), we recommend that the reference population include a distribution of individuals ≥20 years of age if they can be identified as “apparently healthy” on the basis of our proposed criteria. We do not currently recommend specific URLs by age/decade or by ethnicity, although a study has suggested that African-Americans may have a higher 99th percentile URL than Caucasians (31); additional studies are necessary to determine if there are racial differences. Finally, the statistical method used to determine the 99th percentile can substantially influence what 99th percentile URL is determined (33, 36). The 3 most common methods used are the nonparametric method, the Harrell–Davis bootstrap method, and the robust method. We recommend the use of the nonparametric method, which will allow for consistency between all 99th percentile URL determinations and avoid the stringency imposed by the other, more complex, statistical methods. When conducting these analyses, it is critically important to implement an appropriate strategy for removing outliers as the statistical methods used can be affected differently by outliers, leading to different 99th percentiles (36).

In January 2017, the FDA cleared the Gen 5® cTnT assay by Roche Diagnostics, reported to be an hs-cTnT assay, and included sex-specific 99th percentile URLs in the package insert. We recommend that manufacturers be transparent with the criteria for inclusion and exclusion of the apparently healthy reference subjects, as well as describing the statistical method used to determine the 99th percentile. Standardized approaches to establishing sex-specific 99th percentile URLs are necessary to improve comparisons across data sets and allow for consistency in diagnostic accuracy, outcomes risk assessment, and research studies/clinical trials.

While there is no question that there are sex differences in reference intervals for both hs-cTnT and hs-cTnI, the clinical utility of using sex-specific 99th percentiles has not been definitively proven. Relative to the mean value for the combined population, a lower cutoff for women will increase clinical sensitivity at the expense of clinical specificity, and the reverse will be true for men. Shah et al. reported that the use of sex-specific limits doubled the rate of MI diagnoses in women (37). Ultimately, clinical studies will determine the value of sex-specific reference cutoffs. In the TRAPID-AMI study, there was no significant impact when age-specific cutoffs for hs-cTnT were used on 1282 patients presenting to the
emergency department (ED) with suspected AMI (38). In a recent review, Eggers et al. also questioned the medical value of including sex-specific cutoffs (39). These investigators suggested use of a low AMI rule-out cutoff (e.g., 5 ng/L for cTnI) and higher sex-specific hs-cTn cutoffs for ruling in AMI. Both Eggers (39) and Gian-Nitiss (40) recommend that the analytical properties of each hs-cTn assay be considered when assigning cutoff concentrations. For risk stratification purposes, Cullen et al. suggested using a cutoff value that is lower than either the female or overall cutoff concentrations (41).

The studies using hs-cTnT have not examined this issue sufficiently; in addition, the cohorts studied may not have adequately represented the US patient cohort presenting to EDs. However, for the Abbott hs-cTnI assay (42) and other hs-cTn assays that are in development, the role of sex-specific URLs is clear. In addition, it is clear that when hs-cTn assays are used to differentiate chronic from acute disease, sex-specific cutoff values optimize performance. Presently, there are no hs-cTnT assays cleared by the FDA in the US. However, 6 hs-cTnI assays (Abbott, Beckman, Siemens, BioMerieux, Pathfast, and Singluex) have been cleared for use globally outside the US (Conformité Européenne, CE Mark) and have recommended sex-specific 99th percentiles. At this writing, we endorse the GTF-3rd MI recommendation regarding inclusion of sex-specific reference intervals.

**Definition of hs-cTn Assay**

Like many laboratory tests, cTn assays have undergone significant improvements since their initial release. To date, neither the FDA nor the Conformité Européene has identified criteria for labeling cTn assays with a “high-sensitivity” designation. Previously, the IFCC TF-CB has endorsed that hs-cTn assays needed to measure cTn concentrations at or above the LoD in ≥50% of an overall group of combined apparently healthy men and women (10, 24). We recognize that the GTF is based on analytical sensitivity and is subjective; e.g., there are differences in how the LoD for an assay is determined. An evidence-based approach based on improved clinical performance could also be used to define hs-cTn assays. However, there must be consensus regarding the criteria for improvement, either for diagnosis (e.g., statistically higher clinical sensitivity for early detection of AMI) or for risk stratification (e.g., statistically higher odds ratio for predicting short-term outcomes). Until such clinical criteria have been established, we will continue to endorse the use of analytical criteria.

