Clinical Chemistry 63:1
73–81 (2017)

Mini-Reviews

Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care

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BACKGROUND: Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) determinations are fixtures in clinical practice and research. Cardiac troponin testing has been the standard of practice for the diagnosis of acute myocardial infarction (AMI), early rule-out, risk stratification, and outcomes assessment in patients presenting with acute coronary syndrome (ACS) and non-ACS myocardial injury. We recognize from reading the literature over the past several years how poorly understood the analytical characteristics are for cTnI and cTnT assays by laboratorians, clinicians, and scientists who use these assays.

CONTENT: The purposes of this mini-review are (*a*) to define limit of blank, limit of detection, limit of quantification, and imprecision, (*b*) overview the analytical characteristics of the existing cardiac troponin assays, (*c*) recommend approaches to define a healthy (normal) reference population for determining the 99th percentile and the appropriate statistic to use for this calculation, (*d*) clarify how an assay becomes designated as "high sensitivity," and (*e*) provide guidance on determining delta (Δ) change values.

SUMMARY: This review raises important educational information regarding cTnI and cTnT assays, their 99th percentile upper reference limits (URL) differentiated by sex, and specifically addresses high-sensitivity (hs)-assays used to measure low concentrations. Recommendations are made to help clarify the nomenclature and analytical and clinical characteristics to define hs-assays. The review also identifies challenges for the evolving implementation of hs-assays into clinical practice. It is hoped that with the introduction of these concepts, laboratorians, clinicians and researchers can develop a more unified view of how these assays should be used worldwide.

Cardiac troponin I (cTnI)[5] troponin T (cTnT) determinations are the standard of practice for the diagnosis of acute myocardial infarction (AMI) and have been gaining acceptance for early rule-out when using high-sensitivity (hs)-assays *(1–7)*. Cardiac troponin also has been important for risk stratification and outcomes assessment in patients presenting with acute coronary syndrome (ACS) and non-ACS myocardial injury, and may have potential in primary prevention *(8)*. What has become apparent is how poorly understood cTnI and cTnT assays are by those who use these assays. This is evident by reading the methods sections of peer-reviewed studies, recognizing that the analytical characteristics of both cTnI and cTnT assays tend to be misrepresented along with how appropriate cutoffs, 99th percentile upper reference limits (URLs), are defined from study to study *(9)*.

cTnI and cTnT assays have replaced creatine kinase (CK) MB because of their myocardial tissue specificity *(1, 10)*. While cTnI remains true to this concept, studies have recently demonstrated that immuno-reactive proteins, whether cTnT isoforms or other proteins, do appear to cross-react with the cTnT assays that are marketed by Roche in a small subset of neuromuscular disease pathologies that have been studied *(11, 12)*. Clinicians need to be aware of the possibility that noncardiac increases in cTnT may occur (the current prevalence of such increases is likely small but not clear), and may lead to possible false-positive diagnosis of cardiac injury when skeletal muscle pathology is present. Both cTnI and cTnT assays demonstrate a lack of standardization *(13)*.

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Received July 22, 2016; accepted August 17, 2016.
Previously published online at DOI: 10.1373/clinchem.2016.255109

[5] Nonstandard abbreviations: cTnT, cardiac troponin T; cTnI, cardiac troponin I; AMI, acute myocardial infarction; hs, high-sensitivity; ACS, acute coronary syndrome; CK, creatine kinase, POC, point of care; FAB, fragment antigen binding; URL, upper reference limits; LoQ, limit of quantification; LoB, limit of blank; LoD, limit of detection; NPV, negative predictive value; TF-CB, Task Force on Clinical Applications of Cardiac Biomarkers; MI, myocardial infarction; RCV, reference change values.

While hs-cTn assays are starting to receive increasing usage worldwide, in the US they have not yet been cleared by the FDA *(14)*. It is important that cardiac troponin users do not lose sight of the understanding that contemporary and point of care (POC) assays still maintain a large market share in clinical practice *(15)*. The purpose of this mini-review is to address important characteristics of cTnI and cTnT assays. This review does not supplant present guidelines but does offer recommendations in several areas pertaining to the optimal utilization *(16)* of cardiac troponin testing based on assay characteristics.

