

Silvia Masotti, Concetta Prontera, Veronica Musetti, Simona Storti, Rudina Ndreu, Gian Carlo Zucchelli, Claudio Passino and Aldo Clerico*

Evaluation of analytical performance of a new high-sensitivity immunoassay for cardiac troponin I

<https://doi.org/10.1515/cclm-2017-0387>

Received May 6, 2017; accepted August 11, 2017; previously published online September 23, 2017

Abstract

Background: The study aim was to evaluate and compare the analytical performance of the new chemiluminescent immunoassay for cardiac troponin I (cTnI), called Access hs-TnI using DxI platform, with those of Access AccuTnI + 3 method, and high-sensitivity (hs) cTnI method for ARCHITECT platform.

Methods: The limits of blank (LoB), detection (LoD) and quantitation (LoQ) at 10% and 20% CV were evaluated according to international standardized protocols. For the evaluation of analytical performance and comparison of cTnI results, both heparinized plasma samples, collected from healthy subjects and patients with cardiac diseases, and quality control samples distributed in external quality assessment programs were used.

Results: LoB, LoD and LoQ at 20% and 10% CV values of the Access hs-cTnI method were 0.6, 1.3, 2.1 and 5.3 ng/L, respectively. Access hs-cTnI method showed analytical performance significantly better than that of Access AccuTnI+3 method and similar results to those of hs ARCHITECT cTnI method. Moreover, the cTnI concentrations measured with Access hs-cTnI method showed close linear regressions with both Access AccuTnI + 3 and ARCHITECT hs-cTnI methods, although there were systematic differences between these methods. There was no difference between cTnI values measured by Access hs-cTnI in heparinized plasma and serum samples, whereas there was

a significant difference between cTnI values, respectively measured in EDTA and heparin plasma samples.

Conclusions: Access hs-cTnI has analytical sensitivity parameters significantly improved compared to Access AccuTnI+3 method and is similar to those of the high-sensitivity method using ARCHITECT platform.

Keywords: analytical sensitivity; automated immunometric assays; cardiac biomarkers; cardiac troponins; high-sensitivity methods; methods comparison; myocardial infarction; quality control materials; quality specifications.

Introduction

The international guidelines on redefinition of acute myocardial infarction (AMI) published in the year 2000 recommended for the first time that the increase in cardiac troponin I (cTnI) or cTnT levels over the 99th percentile upper reference limit (99th URL) should be considered as clinically relevant, and they also specifically indicated that this cutoff value should be measured with an imprecision ≤ 10 CV% [1]. Of course, these quality specifications for troponin assay require that cTnI and cTnT are also present and can be measured even in blood of healthy subjects [2–4]. Indeed, to measure the 99th URL of circulating cTnI and cTnT levels with an error ≤ 10 CV% is a hard challenge due to the very low biomarker concentrations found in healthy subjects [2–5]. Only after the year 2006, some manufacturers set up the first new generation of cTnI and cTnT immunoassays with improved analytical sensitivity [6–10], in accordance with the quality specifications requested by international guidelines and consensus documents [11–20]. Considering that the plasma/serum volume needed for troponin assay with automated platforms usually ranges from 10 to 100 μ L, an immunoassay for cTnI or cTnT should detect with analytical confidence an amount of 1–5 ng/L (or even less) of protein to be considered a high-sensitivity (hs) method [3–5].

As discussed in detail in several reviews and consensus documents [4, 12, 13, 16–21], there are, at present

*Corresponding author: Aldo Clerico, Laboratory Medicine, Fondazione CNR Regione Toscana G. Monasterio, via Trieste 41, Pisa 56126, Italy; and Scuola Superiore Sant'Anna, Pisa, Italy, E-mail: clerico@ftgm.it

Silvia Masotti and Claudio Passino: Fondazione CNR Regione Toscana G. Monasterio, Pisa, Italy; and Scuola Superiore Sant'Anna, Pisa, Italy

Concetta Prontera and Simona Storti: Fondazione CNR Regione Toscana G. Monasterio, Pisa, Italy

Veronica Musetti, Rudina Ndreu and Gian Carlo Zucchelli: CNR Institute of Clinical Physiology and QualiMedLab, Pisa, Italy

time, only few commercially available methods for cTnI and cTnT assay, which are able to completely satisfy the quality specifications recommended by international guidelines. The IFCC Task Force on Clinical Applications of Cardiac Biomarkers recommends that an hs cardiac troponin assay should be able to measure at least 50% of healthy individuals above the assay's limit of detection (LoD) along with a CV of $\leq 10\%$ at the 99th percentile [16]. Commercially available troponin immunoassays, which do not meet these quality specifications, measuring the 99th URL values with an error $\leq 20\%$ CV%, should be defined to be contemporary sensitive methods [16, 21].

