

Analytical Characteristics of High-Sensitivity Cardiac Troponin Assays

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BACKGROUND: Cardiac troponins I (cTnI) and T (cTnT) have received international endorsement as the standard biomarkers for detection of myocardial injury, for risk stratification in patients suspected of acute coronary syndrome, and for the diagnosis of myocardial infarction. An evidence-based clinical database is growing rapidly for high-sensitivity (hs) troponin assays. Thus, clarifications of the analytical principles for the immunoassays used in clinical practice are important.

CONTENT: The purpose of this mini-review is (a) to provide a background for the biochemistry of cTnT and cTnI and (b) to address the following analytical questions for both hs cTnI and cTnT assays: (i) How does an assay become designated hs? (ii) How does one realistically define healthy (normal) reference populations for determining the 99th percentile? (iii) What is the usual biological variation of these analytes? (iv) What assay imprecision characteristics are acceptable? (v) Will standardization of cardiac troponin assays be attainable?

SUMMARY: This review raises important points regarding cTnI and cTnT assays and their reference limits and specifically addresses hs assays used to measure low concentrations (nanograms per liter or picograms per milliliter). Recommendations are made to help clarify the nomenclature. The review also identifies further challenges for the evolving science of cardiac troponin measurement. It is hoped that with the introduction of these concepts, both laboratorians and clinicians can

develop a more unified view of how these assays are used worldwide in clinical practice.

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Cardiac troponins I (cTnI)⁴ and T (cTnT) have been endorsed internationally as the standard biomarkers for the detection of myocardial injury, risk stratification in patients suspected of acute coronary syndrome, and for the diagnosis of myocardial infarction (1, 2). Thus, clarification of the analytical principles of the immunoassays used in clinical practice is important (3). Furthermore, an evidence-based clinical database is growing rapidly for high-sensitivity (hs) troponin assays (4–8). This database includes risk–outcomes data involving measurements of concentrations within healthy reference populations (6, 7), which heretofore have not been measurable owing to the limited analytical sensitivity (detection limits) of current assays (9). This rapid accumulation of information supports an accompanying need for a better general understanding of the analytical characteristics of cardiac troponin assays. This review does not intend to break new ground or supplant present guidelines, but to help clarify them.

Troponin Biochemistry

CARDIAC TROPONIN T

Three different isoforms of TnT encoded by individual genes have been identified in cardiac muscle, fast-twitch skeletal muscle, and slow-twitch skeletal muscle (10). There is sequence homology between cTnT and skeletal muscle troponin T (sTnT), with a difference of 125 amino acid residues between adult sTnT from fast-twitch skeletal muscle (fast sTnT) and cTnT (56.6% homology) and a 120-residue difference between adult sTnT from slow-twitch skeletal muscle (slow sTnT) and cTnT (58.3% homology). The potential impact of the regeneration of skeletal muscle and of the develop-

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Received August 18, 2011; accepted September 16, 2011.

Previously published online at DOI: 10.1373/clinchem.2011.165795

⁴ Nonstandard abbreviations: cTnI, cardiac troponin I; cTnT, cardiac troponin T; hs, high-sensitivity; sTnT, skeletal muscle TnT; fast sTnT, sTnT from fast-twitch skeletal muscle; slow sTnT, sTnT from slow-twitch skeletal muscle; MAbs, monoclonal antibody; TnC, troponin C; FAB, fragment antigen binding.

ing heart and skeletal muscle on the reexpression of the genes encoding the cross-reacting cTnT isoforms has been reviewed (10). The use of an antibody pair specific to adult cTnT found no evidence of cTnT isoforms corresponding to adult cTnT in skeletal muscle from patients with renal disease (11). The presence of a human fetal exon with complete sequence homology to an exon in fast sTnT and in cTnT may account for previous reports of apparent cross-reactivity in immunohistochemical studies that used anti-cTnT antibodies. The fourth-generation cTnT assay uses monoclonal antibodies (MAbs) that recognize epitopes 5 residues apart in the central part of the molecule: M7 (which recognizes an epitope at residues 125–131) and M11.7 (which recognizes an epitope at residues 136–147). This antibody selection corresponds to the cardiac-specific sequences that have no sequence homology to fetal TnT. A recent analysis has reported, however, that a protein found in skeletal muscle of patients with primary skeletal muscle disease is captured by all of these antibodies, again raising the possibility that the specificity of cTnT for cardiac muscle may not be absolute (12).

