



Analytical and clinical characterization of a novel high-sensitivity cardiac troponin assay in a United States population



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ABSTRACT

Background: Cardiac troponin (cTn) is the keystone for diagnosis of acute myocardial infarction (AMI). We examined the analytical and clinical diagnostic characteristics of the ACCESS hsTnI assay in a United States (US) population.

Methods: All measurements and studies were conducted using a lithium heparin matrix. Sex-specific 99th percentile upper reference limits (URLs) were determined for 1089 healthy women (54.6%) and men using non-parametric statistics. High-sensitivity (hs) performance was assessed to determine if the total CV was $\leq 10\%$ at sex-specific URLs, and if $\geq 50\%$ of cTnI values for each sex exceeded the assay's limit of detection (LoD). Precision, analytical measurement range, high-dose hook effect, and endogenous/exogenous interferences were examined with CLSI guidance. Clinical characterization included serial sampling of 1854 suspected AMI subjects presenting to 14 US Emergency Departments. AMI was adjudicated by a panel of expert cardiologists. The study's only exclusion was end stage renal disease.

Results: 99th percentile URLs were 11.6-, 19.8- and 17.5-ng/L for respective female, male and all-subject populations. Total %CV was $< 8\%$ from 6.8 to 19,000 ng/L, and $< 6\%$ at sex-specific 99th percentiles; $\geq 99\%$ of ACCESS hsTnI values for each sex exceeded the LoD. No high-dose hook effect or endogenous/exogenous interferences were identified. A comparison of Baseline samples collected at ≤ 1 h and any-time after presentation, found 4% lower sensitivity for AMI than with earlier sampling. For 1–9 h post presentation, the sensitivity was $> 90\%$, specificity $> 85\%$; and negative and positive predictive value were $\geq 99\%$ and $> 60\%$, respectively.

Conclusion: Analytical and clinical performance of the ACCESS hsTnI assay meets the definition of a hs cTn method. The ACCESS hsTnI assay has good precision over a wide range, no significant interferences, and sensitivity $> 90\%$ and NPV $\geq 99\%$. Performance is appropriate for aiding in AMI diagnosis.

1. Background

Approximately 7 million people in the United States present to emergency departments (ED) each year with signs and symptoms suggestive of the acute coronary syndromes (ACS), a continuum of cardiac ischemia that spans from stable angina to acute myocardial infarction (AMI). Of these, about 20% of patients (1.3 million) are eventually diagnosed with unstable angina or acute myocardial infarction [1]. Rapid and accurate identification of AMI patients is important for preventing mortality and avoiding unnecessary procedures, lengthy waiting time in EDs, patient anxiety, and associated healthcare costs [1].

During the last decade of the 20th century, evidence demonstrated that cardiac troponin (cTn)T and cTnI have excellent cardiac tissue specificity, as well as high diagnostic accuracy for AMI compared to other biomarkers for assessing myocardial injury [2]. In 1999, the first Global Task Force was assembled and promulgated the consensus recommendation that cTn is the sole biomarker required for diagnosis of AMI [3]. This guidance has been consistent through development of the second [4], third [5], and, most recently in 2018, the fourth [6] Global

Task force documents. The 4th Global Task Force included a focused discussion of myocardial injury and included detailed information on cTn analytic issues, use of Electrocardiogram, and the application of imaging for assessing myocardial injury and AMI [6]. The essential role of cTn is also articulated in AHA/ACCF evidence-based guidelines [7]. Evidence-based guidelines from professional societies including the AACC Academy [8] and the European Society of Cardiology [9] are unanimous in recommendations that cTn should be the sole biomarker for AMI diagnosis and risk stratification.

Currently there are two general classes of cTn assays used in most laboratories worldwide. The first have been coined *Contemporary cTn Assays* to distinguish them from early generation tests having less satisfactory analytic performance [10]. The second type of generally available cTn tests are referred to as *high-sensitivity (hs) cTn assays*, which according to the AACC Academy and IFCC Committee on Clinical Applications of Cardiac Bio-Markers (C-CB) must meet specified criteria [10].

For over 10 years Beckman Coulter has been developing a cardiac troponin assay with enhanced sensitivity [11]. The US final form of this assay is available and has been termed the Beckman Coulter ACCESS

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High Sensitivity Troponin I (hsTnI) assay. The aim of this study was twofold: (i) characterize the analytical performance of the ACCESS hsTnI assay on the Access 2 Immunoassay System (Beckman Coulter, Inc., Chaska, MN); and (ii) assess the clinical performance ACCESS hsTnI assay as an aid in the diagnosis of acute myocardial infarction (AMI)

Methods and materials

1.1. Beckman Coulter ACCESS hsTnI assay

The ACCESS hsTnI test is a sandwich immunoassay that utilizes a capture antibody targeting the 24–40 amino acid region and a detection antibody against a second stable cTnI amino acid region. In the ACCESS hsTnI's first step a tagged detection antibody is added to patient sample followed by an incubation during which binding to a specific epitope on cTnI complex occurs. After this incubation a suspension containing the capture antibody linked to paramagnetic particles is added by the Access 2 system to bind the 24–40 amino acid region cTnI. After a second incubation, a magnetic field is actuated, which pulls the paramagnetic particles having the [capture Ab–cTnI–detection Ab] complex out of solution and holds them to the side of the reaction cuvette. Addition of a substrate that reacts with the detection antibody's tag produces a chemiluminescent signal in proportion to the amount of cTnI in the patient sample. The Access 2 system (Beckman Coulter, Chaska, MN) was used for all hsTnI measurements in this study.

1.2. Analytical performance studies

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were characterized in accordance with CLSI guideline EP17-A2 [12] using a lithium heparin matrix. LoB for the ACCESS hsTnI assay on the Access 2 System was determined by measuring 60 blank samples for cTnI and calculating the mean and standard deviation (SD) according to the following equation: $\text{LoB} = \text{mean} + [1.645 \times \text{SD}]$.

The LoD, also determined in accordance with CLSI guideline EP17-A2 [12], utilized four pooled samples having very low cTnI concentrations; each of these samples was tested 60 times. The SDs were calculated for each sample's set of 60 measurements and then combined and averaged to derive the SD. The LoB and the SD value from the low samples were used to determine the LoD with the equation: $\text{LoD} = \text{LoB} + [1.645 \times \text{SD}]$ [12].

LoQ was designated as equivalent to the 20% total CV for the assay.

