Clinical Chemistry 54:12
1982–1989 (2008)

Analytical Characteristics of Commercial Cardiac Troponin I and T Immunoassays in Serum from Rats, Dogs, and Monkeys with Induced Acute Myocardial Injury

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BACKGROUND: Information is needed regarding analytical characteristics of cardiac troponin (cTn) assays used in preclinical studies.

METHODS: We measured cTnI and cTnT in serum from normal animals and animals with induced myocardial injury [Sprague–Dawley (SD) and Wistar rats, beagle dogs, and rhesus (Rh) and cynomolgus (Cy) monkeys]. We evaluated the following assays: for cTnI, Abbott Architect, Bayer Centaur (first and second generation), Beckman Access, DPC Immulite, Dade Dimension, Ortho Vitros ES, Tosoh AIA, and species-specific enzyme immunoassays; for cTnT, Roche Elecsys.

RESULTS: We found different species-specific responses for the troponin assays evaluated. Abbott, Bayer Ultra, Beckman, and Dade assays gave good responses across all species. In rats, weak responses were observed with DPC and Ortho, and no measurable response with Tosoh. In dogs, weak responses were observed with Tosoh cTnI, Roche cTnT, and species-specific cTnI. In cynomolgus monkeys, weak responses were observed with species-specific cTnI and Roche cTnT. Assay imprecision was ≤20% at 3 or more examined cTn concentrations for Beckman (rat, dog, monkey), Dade (rat, dog, monkey), Abbott (rat, dog, monkey), Bayer first generation (dog), Bayer Ultra (rat, dog, monkey), Roche (monkey), DPC (dog, monkey), Ortho (dog, monkey), and Tosoh (dog, monkey) assays, whereas imprecision was ≤20% at 2 or fewer concentrations for the Bayer first generation (rat, monkey), Roche cTnT (rat, dog), and DPC (rat) assays.

CONCLUSIONS: Not all cTn assays are suitable for monitoring cTn in each animal species or strain. Individual assay characterization by animal species is needed to prevent misinterpretation of myocardial injury–based cardiac troponin findings.

Cardiac troponin I (cTnI)[5] and T (cTnT) are definitive biomarkers for detection of myocardial injury in humans *(1–4)* and have proven utility in preclinical studies for drug-induced cardiac injury in animals *(5–7)*. Increases in serum cTn also correlate with morphological changes in the heart *(7)*. The concordance between results in animal and human studies supports the notion that the cTns are potential bridging biomarkers that can be employed in both preclinical and clinical studies to monitor drug-induced cardiac injury *(5)*.

Numerous commercial assays are available for monitoring cTnI, each with different analytical characteristics, making standardization and harmonization of absolute concentration measurements problematic *(8, 9)*. The IFCC and the National Academy of Clinical Biochemistry (NACB) have developed a quality specifications document for human cTn assays and provided a comparison of their analytical imprecision in human serum *(10–12)*. Substantial heterogeneity ex-

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Received September 13, 2007; accepted August 26, 2008.
Previously published online at DOI: 10.1373/clinchem.2007.097568

[5] Nonstandard abbreviations: cTnI, cardiac troponin I; cTnT, cardiac troponin T; NACB, National Academy of Clinical Biochemistry; ILAR, Institute for Laboratory Animal Research; CLSI, Clinical and Laboratory Standards Institute; LLD, lower limit of detection.

ists between commercial cTn assays in regard to imprecision, sensitivity, and accuracy. These differences exist partly because cTnI assays use different antibody sets that may be directed toward distinct epitopes or fragments of cTnI. Heterogeneity is less of a problem for cTnT assays, since there is only a single assay vendor and the antibody configuration of these assays has not changed between the second- and third-generation assays. Nevertheless, the differences between various commercial cTn assays may lead to uncertainty regarding the validity of the results obtained from animal samples.

