Development and preliminary clinical validation of an ultra-sensitive assay for cardiac troponin using microparticle based immunoassay and single molecule counting

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Abstract

Background: The European Society/American College of Cardiology (ESC/ACC) have established cardiac troponin (cTnI) as the gold standard for diagnosis of acute myocardial infarction (AMI) and risk stratification for adverse cardiac events. We previously reported the development of a highly sensitive cTnI assay and the use of this assay to define cTnI levels in normal subjects. This assay has been further developed to yield greater sensitivity and currently we report its analytical and preliminary clinical performance.

Methods: The Singulex cTnI assay was modified to use paramagnetic microparticles (MP) as the solid phase with a monoclonal capture antibody and a fluorescently-tagged affinity-purified goat detection antibody. After incubations and washing, the fluorescently-tagged antibody is chemically released from MPs and an aliquot is pumped into the Erenna™ Digital Molecule Counting (DMC) System. Individually-labeled antibodies are measured during capillary flow by setting the interrogation volume such that the emission of single fluorescent molecules is detected in a defined space following laser excitation. Total fluorescent signal is determined as a sum of the individual digital events. A 4-log dynamic range is obtained by analyzing digital events as well as total photons.

The limit of detection the Singulex cTnI assay was determined by the mean +3 SD method. The normal range was determined on a population of 150 apparently healthy subjects. We also examined 56 serial samples from 17 patients who presented to the ED with a diagnosis of AMI in a preliminary study. Results were compared to the Centaur cTnI 1st (n=11) and 2nd (n=6) generation (ultra) assays. All had initial Centaur cTnI results that were <0.35 ng/mL (10%CV, 1st gen. Centaur), 6 were <0.1 ng/mL (99th percentile, 1st gen. Centaur), and 2 were <0.04 ng/mL (10% CV 2nd gen. Centaur). The cTnI concentration was positive on at least one subsequent serial sample from these patients on the Centaur, establishing the diagnosis of AMI.

Results: The analytical sensitivity of the Singulex assay was 0.2–0.3 pg/mL. The precision was <10% at 1.6, 12.5 and 50 pg/mL. The assay provided a linear response from 0.39 to 100 pg/mL (r2 = 0.99; y=1x+0.14). The reference population exhibited a normal distribution with a mean at 2 pg/mL (range 0.4–9 pg/mL). We established a preliminary cutpoint at 10 pg/mL, which is 50-fold higher than the analytical sensitivity. In the 9 cases that had initial Centaur cTnI value between 0.1 and 0.35 ng/mL, all were positive for Singulex with values ranging from 37–91 pg/ml (3.7–9 times >cut point). In the 8 cases that had initial Centaur cTnI value <0.1 ng/mL, 3 of 8 cases were Singulex positive. The Singulex assay was positive at least 1 sample earlier than the 10%CV cutoffs for either the 1st gen. (10 of 11 patients) or 2nd gen. Centaur (1 of 6 patients) assays.

Conclusion: This study demonstrated a range for cTnI in a reference population between 0.4 and 9 pg/ml, with a mean at 2 pg/ml which is 10-fold greater than analytical sensitivity. Monitoring increases in cTnI levels above this reference range enabled detection of AMI several hours earlier than the Centaur cTnI assay.

Introduction

- Cardiac troponin is the gold standard for diagnosis of AMI.
- The NACB recommends a cutoff at the 99% of a healthy population with an acceptable precision (<10%).
- Most commercial troponin assays do not have the sensitivity needed to measure troponin in healthy blood with a 10% CV.
- Next generation troponin assays have been developed that detect troponin with <1 pg/mL sensitivity.

Study objectives

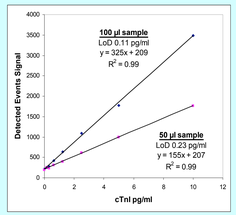
- Evaluate the analytical sensitivity of the Singulex troponin assay.
- Determine the normal range for this assay.
- Measure troponin in patients with acute coronary syndromes admission samples and compare results against the first generation Siemens TnI assay.

Methods

Erenna™ System cTnI Assay Procedure

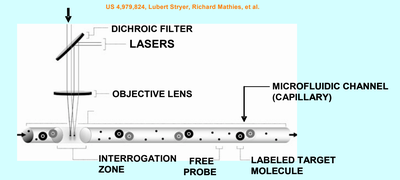
- Assay standardized with NIST cTnI reference material
- 50 µl sample or calibrator + 150 µl assay buffer with streptavidin paramagnetic microparticles coated with biotinylated monoclonal anti cTnI (MPs)
- Incubate 1 hr 25C with shaking
- Wash 1X via magnetic separation
- Add detection antibody (fluorescently labeled affinity purified goat anti-cTnI
- Incubate 30 minutes
- Wash 6X via magnetic separation
- Elute antibody-cTnI complexes from MPs
- Filter MPs away from eluate using 0.2 um 384-well spin plates
- Count dye-labeled molecules and photons in eluate with Erenna™ System

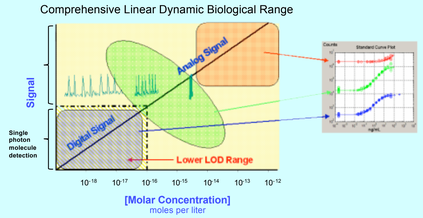
Standard Curves (50 or 100 µl sample volume)



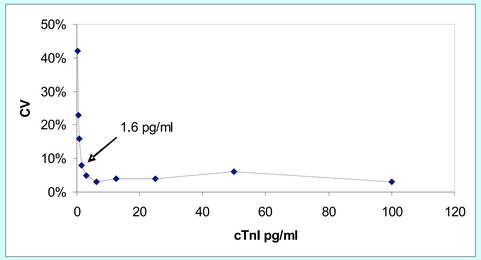
50 µl sample volume used for all other studies

Erenna System optics and data analysis

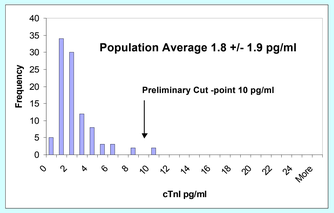




Precision profile



Distribution of cTnI in plasma from 100 apparently healthy blood donors



Preliminary Erenna System cTnI assay performance specifications

Criteria	***Preliminary Specification***
Analytical Sensitivity	0.2 pg/ml
Lower Limit of Quantification based upon Inter-assay 10% CV	1.78 pg/ml
Lower Limit of Quantification based upon dilutional linearity	1.28 pg/ml
Cut point for 99% normal population	10 pg/ml

Summary of Results from 1st specimen at presentation in ED

# Chest Pain Patients at presentation in ED	***Bayer Centaur (First Generation)***	***Singulex Erenna System***
8 Patients	Below detection limit of 0.1 ng/ml	3 of the 8 patients quantifiable for cTnI
9 Patients	Equivocal, between 0.1 and 0.35 ng/ml	All 9 patients quantifiable for cTnI

Conclusions

- Singulex assay is 10–100 fold more sensitive than previous commercial troponin assays.
- Healthy subjects demonstrate a near-gaussian distribution that is skewed slightly to the right.
- The use of a very low cutoff (99th percentile) enables detection of more cases of AMI at presentation than existing generation troponin assays.
- The value of high sensitivity troponin must be validated in a risk stratification.