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A New Season for Cardiac Troponin Assays: It's Time to Keep a Scorecard

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Advancements in cardiac troponin (cTn)[2] assay technology have created a conundrum for clinicians and laboratory scientists, who must determine which assays are best for optimal patient care. Unfortunately, few resources are available to guide the medical and scientific communities in this regard. International guidelines *(1–3)* have defined an increased cTn above the 99th percentile limit as an abnormal result; what is lacking, unfortunately, is an approach to define this limit across the heterogeneity of the assays. In spite of the evidence-based literature demonstrating that cTn concentrations tend to increase in individuals >60 years old *(4)*, 99th percentile reference limits are often determined across wide age ranges using subjects as old as 70 years (convenience samples). Further frustrating the problem of selecting relevant reference subjects, in clinically defined "normal" individuals without known cardiovascular disease, increased cTn concentrations are indicative of a significantly higher risk of death *(4, 5)*. The occurrence of such individuals in reference populations may reflect inadequate screening for comorbidities at the time of sample acquisition. Given such problems, a majority of laboratories either accept the manufacturer's reference limit from the US Food and Drug Administration (FDA)-cleared package insert, perform an underpowered normal range study to establish a reference limit, or accept a reference limit published in the literature. To validate cTn assays, however—to level the playing field for all users—is necessary for the best patient care.

cTnI and cTnT are established as the standard biomarkers for the detection of myocardial injury and prognostic evaluation of patients with acute coronary syndrome (ACS) and without *(1–3)*. The consensus guidelines from the Global Task Force for the Universal Definition of Myocardial Infarction *(1)* and the National Academy of Clinical Biochemistry *(2)*, plus the updated American College of Cardiology/American Heart Association guidelines *(3)*, have recommend that, in patients who present with ischemic symptoms, at least 1 cTn concentration higher than the 99th percentile value during the first 24 h after onset of symptoms indicates myocardial necrosis consistent with myocardial infarction (MI). A rising and/or falling pattern of cTn is typically sought to distinguish increased cTn caused by a chronic, nonischemic pathophysiology from an acute ischemic presentation indicative of an evolving MI. It is been further recommended that only cTn assays with appropriate quality control and optimal total imprecision (CV ≤10%) at the 99th percentile value ("guideline acceptable," Table 1) be used. Better imprecision at low cTn concentrations appears to improve the value of cTn as both diagnostic and risk indicator *(2, 4)*. Use of cTn assays with intermediate imprecision (10% to 20% CV) at the 99th percentile, however, does not lead to significant patient misclassification when interpreting serial cTn results *(6)*. This evidence serves as part of the proposed scorecard designation of "clinically usable" (Table 1).

One challenge that arises as the FDA clears improved cTn assays with higher analytical sensitivity for use in laboratory practice is determining how these new assays compare to older assays already in the marketplace. Diagnostic sensitivities using specimens collected at presentation for detection of MI have improved from 15%–35% for early cTn assays to 50%–75% for contemporary assays *(7–9)*. Over the past 10 years, the science and technology of cTn assays has improved to allow measurement of this biomarker with greatly improved precision at concentrations approaching an assay's limit of detection *(10, 11)*. Much confusion has been generated, however, as cTn assays are neither standardized nor harmonized, and likely never will be—every assay uses a different set of antibodies for capture and detection of circulating cTn forms in blood *(12)*. Using a high-sensitivity (hs) cTn assay, changes over 4 h as small as 2 ng/L at cTn concentrations between 3 and 8 ng/L (which is less than any measurable contemporary FDA-cleared assay) are associated with transient stress-induced myocardial ischemia detected by myocardial perfusion imaging *(11)*. The development of assays able to measure small cTn concentration changes or deltas within the normal

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[2] Nonstandard abbreviations: cTn, cardiac troponin; FDA, US Food and Drug Administration; ACS, acute coronary syndrome; MI, myocardial infarction; hs, high-sensitivity.