The aim of this study is to evaluate the analytical performance of the Access hs-cTnI method using standardized protocols [22, 23]. This method is a chemiluminescent immunoassay for the quantitative determination of cTnI levels using DxI platform. This laboratory test was not commercially available at the moment of this evaluation. We also compared the cTnI concentrations measured with Access hs-cTnI to those obtained in our laboratory with other two cTnI methods: the contemporary sensitive Access AccuTnI+3 immunoassay for DxI platform and the hs TnI method for ARCHITECT platform. Both plasma samples of apparently healthy subjects and patients with cardiac disease and quality control (QC) samples distributed in an external quality assessment (EQA) program [24–26] were used for this comparison.

Materials and methods

Assay methods

This evaluation study began in March 15, 2017, and was completed in July 18, 2017. The Access hsTnI (IUO) (REF B52699) assay is a two-site immunometric assay method (Beckman Coulter, Inc. Brea, CA 92821, USA). Monoclonal anti-cTnI antibody conjugated to alkaline phosphatase is added to a reaction vessel along with a surfactant-containing buffer and sample. After a short incubation, paramagnetic particles coated with monoclonal anti-cTnI antibody are added. The human cTnI binds to the first cTnI antibody on the solid phase, whereas the second cTnI antibody-alkaline phosphatase conjugate reacts with different antigenic sites on the cTnI molecules. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field, whereas unbound materials are washed away. Then the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel, and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of cTnI in the sample (Figure 1). The amount of analyte in the sample is determined from a stored, multipoint calibration curve using seven calibrators from 0 to about 27,000 ng/L (Cat. no. B52700). Beckman Coulter, Inc reports that 99th percentile URL values (95% CI), calculated in a reference population including

1089 healthy individuals (595 females and 494 males), are as follows: overall 17.5 ng/L (12.6–20.7 ng/L); females 11.6 ng/L (8.4–18.3 ng/L); males 19.8 ng/L (14.0–42.9 ng/L) (personal communications from Beckman Coulter, Inc. Clinical Division).

For comparison of cTnI values measured with Access hs-cTnI and AccuTnI+3 methods, two DxI platforms were used, respectively installed in the two clinical laboratories of the Fondazione CNR Regione Toscana G. Monasterio, sited in Pisa and Massa. The alignment between these two DxI instruments was online evaluated with the same QC control materials used in other studies [24–26]. On the other hand, the analytical performances of the Access hs-cTnI method were analyzed only in the laboratory sited in Pisa.

For between-method comparisons with the Access hs-cTnI method, other two methods were also tested: the STAT Architect hs TnI method using the ARCHITECT i1000SR platform (Abbott Diagnostics, Ref. B3P250) [10] and the Access AccuTnI+3 assay for DxI platform (code A98264, Beckman Coulter, Inc. Brea, CA 92821, USA) [24]. Analytical characteristics and clinical results of these two immunoassay methods were previously evaluated in our laboratory using the same standardized protocols [10, 24, 25].

Evaluation of analytical performance

For evaluation of analytical performance of the Access hs-cTnI method, the limits of blank (LoB) and detection (LoD) were calculated following the CLSI EP17-A protocol [22], whereas the reproducibility