Cardiac Troponin Assays

CARDIAC TROPONIN T

The hs-cTnT (fifth generation) assay (Roche Diagnostics) uses fragment antigen binding (FAB) portions of 2 cTnT-specific mouse monoclonal antibodies (MAbs) directed against epitopes in the central region of human cTnT. The capture antibody (M7) is biotinylated and directed against an epitope at amino acid residues 125–131 identical to that in the previous, fourth-generation assay. The detection antibody is directed against an epitope at amino acid residues 136–147. The original antibody (M11.7) has been reengineered, with the constant C1 region of the FAB being replaced by a human IgG C1 region to produce a mouse–human chimeric detection antibody *(15)*. The assay is calibrated against recombinant human cTnT produced in *Escherichia coli* cell culture *(15)*. Assay calibration is not identical to that of the fourth-generation assay, so identical samples measured with the fourth-generation and hs-cTnT assays will give different results. The 99th percentile upper reference limit (URL) of 10 ng/L with the fourth-generation cTnT assay corresponds to 30 ng/L with the hs-cTnT assay *(17)*.

CARDIAC TROPONIN I

MAbs specific to different epitopes of cTnI are able to recognize multiple modifications circulating in the blood. Different forms of the cTnI antigen used as standards or calibrators have helped to improve correlations between different commercial assays by more than 10-fold, but standardization is unlikely *(15)*. An IFCC working group is addressing whether cTnI harmonization can be achieved, predicated on a pooled serum-based secondary reference material *(18)*. Even with the use of a mathematical formula, harmonization between assays remains elusive. The most common reason for the discrepancy in cTnI measurements is the difference in epitope specificity of antibodies used in different assays. Even for assays that employ similar antibodies, different numeric concentrations are found. cTnI measurements are influenced by multiple factors, including proteolytic degradation, phosphorylation, and complexing with cTnC, heparin, heterophile or human antimouse antibodies, and cTnI-specific autoantibodies *(15)*. MAbs are often selected in such a way that if one of the MAbs (capture or detection) is sensitive to the presence of an antigen in the sample, then the other MAb should be insensitive to the same antigen. cTnI has been shown to be cleaved by endogenous proteases during incubation of necrotic myocardial muscle after an AMI *(15)*. The vast majority (>95%) of cTnI in blood occurs as a binary cTnI–cTnC complex. Consideration of assays including an anti-cTnC antibody may be useful in improving the analytical sensitivity. Antibodies used in assays should recognize all circulating cardiac troponin forms on an equimolar basis.

Definitions: Limits of Blank, Detection, and Quantification

With the advent of hs-assays and the emphasis on imprecision (% CV) of assays at the 99th percentile URL and the limit of quantification (LoQ), along with the increasing role of using the limit of blank (LoB) and the limit of detection (LoD) as cutoffs for early rule-out of AMI, understanding what these values mean is important *(19)*; all are analytical parameters used to describe the low concentrations of cardiac troponin measurements. Understanding these terms is related to the emerging clinical evidence suggesting that at "undetectable levels," concentrations less than either LoB or LoD, clinicians can potentially safely rule-out AMI, using a single cardiac troponin, based on very high negative predictive value (NPV) and high clinical sensitivity *(1–7)*. The LoB is the highest cardiac troponin concentration expected to be found when replicates of a sample containing the zero calibrator for a cardiac troponin assay are tested. Statistically, it is often represented as: LoB = mean (zero calibrator) + 1.645 × SD (zero calibrator). In the US, the LoB is not clinically reportable, as high imprecision (% CV >20%) makes a result at this concentration unreliable and one that should not be used. The clinical utilization of the LoB concentration is not clear at present. LoD, a concentration greater than the LoB, is the lowest detectable cardiac troponin concentration reliably distinguished from the LoB in a sample containing a low cardiac troponin concentration that can confidently be reported for clinical use. In the US, laboratories often may not be able to report cardiac troponin at the LoD because available contemporary (generation of assays pre-hs assays) and POC cardiac troponin assays have imprecisions >20% at this concentration, and the FDA only allows manufacturers to report cardiac troponin assay results at concentrations less than the lowest concentration that has a %CV (total imprecision) of ≤20%. Thus, the lowest cardiac troponin concentration that demonstrates a 20% CV is defined as the LoQ.

