Analytical performance characteristics of the Sgx Clarity™ cTnI Assay from Singulex for the detection of cardiac troponin I

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RESULTS

Introduction

More than 15 million Americans have coronary artery disease (CAD) and over 700,000 suffer an acute myocardial infarction (AMI) every year.1-3 Measurements of cardiac troponin T (cTnT) and T (cTnI) are the mainstay in the management of patients with suspected acute coronary syndrome (ACS).2

There is an association between cTnI levels measured within the current reference interval with stress-induced cardiac ischemia4 and with risk of future cardiovascular disease (CVD).4-6

It has been demonstrated that 35% of cardiac stress tests, used in the workup of patients with suspected CAD, may be unnecessary as they are performed in patients without underlying CAD.7 Unnecessary stress test have a potentially increased risk of cancer, estimated at 500 cases annually and a yearly cost of over $500 million in the U.S. alone.4 Measurement of cardiac troponin across the entire reference interval mimics a true high-sensitivity assay may support a more cost-effective and improved patient-safety strategies in CAD diagnostics.

The Sgx Clarity® cTnI assay (Singulex Inc., Alameda, CA, USA) is a fully-automated and ultra-sensitive in vitro diagnostic platform for the measurement of cTnI in EDTA plasma. The system is based on a paramagnetic immunomass powered by single molecule counting technology, which allows for detection of analytes down to femtogram-per-milliliter levels. In patients without previously known CAD who have been referred for stress myocardial perfusion imaging single photon emission computed tomography (SPECT) studies, Sgx Clarity cTnI measurements using the Sgx Clarity cTnI assay combined with clinical judgment had the ability to rule out the presence of cardiac ischemia at a negative predictive value (NPV) of 93%.8 The objective of this study was to validate the analytical performance, specifically the assay’s limit and the functional sensitivity and precision, of the Sgx Clarity cTnI assay in a multisite study.

Materials and Methods

Sgx Clarity cTnI Assay:
The Sgx Clarity cTnI assay measures human cTnI levels in EDTA plasma on the Sgx Clarity system from Singulex. The Sgx Clarity assay is a paramagnetic-based immunoassay powered by single molecule counting technology that uses a single-photon fluorescence detection for analyte quantification. A sample volume of 100 μL is used for analysis, and EDTA-plasma samples are incubated at 37°C with a capture antibody bound to a paramagnetic microparticle and a detection antibody bound to a fluorescent molecule.

After the washing step, remaining fluorescent molecules are eluted from the paramagnetic microparticles and a transferred to a detection vessel for fluorescence measurement. The fluorescence signal is evaluated against a calibration-adjusted master curve and converted into a cTnI concentration.

In this report, the limits of the Sgx Clarity cTnI assay were determined at Singulex, and the analytical performance characteristics were evaluated in a multisite study.

LoD, LoB, and LoQ/Functional Sensitivity:
Testing to determine LoD, LoB, and LoQ was performed following recommendations in CLSI guidelines EP28-A3. The LoD and LoQ were established independently for each of two reagent lots by measuring five replicates of each of five concentrations of cTnI (4.15 pg/mL, 20 pg/mL, 200 pg/mL, 2000 pg/mL, and 20,000 pg/mL), determined to be normal and high concentration, respectively.

Precision:
Testing to determine precision was performed following recommendations in CLSI guidelines EP05-A3. For the first-, second-, and third-order polynomial least-squares regression fits for all samples tested, CV % was calculated for each concentration level, and percent-normal testing on one site (Barcelona), one sample was missing, with analysis performed on 358 samples. On the other site (London), one sample was missing and one sample was removed as an outlier after testing, with analysis performed on 358 samples. The range cTnI concentration for male donors and female donors was 0.66 and 0.37 pg/mL, respectively. There was a significant difference between the central tendencies of the male and female distributions (p<0.05).

Multi-Site Evaluation:
Functional sensitivity was measured by testing ten EDTA-plasma samples near the LoQ, and the 10% functional sensitivity was estimated by analyzing ten EDTA-plasma samples near the LoQ, the 10% functional sensitivity was 0.36, 0.31, and 0.21 pg/mL at 20% CV, and 0.82, 0.71, and 0.49 pg/mL at 10% CV, respectively, at the three testing sites (Table 1). Observed precision was 4.06-5.6% and 2.7%, respectively (Figure 2). During percent-normal testing on one site (Barcelona), one sample was missing, with analysis performed on 359 samples. On the other site (London), one sample was missing and one sample was removed as an outlier after testing, with analysis performed on 358 samples. The range cTnI concentration was 0.08-11.50 and 0.13-12.7 pg/mL, respectively. The 99th URL was estimated to 6.58 (90% CI, 4.13-11.50 pg/mL) and 7.00 pg/mL (90% CI, 4.68-11.27 pg/mL), respectively (Figure 3). The average CV between duplicate determinations was 7.5% and 7.9%, respectively. The median cTnI concentration was 0.42, 0.49, and 0.46 pg/mL among women, and 0.81 and 0.88 pg/mL among men, respectively (Figure 4). The Pearson’s correlation of all replicates between sites was calculated to be 0.945 (95% CI, 0.905-0.952) (Figure 5). Mean Barcelona results were 0.08 pg/mL less than the London results, and limit of agreement was -0.53 to 0.56 pg/mL (Figure 6).

Conclusions

The Sgx Clarity cTnI assay has high analytical sensitivity and low imprecision for the measurement of cTnI in EDTA plasma. The assay has a broad dynamic range and performs well in different concentrations. The overall 99th percentile URL for the Sgx Clarity cTnI assay is 8.67 pg/mL, and the high sensitivity allows for accurate sex-specific URLs to be determined. The performance is reproducible across multiple external sites. The Sgx Clarity cTnI assay is CE marked in the European Union, and is indicated to be used in conjunction with clinical evaluation for ruling out cardiac ischemia in patients suspected of having CAD.

References

7. Singulex Clarity cTnI Assay: Instructions for Use. 06-0019-00. 2017.

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