As sex-specific 99th percentile URLs are now often recommended in clinical practice for hs-cTn assays (7), we recommend that both female-specific and overall reference groups need to meet these detection criteria individually for assays to be designated as an hs-cTn assay.

**Recommendation 5:** We recommend that assays unable to detect cTn at concentrations at or above the LoD in at least 50% of healthy men and women be labeled as contemporary cTn assays.

It is important to understand that the term “high-sensitivity” addresses an assay’s characteristics and not a difference in the forms of cardiac troponin (I or T) being measured (24). The IFCC TF-CB proposed that for an assay to be defined as high-sensitivity, 2 analytical criteria need to be met (10). First, the %CV at the 99th percentile URL should be ≤10%. Second, measurable concentrations should be attainable at a concentration at or above the assay’s LoD for >50% of healthy individuals (10). Our guidelines expand on this second point by requiring both men and women individually attain measurable concentrations, with at least 50% measurable concentrations above the assay’s LoD. The data to support these claims should be published in peer-reviewed journals, as well as by the manufacturer’s package inserts. A point of discussion is whether the LoB should be the discriminator instead of the LoD (43).

**Communication with Clinicians**

The most common issues that confound laboratory blood tests are preanalytic.Clinicians, in general, are not expert at recognizing these issues. This is particularly problematic with all draws from a central line, but most commonly venous central lines, where there can be problems resulting from inadequate removal of residual volume from the catheter when obtaining the sample. Clinicians need to be aware of the need to improve the way in which samples are collected when implementing hs-cTn assays, because at times, minor absolute concentration changes (2–4 ng/L) may translate into differences in clinical triage. For example, hemolysis always lowers cTnT and can increase values with some cTnI assays (44). In addition, interfering substances may adhere to the catheter if it is not flushed appropriately. Clinicians need to be aware of these concerns.

Analytical problems are estimated to occur in <1% of patients (45). These include “flyers” (nonreproducible increases in concentration), interfering antibodies, and macrocomplexes (immunoglobulins linked to cTn) leading to increases of cTn, as well as a variety of other interferences due to drugs such as heparin (45). There is also evidence that occasional increases of hs-cTnT can occur owing to skeletal muscle disease (46). Most often these issues cause increased cTn values that do not change acutely over time. Clinicians should be encouraged to ask for additional laboratory investigations when the reported hs-cTn values are not consistent with the clinical situation. This may include use of commercial products that detect the presence of the interfering antibodies and reduce their interference. When there are interfering sub-
stances in a sample, the apparent analyte concentration may not dilute linearly (47). In addition, cTn antibodies can cause lower values by binding to the troponin ternary complex (containing T, I, and C subunits), thus inhibiting antibody binding (48). On the basis of that understanding, these antibodies should reduce cTnI and cTnT similarly. Samples containing interferences to a cTn assay could be evaluated for evidence of myocardial injury by other cTn assays, which might not exhibit this interference. The in vitro diagnostic industry also has an important role in sensitizing clinicians to these sorts of issues. We advise that package inserts and educational materials as well as laboratory consultation be made available.

The analytical sensitivity of cTn assays varies substantially (24). This relates in part to assay-specific antibodies that differentially detect cTn complexes and fragments as well as to how assays are calibrated. It is important for clinicians to understand the relative sensitivities of the various assays in evaluating patients because many different cTn assays may be in use on different instruments and there may be patient transfers from one hospital to another. One way to evaluate sensitivity, especially with hs-cTn assays, is by determining the number of healthy individuals in whom a value is above the LoD of the assay (10). We endorse the use of this method, as initially published by the IFCC TF-CB, until a better approach, such as looking more directly at clinical sensitivity, can be developed. It is understood that the development of more robust metrics for such assessments are needed and that there may be a subjective component of this analysis until these approaches are developed. Laboratories need to be a conduit for information concerning the relative sensitivity of various assays to help their clinicians optimize clinical care. Directly transferring cTn results measured with different assays should be avoided (24, 45, 49). While there are different commercial assays that produce a reasonable degree of analytic correlation, it is generally not possible to accurately convert a cTn result from one assay to a different cTn assay. Even different cTn tests from the same manufacturers often cannot be compared. Given the importance of serial cTn measurements for the diagnosis of myocardial injury, all measurements from the same individual should be performed with the same assay. Finally, results from cTnT testing cannot be directly compared against cTnI on the same sample or the same patient or at concentrations below 100 ng/L between the fourth generation and Gen 5 cTnT assays (49).