Imprecision represented by repeatability, between run and total within laboratory imprecision was determined in accordance with CLSI EP05-A3 [13] and expressed as %CV for nine Li-Hep plasma pools at cTnI concentrations spanning the assay's reported measuring range (between 6.8 ng/L and 19,310 ng/L).

1.3. High-sensitivity performance assessment and 99th percentile upper reference limits

Two cohorts of healthy individuals were used in these studies. The first was the AACC Universal Sample bank (USB), a publicly available cohort of healthy men and women collected under a protocol approved by Emory University's Institutional Review Board [14]. The AACC USB was utilized to assess high-sensitivity performance of the ACCESS hsTnI assay using the two criteria specified by the AACC Academy and the IFCC C-CACB [10]. The first criterion examined was to determine if the ACCESS hsTnI assay's total %CV is $\leq 10\%$ in the range of the sex-specific 99th percentile URLs. The second criterion was to examine if $\geq 50\%$ of the ACCESS hsTnI measurements for cohorts of > 300 healthy men and healthy women exceeded the assay's LoD. For this effort, specimens from healthy men and healthy women enrolled in the AACC Universal Sample Bank were utilized. Healthy men and women having surrogate biomarker values of NT-proBNP > 125 pg/mL

(if < 75 y) and > 450 pg/mL (if ≥ 75 y), Hemoglobin (Hb) $A_{1c} \geq 6.5\%$ and/or estimated GFR (eGFR) < 60 mL/min/1.73 m² were excluded from high-sensitivity assessment.

The second cohort included was a prospective multicenter study of 1,089 self-reported healthy adults ranging in age from 21 to 99 years; forty-five percent of the subjects were ≥ 60 years of age. This cohort was enrolled at five geographically diverse USA locations and was comprised of 595 healthy women (54.6%) and 494 healthy men. Sex-specific and overall 99th percentile URLs with 95% CI were determined in Li-Hep plasma and serum specimens using the nonparametric method as recommended by the AACC Academy and IFCC Committee of Clinical Application of Cardiac Bio-Markers [10]. Subjects were excluded from calculation of the 99th percentile URL if they met any of the following:

- Disease(s) of/or affecting the cardiovascular system
- Currently taking a medication for cardiovascular disease
- Diabetes Mellitus
- Chronic kidney disease.
- Other serious chronic disease(s) including cancer, Chronic Obstructive Pulmonary Disease, HIV, lupus erythematosus, etc.
- Acute bacterial or viral infection
- Pregnancy

There were no exclusions for surrogate biomarkers.

After collection, samples from the multi-center healthy cohort were centrifuged within 2 h and then frozen and maintained at -70 °C within 4 h. For measurement, the samples were thawed at room temperature, gently mixed, then centrifuged at $3000 \times g$ for 1 min and the supernatant assayed in singlet within 4 h using the ACCESS hs-TnI assay on the Access 2 Immunoassay system.

The respective 99th percentile URLs determined by this study were designated as the clinical decision limits for detecting myocardial injury as per the 4th Universal Definition of Myocardial Infarction [6] and AACC Academy and IFCC C-CACB document [10].

Analytical measurement range (AMR) for the ACCESS hsTnI assay on the Access 2 system was characterized with Li-hep plasma and serum samples according to CLSI EP6-A.33. For AMR assessment, a patient sample with a cTnI value approximately equivalent to highest calibrator at 27,000 ng/L and a sample that was approximately 2.0 ng/L were mixed to produce 7 sample concentrations evenly distributed across the concentration range. Four replicates of each of the 7 mixed samples, 8 replicates of the low sample and 4 replicates of the high sample were then tested. The ACCESS hsTnI's AMR was validated as linear from 2.0 ng/L to the highest calibrator value of 27,000 ng/L, with a maximum percent bias of 10% for samples across the range.

Dilution Process Validation for determining hsTnI concentrations in samples with values exceeding 27,000 ng/L was performed by diluting three separate Li-Hep and serum samples having ACCESS hsTnI values $> 140,000$ ng/L by 1:10, and measurement of each sample was in replicates of 5; i.e. one volume of each high sample was added to 9 volumes of the assay's Sample Diluent A and measured 5 times. Recovery was calculated as per CLSI EP6-A.33 [16].

Analytic specificity and interference testing of the ACCESS hsTnI assay was examined by adding each substance in Table 4 to produce 'test' Li-hep plasma and serum samples having 10 ng/L and 100 ng/L of cTnI at the final concentrations indicated in Table 4. In accordance with CLSI EP7-A2, the impact of each potential interference in test samples was compared to a matched cTnI control that had no potential interfering or cross-reacting substance added [17]. Significant interference was pre-specified as a difference in ACCESS cTnI measurements between the test samples and controls of $\geq \pm 10\%$ or $\geq \pm 2.30$ ng/L, whichever was greater.

High Dose Hook Effect was examined with Li-Hep and serum samples having cTnI values up to 2,000,000 ng/L. Reported ACCESS hsTnI values of ' $> 27,000$ ng/L' were appropriate and considered negative for the high dose hook effect.

1.4. Clinical characterization studies

The patient population for prospectively determining the clinical performance of the ACCESS hsTnI assay consisted of 1854 subjects enrolled at 14 primary care hospital-associated EDs across the USA between Sept, 2010 and Aug 2011 [18]. The integrity of these samples was validated by retesting with the AccuTnI + 3 assay. The rationale for equating AccuTnI + 3 stability with stability of the samples for the ACCESS hsTnI assay is that one of the same antibodies, targeting the very same measurand that was used in both assays. The second antibody used in ACCESS hsTnI is different from AccuTnI + 3, but binds to the same stable cTnI amino acid region as the antibody used in the contemporary assay. Thus if frozen storage causes deterioration of the measurand with AccuTnI + 3 retesting, then the repeat measurements with the AccuTnI + 3 assay in the timeframe of this study would have been impacted. Retested results from 2011 samples were compared with current data using the Passing-Bablok regression analysis at the baseline and endpoints on the AccuTnI + 3 assay. Further, endpoint samples were compared between the AccuTnI + 3 and ACCESS hsTnI results.

Enrollment criteria included presentation to the ED with chest pain or equivalent ischemic symptoms suggestive of acute cardiac ischemia, and a 12-lead ECG at time of presentation. This study was approved by each of the local institutional review boards at each participating centers, and written informed consent was obtained from all subjects. The study population's age was a median of 56 y [95% range 34–85 y]; the race distribution was similar to that of the USA [19]; and approximately 9% of enrolled subjects had chronic renal disease or renal insufficiency.