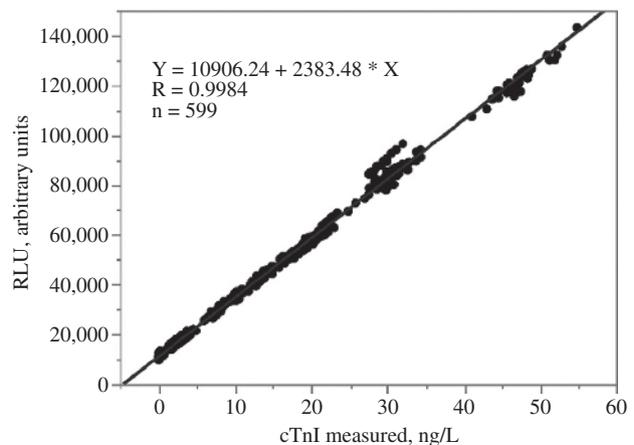


Figure 1: Linear regression analysis between the cTnI concentrations (x-axis), calculated by DxI instrumentation with the Access hs-cTnI method, and the respective RLU values (y-axis), considering the range of cTnI concentrations < 60 ng/L.

For valuation of this linear regression, 599 samples, including calibrators, plasma pools and plasma samples of healthy subjects or patients with cardiac disease, were assayed. The equation of linear regression was also reported in this figure; this equation was used for the calculation of LoB value for Access hs-cTnI, as described in detail in the text. This linear regression should be considered as an accurate estimation of the mean standard curve used by DxI platform for the calculation of cTnI concentrations throughout 3 months, using two different lots of materials and calibrators.

was tested according to the CLSI EP5-A2 protocol [23], using two heparinized plasma pool samples.

To accurately estimate the sampling error near to the zero point of the standard calibration curve [22], authors evaluated the linear relationship between cTnI concentration and RLU values measured in 599 samples (including samples of healthy subjects or patients, QC samples and the first two calibrators of standard curves) by DxI platform considering only the cTnI concentration range between 0 and 60 ng/L (Figure 1). This linear regression should be considered as an accurate estimation of the mean standard curve used by DxI platform for calculation of cTnI concentrations throughout 3 months, using two different lots of materials and calibrators. In particular, two different experimental approaches were used to estimate the LoB value. First, the zero calibrator of the Access hs-cTnI method, which does not contain cTnI, was measured in 92 different runs, using two lots of reagents throughout 3 months and five different curve calibrations. The LoB value was then calculated by interpolation considering this SD value in the linear regression reported in Figure 1. The second approach was based on the direct measurement of zero calibrator, considered as the blank of the method, using the formula: $LoB (ng/L) = \text{median value (ng/L)} + SD (ng/L) \times 1.645$ [22]. According to linear regression reported in Figure 1, it was possible to calculate cTnI concentrations, respectively related to RLU values of 92 measurements of zero calibrator. The LoD value was then calculated according to the formula: $LoD = LoB + 1.645 SD$ [22], where SD was estimated by the distribution of cTnI values measured in a heparinized pool sample collected from healthy women with very low cTnI concentrations.

The imprecision profile was estimated by measuring 10 heparinized plasma pools, which were measured in 42 different runs

throughout 3 months using two different lots of materials and calibrators. These plasma pools were prepared in order to cover a range of cTnI concentrations from about 1 to about 50 ng/L (Table 1). Each plasma pool was prepared using residuals of heparinized plasma samples collected from healthy subjects or patients with cardiac diseases. Each pool of about 20 mL included plasma of 20–40 adult individuals (age range 20–80 years). The pools with lowest cTnI concentrations were obtained especially from healthy women, whereas those with highest concentrations from patients with cardiac disease (in particular heart failure). Plasma pools with intermediate cTnI concentrations were prepared by mixing plasma samples from healthy subjects with those of patients with cardiac diseases. Soon after the preparation, the pools were distributed in several tubes containing 0.5 mL of plasma and then stored at $-20^{\circ}C$ until the assay. To calculate the limit of quantitation (LoQ), the relationship between the error of the measurement (expressed as % CV values) and cTnI concentrations was interpolated by means of a nonlinear regression curve.

Experimental samples

All blood samples used in this study were collected from patients with cardiac disease admitted to clinical ward or ambulatory of the Fondazione CNR Regione Toscana G. Monasterio (Pisa and Massa, Italy) or healthy volunteers, as previously reported in detail [10, 23, 25–27].