The majority of cTnT is found in the contractile apparatus (approximately 10 mg/g tissue) and is released via proteolytic degradation. Although 6%–8% of cTnT has been reported to occur as a free cytosolic component (13), the precise amount is not certain, because studies have used tissues from variable sources, such as organ donor tissue, postmortem tissue, or atrial tissue. Release from a cytosolic pool has been suggested to occur with ischemia; however, the current accepted evidence supports the concept that the release of cTnT (and cTnI) is due to cell death and is irreversible. Cardiac troponin release has also been suggested to be due to reversible ischemic injury. The proposed mechanisms of cardiac troponin release include apoptosis, normal myocyte turnover, cellular release of proteolytic degradation products, increased cell wall permeability, and formation and release of membranous blebs (14). The half-life of cTnT in the circulation is thought to be 120 min (15), with the prolonged window of detection being due to continuous release of cTnT from the myofibrillar pool as the contractile apparatus within the cell undergoes degradation during necrosis. The clearance and degradation of cTnT remains undefined, however. Studies have suggested that intact cTnT is present as a complex with troponin C (TnC) and cTnI, and as free cTnT (16) although these conclusions have been questioned (17). Further studies are required.

The effect of EDTA in the cTnT assay seems to be minimal, whereas differences between serum and plasma values have been reported for a number of cTnI methods. Initial reports showed a variable dif-

ference between cTnT and cTnI values for serum and heparin-treated plasma; however, this variability has been corrected with the current formulation of the cTnT assay. In patient samples, cTnT appears to be very stable, not only at room temperature and at 4 °C, but also when repeatedly frozen and thawed (samples do not exhibit significant changes after 5 freeze–thaw cycles). Assay imprecision varies modestly, depending on the specific instrument used in a given laboratory (18).

The hs-cTnT assay uses fragment antigen binding (FAB) portions of 2 cTnT-specific mouse 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 and is identical to that in the 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. Assay sensitivity has been increased by increasing the sample volume from 15 to 50 μ L, increasing the ruthenium concentration of the detection antibody, and lowering the background signal by buffer optimization. The assay is calibrated against recombinant human cTnT produced in *Escherichia coli* cell culture (19). 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 independently of the influences of imprecision. The 30-ng/L cutoff in the fourth-generation assay corresponds to 50 ng/L in the new hs assay.

CARDIAC TROPONIN I

Human cTnI occurs in cardiac muscle tissue as a single isoform of 209 amino acid residues, with a molecular weight of approximately 23–24 kDa. Three human cTnI isoforms have been described: one is produced in cardiac muscle (cTnI), and 1 isoform each is produced in slow-twitch and fast-twitch skeletal muscles (slow sTnI and fast sTnI, respectively) (10). The overlap in sequence between cTnI and slow sTnI is approximately 40% and somewhat less for fast sTnI. Therefore, antibodies selected for cTnI assays should be tested to ensure that there is no cross-reaction with skeletal isoforms of the protein. The cTnI molecule contains 2 serine residues that can be phosphorylated *in vivo* by protein kinase A. Thus, 4 forms of the protein can coexist in the cell: 1 dephosphorylated, 2 monophosphorylated, and 1 bisphosphorylated. Phosphorylation of cTnI changes the conformation of the protein and modifies its interaction with other troponins, in addi-

tion to its interaction with anti-TnI antibodies. A substantial part of cTnI released into a patient's bloodstream appears to be phosphorylated (20), although other posttranslationally modified TnI isoforms, including those that have been oxidized, reduced, or partially digested by proteases, have been found in the circulation (15). The majority of cTnI is found in the contractile apparatus (approximately 4–6 mg/g tissue) and is released by proteolytic degradation, with 2% to 8% of cTnI reported to occur as a free cytosolic component (21).

The first cTnI immunoassay was described by Cummins et al. in 1987 (22), with the first commercial cTnI assay for the Stratus I analyzer (Dade Behring) appearing in 1996. More than 20 years later, the cTnI immunoassay has been transformed considerably. Current generations of commercially available assays have an analytical sensitivity almost 100-fold higher (1 vs 100 ng/L) than that of the experimental and commercial assays that were initially described (9). MAbs specific to several selected epitopes of cTnI are able to recognize all known modifications circulating in the blood. Different forms of the antigen used as standards or calibrators have helped to improve the correlations between the different commercial assays by more than 10-fold, but standardization has not yet been achieved (23). A working group of the IFCC is cooperating with manufacturers to address the question of whether cTnI standardization or harmonization can be achieved (24). The most common reason for the discrepancy in cTnI measurements is the difference in epitope specificity of the antibodies used in different assays. We now know that cTnI measurements are influenced by multiple factors, among which are the aforementioned posttranslational modifications [proteolytic degradation (25), phosphorylation (20)] and complexing with other molecules [e.g., TnC (26), heparin (20), heterophile or human antimouse antibodies, and cTnI-specific autoantibodies circulating in patients' blood (27)]. Different mono- and polyclonal antibodies used in assays are sensitive to these factors to varying degrees. Therefore, it becomes increasingly important that hs-cTnI assays use antibodies specific to epitopes in the central part of the troponin molecule that are not affected by the numerous modifications found in human blood noted above.