With the increase in development of new pharmaceuticals and the use of laboratory animals in preclinical investigations, the potential for drug-induced myocardial toxicity/injury is an important issue that has gained the attention of both investigators and regulatory agencies *(13, 14)*. cTn measurements have been used as a biomarker for detection of myocardial injury in experimental animals, supplementing histological studies of the myocardium *(14)*. Few studies with animals have systematically addressed the analytical quality of serum/plasma cTn testing as rigorously as in human clinical studies *(15–23)*. Interpretations of cTn concentrations may differ depending on the choice of immunoassay. It is important to consider that data generated from human clinical trials may not necessarily parallel animal findings. One consideration when using human-approved assays for animal studies is whether there is conservation of amino acid sequences across various species. For example, some epitopes of cTnT have been reported to be fully conserved across mammal, rat, mouse, and avian phyla (amino acid 95–181 region, for example) *(24)*. Differences across phyla for cardiac cTnI reactivity, however, can be attributed to species differences or lack of conservation in the primary structure of the protein *(25)*.

In this study, we evaluated selected analytical characteristics of commercially available human cTnI and cTnT assays used for measurement of cTn in sera of laboratory animals.

Materials and Methods

PHASE IA

We obtained cTn-positive serum specimens from experimental animal models in which myocardial damage was induced by drug administration or surgical ligation of one of the coronary arteries. For all in vivo procedures, the animal care and experimental procedures were conducted in compliance with the US Animal Welfare Act and the Institute for Laboratory Animal Research (ILAR) Guide (1996), and all data were in compliance with US FDA Good Laboratory Practices (21 CFR, part 58).

Dogs. Beagle dogs (Marshall Farms, North Rose, NJ), 3 years of age, were given isoproterenol hydrochloride by infusion at a rate of 4 μg/kg/min for total of 80 μg/kg over 20 min. Blood samples (sera) were collected from the jugular vein 2, 4, and 6 h after cessation of isoproterenol administration and frozen at −80 °C. cTnI-negative sera were also collected.

Rats. Male and female Sprague–Dawley [Crl:CD (SD) GS BR] or Wistar [Rat/Crl: WI (Han)] rats, 7–9 weeks old and 166–222 g, were given a single subcutaneous 5 mg/kg injection of isoproterenol hydrochloride. After 2–6 h, the animals were killed via CO_2 or anesthetized with isoflourane/O_2 inhalation, and blood (sera) was collected and frozen at −80 °C.

Monkeys. Adult rhesus monkeys, weighing between 4.8 and 8 kg, bred in captivity, were infused for 2 h via the cephalic or saphenous vein with 20 μg/kg/min norepinephrine at a flow rate of 0.0625 mL/kg/min for 1 day. The animals were killed on day 2, 3, 4, or 5. Immediately before necropsy, blood samples (sera) were obtained and frozen. Adult male and female cynomolgus monkeys were initially anesthetized with ketamine and subsequently with isoflurane. A thoracotomy was performed, after which the heart was exposed and the left anterior descending coronary artery was isolated. A ligature was placed and secured around the left anterior descending coronary. Four hours after the artery was ligated, blood samples were collected and centrifuged, and the serum was frozen at −60 °C.

From each species/strain serum pool, we prepared negative, low, medium, and high cTn pools based on the measurement of cTnT (Roche assay). To evaluate linearity and dynamic range of the selected assays, we diluted high pools with negative species-specific pools for preparation of the medium and low pools. The criteria used to compare immunoreactive responses in the results section were subjective, based on the consensus expert opinion of the authors.

PHASE 1B

We used the following assays according to manufacturer guidelines: Abbott Architect cTnI (second generation), Bayer (now Siemens) Advia Centaur cTnI (first generation, second generation Ultra), Beckman Coulter Access cTnI (second generation), Dade Behring (now Siemens) Dimension RxL cTnI (second generation), DPC (now Siemens) Immulite 1000 cTnI (first generation), Ortho Clinical Diagnostics Vitros ES cTnI (second generation), Tosoh AIA 600 II cTnI (second generation), Roche Elecsys 2010 cTnT (third generation), and species-specific (dog, rat, monkey; Life Diagnostics). We completed imprecision testing following a modified 10-day Clinical and Laboratory Standards Institute (CLSI) protocol EP5-A.

The Dade cTnI assay was used to initially test pools; thus not all low-end pool concentrations were optimized for each immunoassay tested (a limitation of this study). All quality control materials tested (Bio-Rad) were within the expected range before and during experimental testing. In accordance with CLSI precision evaluation guidelines, on day 6 (DPC, Roche, Tosoh) or 7 (Abbott, Bayer first generation, Beckman) of the 10-day precision testing period, we calibrated a second reagent lot used it to complete the study. Not all assays used a new reagent lot on the same day (day 6); this reflected bulk packaging of reagents and the fact that different numbers of tests per kit required different schedules for calibrating and running daily quality controls between assays. The Bayer Ultra assay evaluation was added at a later stage of the study, as it was not available when the study was initiated. Due to limited specimen pool availability, the imprecision protocol carried out on this assay was only 4-day, 1 calibration, 1 lot of reagent.