Blood samples collected were indexed to the documented time of ED presentation. The first sample was designated as baseline, and thereafter at 1–3 h, 3–6 h and 6–9 h after ED presentation [18]. For clinical performance at baseline, analysis was conducted in two ways. The first analysis included early baseline samples collected ≤ 1 h of the ED presentation time; the second analysis included baseline samples collected at all-times after subject-presentation to the ED. At each time after baseline, clinical performance was indexed relative to the subject's documented time of ED presentation. Li-Hep plasma or serum was separated from cells, aliquoted into cryo-tubes, and stored locally at -20 °C or colder. Periodically the samples were shipped on dry ice to four independent external laboratories where they were maintained at -70 °C or colder. The independent testing labs measured cTnI with the ACCESS hsTnI assay and all laboratory investigators and personnel were blinded to all patient information.

The primary clinical outcome for this study was the diagnosis of MI [18]. The clinical endpoint was adjudicated by an independent central endpoints committee consisting of four cardiologists blinded to ACCESS hsTnI assay results and the attending physician's diagnosis at the recruiting center. The cTnI used for the diagnosis at all sites was the ADVIA contemporary Centaur® TnI-Ultra™ (Siemens Healthineers, Newark, DE) standard of care assay. Criteria for the MI diagnosis was from the 2nd Universal Definition of myocardial infarction, which was the current version of guidance from the Global Task force [4] during the conduct of the study, and required a rise and/or fall in TnI values with at least one measurement above the TnI-Ultra 99th percentile URL of 40 ng/L.

1.5. Statistical analysis

Classification of temporal samples from adjudicated subjects was MI or non-MI according to the Centaur TnI-Ultra TnI assay's 99th percentile URL of 40 ng/L. For diagnostic performance calculations, True Positive (TP) results exceeded the ACCESS hsTnI 99th percentile URL for AMI subjects; False Negative (FN) results were less than the 99th percentile URL in subjects adjudicated with AMI; True Negative (TN) results were less than the 99th percentile URL in non-AMI subjects; and False Positive (FP) results exceeded the 99th percentile URL for non-AMI subjects. The SAS 9.4 statistical package was used for analyzing data in this study. A significance level of p-Value < 0.05 was used.

The minimum sample size required for a 95% two-tailed confidence interval about a proportion, assuming diagnostic sensitivity of 90% to 95%, is 73 to 139 MI patients. The Incidence of MI in the USA in patients presenting with signs and symptoms suggestive of AMI is approximately 7% to 15%. In this study's population, the prevalence of AMI was 13%, which is comparable to other published studies using high-sensitivity [20] and next generation troponin assays [21]. Therefore, approximately 1,100 enrolled patients were required to yield the 139 MI patients. Diagnostic sensitivity and specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were determined for females, males and all-subjects at multiple times after ED presentation.

For continuous variables, the most comprehensive description of diagnostic sensitivity and specificity is provided by Receiver Operating Characteristic (ROC) analysis, which examines all possible decision thresholds for all corresponding combinations of sensitivity and specificity. To gain perspective of overall clinical performance, the Area Under each ROC Curve (AUC) was calculated for each of the timeframes indexed to the time of ED presentation.

For the reference interval study, female, male and overall 99th percentile URLs were calculated using the non-parametric statistical method described in CLSI Document EP28-A3c [15]. All data collected were included in the study, and therefore no outlier removal strategy was utilized in this study.

2. Results

Table 1 shows the sex-specific and all-subject 99th percentile URLs and 95% confidence intervals [CI] for the ACCESS hsTnI values derived from 1,089 samples collected from healthy populations of women (54.6%) and men (45.4%). The male 99th percentile URL for Li-Hep plasma was 19.8 ng/L, which is 1.7-fold higher than the female value of 11.6 ng/L (Table 1). Supplemental Table 1 displays the serum data for sex-specific and all-subject values for the healthy 99th percentile URLs and 95% CIs. The serum 99th percentile URLs were 11.8 ng/L for women, 19.7 ng/L for men and 18.2 ng/L (all-subject), which is similar (p-value > 0.5) to the Li-Hep values in Table 1.

The mean for the blank measurements used for LoB calculation was 0.07230 ng/L and the SD of the blank measurements was 0.002869 ng/L for the ACCESS hsTnI assay on the Access 2 Immunoassay System. From the equation in methods: LoB = $0.07230 \text{ ng/L} + (1.645 \times 0.002869 \text{ ng/L}) = 0.077 \text{ ng/L}$. For calculating LoD, the SD was 0.1495 ng/L and with the equation in methods: LoD = $0.07702 \text{ ng/L} + (1.645 \times 0.1495 \text{ ng/L}) = 0.3230 \text{ ng/L}$. Rounded to two significant figures, the assay's LoD is 0.32 ng/L. The LoQ, represented by the 20% total CV for the ACCESS cTnI assay on the Access 2 system, was 0.77 ng/L.

The imprecision profile for the ACCESS hsTnI assay from below the LoD to the Female 99th percentile cutoff is displayed in Fig. 1. Repeatability, within-day and total imprecision for the ACCESS hsTnI assay are displayed in Table 2. As shown, the total CVs were $\leq 7.8\%$ at all nine hsTnI concentrations that ranged from 6.8 ng/L to 19,310.0 ng/L. Further, at concentrations near the sex-specific 99th percentile URLs listed in Table 1, the total CVs were 5.3% and 5.7% at these decision

Table 1

Overall and sex-specific 99th percentile Upper Reference Limits (URLs) of healthy subjects determined in lithium heparin plasma by the recommended non-parametric percentile method (CLSI C28-A3).

Lithium Heparin Matrix	Female	Male	All Subject
Number in Healthy Cohort	595	494	1,089
99th Percentile Healthy URL ¹	11.6 ng/L	19.8 ng/L	17.5 ng/L
90% Confidence Interval, ng/L	8.4 to 18.3	14.0 to 42.9	12.6 To 20.7

¹ URL = Upper Reference Limit.