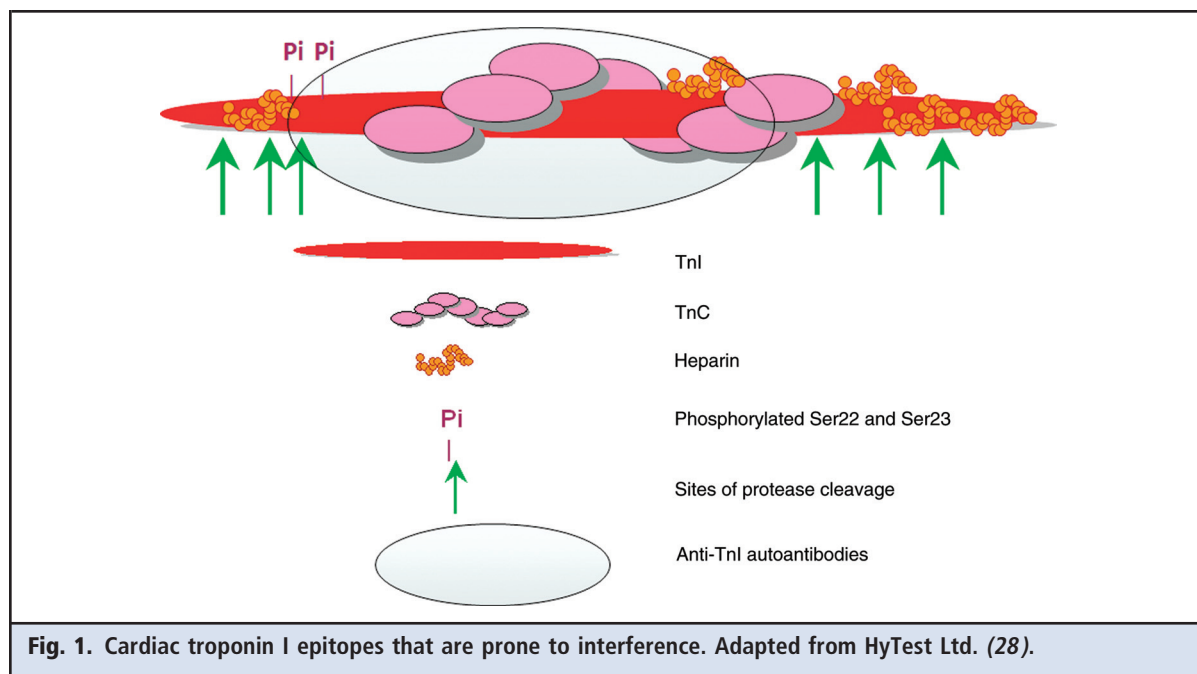
HyTest Ltd. has assessed several hundred different 2-site MAb combinations to find the best one for accurately measuring cTnI by immunoassay (28). This firm has concluded that it is currently not possible to have a single antibody pair (i.e., 1 capture antibody and 1 detection antibody) that would be totally insensitive to all known cTnI modifications and interferences. Given these data, it seems wise to consider the recommendation of this group to use

combinations of >2 MAbs for the development of precise cTnI immunoassays. One could use 2 MAbs for capture and 2 MAbs for detection (conjugated with the specific label)—a “2 + 2” concept (28). In these assays, MAbs should be 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. Thus, the effect of negative or positive interference would be minimized. In addition, the antibodies used in the assay should be specific to the cardiac isoform of troponin and should not show cross-reaction with the 2 skeletal muscle troponin isoforms.

Factors that influence cTnI measurements are presented schematically in Fig. 1. Antibodies specific to different parts of the molecule are sensitive to these factors to different degrees. For example, purified cTnI is well known to be highly susceptible to proteolytic degradation; however, the central part of cTnI closely interacts with TnC in the troponin complex, and TnC protects cTnI from proteolytic degradation. Consequently, the epitopes located in the central part of the cTnI molecule are substantially more stable than the epitopes located in the terminal parts of the molecule. Concurrently, TnC competes with antibodies for binding to cTnI, and only a few antibodies specific to the central part of cTnI can recognize the cTnI–TnC complexed form. cTnI was recently demonstrated to be cleaved by endogenous proteases during incubation of necrotic myocardial muscle after an acute myocardial infarction (25, 26). Consequently, a mixture of the intact cTnI molecule and its proteolytic fragments can be detected in the bloodstream several hours after the onset of the chest pain, although the ratio of fragment molecules to intact molecules, the sizes of fragments, the relationship of the fragmentation with the infarct size, and many other questions need to be addressed. Given that the vast majority (>95%) of cTnI in human blood occurs as a binary cTnI–TnC complex, the antibodies used in the assay should recognize cTnI in complex with TnC. Ideally, antibodies used in assays should recognize all circulating cardiac troponin forms on an equimolar basis.