For each species strain, we initially assayed the pools for cTnI using the Dade Behring Dimension assay. We prepared 4 sets, 1 for each species-strain evaluated, of 5 pools [pools A (highest concentration) to E (lowest concentration)] to span the diagnostic range of the assay. For each species, we calculated separate dilutions, since the absolute amount of cTn released at the time of experimental sampling was not uniform among species/strains. The targeted concentrations for the 5 pools, based on the Dade cTnI assay, were 0.8, 0.4, 0.2, 0.1, and 0.05 μg/L. After the pools were made, aliquots were immediately prepared and frozen in sufficient quantities to allow for a new vial to be thawed for each testing run. The aliquots used in the precision evaluation were stored at −20 °C for 11–12 days before testing. During the imprecision evaluation, we tested each pool (A–E) for each species/strain in duplicate, twice a day for 10 days, with at least 2 h between runs for all assays. The order of the pools tested was randomized each day, and at least 2 different lots of reagents were used during the 10-day testing period. The species/strains tested were SD rat (not tested on Ortho Vitros ES and Tosoh cTnI assays due to nonresponsive results from Phase Ia), beagle dog, and rhesus monkey. Given the comparable reproducibility of responses observed in the SD and Wistar strains and rhesus and cynomolgus species in phase 1A, only 1 strain of rat (SD) and monkey species (rhesus) was carried forward into phase 1B. This was further supported by the fact that we experienced short-sample problems when we attempted to test the cynomolgus monkey specimens; the data were not reliable and therefore are not reported. Imprecision profiles were defined as good (G), imprecision profile with ≥3 pools having CV ≤20% (functional sensitivity), and poor (P), imprecision profile with <3 pools having CV ≤20%. We recognize that our criteria were not an evidence-based objective tool, but an assessment based on the expert opinions of the authors. Assays that met a 10% CV at the 99th percentile value as recommended by guidelines for analytical quality specifications of assays were so designated *(1, 2, 11, 12)*.

We determined the lower limit of detection (LLD) for each assay in each species/strain tested by measuring 20 replicates of the negative serum pools on day 1 of the precision testing. The mean (2SD) is reported as the LLD for each assay. For the 3 assays for which some data were reported as being less than the LLD of the assay, the number just below the LLD was substituted (Roche Elecsys <0.010 = 0.009; Tosoh <0.06 = 0.05; DPC <0.200 = 0.199 μg/L).

We calculated total imprecision profiles for each pool following a modified CLSI EP5-A guideline, as described *(12)*. Three assays, Roche cTnT, Tosoh cTnI, and DPC cTnI, do not report numerical data down to 0, but instead have a "<LLD" result. Mean cTn pool concentrations reported in the imprecision studies were based on aliquots with measurable cTn only. For imprecision calculations, if >25% of the results for a single pool were <LLD, the % CV was not calculated (underpowered). For those pools with fewer than 25% of the measurements <LLD, the % CV was calculated after eliminating the LLD numbers and using just the remaining data. We excluded outlier pairs from the analysis according to CLSI guidelines for the determination of outlier replicates during total imprecision evaluations.

Results

PHASE IA

The varying responses of all cTn assays compared for all species/strains to the negative, low, medium, and high pools for each animal species tested are shown in Supplemental Fig. 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol54/issue12. Table 1 summarizes the regression analysis data (linearity study) for all cardiac troponin assays compared to the negative, low, medium, and high pools with assigned values of 0, 1, 2, and 4, respectively. The concentration determined by each assay depends, in part, on the amount of circulating cTn at the time of blood sampling. As the extent of myocardial injury was not uniform for each species/strain, direct comparison of absolute values cannot be made among species/strains. Results vary across the 5 animal groups ($P < 0.001$) by analyzer ($P < 0.001$) and dilution level ($P < 0.001$), with a significant interaction between animal and analyzer ($P < 0.005$). Results do not vary by rat strain ($P < 0.5$) but do vary by monkey strain ($P < 0.001$).