Considering the healthy volunteers, heparinized blood samples were collected from healthy subjects, enrolled from laboratory and clinical staff, blood donors and volunteers enrolled in multicenter studies [27] in order to prepare some plasma pools with low cTnI concentrations for estimation of analytical sensitivity and between-method comparison. In particular, heparinized blood samples from 78 healthy subjects, enrolled from the laboratory and clinical staff, were also collected (mean age 45.7 years, SD 10.8 years, median 45.7 years; 51 women and 27 men). The presence of acute or chronic diseases of cardiovascular and other apparatus was excluded by means of medical history and physical examination, as well as laboratory tests (including NT-proBNP and hs-cTnI or hs-cTnT assay) and non-invasive investigations (including ECG and echocardiography) [27].

Heparinized blood samples were also collected from patients with acute or chronic cardiac diseases, referred to the Division of Cardiovascular Medicine of Fondazione CNR-Regione Toscana G. Monasterio (Pisa, Italy). The diagnosis of acute or chronic heart failure was made according to ESC [28] and ACCF/AHA guidelines [29]. The diagnosis of AMI was made according to the Third Universal Definition of Myocardial Infarction [15] and to the most recent ESC guidelines [20].

In order to reduce the volume of collected blood, cTnI was measured in plasma remaining after routine clinical analyses. Samples were collected in polypropylene tubes with lithium heparin (heparinized plasma) (VACUTEST Lithium Heparin, code 12010, Vacutest Kima, Italy); serum plasma EDTA (VACUTEST K₃ EDTA, code 13040, Vacutest Kima, Italy) samples were also used in order to test some possible matrix effects. Plasma was obtained shortly after venipuncture by centrifugation for 10 min at room temperature (about $22^{\circ}C$), and then the samples, if not immediately assayed, were frozen and stored at $-20^{\circ}C$ in 0.5-mL aliquots in polypropylene tubes if the assay was expected within some weeks, or alternatively, at $-80^{\circ}C$ for long term storage.

Table 1: Heparinized plasma pools for evaluation of imprecision profile.

Pool	Subjects/ patients ^a	Mean, ng/L	Median, ng/L	SD, ng/L	CV, %
1	Healthy subjects	1.48	1.55	0.42	28.62
2	Healthy subjects	2.18	2.30	0.40	18.45
3	Healthy subjects	3.10	3.10	0.44	14.30
4	Healthy subjects	3.21	3.20	0.44	13.7
5	Healthy subjects	7.34	7.35	0.48	6.59
6	Healthy subjects	9.80	9.90	0.81	8.24
7	Healthy subjects	13.42	13.30	0.79	5.87
8	Subjects/patients	20.58	20.70	1.13	5.47
9	Patients	30.58	30.35	1.32	4.30
10	Patients	46.98	46.86	2.25	4.78

^aSubjects/patients: in this column the characteristics of individuals included in heparinized samples pools are indicated. Each sample pool included plasma of about 20–40 adult normal subjects or patients with cardiac disease (age 20–80 years). The pools with the lowest cTnI concentrations were obtained especially from healthy women (age <65 years), whereas those with highest concentrations were from patients with cardiovascular disease (especially heart failure). Plasma pools with intermediate cTnI concentrations were prepared by mixing plasma samples of healthy men and patients of both sexes. Each pool was measured in 42 different runs using two different lots of materials and calibrators throughout 3 months.

For between-method comparisons, heparinized plasma samples of healthy subjects and patients with AMI were used in order to cover all clinical working range of cTnI concentrations below the LoD value of Access hs-cTnI method (i.e. 1.3 ng/L) to about 75,000 ng/L. All the plasma samples with cTnI concentrations higher than 20,000 ng/L were diluted 1:2 and 1:4 using the diluent buffer supplied by manufacturer, following the instructions recommended by the reference manual (UniCel DxI, Reference Manual, P/N B13864E, June 2015, Beckman Coulter, Inc.).