How Does an Assay Become Designated “High Sensitivity”?

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. Several names have been used in the literature for these assays, including “high-performance,” “highly



sensitive,” “high-sensitive,” “ultrasensitive,” “novel highly sensitive,” “sensitive,” and “high sensitivity.” We propose that the term “high-sensitivity” be uniformly used for publication in *Clinical Chemistry* and throughout the scientific literature. This term, however, begs the question: How does one define an hs assay? In a scorecard concept, an assay was proposed to be hs if it met 2 basic criteria (9). First, the total imprecision (CV) at the 99th percentile value should be $\leq 10\%$. Second, measurable concentrations below the 99th percentile should be attainable with an assay at a concentration value above the assay’s limit of detection for at least 50% (and ideally $>95\%$) of healthy individuals to attain the highest level of scorecard designation. It is unclear which data in the literature should be used for such an analysis, as study findings are variable. For hs-cTnT, the initial validation study showed measurable concentrations above the limit of detection for a large number of healthy individuals (29). A later study, however, showed much lower rates of measurable concentrations (18), as did a study of a large population that included patients with cardiovascular disease, for whom one would expect higher percentages of samples with measurable cTnT (6). As Table 1 shows, none of the currently marketed assays for both central laboratory and point-of-care testing met the 2-fold criteria for an hs-designated level 4 assay. Table 2 shows that the reformulated and newer hs assays (5 hs-cTnI assays and 1 hs-cTnT assay) potentially meet these criteria. The one hs assay that is commercially available worldwide (except in the US, because it has not yet obtained

US Food and Drug Administration clearance) is the Roche hs-cTnT assay. Concentrations for hs assays are expressed in nanograms per liter (picograms per milliliter) instead of the commonly published units of micrograms per liter. We propose that all journals, manufacturers, and laboratories universally adopt the former unit of measure to avoid confusion and decimal points followed by unnecessary zeros. For example, a concentration of $0.0015 \mu\text{g/L}$ would need to be reported as 1.5 ng/L . Adoption of this convention would also help avoid clinical errors in data reporting for both electronic medical records and electronic data transfer, for which the rounding of decimals to zero is a real risk. It should be appreciated that using nanograms per liter as the standard unit will produce very high cardiac troponin values ($>1000 \text{ ng/L}$) after large cardiac insults.

Defining Healthy Reference Populations for Determining the 99th Percentile

Tables 1 and 2 present 99th percentile reference values for each assay, as designated by the manufacturer of each assay. A close examination of the package inserts, however, will reveal very little consistency in the types or numbers of individuals enrolled for determining the 99th percentile. No clear standard has been set for the statistical analysis used to calculate the 99th percentile. Defining what constitutes a healthy reference individual is currently a topic of debate. Should these individuals be apparently healthy individuals younger than 30

Table 1. Analytical characteristics of contemporary sensitive and point-of-care cardiac troponin assays.

Company/platform/assay	Cardiac troponin concentration at:			Amino acid residues of epitopes recognized by capture (C) and detection (D) MAbs
	LoD, ^a $\mu\text{g/L}$	99th Percentile, $\mu\text{g/L}$ (CV) ^b	10% CV concentration, $\mu\text{g/L}$	
Abbott AxSYM ADV	0.02	0.04 (14%)	0.16	C: 87–91, 41–49; D: 24–40
Abbott ARCHITECT	0.009	0.028 (14%)	0.032	C: 87–91, 24–40; D: 41–49
Abbott i-STAT	0.02	0.08 (16.5%)	0.10	C: 41–49, 88–91; D: 28–39, 62–78
Alere Triage	0.05	<0.05 (NA)	NA	C: NA; D: 27–40
Alere Triage Cardio3 ^c	0.01	0.02 (17%)	NA	C: 27–39; D: 83–93, 190–196
Beckman Access AccuTnl	0.01	0.04 (14%)	0.06	C: 41–49; D: 24–40
bioMérieux Vidas Ultra	0.01	0.01 (27.7%)	0.11	C: 41–49, 22–29; D: 87–91, MAb 7B9
Mitsubishi Pathfast	0.008	0.029 (5.0%)	0.014	C: 41–49; D: 71–116, 163–209
Ortho Vitros Eci ES	0.012	0.034 (10%)	0.034	C: 24–40, 41–49; D: 87–91
Radiometer AQT90 cTnl	0.009	0.023 (17.7%)	0.039	C: 41–49, 190–196; D: 137–149
Radiometer AQT90 cTnT	0.008	0.017 (15.2%)	0.026	C: 125–131; D: 136–147
Response RAMP	0.03	<0.01 (18.5% at 0.05)	0.21	C: 85–92; D: 26–38
Roche cobas h232 Cardiac T ^{c,d}	0.05	NA	NA	C: 125–131; D: 136–147
Roche Elecsys TnT Gen 4	0.01	<0.01	0.030	C: 136–147; D: 125–131
Roche Elecsys Tnl	0.16	0.16 (10%)	0.30	C: 87–91, 190–196; D: 23–29, 27–43
Roche Cardiac Reader cTnT ^e	0.03	NA	NA	C: 125–131; D: 136–147
Siemens Centaur Ultra	0.006	0.04 (8.8%)	0.03	C: 41–49, 87–91; D: 27–40
Siemens Dimension RxL	0.04	0.07 (20%)	0.14	C: 27–32; D: 41–56
Siemens Immulite 2500	0.1	0.2 (NA)	0.42	C: 87–91; D: 27–40
Siemens Stratus CS	0.03	0.07 (10%)	0.06	C: 27–32; D: 41–56
Siemens Vista	0.015	0.045 (10%)	0.04	C: 27–32; D: 41–56
Tosoh AIA	0.06	<0.06 (NA)	0.09	C: 41–49; D: 87–91