Total Imprecision (% CV) at 99th Percentile

Day-to-day imprecision of cardiac troponin assays is defined by the % CV and is determined using multiple lots of both reagents and calibrators over multiple days *(19)*. The CLSI EP5-A2 document details the evaluation protocol that spans 20 days, 2 repeats a day, with 2 different lots used for reagents and calibration materials. For clinical use, cardiac troponin assays have been deemed "guideline acceptable" if they have a % CV of ≤10% at the 99th percentile, "clinically usable" if the % CV is >10% to ≤20%, and "not clinically acceptable" if the % CV is >20% *(20, 21)*. The hs-assays have less analytical noise and meet the highest standard of clinical-practice guideline precision recommendations (% CV ≤10%) at the 99th percentile, whereas contemporary and POC cardiac troponin assays have a % CV between 10% and 20% at the 99th percentile. Using hs-cTn assays decreases analytical noise, allowing reporting of real cardiac troponin increases above the 99th percentile indicative of myocardial injury, rather than increases in cardiac troponin resulting from analytical imprecision, thereby improving diagnostic accuracy. The Third Universal Definition of MI guidelines recommends preferential use of assays that demonstrate a total CV of ≤10% at the 99th percentile *(1)*, but also supports the use of assays with a CV of ≤20%, because a 20% CV does not lead to misclassification of patients in diagnostic or risk-assessment management *(20)*.

Table 1 shows that all hs-cTn assays attain a CV at the 99th percentile ≤10%. Such information is useful to laboratorians and clinicians because it allows them to confidently report hs-cTn values and serial changes in cardiac troponin results over time that are unaffected by analytical noise. The true test of how well the % CV of assays will hold up is when assays are used daily in clinical practice and QC materials are evaluated over weeks. Presently, only the Abbott hs-cTnI and the Roche hs-cTnT assays are used in clinical practice, and appear to have maintained the <10% CV quality characteristic at the 99th percentile *(22, 23)*. Contemporary assays often reported in the manufacturers' package insert as being able to achieve a 10% CV at the 99th percentile are rarely able to do so in clinical laboratory practice.

99th Percentile

The cardiac troponin 99th percentile URL corresponds to the clinical-practice guideline concentration for the diagnosis of AMI *(21)*. This URL is typically derived from apparently healthy individuals enrolled in studies by manufacturers or investigators *(9)*. For hs-cTn assays, the IFCC Task Force on Clinical Applications of Cardiac Biomarkers (TF-CB) has proposed recommendations for determining 99th percentiles *(21)*. Sex is an important factor influencing the 99th percentile, and sex-specific 99th percentiles are recommended to be reported for clinical use when using hs-assays. Contemporary and POC *(24)* assays do not have the analytical sensitivity to adequately differentiate 99th percentiles by sex, and thus only require a single over-all (men plus women) 99th percentile. The use of a single diagnostic cardiac troponin threshold for hs-cTnI assays, as studies for hs-cTnT are limited, has been shown to contribute to the under diagnosis of AMI in women, whereas with sex-specific cutoffs the proportion of men and women with the diagnosis of AMI is similar *(22, 25)*. For hs assays, cardiac troponin results should be reported in whole numbers in nanograms per liter (ng/L) to distinguish them from contemporary and POC assays. While globally endorsed, the 99th percentile is still not uniformly applied in clinical practice. Only 30% to 50% of laboratories have reported using the 99th percentile, thereby compromising the diagnosis of AMI in practice as well as myocardial infarction (MI) endpoints in clinical trials *(26)*. Although concentrations with hs-cTn assays increase with age, this is likely due to comorbidities, and changing URLs with age for clinical use would add complexity and disadvantage the more healthy elderly.

Defining Healthy Reference Populations for Determining 99th Percentile

99th Percentiles for contemporary, POC, and hs-assays are shown in Table 1. There is poor consistency in the composition or numbers of individuals enrolled for determining the 99th percentile. Defining what constitutes a healthy reference individual is a topic of debate *(21)*. Should individuals be apparently healthy, young individuals, or should they be age-matched patients hospitalized without known cardiovascular disease, similar to the demographics of patients who rule in for an AMI, with ages of 30–90 years? How does one determine who is healthy? Should individuals be selected: (*a*) via personal interview with questions addressing known cardiac medications, such as statins; (*b*) after obtaining information about known diseases associated with cardiovascular disease, such as renal disease or diabetes; or (*c*) via definitive physician evaluation of an individual after taking a history and physical examination, including an electrocardiogram, echocardiogram or imaging? The third option, while ideal, is cost-prohibitive. Sequential selection of a reference population on this basis has been shown to shift the derived 99th percentile to lower values *(27)*. We recommend a 2-fold approach in which both younger (<30 years) and older (>30 years, with median age of 60–65 years to be representative of cardiac patients), apparently healthy reference groups are recruited. Inclusion criteria should be based on data obtained from a history of medications (not on cardiac drugs), known