In our laboratory, 31 QC samples with different cTnI concentrations according to the ILAC G13 guidelines were also prepared. These QC samples were measured by all laboratories (about 90 Italian laboratories for each annual cycle) participating in the Immunocheck QualiMedLab EQA annual cycles from 2014 to 2016, as previously described in detail [24–26]. Sample pools were prepared using residuals from heparinized plasma samples collected from healthy subjects or patients with cardiac diseases. For preparation of these QC samples, several heparinized plasma specimens, containing different cTnI concentrations, were pooled together (about 30–60 individuals for each pool) to obtain several sample pools with a final volume of about 100 mL. After the preparation, these pools were immediately stored at -20°C . All samples were tested for absence of HBsAg, antiHCV and antiHIV. QC samples were sent by mail as lyophilized materials. Stored sample pools were thawed then distributed in approximately 150 vials (each containing a plasma volume of 0.5 mL) and finally lyophilized, within 2 weeks after preparation of sample pools, as previously reported [24–26]. The lyophilized materials were reconstituted with 0.5 mL of distilled water by participant laboratories before the assay. The consensus mean values of cTnI concentrations, measured in QC samples by the laboratories participating of EQA cycles (about 30 laboratories/year) and using the Access AccuTnI + 3 assay with DxI platform, were used for comparison with the results of Access hs-cTnI method.

All subjects and patients gave the informed consensus for the use of their residual blood samples in the study.

Statistical analyses

For the evaluation and comparison of the analytical performance of tested cTnI immunoassay methods, standard statistical analyses were carried out using the JMP program (version 12.1.0, SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). Because cTnI circulating levels are not normally distributed, both non-parametric and parametric tests after logarithmic transformation of data were used for statistical analysis. Accordingly, for linear regression analyses between the cTnI values measured by the two Access methods and the ARCHITECT method, both the original data and log-transformed values were used. For between-method comparison, both original data (using non parametric tests) and log-transformed data were used. The log₁₀-transformed values were also used for regression analyses because: (1) cTnI concentrations are not normally distributed in both healthy subjects and cardiac patients, but these values approximate a log-normal distribution; (2) the use of log-transformed values allows a more accurate evaluation and detection of all the working clinical range, especially for cTnI concentrations within the normal range. In particular, the log-transformed values allowed an accurate detection and comparison of results related to both clinical and QC samples. (3) Better correlations (R-values)

Table 2: Values of limit of blank (LoB), limit of detection (LoD), limit of quantitation (LoQ) at 10% CV and 20% CV of the cTnI immunoassay systems evaluated in the study.

Method	LoB, ng/L	LoD, ng/L	LoQ 20% CV, ng/L	LoQ 10% CV, ng/L	Reference
Access AccuTnI + 3	4.5	10.9	17.1	30.4	[24]
hs-cTnI DxI	0.6	1.3	2.1	5.3	Present study
hs-cTnI Architect	0.7	1.3	1.8	4.7	[10]

were obtained when log-transformed data were used and compared to original cTnI data. Indeed, the best fit for comparisons between two sets of original cTnI data should be a non-linear regression (for example, using a spline function), but results derived from non-linear curves are less easily interpretable than those from linear regression equations using log₁₀-transformed data. In the regression analyses for method comparisons, all samples with cTnI values below the LoD values were equalized to the LoD values of the respective method (i.e. 1.3 ng/L for both hs-cTnI Access and hs-cTnI ARCHITECT methods, and 4.5 ng/L for Access AccuTnI + 3 method, respectively) (Table 2).

For EQA program, the statistical analyses of immunoassay results used for the periodical and cumulative reports were previously described in detail in other studies from our laboratory [24–26].

Results

Evaluation of analytical performance

Estimation of LoB and LoD values

The LoB value of the Access hs-cTnI method was estimated by means of two different experimental approaches. The first approach is based on estimation of measurement error of standard curve zero calibrator, expressed as RLU arbitrary units, according to the following formula: $\text{LoB} = \text{blank of the method} + \text{SD} \times 1.645$ [22]. SD value (i.e. 796.404 RLU) was estimated by measuring zero calibrator in 92 different runs, using two lots of reagents throughout 3 months and five different curve calibrations. According to the linear equation reported in Figure 1, the y-intercept to 0 was 10906.24 RLU. Therefore, the estimated error of the blank of the method (i.e. LoB value), expressed as RLU, was calculated by the following formula [22]: $\text{LoB (RLU)} = 10906.24 + [\text{SD of zero calibrator (i.e. 796.404 RLU)} \times 1.645] = 12216.32 \text{ RLU}$. The LoB value (expressed as ng/L) was then calculated by interpolation of this value (i.e. 12216.32 RLU) using the linear equation $Y = 10906.24 + 2383.48 X$ (Figure 1). The interpolated LoB value was 0.55 ng/L.