^a LoD, limit of detection; NA, not available; Gen 4, fourth-generation assay.
^b CV at 99th percentile.
^c Not cleared by the US Food and Drug Administration.
^d Standardized against hs-cTnT assay.
^e Standardized against Gen 4 cTnT assay.

years, or should they be age-matched patients hospitalized without known cardiovascular disease, similar to the demographics of patients who rule in for an acute myocardial infarction, likely between the ages of 30 and 90 years? How does one determine who is apparently healthy, i.e., normal? Should these individuals be selected: (a) via personal interview with questions addressing known medications, such as the use of statins for hyperlipidemia; (b) after obtaining information about known diseases associated with cardiovascular disease, such as renal disease or diabetes; or (c) via definitive evaluation of an individual by a physician taking a history and conducting a physical examination, including an electrocardiogram and an echocardiogram? The third option, in our opinion, would be ideal but is likely cost-prohibitive. Sequential selection of a

reference population on this basis can be shown to shift the derived 99th percentile (30). For deriving reference intervals for each assay, we recommend a 2-fold approach in which both younger (<30 years) and older (>30 years, with a median age of 60 to 65 years to be representative of the ages of cardiac patients) apparently healthy reference groups are recruited. Inclusion criteria should be based on data obtained from an interview for a history of medications and known underlying disease, as well as a blood measurement of a natriuretic peptide (N-terminal pro-B-type natriuretic peptide or B-type natriuretic peptide) interpreted vis-à-vis a cutoff value for the exclusion of ventricular dysfunction (31) to serve as a surrogate biomarker for underlying myocardial dysfunction. In addition, the groups should be split equally by sex and include a

Table 2. Analytical characteristics of hs cardiac troponin assays.

Company/ platform/assay	Cardiac troponin concentration at:			Amino acid residues of epitopes recognized by capture (C) and detection (D) MAbs
	LoD, ^a ng/L	99th Percentile, ng/L (CV) ^b	10% CV concentration, ng/L	
hs-cTnI				
Abbott ARCHITECT ^c	1.2	16 (5.6%)	3.0	C: 24–40; D: 41–49
Beckman Access ^c	2–3	8.6 (10%)	8.6	C: 41–49; D: 24–40
Nanosphere MTP ^c	0.2	2.8 (9.5%)	0.5	C: 136–147; D: MAb PA1010
Singulex Erenna ^c	0.09	10.1 (9.0%)	0.88	C: 41–49; D: 27–41
Siemens Vista ^c	0.5	9 (5.0%)	3	C: 30–35; D: 41–56, 171–190
hs-cTnT				
Roche Elecsys ^d	5.0	14 (8%)	13	C: 136–147; D: 125–131

^a LoD, limit of detection; MTP, microtiter plate.
^b CV at the 99th percentile.
^c Under development and not available for commercial use.
^d Available for use worldwide but not cleared by the US Food and Drug Administration for use in the US.