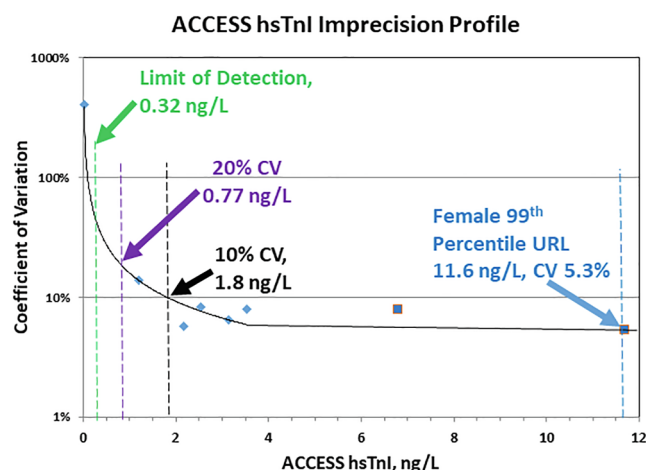


Fig. 1. Precision study at low concentrations for the ACCESS hsTnI assay.

points for women and men, respectively. These total CV data of $\leq 10\%$ demonstrate that the ACCESS hsTnI assay meets the high-sensitivity precision requirement at the female and male 99th percentile URLs.

Table 3 displays data for the female and male healthy populations from the AACC USB both before and after exclusion of 110 participants (13.2% of the healthy population), because of NT-proBNP, HbA_{1c} and/or eGFR values that were suggestive of underlying disease. Table 3 shows that the final female and male healthy cohorts each consisted of > 300 members, and that $> 99\%$ of the subjects from each sex cohort exceeded the Access 2 hsTnI assay's LoD. Together with the total CVs of $\leq 10\%$ at the sex-specific 99th percentile URLs, these data confirm that the ACCESS hsTnI assay demonstrated performance that confirms the ACCESS hsTnI test is a true high-sensitivity cTn assay.

A study of the ACCESS hsTnI assay's AMR and linearity demonstrated a maximum deviation from linearity of $< 10\%$ for samples across the range from 2.0 ng/L to approximately 27,000 ng/L; thus, the range from 2.0 ng/L to 27,000 ng/L defines the assay's AMR. For samples exceeding the assay's upper limit of linearity, 10-fold dilution of Li-Hep and serum samples having hsTnI values between 140,000 ng/L and 150,000 ng/L with the assay's Sample Diluent A demonstrated recovery of 100% and 102%, respectively. Thus, the strategy of diluting samples above the AMR 1:10 with the assay's Sample Diluent A was validated.

Studies of the ACCESS hsTnI assay for interference with cardiac troponin C, cardiac troponin T, skeletal muscle troponin I, fibrinogen, myosin, myoglobin, tropomyosin and CK-MB showed no significant cross reactivity (Table 4). Interference studies conducted with other substances at the concentrations are also listed in Table 4. All but four of the substances in Table 4 demonstrated a change in concentration

Table 3

Results for healthy population from the AACC Universal Sample Bank before (Section A) and after (Section B) with exclusions for Hemoglobin A_{1c} $\geq 6.5\%$, NTproBNP > 125 pg/mL if < 75 years old or > 450 pg/mL if ≥ 75 years old or estimated glomerular filtration rate < 60 mL/min per 1.73 m².
^aLoD = Limit of Detection.

Sex	Number in Healthy Population	Number $\geq$ LoD ^a	Proportion of Population $\geq$ LoD
Before Exclusion for Biomarkers			
Male	421	421	100%
Female	415	412	99.3%
All-subject	836	833	99.6%
After Exclusion for NT-proBNP, Hemoglobin A _{1c} and Creatinine (eGFR)			
Male	376	376	100%
Female	350	349	99.7%
All-subject	726	725	100%

^a LoD = Limit of Detection. The LoD was 0.323 ng/L.

Table 4

Interfering or cross-reacting substances and concentration evaluated. The change in concentration between test and control samples was $\leq 5\%$ for all but four substances. Concentration changes were $> 5\%$ but less than 10% for the following 4 substances; Fibrinogen, TPA, Human Serum Albumin and Unconjugated Bilirubin.

Acetaminophen	50 mg/dL	Fibrinogen	1000 mg/dL
Acetylsalicylic Acid	65 mg/dL	Furosemide	40 mg/dL
Actin	1,000 ng/mL	Hemoglobin	4 mg/mL
Atenolol	1 mg/dL	Human Serum Albumin	6,000 mg/dL
Atorvastatin	20 µg/mL	Ibuprofen	50 mg/dL
Bilirubin (conjugated)	40 mg/dL	Intralipid	3,000 mg/dL
Bilirubin (unconjugated)	20 mg/dL	Sodium Heparin	28.8 U/mL
Bivalirudin	42 µg/mL	Methyldopa	2.5 mg/dL
Caffeine	10 mg/dL	Myoglobin	1,000 ng/mL
Captopril	5 mg/dL	Myosin	1,000 ng/mL
Cardiac troponin C	250 ng/mL	Nitrofurantoin	6.4 mg/dL
Cardiac Troponin T	125 ng/mL	Nystatin	2 mg/dL
Cinnarizine	40 mg/dL	Phenobarbital	20 µg/mL
CK-MB	1,000 ng/mL	Rifampicin	60 µg/mL
Clopidogrel	75 µg/mL	Rosuvastatin	20 µg/mL
Cocaine	2 mg/dL	Skeletal troponin I	250 ng/mL
Cyclosporine	5 µg/mL	Tissue Plasminogen Activator (TPA)	2.5 µg/mL
Digoxin	200 ng/mL	Tropomyosin	1,000 ng/mL
Dopamine	65 mg/dL	Verapamil	16 mg/dL

that was $\leq 5\%$ after addition of potential interfering substances. Concentration changes were $> 5\%$, but $< 10\%$ for Fibrinogen, TPA, Human Serum Albumin and Unconjugated Bilirubin. None of the substances listed in Table 4 showed interference exceeding the pre-specified unacceptable value of 10%

Table 2

Imprecision expressed as repeatability, between run and total within laboratory imprecision of at various concentrations was determined in accordance with CLSI EP05-A3. Note that the lower concentrations are in the range of the 99th percentile upper reference limit for females of 11.6 ng/L and males of 19.6 ng/L are well below the $\%$ CV of $\leq 10\%$ required for high-sensitivity performance.

Sample	N	Concentration (ng/L)	Repeatability†		Between Run		Total Within Laboratory Imprecision	
			SD	%CV	SD	%CV	SD	%CV
Sample A	80	6.8	0.22	3.3	0.20	2.9	0.53	7.8
Sample B	80	11.7	0.38	3.3	0.35	3.0	0.62	5.3
Sample C	80	19.3	0.70	3.6	0.37	1.9	1.09	5.7
Sample D	80	29.8	0.93	3.1	0.56	1.9	1.09	3.7
Sample E	80	53.1	1.45	2.7	1.07	2.0	2.51	4.7
Sample F	80	97.5	2.52	2.6	1.95	2.0	3.19	3.3
Sample G	80	1,043.0	27.00	2.6	14.00	1.3	51.00	4.9
Sample H	80	14,516.0	408.00	2.8	361.00	2.5	722.00	5.0
Sample I	80	19,310.0	794.00	4.1	0.00	0.0	1153.00	6.0

† Repeatability, between run and within laboratory imprecision determined in accordance with CLSI EP05-A3.