Table 1. Linear regression analysis statistics for dilution studies by animal species/strain for each cTn assay compared to the relative dilution factor of each pool tested.[a]

	SD rat		Wistar rat		Beagle dog		Rhesus monkey		Cynomolgus monkey	
Analyzer	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Architect	3.60	−0.91	4.30	−5.7	0.96	0.11	6.38	−0.43	1.28	0.10
Centaur first generation	5.10	0.63	5.91	0.08	4.52	−0.69	20.0	−0.18	6.07	0.50
Access	5.02	−0.74	6.39	−0.92	1.81	−0.17	9.12	−1.35	2.03	0.23
Dimension	5.89	−1.00	6.20	0.30	1.13	−0.09	7.10	0.61	2.36	0.03
Vitros	0.10	−0.07	0.10	−0.09	1.77	−0.21	7.21	0.20	2.12	0.57
Immulite	0.18	0.13	0.17	0.29	5.64	−3.63	23.5	−3.20	3.01	0.04
Elecsys	1.23	0.12	1.08	0.11	0.06	−0.04	0.89	0.05	0.73	0.28
Enzyme immunoassay	1.22	0.59	1.05	0.67	1.40	−0.34	6.23	−2.01	2.65	−2.27
Tosoh[b]					1.03	−0.65	7.08	0.66	1.84	0.19

[a] Negative, low, medium, and high pools were assigned values of 0, 1, 2, and 4, respectively, for this linearity study. All r values >0.99.
[b] No data reported for Tosoh assay for rats owing to lack of response (all values <0.6).

The comparative immunoreactive responses rated as good (G), adequate (A), weak (W), or none (N) of the respective assays (assessed by the comparative magnitude of the response) are summarized in Table 2. Based on these findings by assay and species/strain as well as limited specimens for the cynomolgus monkey, we decided that phase IB imprecision studies would be carried out only on SD rat, dog, and rhesus monkey.

PHASE IB

The LLD values for each assay and species tested are shown in Table 3. Fig. 1 shows the graphic display of % CV vs pool concentrations, along with each assay's 99th percentile reference value. The mean cardiac troponin concentration for pools A (highest concentration) to E (lowest concentration) grouped by species for each assay show marked differences between the results ob-

Table 2. Assessment of immunoreactivity and imprecision profiles in animal species/strains across cTn analytical platforms.[a]

	cTn immunoreactivity[b]				Imprecision profile		
	SD and Wistar rats	Beagle dog	Rhesus monkey	Cynomolgus monkey	SD rat	Beagle dog	Rhesus monkey
Abbott Architect	G	G	G	G	G	G	G
Bayer Centaur	G	G	G	G	P	G	P
Bayer Centaur Ultra	G	G	G	G	G	G	G
Beckman Access	G	G	G	G	G	G	G
Dade Dimension	G	G	G	G	G	G	G
DPC Immulite	W	G	G	G	P, N	G	G
Ortho Vitros ES	W	G	G	G	ND	G	G
Roche Elecsys	G	W	G	G	P	P, N	G
Tosoh AIA 60011	N	A	G	G	ND	G	G
Species-specific enzyme immunoassay	A	A	G	A	ND	ND	ND

[a] Immunoreactivity: G, good response; A, adequate response; W, weak response; N, no response. Imprecision profile: G, good profile (>3 values <20% CV); P, poor profile (<3 values <20% CV); ND, not done; N, no response.
[b] Comparative magnitude (fold) and proportionality of response from negative pool or LLD.

Table 3. LLD in μg/L (based on human cTn calibrators) for cTn assays by animal species/strain.[a]

	SD rat	Beagle dog	Rhesus monkey
cTnI			
Abbott Architect	0.01	0.01	0.02
Bayer Advia	0.02	0.02	0.02
Centaur			
Beckman Access	0.03	0.02	0.02
DPC Immulite 1000	0.20	0.20	0.20
Dade Dimension	0.02	0.03	0.02
RxL			
Ortho Vitros ES	ND[b]	0.068	0.008
Tosoh AIA 600 II	ND	0.06	0.06
cTnT			
Roche Elecsys 2010	0.010	0.010	0.010

[a] Bayer Advia Centaur Ultra cTnI LLD not performed.
[b] ND, not done.

tained with the cTnI and cTnT assays. The analytical response for each pool varied >20-fold for the 9 participating cTnI assays, underscoring both the lack of standardization and the differences in detection of cTn among the various species in the examined assays, and confirming the observations made in phase 1A. The data also indicate that the different methods had different analytical sensitivities for measuring cTnI in the pools with the lowest concentrations of this biomarker.