diverse racial and ethnic mix. The CLSI recommends a minimum of 120 individuals per group of healthy individuals for an appropriate statistical determination of a normal reference limit cutoff. This number was suggested as the minimum number of individuals necessary for estimating the limits of a central 95% reference interval (with limits at the 2.5th and 97.5th percentiles) by use of the nonparametric estimation method. This number of individuals is not adequate for determining a 99th percentile value, for which 300–500 individuals have been recommended to allow an appropriately powered analysis. To date, no study has compared all contemporary sensitive assays and/or hs assays within the same reference or disease population. For the hs assays, data indicate a higher 99th percentile value for males than for females, for hs-cTnI [Singulex assay (32)] and hs-cTnT (18), but not for hs-cTnI measured with the Beckman Coulter assay (8). Unpublished data for the Abbott ARCHITECT prototype STAT hs-TnI assay showed that concentrations were significantly sex and age dependent (F.S. Apple, personal communication). In addition, the percentage of individuals within each reference study with a measurable value above the limit of detection has been shown to vary from study to study. This variation is likely dependent on sample type, sample handling and storage, and whether samples were analyzed fresh or after a freeze–thaw cycle.

Biological Variability

Determining the biological variation is not possible for both cTnI and cTnT with the contemporary sensitive

assays in clinical practice today, because these assays cannot reliably measure concentrations in healthy individuals. Most of these assays detect measurable values in <15% of healthy individuals (33, 34). In contrast, Table 3 demonstrates the biological-variation characteristics of 5 hs assays (35–38), with the same samples used for measuring short-term variation as initially collected by Wu et al. (35) for all but 1 study (36). Within-individual mean cardiac troponin concentrations ranged between 2.2 ng/L and 4.9 ng/L for both cTnI and cTnT, and reference change values ranged from $\pm 32\%$ to 69.3%. In a comparison of 2 independently published studies of hs-cTnT, characterization of the biological variation yielded positive reference change values of 84.5% (35) and 47% (36). These data, along with imprecision data at the 99th percentile (discussed below), will need appropriate statistical evaluation to establish guidelines to assist in the clinical interpretation of cardiac troponin results, both for single test results and for evaluating what changes in cardiac troponin in serial testing represent actual abnormality and change over time, and what changes are merely noise.

Imprecision at the 99th Percentile

The 2007 universal definition of myocardial infarction guidelines published by the Joint Task Force (1) recommended preferential use of assays that demonstrated a total CV of $\leq 10\%$ at the 99th percentile, on the assumption that better precision will improve the ability to detect a significant serial change. Evidence-based data, however, now support the use of assays

Table 3. Short-term analytical and biological variation by hs-cTnI assays.

	Abbott ^a	Beckman ^a	Roche (E170) ^b	Siemens ^a	Singulex ^c
CV-A, ^d %	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, % ^e	NA	NA	47.0	NA	NA
RCV increase, % ^f	69.3	63.8	NA	57.5	46.0
RCV decrease, % ^f	-40.9	-38.9	NA	-36.5	-32
Within-individual mean, ng/L	3.5	4.9	NA	5.5	2.8

^a Apple et al. (38).
^b Vasile et al. (36).
^c Wu et al. (35).
^d CV-A, analytical CV; CV-I, within-individual CV; CV-G, between-individual CV; NA, not available; RCV, relative change value.
^e RCV percentage applies to the parametric data.
^f RCV increase and decrease percentages refer to nonparametric data and are log-transformed.

with a CV of $\leq 20\%$ at the 99th percentile (39, 40), because a 20% CV does not lead to misclassification of patients in diagnostic or risk-assessment management. This information is promising for the contemporary sensitive cTnI assays used in current practice, because the large majority of assays used worldwide fall into this category.

Table 2 shows both the CVs at the 99th percentile concentration for the 6 hs cardiac troponin assays and the lowest concentrations reported for which a 10% CV was obtained. All hs assays demonstrated a CV $\leq 10\%$ at the 99th reference percentile, and several assays demonstrated a 10% CV at a concentration an order of magnitude lower. Such information is useful to laboratorians and clinicians because it allows them to confidently report hs cardiac troponin values and changing results over time that are unaffected by analytical noise. The true test of how well the total imprecision of cardiac troponin assays (as reported in Tables 1 and 2) from manufacturer studies will hold up is when assays are used daily in clinical practice and QC materials are evaluated over weeks, after numerous calibrations and reagent lot changes. For now, only the Roche hs-cTnT assay has been used in clinical practice, and it appears to have maintained the 10% total CV quality characteristic at the 99th percentile concentration (18, 19). Comparison of the new hs-cTnT assay with the previous fourth-generation cTnT assay has shown good agreement for values >100 ng/L in 2 studies (slope, 0.962–1.087) (18) but higher discordance in another study (slope, 0.77) (29).

Conclusion

This review has attempted to clarify the important analytical and reference-limit issues pertaining to cTnI and cTnT assays and has specifically addressed hs assays used to measure low concentrations (i.e., nanograms per liter or picograms per milliliter). Recommendations have been made to help clarify the nomenclature. The review also has identified additional challenges for the evolving science of cardiac troponin measurement. It is hoped that by the introduction of these concepts, both laboratorians and clinicians can develop a more unified view of how these assays are used worldwide in clinical practice.

