Temporal Variability of Cardiac Troponin-I Concentrations in Rats Under Standard Laboratory Conditions

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Abstract

Background: The use of cardiac troponin I (cTnI) as a biomarker for cardiotoxicity has become a standard, and is supported by the European Society/ American College of Cardiology. Cross-species reactivity of cTnI has recently been shown in rats, dogs, and monkeys. However, for use in rodent model systems an issue with many clinical assays for cTnI measurement is that the limit of quantification and required serum volume are too high. This hinders studies in rodent model systems which observe changes in cTnI concentrations in individual animals over time. Additionally, it has been difficult to measure baseline concentrations and document biological variability of cTnI concentrations in healthy rodents.

Objective: In this study we investigated the use of a highly sensitive cTnI assay, the Erenna™ cTnI Immunoassay (Singulex), utilizing microparticles (MP) to quantify concentrations of cTnI, longitudinally in individual rats over time under standard laboratory conditions.

Methods: Sera were collected hourly, over a 24 hour time period, from 18 healthy rats handled under three standard laboratory conditions: resting, oral dosing with placebo, and simulated transportation. Blood was collected from individual rats at each time point. Serum aliquots of 30 µL were split and analyzed in duplicate using the Erenna MP-based cTnI Immunoassay.

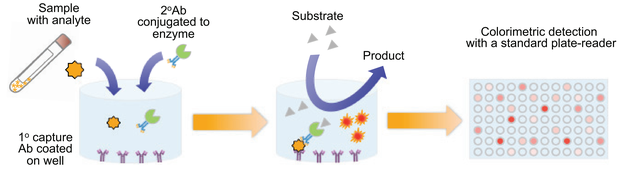
Results: Average concentrations of cTnI in healthy rats ranged from 2.8–6.3 pg/mL (n=3; 3 provided non-detectable cTnI), 3.6–8.7 pg/mL (n=5), and 2.7–6.9 pg/mL (n=6) in resting, orally dosed and transported rats, respectively. No trends in the increase or decrease of cTnI concentration over time were apparent. The median (+/– STDEV) concentrations of cTnI over all time points was 4.2 +/– 1.5 pg/mL (n=27), 5.9 (+/– 2.2) pg/mL (n=28), and 4.3 (+/– 1.4) pg/mL (n=29) for resting, orally dosed and transported rats, respectively. There was no statistical difference (95% CI) observed in cTnI concentrations between rats handled under these three standard laboratory conditions.

Conclusion: In this study, we demonstrated use of a highly sensitive cTnI Immunoassay to detect concentrations of cTnI in live, healthy rats handled under standard laboratory conditions using 15 µL of serum/determination. Further we demonstrated that rat blood cTnI concentrations show minimal variability over a 24 hr interval. This understanding of baseline and biological variability in rats will be fundamental for designing and analyzing future studies that assess potential cardiotoxicity in drug development.

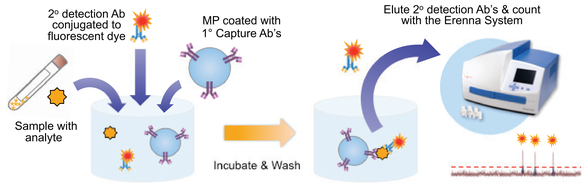
Introduction

Figure 1: Comparison of traditional ELISA immunoassays with the novel Erenna Immunoassay System.

(A) Traditional ELISA immunoassays consist of 3 major steps: a sandwich immunoassay, enzymatic amplification, and colorimetric detection.



(B) The Erenna Immunoassay System consists of 2 major steps: a modified microparticle (MP) based sandwich immunoassay followed by single-molecule counting (SMC) technology.



Methods

Animal Handling: Healthy rats (n=18) were handled under three standard laboratory conditions: resting (n=6), orally dosed with placebo (n=6), or simulated transportation (n=6).

Specimens: Rat sera (30 µL) were collected hourly from individual rats, over a 24-hour time period that began approximately 4–6 hours before the specified standard laboratory handling event ensued.

Sample Analysis: Rat cTnI concentrations were quantified in duplicate with the Erenna IA System (Figure 1) using 15 µL of rat serum per sample.

Results

Figure 2: Comparison of Average cTnI Levels in Healthy Rats After Standard Laboratory Handling. Rat sera were collected hourly over a 24 hour time period from healthy rats that were either resting, given an oral dose of placebo, or undergoing simulated transport. Time of handling is indicated by the vertical bar at time=0. No significant differences between cTnI levels in individuals over time were observed.