Some laboratories have performed cTn testing on point-of-care platforms to achieve turnaround time goals and then test the same sample on a central laboratory platform that might be more sensitive. This “rebaselining” of the central laboratory result is an acceptable practice so long as delta changes (i.e., change in cTn results of the second sample from the first) are not computed between instruments or devices. If multiple platform testing is conducted, it is important to indicate the methodology used on the laboratory report as well as the assay-specific 99th percentile URL on this report. Communication between the laboratory, the provider, and the individual ordering these tests is essential. Explicitly stating the type of cTn assay and manufacturer (as currently recommended for tumor markers) will avoid confusion and aid in the interpretation of results as the LoD, LoQ, and the 99th percentiles will vary between assays. Finally, when transitioning to an hs-cTn assay from a contemporary assay, communication to the clinical users before the transition and specification that the test result was generated by use of an hs-cTn assay enables adoption of clinical guidelines associated with these specific assays. Providing this information will be useful for all healthcare organizations but is particularly important for institutions utilizing point-of-care cTn assays in addition to the laboratory-based assays.

**Recommendation 6: Laboratories should communicate with clinicians on the influence of preanalytic and analytic problems that confound hs-cTn assays. For institutions or health systems using 2 or more cTn assays, differences in the sensitivity of the various cTn assays should be explained to assist clinicians in understanding discrepancies when patients are transferred from other facilities.**

We acknowledge that while it is desirable to have the type (i.e., manufacturer, platform, version/generation) of cTn assay accompanying the result, this may not be feasible. However, all laboratories should, at the least, communicate to their clinical users the specific cTn assay in use prior to implementation and further communicate when versions or other changes that affect the analytical performance have occurred. Communicating the specific cTn assay maintained and utilized within the clinical laboratory to the healthcare providers will help mitigate confusion on interpretation and may facilitate uptake of guideline recommendations.

**Assay Variables**

There is substantial heterogeneity in cTn assays, and different cutoff values are used from one assay to the other and from one laboratory to another (50, 51). In addition, there are preanalytic and analytic issues associated with samples for the measurement of cTn (24). Accordingly, to facilitate comparisons across studies and to provide information critical for local implementation of suggested approaches, all studies must report the specific metrics utilized for the evaluation of cTn in all biomarker papers, compliant with Standards for Reporting Diagnostic Accuracy guidelines (52).

**Recommendation 7: Authors of studies using cardiac biomarkers, including hs-cTn, should document preanalytical and analytical variables important to the study and...**
be explicit concerning their postanalytical interpretative approaches.

The ability to compare data across studies is critical to effectively utilize any biomarker, including cTn. This is difficult because of the observed heterogeneity across cTn assays. Publications that exclude critical analytic parameters, such as assay manufacturer, instrument model, 99th percentile URL, LoB, LoD, LoQ, overall assay imprecision, sample type, and reagent lot number(s), are incredibly difficult to interpret because there is no ability to know whether or not the appropriate metrics were used (21, 24, 53). Accordingly, there is a need for consistency within the literature in reporting all these parameters used in any given study. They should specifically include the metrics listed above and further include how the samples were obtained, how long they were stored before they were processed, storage time and temperature, and the specifics for the statistical evaluations used for the interpretation of results.

Harmonizing and Standardizing

In 2001, an AACC subcommittee on standardization determined a degree of harmonization could be produced with use of a single reference material. The availability of this standard reference material (SRM 2921) has resulted in reducing the interassay variability between commercial cTn assays from as high as 88% down to 16% (54). We recognize that although additional standardization attempts are in progress, complete standardization will likely not be possible unless antibodies with the same characteristics are used across all manufacturers of cTn assays (24, 55, 56).

SRM 2921 was determined not to be ideal for use as a common calibrator for cTnI field methods (54). For this reason, a commutable serum-based SRM termed SRM 2922 is being developed and validated by the National Institute of Standards and Technology for use in standardization and harmonization of cTn methods. A strategy for the use of such a material was piloted by the IFCC WG on Standardization of Troponin I and demonstrated a significantly higher degree of measurement equivalence after mathematical recalibration. This study indicated that measurement harmonization or standardization would be effective at reducing interassay bias (57).