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 Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: F.S. Apple, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: F.S. Apple, Instrumentation Laboratories, Abbott Diagnostics, and Ortho Clinical Diagnostics.

Stock Ownership: None declared.

Honoraria: F.S. Apple, Instrumentation Laboratories and Abbott Diagnostics.

Research Funding: F.S. Apple, Siemens, Abbott, Radiometer, B.R.A.H.M.S., Roche, Alere, BD, Instrumentation Laboratories, and Mitsubishi.

Expert Testimony: None declared.

References

1. Thygesen K, Alpert JS, White HD, on behalf of the Joint ESC/ACCF/AHA/WHF Task Force for the Re-definition of Myocardial Infarction. Universal definition of myocardial infarction. *Eur Heart J* 2007; 28:2525–38.
2. Morrow DA, Cannon CP, Jesse RL, Newby LK, Ravkilde J, Storrow AB, et al. National Academy of Clinical Biochemistry practice guidelines: clinical characteristics and utilization of biomarkers in acute coronary syndromes. *Clin Chem* 2007;53: 552–74.
3. Apple FS, Jesse RL, Newby LK, Wu AHB, Christenson RH. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage laboratory medicine practice guidelines: analytical issues for biomarkers of acute coronary syndromes. *Clin Chem* 2007;53:547–51.
4. Jaffe AS, Apple FS. High-sensitive cardiac troponin: hype, help and reality. *Clin Chem* 2010; 56:342–4.
5. Reichlin T, Irfan A, Twerenbold R, Reiter M, Hochholzer W, Burkhalter H, et al. Utility of absolute and relative changes in cardiac troponin concentrations in the early diagnosis of acute myocardial infarction. *Circulation* 2011;124:136–45.
6. de Lemos JA, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. *JAMA* 2010;304:2503–12.
7. de Flippi CR, de Lemos JA, Christenson RH, Gottdiener JS, Kop WJ, Zhan M, et al. Association of serial measures of cardiac troponin T using a sensitive assay with incident heart failure and cardiovascular mortality in older adults. *JAMA* 2010;304:2494–502.
8. Venge P, Johnston N, Lindahl B, James S. Normal plasma levels of cardiac troponin I measured by the high-sensitivity cardiac troponin I Access prototype assay and the impact on the diagnosis of myocardial ischemia. *J Am Coll Cardiol* 2009;54: 1165–72.
9. Apple FS. A new season for cardiac troponin assays: It's time to keep a scorecard. *Clin Chem* 2009;55:1303–6.
10. Gaze DC, Collinson PO. Multiple molecular forms of circulating cardiac troponin: analytical and clinical significance. *Ann Clin Biochem* 2008;45: 349–55.
11. Ricchiuti V, Voss EM, Ney A, Odland M, Anderson PA, Apple FS. Cardiac troponin T isoforms expressed in renal diseased skeletal muscle will not cause false-positive results by the second generation cardiac troponin T assay by Boehringer Mannheim. *Clin Chem* 1998;44:1919–24.
12. Jaffe AS, Vasile VC, Milone M, Saenger AK, Olson KN, Apple FS. Diseased skeletal muscle: a non-cardiac source for increased circulating concentrations of cardiac troponin T. *J Am Coll Cardiol* 2011;58:1819–24.
13. Voss EM, Sharkey SW, Gernert AE, Murakami MM, Johnston RB, Hsieh CC, Apple FS. Human and canine cardiac troponin T and creatine kinase-MB distribution in normal and diseased myocardium. Infarct sizing using serum profiles. *Arch Pathol Lab Med* 1995;119:799–806.
14. White HD. Pathobiology of troponin elevations: Do elevations occur with myocardial ischemia as well as necrosis? *J Am Coll Cardiol* 2011;57: 2406–8.
15. Katus HA, Remppis A, Scheffold T, Diederich KW, Kuebler W. Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. *Am J Cardiol* 1991;67:1360–7.
16. Wu AH, Feng YJ, Moore R, Apple FS, McPherson PH, Buechler KF, Bodor G, for the American Association for Clinical Chemistry Subcommittee for, and Clinical Chemistry Subcommittee on cTnl Standardization. Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. *Clin Chem* 1998;44:1198–208.
17. Michielsen EC, Diris JH, Wodzig WK, Dieijen-Visser MP. Size-exclusion chromatography of circulating cardiac troponin T. *Clin Chem* 2006;52: 2306–7.
18. Saenger AK, Beyrau R, Braun S, Cooray R, Dolci A, Freidank H, et al. Multicenter analytical evaluation of a high-sensitivity troponin T assay. *Clin Chim Acta* 2011;412:748–54.