Table 1. Scorecard designations of cTn assays.

Acceptance designation	Total imprecision at the 99th percentile, CV%
Guideline acceptable	≤10
Clinically usable	>10 to ≤20
Not acceptable	>20
Assay designation	**Measurable normal values below the 99th percentile, %**
Level 4 (third generation, hs)	≥95
Level 3 (second generation, hs)	75 to <95
Level 2 (first generation, hs)	50 to <75
Level 1 (contemporary)	<50

range, with acceptable imprecision, will improve the ability to distinguish patients with acute disease (rising cTn) from those with more chronic disease (static cTn) *(9, 12)*.

Since 1996, numerous formulations of cTn assays have been released with improvements in reagent and antibody configurations. Manufacturers are now developing a generation of hs cTn assays that are more precise at low concentrations and measure cTn concentrations less than 1 ng/L (0.001 μg/L) *(10)*, which is lower than possible with the current FDA-cleared assays. Recent discussions between the FDA and leaders representing laboratory medicine, cardiology, emergency medicine, and industry have focused on the need to better define how these new hs cTn assays compare with the current and older generations of cTn assays and the need to set standards of performance to better define the quality specifications of new assays that will be submitted for FDA clearance. The goal is to better define the playing field for assays to aid in the diagnosis of MI and better stratify patients by risk of adverse events. To summarize these discussions, the optimal goals that the FDA would like to achieve include (1) to transition from the current practice of approving assays based on higher ROC-optimized cutoffs to use of the 99th percentile value; (2) to ensure that cTn assays are accurate enough at the 99th percentile values for clinical use; and (3) to define the effect of implementing the 99th percentile value in clinical practice on minimizing false-negative and -positive findings. Understanding what is truly normal for cTn, once incorporated into clinical practice, will be a major step forward in the cardiac biomarker field.

To solve the conundrum of assay-to-assay differences, a head-to-head comparison must be made of the leading marketshare assays used in clinical practice, as well as hs cTn assays that will enter the marketplace within 2 years. One rational approach would be to use a common normal reference population within a focused, young, healthy age group to establish 99th percentile values. Multiple studies using contemporary, first-generation, and newer hs cTn assays have demonstrated that the cTn 99th percentile value strongly depends on the composition of the individuals within the reference population *(13, 14)*. In 1 study examining 4 contemporary cTn assays, the absolute 99th percentile reference concentrations and the number of measurable concentrations below the 99th percentile concentration varied from 1% to 67% across the assays *(14)*. Studies using hs assays detect cTn in almost 100% of normal samples, however, appearing near-gaussian in distribution *(4, 10)*.

To overcome the barrier for accurate interpretation of cTn values in clinical practice, I propose a 2-tier system of analysis using both the 99th percentiles and imprecision values at the 99th percentile, based on a young, healthy reference population that is diversified by sex, race, and ethnicity. This approach establishes a real scorecard, as described below, to capture the essence of which assays are acceptable for use in clinical practice, thereby assisting the FDA in assay clearance criteria, as well as assisting in the transition to the future generations of hs cTn assays. The proposed assay-dependent scorecard shown in Table 1 is based on designations of the total imprecision (CV, %) of each assay at the 99th percentile and how many specimens from normal individuals have cTn concentrations that are actually measurable below the 99th percentile. The ultimate goal is to have all assays be "third generation (level 4), guideline acceptable." In this issue of *Clinical Chemistry,* Collinson et al. *(15)*, using the Siemens cTnI Ultra assay, describe the 99th percentile value from 309 fully characterized normal individuals to be 0.039 μg/L, with 46% of subjects having measureable concentrations. Further, they determined the 10% CV to be 0.045 μg/L. Using the scorecard approach, this assay would be designated a "level 1 (contemporary), clinically useful" assay, not a high-sensitivity assay as claimed. Table 2 provides scorecard designations for all cTn assays posted on the IFCC website (http://www.ifcc.org/PDF/IFCC_Troponin_Web_Page_Table_of_Assays_Oct_2008.pdf), based on the manufacturer's package insert claims and the published literature. The importance for peer-reviewed literature studies to validate the manufacturer's claims is demonstrated by the difference in designation between the Collinson study *(15)* and the manufacturer's data that places the Siemens Ultra assay in a more favorable light (as guideline acceptable). The scorecard approach allows users to verify whether a manufacturer's claims—guideline acceptable vs clinically usable, "contemporary" vs "high sensitivity"—are valid.