Table 1. Analytical characteristics of commercial and research contemporary, POC, and hs-cTnI and hs-cTnT assays.[a]

Company/platform/assay	LoD, µg/L	99th, µg/L	% CV at 99th	10% CV, µg/L	Epitopes/antibodies[b]	Detection tag
Contemporary assays						
Abbott ARCHITECT	<0.01	0.028	15	0.032	C: 87–91, 24–40; D: 41–49	Acridinium
Beckman Access 2	0.01	0.02	14	0.040	C: 41–49; D: 24–40	ALP
Beckman Coulter DxI	0.01	0.03	14	0.040	C:41–49; D:28–80	ALP
Ortho-Clinical Diagnostics Vitros	0.012	0.034	10	0.034	C: 24–40, 41–49; D: 87–91	HRP
Siemens Centaur Ultra	0.006	0.04	10	0.030	C: 41–49, 87–91; D: 27–40	Acridinium
Siemens Dimension RxL	0.04	0.07	20	0.140	C: 27–32; D: 41–56	ALP
Siemens VISTA	0.015	0.045	10	0.040	C: 27–32; D: 41–56	Chemiluminescent
Tosoh AIA	0.06	<0.06	8.5	0.090	C: 41–49; D: 87–91	ALP
Roche 4th generation cTnT	0.01	0.01	18	0.03	C: 125–131; D: 136–147	Ruthenium
POC assays						
Abbott i-STAT	0.02	0.08	16	0.10	C: 41–49, 88–91; D: 28–39,62–78	ALP
Alere Triage	0.05	<0.05	NA	NA	C: NA; D: 27–40	Fluorophor
bioMérieux Vidas	0.01	0.01	27	0.11	C: 41–49, 22–29; D: 87–91, 7B9	ALP
LSI Medience PATHFAST	0.008	0.029	5.1	0014	C: 41–49; D:71–116, 163–209	ALP
Radiometer AQT90 cTnI	0.0095	0.023	17	0.039	C: 41–49, 190–196; D: 137–149	Europium
Radiometer AQT90 cTnT	0.01	0.017	20	0.03	C: 125–131; D:136–147	Europium
Response Biomedical RAMP	0.03	<0.1	18	0.21	C: 85–92; D: 26–38	Flourophor
Roche Cardiac Reader	<0.05	<0.05	NA	NA	C: 125–131; D:136–147	Gold particules
Siemens Stratus CS	0.03	0.07	10	0.06	C: 27–32; D: 41–56	ALP
Trinity Meritas	0.019	0.036	17	NA	C: 24–40; 41–49, D: 88–90, 137–148,190–196	Fluorophor
ET Health	0.1	0.2	NA	0.42	C: 87–91:D: 27–40	ALP
Nanomix	0.15	NA	NA	0.64	C: 87–91:D: 27–40	ALP

hs-Assays	ng/L	M/F, ng/L	% CV at 99th	10% CV, ng/L	Epitopes/antibodies	Detection tag
Abbott ARCHITECT hs-cTnI	1.2/1.9	34/16	5	3	C: 24–40; D: 41–49	Acridinium
Beckman Coulter Access hs-cTnI	2.5	52/23	<10	8	C: 41–49; D: 24–40	ALP
Ortho-Clinical Diagnostics hs-cTnI	1.0	19/16	<10	3	C:24–40, 41–49; D: 87–91	HRP
Roche E170 hs-cTnT	5	20/13	8	13	D: 125–131; C: 136–147	Ruthenium
Siemens Vista hs-cTnI	0.5	55/33	5	3	C: 30–35; D: 41–56,171–8	Luminescence
Singulex Errena hs-cTnI	0.09	36/30	5	0.9	C: 41–49; D: 27–41	Fluorescence

[a] Assays not designated as cTnT are cTnI.
[b] C, capture antibody; D, detection antibody; NA, not available.

