Prototype digital immunoassay for troponin I with subfemtomolar sensitivity

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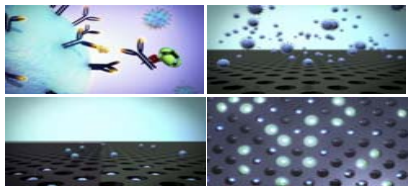
INTRODUCTION

High-sensitivity cardiac troponin I (cTnI) measurement offers a promising new tool for early detection and monitoring of cardiovascular disease. The ability to reliably assign TnI values to all normal subjects tested represents a newly desired assay capability in many applications. We report preliminary analytical data from a prototype Single Molecule Array (Simoa, or digital immunoassay) for serum TnI that is capable of 2—3 logs greater sensitivity than the clinically used cTn assays and 1—2 logs greater sensitivity than the latest high-sensitivity troponin assays, most of which are not yet commercially available.

METHODOLOGY

The Simoa assay used reagents similar to those for conventional ELISAs. TnI-specific capture antibody was immobilized on paramagnetic beads, and detection antibody was biotinylated. Sandwich immunocomplexes were formed by incubating TnI antigen and antibodies together and then labeling with streptavidin-conjugated beta-galactosidase. Beads with the labeled immunocomplexes were isolated and sealed in individual microwells of the array containing fluorescent substrate. Well arrays were imaged with a CCD camera. Enzyme-labeled beads that converted substrate into fluorescent product over time were considered to be "on" for purposes of digital counting. The entire range of signal was determined using imaging analysis software to determine the Average Enzyme per Bead (AEB), the unit of measurement of Simoa. A standard curve relating the AEB output to TnI concentration was used to determine the sample concentration. The digital TnI immunoassay was evaluated for recovery, linearity, precision, analytical sensitivity, and ability to measure cTnI in normal serum samples. Discrimination of normal subjects from those with mild to moderate heart failure was also preliminarily assessed.



RESULTS

Fig. 1. Dose-response of Simoa over 3 logs of spiked TnI in human serum. Each data point represents the mean of 3 replicates. The insert highlights the low end of the curve obtained with digital quantification. The line shows linear fitting with an $R^2 > 0.999$, from which the 3SD estimated LoD was 17.8 fg/mL.

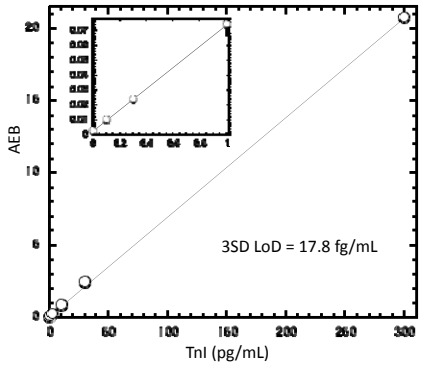


Fig. 2. Analytical LoD estimate for TnI assay. A mean LoD of 17 fg/mL (SD, 6.9) was estimated from 4 calibration runs performed on different days with 2 reagent lots. The mean signal-to-background ratio at the lowest dose is 3.87 (SD, 0.24), indicating excellent curve shape at the low end.

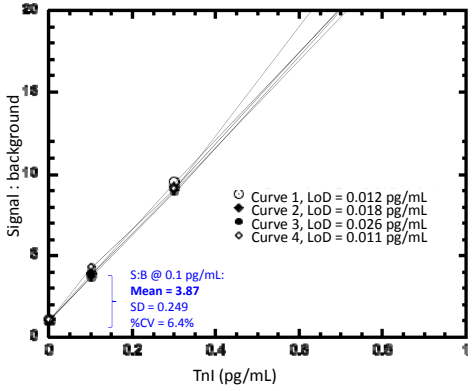


Fig. 3. Linearity by mixing high and low TnI samples. Admixtures were prepared per CLSI EP6-A using a high TnI sample (76.9 pg/mL) and a low TnI sample (2.58pg/mL). Data fit a linear model (R2 = 0.989), with an average deviation from linearity of 11%.

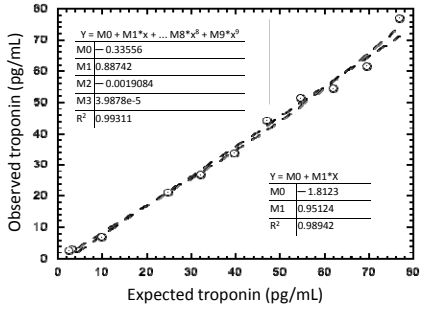


Fig. 4 . Test of TnI in normal control and heart failure samples. Serum samples from 46 control individuals and 34 mild/moderate heart failure patients (New York Heart Association classes II and III) were tested. cTnI values from the 46 normal control samples ranged from 0.13 to 12.25 pg/mL, with a mean, median, and 75 percentile of 1.92, 1.05, and 2.36 pg/mL, respectively. Total imprecision from a normal serum sample tested on 4 separate runs was 9.5% CV with a mean cTnI of 1.45 pg/mL. cTnI values from 34 mild to moderate heart failure patients ranged from 2.27 to 388 pg/mL, with a median of 12.52 pg/mL. Findings for the cohort of heart failure samples were significantly elevated relative to those of the normals ($p = 0.0067$).

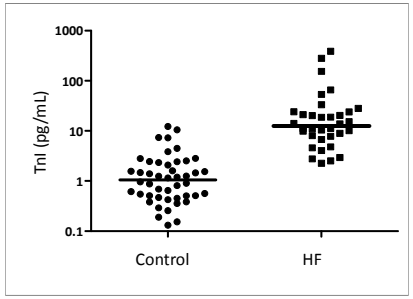


Table 1. Analysis of control and heart failure patients

TnI value ng/L	Ctrl	HF
Number of values	46	34
Minimum	0.1309	2.27
25% Percentile	0.4966	7.538
Median	1.054	12.52
75% Percentile	2.362	23.91
Maximum	12.25	388.8
Mean	1.927	38.63
Std. deviation	2.59	81.02
Std. error	0.3819	13.9
Lower 95% CI	1.158	10.36
Upper 95% CI	2.697	66.9
P value	0.0067	

CONCLUSIONS

The assay reliably quantified cTnI in normal individuals, with an LoD well below the lowest normal sample tested. These data suggest the assay could represent an advance in sensitivity relative to current high-sensitivity cTnI methods and could be a new enabling tool for high definition cTnI measurement.

REFERENCES

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