The possibility of a high dose hook effect with the ACCESS hsTnI assay was examined with samples having > 2,000,000 ng/L concentrations of cTnI. All of these elevated Li-Hep and serum samples reported the appropriate concentration of ' $> 27,000$ ng/L', which validated the absence of a high-dose hook effect up to this extremely high concentration.

Stability studies were designed to assess changes in the measurand targeted by the ACCESS hsTnI assay. Index measurements in 2010 and 2011 were conducted with the AccuTnI +3 assay, which targets the same measurand that is quantified in the ACCESS hsTnI assay. Passing-Bablok regression showed that the sample results for the index AccuTnI +3 assay results were not significantly different from the AccuTnI +3 measurements at the time of this study. Also, there was no significant difference for the comparison between the initial or current set of AccuTnI +3 results compared to the ACCESS hsTnI assay measurements. These data indicate that no material change occurred in the cTnI measurand with frozen storage, and serves to validate the stability of samples used for the ACCESS hsTnI study.

Demographics for the 1854 subjects enrolled in the clinical characterization study for suspected AMI are summarized in Table 5. The overall prevalence of Non-ST-elevation MI (NSTEMI) in this population was 12.8%. As shown, this population was comprised of approximately 59% Caucasian, 32% individuals of African descent and 2% Asian. As shown in Table 5, the NSTEMI patients were typically older, more frequently male, and had more risk factors, including renal insufficiency and abnormal ECG findings. These MI subjects had cardiac medications prescribed more often and were more likely to have a history of coronary artery disease and previous MI compared to subjects who did not have an AMI. Also, the NSTEMI patients had their first blood collection about 2 h earlier after symptom onset, median 5.9 h, Interquartile Range (IQR): 3.3–21.1, compared to those without AMI who had their first sampling at a median of 7.6 h (IQR: 3.5–29.1) after symptoms onset ($p = 0.018$).

Table 5

Demographics of subjects enrolled in the study. *IQR = Interquartile range; [§] All subjects with a baseline sample. [†] Subjects with baseline sample collected ≤ 1 h from hospital presentation.

Demographics	Category	Subjects: N (%)	MI Subjects	Non-MI Subjects
Number of Subjects	n (% of total enrollment)	1854 (100.0%)	238 (12.8%)	1616 (87.2%)
Age	Age, Median [95percentrange]	56 years [34–85]	62 [38–88]	56 [33–85]
	< 60 yrs	1100/1854 (59.3%)	104/238 (43.7%)	996/1616 (61.6%)
	≥ 60 yrs	754/1854 (40.7%)	134/238 (56.3%)	620/1616 (38.4%)
Sex	Male	1015/1854 (54.7%)	167/238	848/1616
	Female	839/1854 (45.3%)	71/238	768/1616
Timing	Median Time from Symptom Onset to presentation (IQR)*	4.0 hrs IQR: 14.5 hrs [1.6–16.0]	3.1 hrs IQR: 11.2 hrs [1.4–12.6]	4.0 hrs IQR: 14.8 hrs [1.6–16.5]
	Median time, presentation to baseline collection, Interquartile Range [IQR]	Median: 1.2 hrs, IQR: [0.8–1.7] N = 1765 with baseline data	Median: 1.0 hrs IQR: [0.6–1.6] N = 221 baseline data	Median: 1.3 hrs IQR: [0.8–1.7] N = 1544 baseline data
	≤ 1 hr [†] from Presenting	Median: 0.6 hrs IQR: [0.4–0.8] N = 668 with baseline data	Median: 0.5 hrs IQR: [0.3–0.7] N = 101 baseline data	Median: 0.7 hrs IQR: [0.5–0.8] N = 567 baseline data
Race	White	1093/1854 (59.0%)	140/238 (58.8%)	953/1616 (59.0%)
	Black/African American	595/1854 (32.1%)	68/238 (28.6%)	527/1616 (32.6%)
	Unknown	80/1854 (4.3%)	15/238 (6.3%)	65/1616 (4.0%)
	Asian	37/1854 (2.0%)	5/238 (2.1%)	32/1616 (2.0%)
	Other	49/1854 (2.6%)	10/238 (4.2%)	39/1616 (2.4%)
Risk Factors	Hypertension	1311/1848 (70.9%)	184/237 (77.6%)	1127/1611 (70.0%)
	Dyslipidemia or Hypercholesterolemia	989/1821 (54.3%)	135/233 (57.9%)	854/1588 (53.8%)
	Diabetes Mellitus	548/1848 (29.7%)	81/238 (34.0%)	467/1610 (29.0%)
	Current smoker	509/1839 (27.7%)	69/236 (29.2%)	440/1603 (27.4%)
	Past smoker	615/1808 (34.0%)	95/230 (41.3%)	520/1578 (33.0%)
	Known coronary artery disease (documented stenosis $> 50\%$)	571/1792 (31.9%)	107/228 (46.9%)	464/1564 (29.7%)
	Prior Acute Myocardial Infarction	444/1833 (24.2%)	81/234 (34.6%)	363/1599 (22.7%)
	Prior coronary artery stent or angioplasty	436/1840 (23.7%)	81/235 (34.5%)	355/1605 (22.1%)
	Prior coronary artery bypass graft (CABG)	209/1851 (11.3%)	35/238 (14.7%)	174/1613 (10.8%)
	Heart Failure	299/1844 (16.2%)	58/237 (24.5)	241/1607 (15.0%)
	Chronic Kidney Disease or chronic renal insufficiency	166/1847 (9.0%)	32/238 (13.4%)	134/1609 (8.3%)

Supplemental Table 2 shows the AUC for the respective Baseline, 1 to 3-hour, 3–6 h and 6–9 h ROC Curves and 95% CIs for the overall population. The ROC AUC values for all the time points were ≥ 0.950 , demonstrating the ACCESS hsTnI assay's high diagnostic utility for AMI diagnosis.

