Fully automated ultrasensitive digital immunoassay for troponin using single molecule array technology

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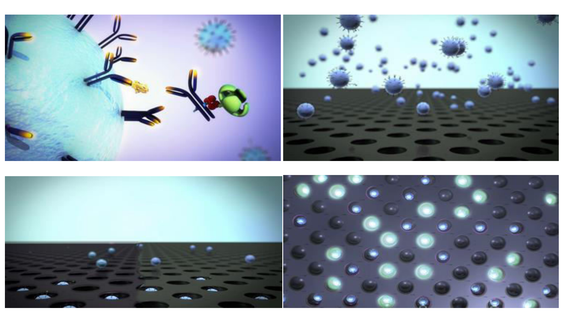
INTRODUCTION

Ultra-sensitive cardiac troponin measurement offers a promising new tool for early detection and monitoring of cardiovascular disease. With growing interest in exploring as an early indicator of adverse heart health trends, the ability to quantitate troponin in healthy control populations is emerging as a highly desirable assay capability. We report analytical data from a fully automated digital immunoassay for cardiac troponin I (cTnI) based on Single Molecule Array (Simoa) technology with a limit of detection 2 logs lower than contemporary high sensitivity troponin assays.

METHODOLOGY

Simoa TnI assay reagents were developed for a paramagnetic bead-based ELISA for use in the Simoa HD-1 Analyzer. Anti-cTnI capture beads were prepared by covalent coupling of antibody to carboxy paramagnetic microbeads, detector antibody was biotinylated by standard methods, and an enzyme conjugate was prepared by covalent coupling of streptavidin and beta–galactosidase. The HD-1 Analyzer first performs a 2-step sandwich immunoassay using 42 µL of serum or plasma sample, then transfers washed and labeled capture beads to a Simoa disc where the beads are singulated in 50-femtoliter microwells, sealed in the presence of substrate, and interrogated for presence of enzyme label. A single labeled cTnI molecule provides sufficient fluorescence signal in 30 seconds to be counted by the HD-1 optical system. At low cTnI concentration, the percentage bead-containing wells in the array with a positive signal is proportional to the amount of cTnI present in the sample. At higher cTnI concentration, the total fluorescence signal is proportional to the cTnI in the sample. The concentration of cTnI is then interpolated from a standard curve (range to 300pg/mL). This fully automated assay has time-to-first-result of 45 minutes.



RESULTS

Fig. 1. Representative dose response of Simoa cTnI assay across a 4.5 log range. Each data point represents the mean of 3 replicates. The insert highlights the low end of the curve obtained with digital quantification

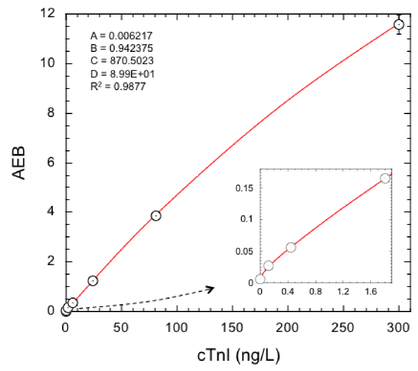


Fig. 2. Sensitivity of Simoa cTnI assay. Limit of detection (2.5 SD) was 0.010 pg/mL across 26 runs. Limit of quantification (20% dose CV from diluted serum samples) was 0.079 pg/mL across 16 runs and 144 determinations.

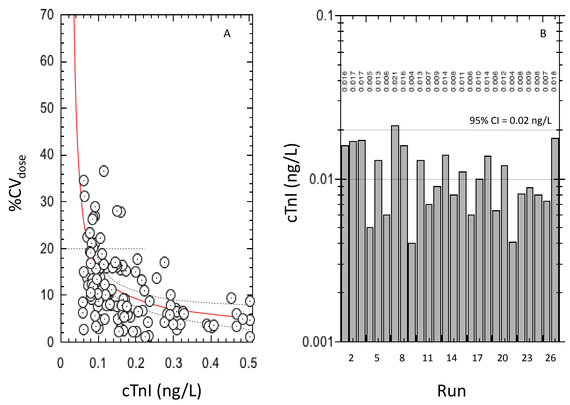


Fig. 3. Linearity and recovery of Simoa cTnI assay. Linearity was conducted per CLSI EP6-A using admixture of serum supplemented with NIST cTnI complex and a normal serum. The mean linearity was 89.5%. The mean spike recovery from 20 samples was 80.5% (data not shown).