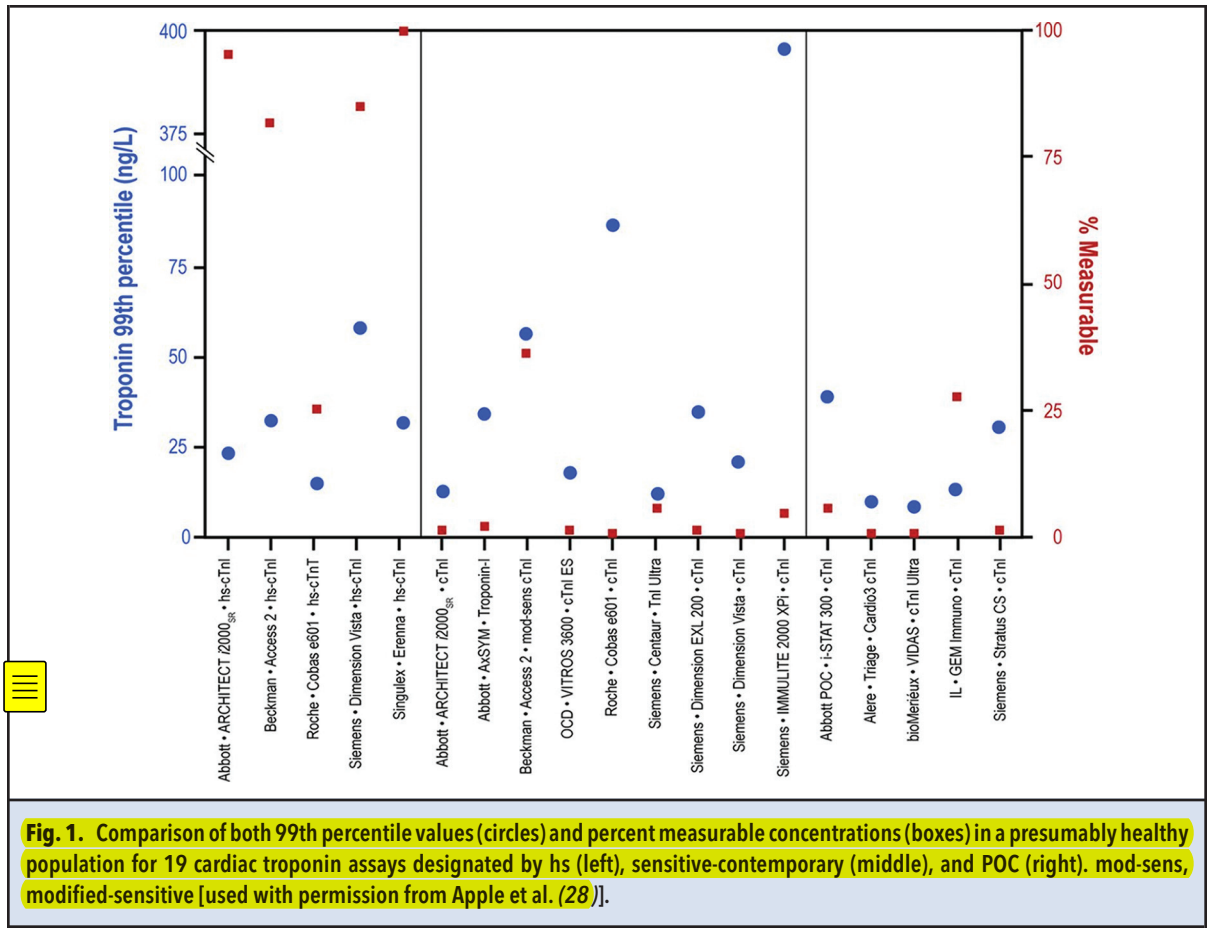

Fig. 1. Comparison of both 99th percentile values (circles) and percent measurable concentrations (boxes) in a presumably healthy population for 19 cardiac troponin assays designated by hs (left), sensitive-contemporary (middle), and POC (right). mod-sens, modified-sensitive [used with permission from Apple et al. *(28)*].

underlying diseases, and blood measurements, using a natriuretic peptide concentration (assay dependent) that would provide a high NPV for ruling out heart failure to serve as a surrogate biomarker for underlying myocardial dysfunction, and an estimated glomerular filtration rate (eGFR) >60 mL · min^{-1} m^{-2} for defining underlying renal insufficiency. At present, no definitive peer-reviewed surrogate biomarker recommendations have been published. Men and women should be equally represented, with a diverse racial and ethnic mix. The number of individuals for determining a 99th percentile has been defined by the IFCC TF-CB to be a minimum of 300 for men and 300 for women. No study has compared all contemporary sensitive assays and/or hs-assays within the same reference or disease population *(28)*. At the 2016 national AACC meeting, a reference interval blood draw study was performed that allowed manufacturers to purchase 535 plasma or serum samples for use in determination of a 99th percentile.