Table 6 displays the Access hsTnI assay's diagnostic performance at the 99th percentile URLs for the female, male and all-subject populations using a lithium heparin sample matrix. Panel A of Table 6 lists Baseline diagnostic performance examined in two ways. The first included samples collected ≤ 1 h after ED presentation, $n = 664$, median time after ED presentation 0.6 h, (IQR: 0.4–0.8 h). The second analysis examined diagnostic performance of the all-time Baseline samples collected at any time after ED presentation; $n = 1,758$, median time 1.2 h, (IQR: 0.8–1.7 h). Note that in Panel A, the Baseline samples collected ≤ 1 h from presentation demonstrated approximately 4% lower sensitivity (p -value > 0.5) and 1% lower NPV for both sexes and overall compared to all-time Baseline samples. None of these differences between the ≤ 1 h and all-time baseline samples achieved statistical significance (p -values > 0.12).

Table 6 shows that samples collected at presentation times after baseline for both sexes and for all-subjects showed excellent sensitivity and $\geq 99\%$ NPVs. Further note, however, that despite the respectable specificity values of 83 – 90%, the PPVs were modest at values of 37–62%.

Supplemental Table 3 displays the ACCESS hsTnI assay's diagnostic performance at the 99th percentile URLs for the female, male and all-subject populations using a serum matrix.

Table 7 displays data for subjects with comorbidities, e.g. heart failure, renal insufficiency, etc. that were adjudicated as non-AMI or MI and had ACCESS hsTnI values that exceeded the relevant sex-specific 99th percentile URL in one or more serial sample(s). The subjects in the left panel of Table 7 were classified as False Positives in the data reported in Table 6. Thus when calculating the specificity and PPV in

Table 6

Diagnostic performance of the Access 2 hsTnI assay at different times after presentation. Panel A shows performance of samples collected ≤ 1 hour after presentation to the emergency department and also performance of baseline samples collected at any time after presentation for each sex and for all baseline collections. Panel B shows diagnostic performance at the subsequent times after presentation.

Presentation	Sensitivity						Positive Predictive			Negative Predictive		
	Value			Value			Value			Value		
	N ^a	%	95% CI ^b	N	%	95% CI	N	%	95% CI	N	%	95% CI
Panel A												
Female ≤ 1 h ^d	30	90.0	73.5–97.9	254	89.8	85.4–93.2	53	50.9	36.8–64.9	231	98.7	96.3–99.7
Female all ^e	70	94.3	86.0–98.4	738	90.2	87.9–92.2	138	47.8	39.3–56.5	670	99.4	99.5–99.8
Baseline ^e Male ≤ 1 h	70 ^d	87.1	77.0–93.9	310	87.7	83.6–91.2	99	61.6	51.3–71.2	281	96.7	94.0–98.5
Male all	149 ^e	91.3	85.5–95.3	801	88.3	85.8–90.4	230	59.1	52.5–65.5	720	98.2	96.9–99.0
All-Subject ≤ 1 h	100 ^d	86.0	77.6–92.1	564	90.4	87.7–92.7	140	61.4	52.8–69.5	524	97.3	95.6–98.5
All-Subject all	219 ^e	90.4	85.7–94.0	1,539	90.3	88.7–91.8	347	57.1	51.7–62.3	1,411	98.5	97.7–99.1
Panel B												
Female	43	97.7	87.7–99.9	533	90.4	87.6–92.8	93	45.2	34.8–55.8	483	99.8	98.9–100.0
1 to 3 hours Male	92	94.6	87.8–98.2	557	88.7	85.8–91.2	150	58.0	49.7–66.0	499	99.0	97.7–99.7
All Subject	135	94.8	89.6–97.9	1090	90.4	88.5–92.1	233	54.9	48.4–61.4	992	99.3	98.6–99.7
Female	49	98.0	89.1–99.9	557	89.2	86.4–91.7	108	44.4	34.9–54.3	498	99.8	98.9–100.0
3 to 6 hours Male	102	92.2	85.1–96.6	604	88.6	85.8–91.0	163	57.7	49.7–65.4	543	98.5	97.1–99.4
All-Subject	151	92.7	87.3–96.3	1161	90.0	88.1–91.7	256	54.7	48.4–60.9	1056	99.0	98.1–99.5
Female	22	100.0	84.6–100.0	225	83.6	78.1–88.1	59	37.3	25.0–50.9	188	100.0	98.1–100.0
6 to 9 hours Male	49	98.0	89.1–99.9	226	83.2	77.7–87.8	86	55.8	44.7–66.5	189	99.5	97.1–100.0
All-Subject	71	98.6	92.4–100.0	451	85.8	82.2–88.9	134	52.2	43.4–60.9	388	99.7	98.6–100.0

^aN = number of patient samples; ^bCI = Confidence Interval; ^cBaseline = first sample collected. Diagnostic performance was analyzed in two ways: ^dthe first included samples collected ≤ 1 h after ED presentation, median 0.6 h, Interquartile Range (IQR): 0.4–0.8 h; ^ethe second examined diagnostic performance of Baseline samples collected at all times after ED presentation, median 1.2 h, IQR: 0.8–1.7 h. Subsequent times are indexed relative to the subject's ED presentation time.

Table 6, these results lowered the diagnostic specificity based on ACCESS hsTnI result; however they do not impact the diagnostic sensitivity or NPV reported in Table 6. The data reported shows the modest absolute, i.e. ng/L, and percent increase expected in serial samples for non-MI patients having the comorbidities listed. Also, data in Table 7 allow a comparison of serial ACCESS hsTnI changes in non-AMI patients with comorbidities, with the absolute and percent serial changes observed in AMI subjects with the same comorbidities.

3. Discussion

We characterized the analytical and clinical characteristics for the ACCESS hsTnI assay performed on the Access 2 System in a US population undergoing evaluation for possible AMI. High-sensitivity assays detect lower cardiac troponin concentrations than was previously possible in the US because they are both more precise and have lower LoD and LoQ compared to contemporary cardiac troponin assays currently in broad use in the US. These characteristics allow resolution of the distinctly different 99th percentile URLs for healthy men and women. High-sensitivity assays also allow the reliable measurement of cardiac troponin values well below the 99th percentile URLs, which facilitates identification of rising and/or falling biomarker values with precision that was not possible previously with contemporary assays. Clinically, the excellent precision of high-sensitivity cTnI assays at low levels has led to their use in Accelerated Diagnostic Protocols (ADP) for early rule out of AMI [22,23,24]. Use of such an ADP with the ACCESS hsTnI assay was recently investigated in patients presenting > 3 h after their onset of suspected ACS symptoms [25]. With this ADP, 100% NPV for AMI was documented with two successive ACCESS hsTnI measurements, one at baseline and a second 1 to 3 h later, when one ACCESS hsTnI result was < 4.1 pg/mL and the other was < 99 th percentile URL.