Recommendation 8: Commutable materials should be developed for use in harmonizing and standardizing cTn measurements.

To achieve harmonization or standardization, a system is required that provides reliable transfer of the measurement values from the highest available hierarchical concentration to field methods that are routinely used in clinical laboratories (58). Key elements of the system are the reference measurement procedure and appropriate reference material, which are assigned certified values with known uncertainty. Once a suitable SRM is available and has been value assigned, it can be used with manufacturers’ cTn-testing procedures to transfer values to the commercial calibrators intended for field use. However, to transfer values to the manufacturers’ procedures, the SRM used for this purpose must be commutable, i.e., demonstrate interassay properties similar to human samples. Although SRM 2921 has been available from the National Institute of Standards and Technology for over a decade, no truly commutable SRM for cTn is available. SRM 2922 is a serum-based, commutable material in development that could be used for standardization or harmonization.

Turnaround Time

The previous LMPG have recommended a turnaround time of 60 minutes or less from the time of blood collection to the reporting of results through the laboratory information system (1, 2). Clinical laboratories often track turnaround times from the moment samples are received in the laboratory to the reporting of results. Clinical staff who are not accountable to clinical laboratory personnel are generally responsible for blood collection in the ED and subsequent sample transport to the laboratory. However, the overall turnaround time is a measure of laboratory quality, and therefore collaborative approaches should be pursued to decrease the overall collection-to-result timing interval. To meet these requirements, laboratories have instituted fast-track protocols, including use of pneumatic tubes for sample delivery, computer order entry by physicians in the ED, and dedicated pathways to quantify cardiac troponin (59).

Recommendation 9: Cardiac troponin results should be reported within 60 minutes or less of when a sample is received. There should be continued efforts to improve this to a time of 60 minutes from when the sample was collected.

Changes in cTn Over Time

In the appropriate clinical setting, improved analytics of hs-cTn assays for detection of myocardial injury have led some ED physicians to rule out AMI based on a single hs-cTn result below the LoD, as well as rule in AMI by use of a 0 h to ≤3 h serial protocol of rising cTn values (60). However, because several other medical conditions increase cTn in the absence of MI, serial cTn testing remains relevant.

Cutoff concentrations for denoting a statistically significant change in serial cTn results must be established from biological variability studies by calculating the reference change value (RCV). These estimates, derived from studies of healthy individuals, could not be determined accurately by cTn assays with analytical sensitivity
incapable of measuring the marker concentrations in all samples from the recruited individuals. Using hs-cTn assays, biological variation studies have shown that cTn has a low index of individuality; i.e., intraindividual variances are only a fraction of the total group variances (61). This implies that serial testing provides a better means for diagnosis than use of a population-based upper reference interval. A significant change in cTn concentrations on samples collected over time improves the clinical specificity of cTn.

Recommendation 10: The laboratory should help educate clinicians on the importance of specific metrics by which true clinical changes in cTn concentrations can be distinguished from analytical and biological variabilities.

When “contemporary” cTn assays are used (and to a lesser extent for hs-cTn assays), serial cutoff concentrations cannot be determined for patients who present to the ED with chest pain symptoms when the initial cTn results are below the assay LoD. While biological variation studies can be used as a starting point for the RCV that is statistically significant, the actual RCV must be empirically determined through a clinical trial for each commercial assay with the understanding that RCVs differ between rising and falling cTn concentrations. Currently, absolute changes in cTn concentrations, rather than relative (percent) changes, appear to be preferred for hs-cTn assays when concentrations are low (8, 12, 62). When cTn concentrations are high, a relative RCV cutoff may be more appropriate.

Summary

These recommendations have been developed to provide consistency and knowledge in areas where formal guidelines and/or a substantial data-driven literature was lacking. They were crafted by a core group from the AACC Academy with expertise in laboratory medicine and vetted extensively through the IFCC Committee tasked with the development of educational materials to assist in the implementation of cardiac biomarker assays, including cTn. This group contains experts in laboratory medicine and cardiology, and representation from the in vitro diagnostic industry. It is our hope that by providing suggestions in these areas, we can help to fill gaps in our practices and by doing so, move the field forward. As with all consensus statements, we welcome the field’s agreement and, particularly, new data that will move the field forward.

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References

Special Report