Table 2 summarizes the differences in the respective imprecision profiles as good or poor within each set of species serum pools by immunoassay. Only the Bayer Ultra assay demonstrated a ≤10% CV at the 99th percentile value in the monkey. As noted in "Methods," however, design of the low-concentration pools precluded the ability to determine whether the Bayer Ultra and Ortho ES assays in the dog and the Ortho ES assay in the monkey could meet a 10% CV requirement at the 99th percentile value.

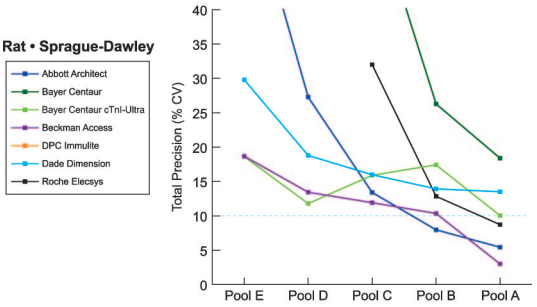
Discussion

The results of this study demonstrate that the performance of commercially available cTn assays varies widely between laboratory animal species. The current study is the most comprehensive evaluation of commercially available cTn assays, with specimens from 3 laboratory species, and complements studies by O'Brien et al. *(6, 20)* that also addressed the performance differences between cTn assays with samples from different laboratory animal species. It is not surprising that our imprecision findings, for example for the Bayer assay, differ from other studies *(6)*, as our results represent measurement of cTnI on the Advia Centaur system for both the first generation and Ultra assays and not the ACS100 system and use the CLSI protocol, although it is modified for a 10-day imprecision study; both factors are unique to this study. Our study is also unique in defining the imprecision profiles at low cTn concentrations using species/strain-specific sera, and in providing direct comparisons among assays independently of the commercial information provided with each assay. Good imprecision profiles were demonstrated for Beckman (rat, dog, monkey), Dade (rat, dog, monkey), Abbott (rat, dog, monkey), Bayer first generation (dog), Bayer Ultra (rat, dog, monkey), Roche (monkey), DPC (dog, monkey), Ortho (dog, monkey), and Tosoh (dog, monkey).

Our data with different animal species/strains confirm the large diversity among cTn assays with respect to total imprecision, as demonstrated using human serum *(12)*, and underscore the need to understand the limitations of cTn assays when used in experimental animal models. The demand for very precise cTn assays undoubtedly presents a difficult challenge. The results obtained with more recently released second- and third-generation assays show that there has been substantial improvement in the precision and sensitivity offered. This is well demonstrated based on our preliminary comparison of data between the Bayer first-generation and the substantially improved second-generation Ultra assay. Low-end analytical improvement is considered by manufacturers as the main goal in the design and development of new-generation assays. Our study was not designed to evaluate accuracy, i.e., the closeness of agreement of a single measurement with true value, as no reference method or reference material is available.

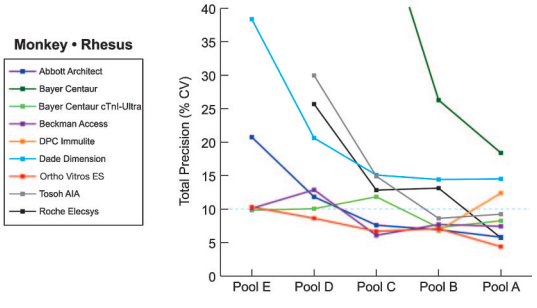
Several investigators have examined the differential reactivity of cardiac tissue to both cTnT (Roche second generation) and cTnI (Dade Stratus I, first generation) assays *(18, 23)*. For cTnT, a 20-fold difference in cardiac tissue reactivity was found, in order of decreasing levels, in rat, dog, cat, turkey, pig, horse, rabbit, sheep, chicken, and fish. For cTnI, a 110-fold difference in cardiac tissue reactivity was present in dog, calf, horse, sheep, pig, rabbit, rat, mouse, turkey, chicken, and trout. A recent study in dogs, rats, and mice demonstrated that histopathological and pathophysiological cardiac changes induced by exposure to various inotropic agents and cardiotoxic drugs correlated with increased serum cTnI concentrations *(6)*. This study also showed that the first-generation DPC Immulite cTnI and the species-specific enzyme immu-