19. Hallermayer K, Klenner D, Vogel R. Use of recombinant human cardiac troponin T for standardization of third generation troponin T methods. *Scand J Clin Lab Invest Suppl* 1999;230:128–31.
20. Katrukha A, Bereznikova A, Filatov V, Esakova T. Biochemical factors influencing measurement of cardiac troponin I in serum. *Clin Chem Lab Med* 1999;37:1091–5.
21. Adams JE, Bodor GS, Davila-Roman VG, Delmez JA, Apple FS, Ladenson JH, Jaffe AS. Cardiac troponin I: a marker with high specificity for cardiac injury. *Circulation* 1993;88:101–6.
22. Cummins B, Auckland ML, Cummins P. Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. *Am Heart J* 1987;113:1333–44.
23. Christenson RH, Duh SH, Apple FS, Bodor G, Bunk D, Dalluge J, et al. Standardization of cardiac troponin I assays: round robin performance of ten candidate reference materials. *Clin Chem* 2001; 47:431–7.
24. Tate JR, Bunk DM, Christenson RH, Katrukha A, Noble JE, Porter RA, et al. Standardisation of cardiac troponin I measurement: past and present. *Pathology* 2010;42:402–8.
25. Katrukha AG, Bereznikova AV, Filatov VL, Esakova TV, Kolosova V, Pettersson K, et al. Degradation of cardiac troponin I: implication for reliable immunodetection. *Clin Chem* 1998;44: 2433–40.
26. Katrukha AG, Bereznikova AV, Esakova TV, Pettersson K, Lovgren T, Severina ME, et al. Troponin I is released in bloodstream of patients with acute myocardial infarction not in free form but as complex. *Clin Chem* 1997;43:1379–85.
27. Eriksson S, Halenius H, Pulkki K, Hellman J, Pettersson K. Negative interference in cardiac troponin I immunoassays by circulating troponin autoantibodies. *Clin Chem* 2005;51:839–47.
28. HyTest Ltd. Troponins. <http://www.troponins.com/troponins> (Accessed July 2011).
29. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. *Clin Chem* 2010;56:254–61.
30. Collinson PO, Heung YM, Gaze D, Boa F, Senior R, Christenson R, Apple FS. Influence of population selection on the 99th percentile reference value for cardiac troponin assays. *Clin Chem* 2012;58:219–25.
31. de Lemos JA, McGuire DK, Khera A, Das SR, Murphy SA, Omland T, Drazner MH. Screening the population for left ventricular hypertrophy and left ventricular systolic dysfunction using natriuretic peptides: results from the Dallas Heart Study. *Am Heart J* 2009;157:746–53.
32. Apple FS, Simpson PA, Murakami MM. Defining the serum 99th percentile in a normal reference population measured by a high-sensitivity cardiac troponin I assay. *Clin Biochem* 2010;43:1034–6.
33. Apple FS, Murakami MM. Serum and plasma cardiac troponin I 99th percentile reference values for 3 2nd-generation assays. *Clin Chem* 2007; 53:1558–60.
34. Collinson PO, Clifford-Mobley O, Gaze D, Boa F, Senior R. Assay imprecision and 99th-percentile reference value of a high-sensitivity cardiac troponin I assay. *Clin Chem* 2009;55:1433–4.
35. Wu AHB, Quynh AL, Todd J, Moecks J, Wians F. Short- and long-term biological variation in cardiac troponin I measured with a high-sensitivity assay: implications for clinical practice. *Clin Chem* 2009;55:52–8.
36. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and analytical variability of a novel high-sensitivity cardiac troponin T assay. *Clin Chem* 2010;56:1086–90.
37. Frankenstein L, Wu AHB, Hallermayer K, Wians FH Jr, Giannitsis E, Katus HA. Biological variation and reference change value of high-sensitivity troponin T in healthy individuals during short and intermediate follow-up periods. *Clin Chem* 2011; 57:1068–71.
38. Apple FS, Murakami MM, Wians FH, Ler R, Kaczmarek JM, Wu AHB. Short-term biological variation of cardiac troponin I measured with three high-sensitivity assays [Abstract]. *Clin Chem* 2011;57:C05.
39. Apple FS, Parvin CA, Buechler KF, Christenson RH, Wu AHB, Jaffe AS. Validation of the 99th percentile cutoff independent of assay imprecision (CV) for cardiac troponin monitoring for ruling out myocardial infarction. *Clin Chem* 2005; 51:2198–200.
40. Kupchak P, Wu AHB, Ghani F, Newby K, Ohman EM, Christenson RH. Influence of imprecision on ROC curve analysis for cardiac markers. *Clin Chem* 2006;52:752–3.