The second experimental approach is based on direct measurement of zero calibrator, considered as the blank of

the method [22]. According to linear regression reported in Figure 1, the distribution of cTnI concentrations related to RLU values of 92 measurements of zero calibrator was calculated. These values approximated the normal distribution: mean 0.103 ng/L, SD 0.328 ng/L, median 0.043 ng/L, minimum value -0.58 ng/L, maximum value 0.96 ng/L. The following formula was then used to estimate the LoB value: mean (0.103 ng/L) + [SD (0.328 ng/L) × 1.645] = 0.64 ng/L [22].

It is important to note that similar results (i.e. 0.55 ng/L and 0.64 ng/L, mean value 0.595 ng/L) for the estimation of LoB value were obtained with these two approaches. Accordingly, a value of 0.6 ng/L was considered to be a reliable estimation of the LoB for the Access hs-cTnI, and this value is reported in Table 2.

LoD was then calculated according to the following formula [22]: $LoD = LoB (0.6 \text{ ng/L}) + 1.645 \text{ SD } (0.44 \text{ ng/L}) = 1.3 \text{ ng/L}$. SD was estimated considering the distribution of cTnI values measured in a heparinized sample pool collected from healthy women with very low cTnI concentrations (mean 1.48 ng/L, SD 0.44 ng/L, n = 42).

LoB and LoD values of Access hs-cTnI method using the DxI 800 platform, evaluated in our laboratory according to the CLSI EP17-A protocol [22], are reported in Table 2. For comparison, the analytical parameters, previously evaluated in our laboratory with the same standardized protocol [22] for Access AccuTnI +3 [24] and ARCHITECT hs-cTnI [10] methods, are also reported in the Table 2.

Evaluation of LoQ values by means of imprecision profile

The imprecision profile of Access hs-cTnI method is reported in Figure 2. The imprecision profile was estimated by measuring 10 heparinized plasma pools assayed in 42 different runs with mean cTnI concentrations ranging from about 1 to about 50 ng/L, and prepared from healthy subjects and patients with cardiovascular diseases, as reported in Table 1. To calculate the LoQ value at 10% (i.e. 5.3 ng/L) and 20% CV (i.e. 2.1 ng/L), the relationship between the error of the measurement and cTnI concentrations (expressed as CV) was interpolated by means of a reciprocal regression equation (Figure 2); for comparison with the two other cTnI methods, the calculated LoQ values are also reported in Table 2.

It is important to note that there were significant differences ($p < 0.0001$ by Wilcoxon test) between the cTnI values of plasma pools 1, 2 and 3 (Table 1). These results confirm the data of LoD value (i.e. 1.3 ng/L) that the Access hs-cTnI method is able to detect on average about 1 ng/L in plasma pools collected from healthy subjects (especially

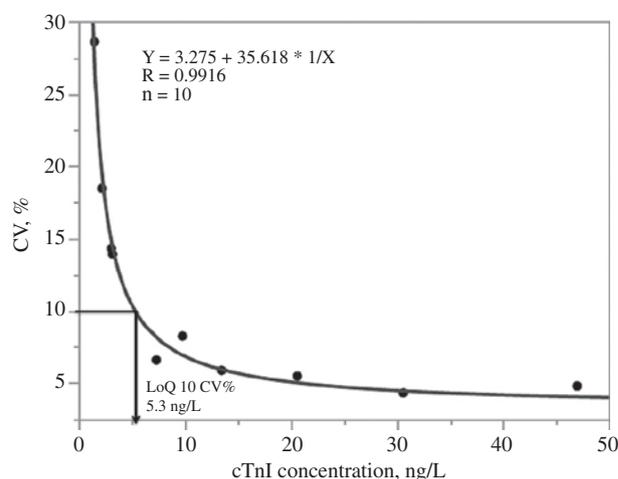


Figure 2: Imprecision profile of the Access hs-cTnI method using the DxI platform.

The imprecision profile was estimated by measuring 10 heparinized plasma pools in at least 40 different runs, collected from healthy subjects and patients with cardiovascular diseases, using two different lots of reagents and calibrators throughout 2 months. The characteristics of these 10 plasma pools were reported in detail in Table 1. The interpolated reciprocal curve is reported. The calculation of the LoQ value at 10% CV was also indicated.

women), at least under experimental protocols used in this study.