A



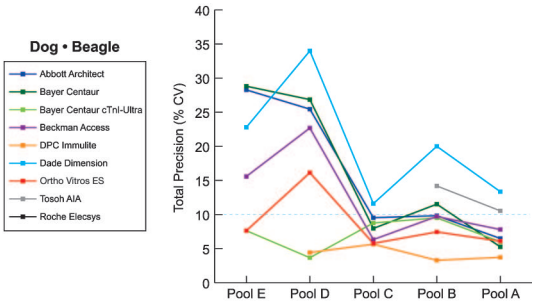
Mean Troponin (µg/L)	99th	Pool E	Pool D	Pool C	Pool B	Pool A
Abbott Architect cTnI	*0.012*	0.018	0.043	0.097	0.207	0.449
Bayer Centaur cTnI	*0.10*	0.004	0.026	0.083	0.252	0.559
Bayer Centaur cTnI-Ultra	*0.04*	0.017	0.029	0.052	0.113	0.224
Beckman Access cTnI	*0.04*	0.052	0.091	0.158	0.316	0.633
DPC Immulite cTnI	*0.20*	<0.200[f]	<0.200[f]	<0.200[f]	<0.200[f]	<0.200[f]
Dade Dimension cTnI	*0.07*	0.046	0.086	0.173	0.370	0.806
Roche Elecsys cTnT	*0.01*	<0.010[f]	0.012[g]	0.040	0.106	0.237

B



Mean Troponin (µg/L)	99th	Pool E	Pool D	Pool C	Pool B	Pool A
Abbott Architect cTnI	*0.012*	0.058	0.121	0.251	0.475	0.990
Bayer Centaur cTnI	*0.10*	0.031	0.072	0.172	0.369	0.807
Bayer Centaur cTnI-Ultra	*0.04*	0.029	0.047	0.094	0.170	0.326
Beckman Access cTnI	*0.04*	0.070	0.125	0.225	0.403	0.805
DPC Immulite cTnI	*0.20*	<0.200[f]	<0.200[f]	0.215[f]	0.290	0.608
Dade Dimension cTnI	*0.07*	0.047	0.072	0.135	0.259	0.514
Ortho Vitros ES cTnI	*0.034*	0.062	0.114	0.220	0.407	0.882
Tosoh AIA 600 II cTnI	*0.06*	0.10[g]	0.107[g]	0.238	0.454	1.010
Roche Elecsys cTnT	*0.01*	0.011[g]	0.037	0.091	0.194	0.375

C



Mean Troponin (µg/L)	99th	Pool E	Pool D	Pool C	Pool B	Pool A
Abbott Architect cTnI	*0.012*	0.031	0.070	0.155	0.317	0.641
Bayer Centaur cTnI	*0.10*	0.156	0.289	0.609	1.200	2.355
Bayer Centaur cTnI-Ultra	*0.04*	0.114	0.216	0.425	0.743	1.620
Beckman Access cTnI	*0.04*	0.082	0.137	0.275	0.498	1.015
DPC Immulite cTnI	*0.20*	0.201[g]	0.312	0.586	1.122	2.255
Dade Dimension cTnI	*0.07*	0.058	0.094	0.181	0.350	0.703
Ortho Vitros ES cTnI	*0.034*	0.099	0.137	0.225	0.383	0.769
Tosoh AIA 600 II cTnI	*0.06*	<0.06[f]	<0.06[f]	0.06[f]	0.150	0.387
Roche Elecsys cTnT	*0.01*	<0.010[f]	<0.010[f]	<0.010[f]	<0.010[f]	<0.010[f]

Fig. 1. Total imprecision profiles (graphs) based on mean cardiac troponin concentrations (tables) for cardiac troponin assays evaluated for Sprague–Dawley rat (A), beagle dog (B), and rhesus monkey (C).