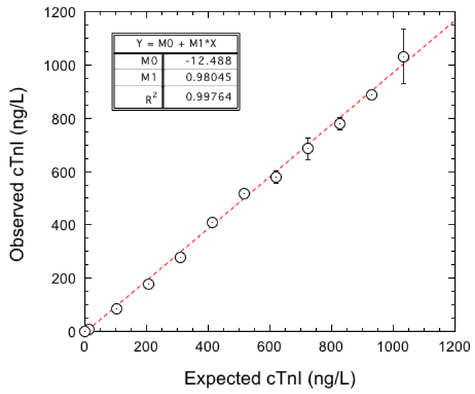


Fig. 4. Imprecision of Simoa cTnI assay. Precision per EP5-A guideline included two serum-based panels, 1 plasma-based panel and two cTnI controls assayed in replicates of three twice per day for five days using a single calibration curve. ANOVA gave CV's < 10% for all levels.

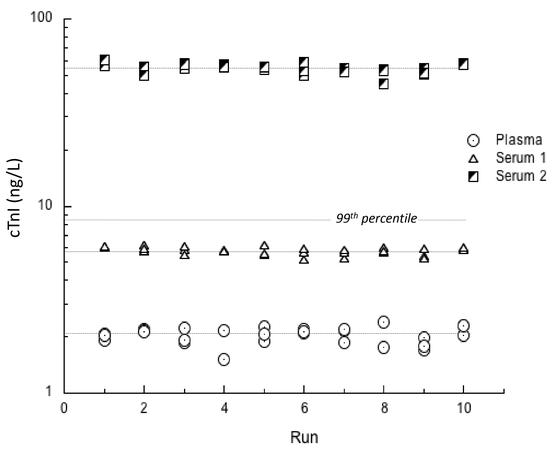
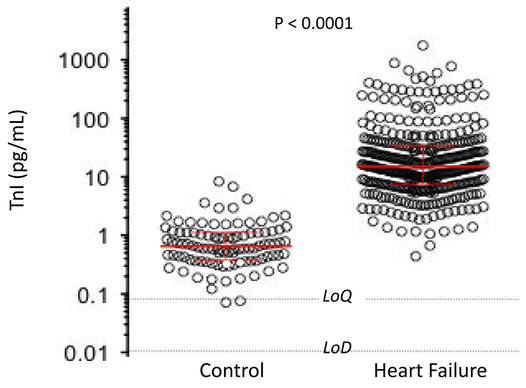


Table 1. Imprecision of Simoa cTnI assay.

Sample	Mean TnI ng/L	Within-run %CV (n=3)	Between-run %CV (n=10)	Between-day %CV (n=5)	Total %CV (n=30)
Plasma	2.0	9.7	3.0	0.0	10.2
Serum 1	5.7	5.9	0.0	1.0	6.0
Serum 2	54.7	4.8	3.8	1.8	6.4

Fig. 5 . Test of TnI in normal control and heart failure samples. Serum cTnI values from 97 healthy control samples ranged from 0.072 to 8.40 pg/mL, with a mean and 99th percentile of 1.01 and 8.40 pg/mL. Serum cTnI values from 375 patients with mild to moderate heart failure ranged from 0.440 to 1770 pg/mL, with a median of 15.1 pg/mL. The heart failure samples had significantly higher cTnI concentrations than healthy subjects (p=0.0002).



CONCLUSIONS

The assay was evaluated for sensitivity, recovery, linearity, precision and normal range. Discrimination of healthy subjects from those with mild to moderate heart failure was also preliminarily assessed. The results show the digital Simoa cTnI assay exhibited good general analytical properties and cTnI levels from healthy subjects were above the sensitivity limits. The assay represents a new enabling tool for ultra-sensitive cTnI measurement.

REFERENCES

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