To solve the conundrum of assay-to-assay differences, direct comparisons of contemporary assays used in clinical practice with hs-cTn assays already in the marketplace are needed. In 1 study examining multiple contemporary, POC, and hs-assays, the ability of the various assays to measure concentrations below the 99th percentile ranged from 1% to 98% of samples (Fig. 1). The 99th percentile variability between assays was substantial, exemplifying the lack of cTnI and cTnT assay standardization. The hs-cTn assays with an ability to measure concentrations in all or nearly all reference individuals demonstrated nearly gaussian distributions of cardiac troponin results.

Analytical Designation for hs-Assay

It is important to understand that the term "high-sensitivity" reflects the assay's characteristics and does not refer to a difference in the form of cardiac troponin being measured. There is a need for a consensus on defining what nomenclature should be used for an hs-assay. The term "high-sensitivity" has uniformly been used for publication in *Clinical Chemistry* and more frequently throughout the scientific/medical literature. This term, however, begs the question: how does one define an hs-assay? The IFCC TF-CB has proposed that for an assay to be defined as hs, 2 analytical criteria need to be met *(21)*.

Table 2. Measurable values among hs-cardiac troponin assays using sex-specific cutoffs.

Manufacturer–analyzer–assay	No. of results	LoD, ng/L	Measurable values ≥LoD	Proportion of undetectable values (<LOD)
Abbott ARCHITECT hs-cTnI	F: 252[a]	1.9	F: 67% (170/252)	F: 33% (82/252)
	M: 272		M: 80% (217/272)	M: 20% (55/272)
Beckman Coulter Access hs-cTnI	F: 252	2.5	F: 74% (187/252)	F: 27% (65/252)
	M: 272		M: 87.5% (238/272)	M: 13% (34/272)
Roche Cobas e601 hs-cTnT	F: 252	5	F: 7% (18/252)	F: 93% (234/252)
	M: 272		M: 43% (117/272)	M: 57% (155/272)
Siemens Dimension Vista hs-cTnI	F: 239	0.5	F: 82% (196/239)	F: 18% (43/239)
	M: 264		M: 90% (237/264)	M: 10% (27/264)
Singulex Erenna hs-cTnI	F: 252	0.09	F: 100% (252/252)	F: 0% (0/0)
	M: 272		M: 100% (272/272)	M: 0% (0/0)

[a] F, female; M, male.

First, the % CV at the 99th percentile value should be ≤10%. Second, measurable concentrations should be attainable with at a concentration above the assay's LoD for at least 50% of healthy individuals. As shown in Table 2 and Fig. 1, the hs-cTnT assay showed lower than recommended rates of measurable concentrations when using the IFCC recommendation. None of the currently FDA cleared assays for either contemporary or POC testing met the 2-fold criteria for an hs designation. One commercial hs-cTnI assay (Abbott) is available worldwide (except in the US, where it is not yet FDA cleared). For hs-assays all journals, manufacturers, and laboratories should adopt the ng/L unit of measure to avoid confusion and decimal points followed by unnecessary zeros as used for contemporary and POC assays. Thus, a concentration of 0.0015 μg/L (contemporary assay) would need to be reported as 2 ng/L (hs-assay).

Sex-Derived 99th Percentiles

For hs-cTn assays, sex-specific 99th percentiles are recommended *(21)*. Numerous studies have demonstrated distinct 99th percentiles according to sex, with men having higher concentrations than women, justified by men having larger left ventricular mass than women *(25, 29)*. In a large, healthy population cohort study in which hs-cTn was measured using 5 hs-assays *(28)*, men had a higher proportion of measurable cardiac troponin concentrations than women (Table 3). The hs-cTnI assays demonstrated a heterogeneous ability to measure values above the LoD, but all above 50%. The proportion of undetectable cardiac troponin values varied substantially across assays with women having a higher proportion of undetectable values as compared with men. By using the hs-cTnT assay, marked differences were observed be-