Shown are 99th percentile concentrations as per manufacturer FDA-cleared package insert for normal population studies in humans.

noassays had poor sensitivity and demonstrated <1% of the dynamic range of the Bayer Centaur cTnI (first generation) and Roche cTnT assays in the rodent; in the dog, the DPC assay was effective. Our current observations support and extend these findings. There is no dispute of the ability to measure cTnT in dogs at low concentrations. This is exemplified by the study of O'Brien et al. *(20)*, who measured dog cTnT concentrations at 0.3 μg/L, a value however 30-fold higher than the 99th percentile reference value, after ischemic-induced injury with reperfusion. Our observations demonstrate cTnT to be less sensitive than several cTnI assays in the dog model, with the cTnI assays providing a measurable signal in the absence of a cTnT signal in the examined specimen. Collectively, the results of our studies emphasize how individual cTn assay characteristics for each animal species/strain need to be uniquely examined. Animal data should not be compared to and correlated with different animal species or human data. The majority of anti-cTnI monoclonal antibodies possess wide specificity, recognizing both human and animal cTnI. Differences in antibody response across species have been documented, however *(26)*. For example, it appears that in the case of cTnI, assays that use 2 monoclonal antibodies (1 for capture and 1 for detection), and 1 of these anti-cTnI antibodies has specificity against the region 87–91, will show no cross-reactivity in the rat. This probably explains the lack of response observed in the evaluation of the Tosoh assay in the rat. The Architect and Bayer Ultra assays also have anti-cTnI antibody directed against the 87–91 amino acid epitope, but are 3-antibody assays employing an additional capture antibody with demonstrated cross-reactivity with rat cTnI. Additionally, it has been reported that the choice of antibodies has a great impact on the clinical performance of cTnI assays, with the inclusion in the assay of at least 2 monoclonal antibodies against epitopes in the N-terminal part of the heart-specific region of the molecule seeming critical to obtaining optimum performance *(27)*. Thus observations made at the clinical level may well translate into animal studies at the preclinical level.

Several limitations of our study should be noted. First, since each method was assessed in only 1 laboratory, it was not possible to definitively determine the effect, if any, on the imprecision of an assay by type or training of laboratory personnel performing the measurements. Second, although animal serum pools are better than artificial protein matrices, pooling may mask problems related to the performance for individual samples because interfering compounds in individual sera, e.g., heterophile antibodies, are diluted and their effects may escape attention. The addition of synthetic proteins to serum does not mimic the complexity of cTn forms found in the circulation following myocardial injury. Third, it is difficult to accurately estimate 10% and 20% CV concentrations based on an interpolation between relatively few points, compounded with the reality that several analysis measurements were not valid for statistical calculations. We used strict classification criteria based on the recommendations that the most robust cTn assays should obtain a ≤20% CV total imprecision at the 99th percentile reference value *(1, 2, 11, 12)*. Because reference values were not determined in the study by species, we used the human 99th percentiles as published in the FDA-cleared package insert of each manufacturer. Finally, design of the pool of low concentrations precluded the ability to determine whether the Bayer Ultra and Ortho ES assays in the dog and the Ortho ES assay in the monkey could meet the a 10% CV at the 99th percentile value.

In conclusion, not all cTn assays are equally effective in animals. We have demonstrated variable cTnI concentration responses and total imprecision characteristics at and above the 99th percentile reference values across the 3 laboratory animal species examined. We recommend that each laboratory be cognizant that responses vary by species and possibly strain, and that differences in relative cTn concentrations between animals and human can occur and need to be interpreted with caution.

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 of Potential Conflicts of Interest: *Upon submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: F.S. Apple, Abbott Laboratories, Ortho-Clinical Diagnostics, and Sensera.
Stock Ownership: None declared.
Honoraria: F.S. Apple, Abbott Laboratories, Ortho-Clinical Diagnostics, and Sensera.
Research Funding: F.S. Apple, Abbott Laboratories, Siemens (Bayer, Dade-Behring, DPC), Mitsubishi Kagaku Iatron, Ortho-Clinical Diagnostics, bioMerieux, Response Biomedical, Roche Laboratories, Radiometer Medical, Bio-Rad, Biosite, and Wyeth Pharmaceuticals. The project was conducted with funding and technical input from the Health and Environmental Sciences Institute (HESI) Biomarkers Committee; HESI is a nonprofit organization that sponsors collaborative research programs involving industrial, academic, and government scientists (http://hesiglobal.org).
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played a direct role in the approval of manuscript.

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