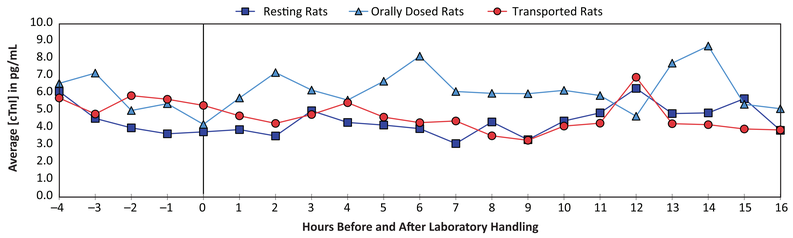
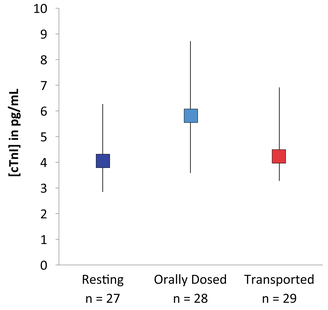
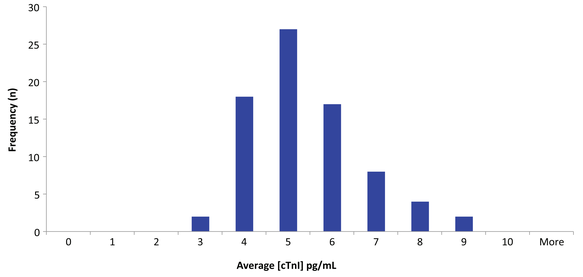


Figure 3: Comparison of cTnI Levels in Healthy Rats Under Different Laboratory Handling Conditions. Data across all time-points for each condition were analyzed, and the median (+/– SD) and range for each laboratory handling cohort were determined. No significant differences in [cTnI] were observed between the three different handling conditions.



Parameter	Resting Rats	Orally Dosed Rats	Transported Rats
Median [cTnI] pg/mL	4.15	5.91	4.33
Range [cTnI] pg/mL	2.8–6.3	3.6–8.7	3.3–6.9
SD	0.87	1.28	0.92
(n)	27	28	29

Figure 4: Summary of [cTnI] Distribution in a Laboratory Population of Healthy Rats. Results for all rats (resting, orally dosed and transported) were pooled, and the frequency of [cTnI] distribution was determined.



Summary of [cTnI] Distribution	
Average	4.95 pg/mL
+/– SD	+/– 1.27 pg/mL
Range	2.7–8.7 pg/mL

Table 1: Healthy levels of [cTnI] in Rats compared to Humans, Dogs and Monkeys. Average [cTnI] levels and the range of [cTnI] quantified in serum from healthy rats were compared to previously reported ranges of [cTnI] in humans[1], dogs[2] and monkeys[2] as quantified by the Erenna Immunoassay System.

Species	Average Healthy [cTnI]	Healthy Range of [cTnI]
Rat	5 pg/mL	2 – 10 pg/mL
Human[1]	2 pg/mL	1 – 12 pg/mL
Dog[2]	2.5 pg/mL	1 – 4 pg/mL
Monkey[2]	4.4 pg/mL (female) 5.3 pg/mL (male)	4 – 5 pg/mL

[1]Todd J., Freese B., Lu A., Held, D., Morey J., Livingston R., Goix P. (2007) **Ultrasensitive Flow-based Immunoassays by Use of Single-Molecule Counting.** *Clin Chem* 53(11):1990-5. doi:10.1373/clinchem.2007.091181 [PMID: 17890441]
[2]Schultze A.E., Konrad R.J., Credille K.M., Lu Q.A., Todd J. (2008) **Ultrasensitive Cross-Species Measurement of Cardiac Troponin-I Using the Erenna Immunoassay System.** *Tox Path*, in press. doi: 10.1177/0192623308322016

Conclusions

- The Erenna Immunoassay System can accurately quantify cTnI from healthy rats (LoD = 0.2 pg/mL) using small sample volumes of only 15 µL.
- There is little biological variation in baseline levels of cTnI in healthy laboratory rats.
- Measurements of [cTnI] are consistent between individual rats over long periods of time and under standard laboratory handling conditions.
- Currently, we are applying this novel technology towards establishing a reference range for baseline [cTnI] in a larger population of healthy rats.
- Studies like these are essential for developing reference ranges for cTnI which serve as a baseline for cardiotoxicity studies in commonly used animal model systems.