The laboratory impact of applying such a scorecard approach in an assay-to-assay comparison using

Table 2. cTn assay scorecard designations by individual assays.

Company/platform/assay[a]	99th percentile, μg/L	10% CV, μg/L	Acceptance designation	Assay designation
Abbott AxSYM ADV	0.04	0.16	Not acceptable	Level 1
Abbott Architect	0.028	0.032	Clinically usable	Level 1
Abbott i-STAT	0.08	0.1	Clinically usable	Level 1
Beckman Access Accu	0.04	0.06	Clinically usable	Level 2
bioMerieux Vidas Ultra	0.01	0.11	Not acceptable	Level 1
Innotrac Aio!	0.025	0.06	Clinically usable	Level 1
Inverness Biosite Triage	<0.05	NA[b]	NA	Level 1
Inverness Biosite Triage (r)	0.056	NA	Clinically usable	Level 1
Mitsubishi PATHFAST	0.029	0.014	Guideline acceptable	Level 1
Ortho-Clinical Diagnostics Vitros ECi ES	0.034	0.034	Guideline acceptable	Level 1
Radiometer AQT90	0.023	0.039	Clinically usable	Level 1
Response Biomedical RAMP	<0.1	0.21	Clinically usable	Level 1
Roche Elecsys 2010	<0.01	0.03	Clinically usable	Level 1
Siemens Centaur Ultra	0.04	0.03	Guideline acceptable	Level 1
Siemens Dimension RxL	0.07	0.14	Clinically usable	Level 1
Siemens Immulite 2500 STAT	0.2	0.42	Not acceptable	Level 1
Siemens Stratus CS	0.07	0.06	Guideline acceptable	Level 1
Siemens VISTA	0.045	0.04	Guideline acceptable	Level 1
Tosoh AIA II	<0.06	0.09	Clinically usable	Level 1
Research hs assays[c]				
Beckman Access hs-cTnI	0.0086	0.0086	Guideline acceptable	Level 4
Roche Elecsys hs-cTnT	0.013	0.012	Guideline acceptable	Level 4
Nanosphere hs-cTnI	0.0028	0.0005	Guideline acceptable	Level 3
Singulex hs-cTnI	0.0101	0.00088	Guideline acceptable	Level 4

[a] Per manufacturer's package insert.
[b] NA, insufficient information to designate.
[c] Per published literature.

the same population would be to gain a better understanding of what is considered a normal cTn concentration, using either a single cutoff or a cutoff defined by sex, race, and/or ethnicity. The likely clinical effects of using assays rated as guideline acceptable or clinically usable by the scorecard are (1) emergency medicine physicians will achieve improvements in triage through earlier ruling out (improved specificity) and ruling in (improved sensitivity) of MI patients; (2) cardiology and internal medicine physicians will see improved outcomes for both inpatients (hospitalized, short-term risk) and outpatients (posthospitalization, long-term risk); (3) other medical specialty physicians will be better able to identify patients, often without clinical symptoms, who may be at risk of cardiac-related adverse outcomes; and (4) clinical trial investigators will be able to identify appropriate and optimal patient enrollment and outcome measures.

The season is here, now, to provide an evidence-based scorecard for educating clinicians and laboratories on the strengths and weakness of each cTn assay used in clinical practice, and in applied and translational studies and trials.

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