Here we demonstrated that the ACCESS hsTnI assay on the Access 2 Immunoassay system meets the two criteria established for classification as an hs-cTn method [10]. For consistency with other studies, we used the AACC USB for this purpose because this cohort is publicly accessible [14]. This is important because different populations demonstrate different distributions, reflected by the 99th percentile URLs, as shown by Wildt et al. [26]. Further, Gore et al. demonstrated that

differences in populations run parallel with age and sex of the cohort, with particularly high values in older men [27]. These differences can be clinically meaningful because more than 10% of men 65 to 74 years of age with no cardiovascular disease had cardiac troponin values above the MI threshold for the 99th URL [27].

We characterized the ACCESS hsTnI assay's imprecision across the validated AMR, and demonstrated that this assay's total CV was $< 6\%$ at the sex-specific 99th percentile URLs of 11.6 ng/L and 19.8 ng/L for women and men, respectively [10]. We were reassured that this total CV is accurate by the reporting of a nearly identical 6% total CV in a separate analytical report for the ACCESS hsTnI assay [28]. We also determined the ACCESS hsTnI assay on the Access 2 system's LoD and 10%CV. The LoD and 10% CV were very close to an earlier independent publication, i.e. 0.32 ng/L (here) versus 0.34 ng/L [28] and 1.8 ng/L (here) versus 1.35 ng/L [28], respectively [28]. The Access 2 hsTnI assay successfully measured well over 50% of samples from healthy women and men above the assay's LoD in cohorts of > 300 individuals of each sex with the AACC USB (Table 3).

We determined the sex-specific 99th percentile URLs for men and women in a study that included nearly 500 healthy individuals of each sex, which exceeded the minimum number of > 300 of each sex specified in expert recommendations [10]. We did not exclude subjects based on biomarker criteria in this study after finding that doing so made no significant difference in sex specific or all subject 99th percentile URLs. We were reassured that these 99th percentile URLs were appropriate by comparison earlier analytical study of the ACCESS hsTnI assay that reported an all-population 99th percentile URL of 15.8 ng/L [28], which was well within the 95% CI for the all-population value in our study, i.e. 17.5 ng/L [95% CI:12.6–20.7] in lithium heparin, and 18.2 ng/L [95% CI 13.1 to 23.1] in serum samples. Also, while expert opinion documents have been promulgated that advocate use of biomarker surrogates [10], there is no universal consensus or evidenced-base guidance for how 99th percentile URLs should be determined [6,29].

Analytical confounders including cross-reactivity and potential interferences were examined for 38 substances; none of these were determined to be significant for at the concentrations assessed. Also, the ACCESS hsTnI assay does not have a high dose hook effect up to the extremely high TnI concentrations.

Table 7
Patients with comorbidities and serial samples having at least one ACCESS 2 hsTnI result that exceeded the sex-specific 99th percentile URLs. Panel A (left) displays data for Non-Acute Myocardial Infarction (i.e. Rule-out) patients with comorbidities; Panel B (right) lists data for patients with comorbidities that Ruled-in for Acute Myocardial Infarction.

Comorbidity	PANEL A						PANEL B					
	Non-Acute Myocardial Infarction (Rule-out)						Acute Myocardial Infarction Patients(Rule-in)					
	N	Baseline Tn (ng/L)	Max Tn change between serial measurements (ng/L)	Max % Change between consecutive serial measurements	Median (IQR)	Median (IQR)	N	Baseline Tn (ng/L)	Max Tn change between serial measurements (ng/L)	Max % Change between consecutive serial measurements	Median (IQR)	Median (IQR)
Heart failure	82	26.2 (20.3–44.3)	1.9 (–0.1–4.8)	6.6% ((0.7%)–15.9%)	6.6% ((0.7%)–15.9%)	6.6% ((0.7%)–15.9%)	51	44.0 (26.8–137.9)	8.4 (1.1–159.1)	16.0% (2.5%–148.3%)	8.4 (1.1–159.1)	16.0% (2.5%–148.3%)
Renal failure	49	31.0 (20.8–55.7)	1.9 (–0.4–4.8)	5.5% ((1.8%)–13.0%)	5.5% ((1.8%)–13.0%)	5.5% ((1.8%)–13.0%)	25	56.2 (27.8–189.4)	15.9 (–0.4–64.1)	23.7% (1.8%–134.5%)	15.9 (–0.4–64.1)	23.7% (1.8%–134.5%)
Atrial fibrillation/flutter	13	23.9(13.8–29.7)	2.3 (1.3–7.0)	11.9% (6.3%–38.8%)	11.9% (6.3%–38.8%)	11.9% (6.3%–38.8%)	14	61.9 (33.6–96.0)	32.6 (7.7–70.7)	43.5% (10.3%–115.7%)	32.6 (7.7–70.7)	43.5% (10.3%–115.7%)
Infection/Cardiotoxic	8	36.8(26.1–39.1)	1.7 (–1.3–3.9)	4.2% ((4.1%)–12.1%)	4.2% ((4.1%)–12.1%)	4.2% ((4.1%)–12.1%)	6	114.5 (32.8–912.4)	12.2 (3.5–14.6)	10.9% (10.6%–23.5%)	12.2 (3.5–14.6)	10.9% (10.6%–23.5%)
Drugs												
No specified comorbidities	76	20.3 (13.7–31.6)	3.0 (0.0–6.6)	16.5%(0.0%–32.5%)	16.5%(0.0%–32.5%)	16.5%(0.0%–32.5%)	119	76.2 (34.8–330.8)	45.6 (3.3–332.0)	41.5% (6.9%–231.1%)	45.6 (3.3–332.0)	41.5% (6.9%–231.1%)
Overall		24.1(15.2–39.7)	2.1 (–0.1–5.6)	7.6% (0.2%–22.9%)	7.6% (0.2%–22.9%)	7.6% (0.2%–22.9%)	Overall	65.1 (31.8–247.0)	24.0 (3.0–231.9)	28.9% (4.1%–156.8%)	24.0 (3.0–231.9)	28.9% (4.1%–156.8%)

Recent literature has investigated potential interference of macro-complexes in cTn assays [30,31,32]. Lam et al [30] examined macro-cTnI interference before and after immunoglobulin depletion in a cohort of troponin positive patients and found 55% prevalence of macro-TnI. This study showed that ACCESS hsTnI values were impacted by the macro-complex in 21% of these samples, which was lower than the 80% impact that the macrocomplex samples had on several other assays [30]. It is curious that prevalence of macrocomplexes in the Lam study [30] was about 10-fold higher than the 5% reported previously [31]. Further, a study by Kavsak et al [32] documented a 3.5% prevalence of macro-complex interference. This study showed that specimens with macro-complexes showed interference with some high-sensitivity cTn assays, but no interfere with the ACCESS hsTnI assay [32]. In any case, it appears that macrocomplexes are a relatively common finding, and that more studies on the impact of these substances on high-sensitivity assays are needed.