Table 3. Short-term analytical and biological variation of hs-cTn assays.[a]

	Abbott	Beckman	Roche (E170)	Siemens	Singulex
CV-A, %[b]	13.8	14.5	7.8	13.0	8.3
CV-I, %	15.2	6.1	15.0	12.9	9.7
CV-G, %	70.5	34.8	NA	12.3	57
Index of individuality	0.22	0.46	NA	0.11	0.21
RCV, %[b]	NA	NA	47.0	NA	NA
RCV increase, %[c]	69.3	63.8	NA	57.5	46.0
RCV decrease, %[c]	−40.9	−38.9	NA	−36.5	−32
Within-individual mean, ng/L	3.5	4.9	NA	5.5	2.8

[a] Adapted with permission from Apple and Collinson *(15)*.
[b] CV-A, analytical CV; CV-I, within-individual CV; CV-G, between-individual CV; NA, not available.
[c] RCV percentage applies to the parametric data.
[d] RCV increase and decrease percentages refer to nonparametric data and are log transformed.

tween men and women in both the proportion of measurable (F, 7%, vs M, 43%) and undetectable (F, 93%, vs M, 57%) values.

From a clinical perspective these data are informative owing to the emergent interest in using undetectable cardiac troponin values to expedite the rule-out of AMI with a single measurement at presentation, which if under the LoD, safely identifies a subset of patients at very low risk for adverse events *(3–7, 30, 31)*. A balance needs to be struck between using this rule-out strategy in the highest proportion of patients possible vs the interest in using assays with high analytical sensitivity that facilitate the use of following serial concentration changes, particularly at low concentrations, to rule in/out acute MI. Again, it must be acknowledged that different assays will have different LoD concentrations *(15)*.

Statistical Approach to Define 99th Percentile

There is a need for a uniform, standardized statistical approach to calculate cardiac troponin 99th percentiles. A recently published study *(32)* is the first to demonstrate and support the use of the nonparametric method and not the Harrell–Davis Bootstrap Method (or nonparametric Bootstrap Method) or the Robust method. The nonparametric method is a distribution-free method using ranks of observed values to determine percentiles for a given reference interval/cutoff. It is simple to calculate and easy to determine a sample size. For a 99th percentile and 90% CI, n $\geq$299 study participants are required, as recommended by the IFCC TF-CB. The Harrell-Davis Bootstrap method uses a linear combination of order statistics to estimate a percentile/reference interval and weights observed values using the incomplete β distribution. However, it involves a more complicated calculation. It can provide similar results obtained by the traditional nonparametric method, but the extra effort is not seen as worthwhile. This method also can be highly influenced by outliers. The robust method uses estimates of location and scale, iteratively weights-observed values, and smooths/down-weights observations the further they are from the center. It minimizes effect of outlying/extreme observations, requires data to be symmetric and is computationally intensive.

Biological Variability of hs-cTn Assays

Determining biological variation is not possible for both cTnI and cTnT with contemporary and POC assays in clinical practice today, because these assays cannot reliably measure concentrations in healthy individuals where they detect measurable values in <25% of healthy individuals *(15)*. In contrast, Table 3 demonstrates the biological-variation characteristics of 5 hs-assays. Within-individual mean cardiac troponin concentrations ranged between 2 ng/L and 5 ng/L for both cTnI and cTnT, respectively, and reference change values (RCV) ranged from ±32% to ±69.3%. For hs-cTnT differences in RCVs have also been found between different analyzers used for the same assay.

Characterizing hs-Assays by Using Clinical Criteria

While criteria has been provided by the IFCC TF-CB for defining hs-cTn assays using analytical characteristics *(21)*, investigators have opined that clinical criteria may also be of importance in defining hs-assays. The IFCC TF-CB is currently evaluating whether the following clinical criteria would provide hs-assay clarity. First, use a rule-out MI criterion predicated on an hs-cTn measurement <LoD concentration that coincides with a NPV at baseline sample >99.0% and a clinical sensitivity at baseline sample >99.0%. Second, use a risk outcomes criterion predicated on a NPV of >99% for major adverse cardiac events or all-cause death at 30 days using either a concentration <LoD or a 0–1 h or 0–2 h absolute concentration Δ (assay dependent). Additional studies will be needed to determine whether these risk criteria can also be used with contemporary assays.