For clinical characterization, a diverse patient population of > 1850 male and female subjects with suspected of ACS was recruited from across the US. The AMI prevalence of this population was ~13%, which is very similar to previous US clinical performance studies of high-sensitivity assays [20,21]. Other than patients on renal replacement, there were no exclusions for comorbidities in this study. Diagnosis of subjects was adjudicated by an independent central endpoints committee, who were blinded to ACCESS 2 hsTnI assay results, and the local attending physicians' diagnosis.

ROC analysis of data for the ACCESS hsTnI assay demonstrated AUCs exceeding 0.95 from the time of baseline collection up to 9 h after ED presentation. This performance translates into high diagnostic sensitivity and NPV up to 9 h after presentation.

It is very common in assay performance trials that the timing of baseline study sample collection occurs sometime after the specimen used for clinical care. This delay is due, in part, to the time required for ethical recruitment and the informed consent process. However, efforts must be made to minimize this delay or there is risk of over-estimating a cardiac troponin assay's early diagnostic sensitivity in routine clinical use. To estimate the impact of later baseline sampling, we compared the ACCESS hsTnI assay's diagnostic performance using early baseline samples collected ≤ 1 h (median: 0.6 h from ED presentation) with later all-time baseline study samples, (median: 1.2 h from presentation). Clinical sensitivity for the ≤ 1 h baseline group exceeded 90% for the females only, and sensitivity of ≤ 1 h baseline cohort was about 4% lower for both sexes and all-subjects compared to the later all-subject baseline collection cohort. This highlights the importance of designing studies that collect study samples very close to those used clinically, even though the difference in diagnostic parameters of sensitivity, specificity, NPV and PPV between the ≤ 1 h and all-time baseline groups was not statistically significant (p -value > 0.12) in this study. Later samples in the timeframe of 1–9 h after presentation demonstrated diagnostic sensitivity > 92%, NPV $\geq 99\%$ and specificity 83–90% and PPVs of 37–62%. This is not surprising; it is common knowledge that disease prevalence influences both NPV and PPV, and that as the disease prevalence decreases, PPV also decreases and NPV increases. Given the prevalence of AMI in the US, the modest PPV values and the high NPVs are not surprising. It is equally unsurprising that the ACCESS 2 hsTnI assay is a more efficient rule-out test than a rule-in test for AMI, although the assay can be an effective and valuable aid for both clinical uses.

A number of comorbidities are known to cause cardiac troponin release above the 99th percentile URL, including heart failure, renal failure and atrial fibrillation [33]. Table 7 provides insight into the absolute (ng/L) and percent changes in Access 2 hsTnI assay values that may be observed in serial samples from patients with comorbidities who had baseline values exceeding the 99th percentile URL. For comparison, baseline values, absolute and percent change in Access 2 hsTnI values for subjects with the same comorbidities who had a diagnosis of AMI are also displayed. Five- to 10-fold differences in absolute change and substantial percent differences were observed. These data will be useful to practitioners who are faced with ruling-out or ruling-in AMI in

patients with these morbidities who have baseline values above the 99th percentile URL.

This study had limitations for application in real-world practice. First, like all cTn assays, the diagnostic specificity and PPV of the ACCESS hsTnI assay is likely underestimated because 'FP' results are classified as above or below the 99th percentile URL only, whereas the study's adjudicators also considered the presence of a rising and/or falling pattern for classifying the subject for the AMI diagnosis in accordance with the Global Task Force's redefinition of acute MI guidance [6]. As shown in Table 7, there were 188 subjects with hs-cTnI values exceeding the 99th percentile that may not have demonstrated an overall clinical picture and rising and/or falling cTn pattern sufficient for diagnosis of AMI during adjudication. Thus, these non-MI results would have been more appropriately classified as myocardial injury, rather than 'FP' results for AMI. This could be particularly important in this study because there were no exclusions for comorbidities except end stage renal disease, which is known to cause chronic cTn increases. Second, this report was purely a diagnostic study because the gold standard for classification was expert adjudication without knowledge of patient mortality or adverse cardiac events at 30 d or later. Thus, this study was not informative as to how knowledge of ACCESS hsTnI results may have impacted clinical outcomes. Third, clinicians managing the subjects enrolled in this study were blinded to ACCESS hsTnI assay results and no clinical decisions took these data into account. It is possible that some patients with a negative hs-cTnI result were hospitalized and may have benefitted clinically, or clinical management may have been optimized had the ACCESS hsTnI assay results been available. Fourth, a contemporary cTn assay was used by the adjudicators as the comparator to classify subjects as AMI or non-MI. Fifth, this study utilized a sample bank collected from 2010 to 2011 for which stability validation was based on evidence demonstrating that the measurand targeted by the ACCESS hsTnI assay did not deteriorate on frozen storage. Evidence of stability was established by repeat measurements of the frozen samples with the contemporary AccuTnI +3 assay because it uses one of the same antibodies to target the very same measurand as in the ACCESS hsTnI test. The second antibody used in ACCESS hsTnI is different from AccuTnI +3, but binds to the same stable cTnI amino acid region as the antibody used in the contemporary assay. Unfortunately, there is a paucity of guidance specifying how such stability studies should be conducted and analyzed. Although the US FDA accepted this method of validating stability, we believe it would benefit the field if CLSI, a metrology institute, a professional association and/or regulatory authority would collaborate to develop evidence-based guidance specifying the acceptable way of conducting validation studies of frozen stability and analyzing results.

In Conclusion, the ACCESS hsTnI assay on the ACCESS 2 Immunoassay System demonstrated high-sensitivity analytical performance according to established criteria [10]. Clinically, the ACCESS hsTnI assay has high diagnostic sensitivity and specificity for AMI diagnosis in the early hours after ED presentation. This assay demonstrated high NPV, with a PPV expected from the prevalence of AMI in USA populations. Overall, analytical and clinical performance of the ACCESS hsTnI promise that it will contribute positively to care delivered to suspected ACS patients in the US.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clinbiochem.2020.05.014>.

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