Implementing Δ Cardiac Troponin Values

There is no universal Δ value for cTnI or cTnT values to best optimize clinical specificity *(21)*. Deltas will be assay dependent and vary between a percentage change for contemporary and POC assays *(33)* as compared to absolute concentration changes for hs-assays *(5)*. Further, the calculation of Δ values will vary depending on the timing between serial samples, i.e., 0–1, 2 or 3 h (hs-assays) vs 0 to 6 h (contemporary assays), as well as whether the initial cardiac troponin concentration is within the reference range below the 99th percentile (at the time of the disease evolution) or above the 99th percentile. Small concentration changes may also result from poor analytical imprecision for contemporary and POC assays that tend to have poor imprecision at low cardiac troponin concentrations, particularly those near the 99th percentile. Defining a consistent period of time for analysis is also important for the development of an accurate approach to this analysis if different assays are to be compared. A recommendation has been made for hs-cTnT to use a 50% change near the 99th percentile URL and a 20% change when the baseline value is increased above the 99th percentile within a 3 h interval *(34)*. Shorter time intervals ($\leq$3 h) will be required with hs-cTn assays to assist in ruling out AMI. Consideration must always be given to late presenting MIs, which may confound the use of both absolute and percentage Δ values *(35)*.

Preparing for Implementation of hs-Assays

As global change occurs, laboratories need to start preparing their services for the eventuality of going live with hs-assays *(36, 37)*. The following draft check-list, derived from the evidence based literature, will help educate laboratories currently using and prepare laboratories not using hs-assays for the conversion from contemporary and POC assays to hs-assays. First, education of laboratory medicine and clinical staff on relevant literature pertaining to the manufacturers' assay being implemented will be necessary. Distribute analytical, diagnostic and risk assessment outcomes studies to your clinical colleagues, and present information on the new hs-assay at their staff meetings and clinical conferences. Second, develop understanding among users of the new hs-assay that the concentration numbers are going to change, and that they should not expect a conversion factor. Third, a URL at the 99th percentile will need to be established, following the IFCC TK-LB educational materials. Fourth, preparation will be needed for changing from a single to sex-specific 99th percentile, recognizing that this value for women will be less than for men. Fifth, conversion to reporting only whole numbers in ng/L will be needed. Sixth, define a QC material at the 99th percentile to monitor % CV. Seventh, consider using cardiac troponin values <LoD of the hs- assay as a potential rule-out characteristic. Work closely with your emergency medicine colleagues to explore this financial savings utilization. Eighth, provide serial testing protocols that consider earlier measurements such as at baseline, 1.5h and 3h for diagnostic determinations *(38)*. Work conjointly with emergency department physicians and cardiologists to define clinical protocols to facilitate optimal use. Ninth, educate your clinicians that although an increased cardiac troponin is reflective of MI it does not mean there was an MI, as each patient's cardiovascular history must be reviewed in this context *(39)*. Tenth, work on assuring good preanalytical sampling as the hs-assays are so sensitive that poor sample quality can be a problem.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: F.S. Apple, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: F.S. Apple, HyTest, Philips Healthcare Incubator, and Metanomics Health; Y. Sandoval, Roche Clinical Diagnostics; A. Jaffe, Beckman Coulter, Alere, Abbott, Roche, Siemens, ET Healthcare, NeuorgenomiX, Lpath, Singluex, and Novartis; J.Ordonez-llanos, Roche Diagnostics, Singulex, bioMerieux, and Stat Diagnostics.
Stock Ownership: None declared.
Honoraria: F.S. Apple, Instrumentation Laboratory and Abbott POC; J.Ordonez-llanos, bioMérieux and Roche Diagnostics.
Research Funding: Roche Diagnostics and Singulex, Inc. F.S. Apple, Abbott Diagnostics, Siemens Healthcare, Singulex, Roche Diagnostics, and Beckman Coulter.
Expert Testimony: None declared.
Patents: None declared.

Acknowledgments: Justin L Rogers, PhD, Abbott Diagnostics; IFCC Task Force on Clinical Applications of Cardiac Biomarkers committee members: Paul Collinson, Mario Plebani, Martin Than, Judd Hollander, Bertil Lindahl, and Martin Mockel; and numerous representatives of the in vitro diagnostic industry that manufacture high sensitivity contemporary and POC cardiac troponin assays who serve as consultants to the TF.

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