Evaluation of reproducibility

Results of reproducibility evaluation for Access hs-cTnI method using DxI 800 platform, according to the CLSI EP5-A2 protocol [23], are reported in Table 3. Reproducibility was evaluated using two heparinized plasma pools collected from healthy subjects with mean cTnI concentrations of 3.25 ng/L (sample A) and 18.33 ng/L (sample B), respectively. These two plasma pools were measured in double in every morning and afternoon of 20 consecutive working days [23]. The formulas recommended by the CLSI EP5-A2 document [23] were used for evaluation of within-run and between-run variability.

Table 3: Reproducibility evaluation of the ACCESS hs-cTnI method for DxI platform.

Sample	Mean cTnI, ng/L	Within-run variability, CV%	Between-run variability, CV%
Sample A	3.25	11.21	14.96
Sample B	18.33	3.76	7.24

Matrix effects

Matrix effects were evaluated in 109 couples of serum samples and heparinized plasma samples collected from the same healthy subjects and patients with myocardial infarction (range of cTnI concentration from about 2 to about 20,000 ng/L). There was no difference between cTnI values measured by Access hs-cTnI in heparinized plasma and serum samples (mean serum concentration: 5022.8 ng/L; mean heparin concentration: 4467.7; mean difference 555.1 ng/L, $p=0.5824$ by Wilcoxon test; linear regression: $\log\text{-heparin}=0.0186+0.9919 \log\text{-serum}$, $R=0.9994$, $n=109$). On the contrary, there was a significant difference between cTnI values measured by the Access hs-cTnI, respectively measured in EDTA and heparin plasma samples (mean EDTA concentration: 3205.34 ng/L; mean heparin plasma concentration: 3850.86 ng/L; mean difference 645.52 ng/L; $p<0.0001$ by Wilcoxon test; linear equation: $\log\text{-EDTA}=-0.0448+0.9756 \log\text{-heparin}$, $R=0.9772$, $n=46$).

Between-method comparisons

Comparison between the two access methods

In Figure 3, it is reported the close linear regression between cTnI concentrations measured with the two Access cTnI methods for DxI platform, using both plasma samples collected from 257 healthy subjects and patients with cardiac disease and 31 QC samples, distributed in an EQA study

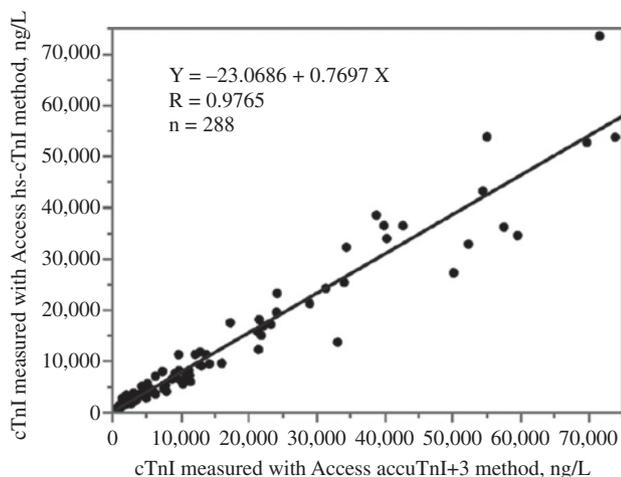


Figure 3: Linear regression between cTnI concentrations, measured by the Access AccuTnI + 3 (x-axis) and hs-cTnI methods (y-axis). For this regression analysis, 257 heparinized plasma samples of healthy subjects and patients with cardiac disease (including some patients with AMI) and 31 QC samples were evaluated. The calculated linear equation is reported in figure.

with cTnI concentrations from about 20 to about 1700 ng/L. The cTnI concentrations were measured by Access hs-cTnI method in our laboratory, including those concerning the QC samples. cTnI values were measured in our laboratory with Access AccuTnI + 3 method measured for the 257 clinical samples, whereas the cTnI values of the 31 QC samples represent the mean values measured by laboratory participant to EQA study (about 30 Italian laboratories), as previously described in detail [23–25]. It is important to note that the regression between the two Access cTnI methods was not significantly different when the 31 QC samples were excluded by regression analysis ($Y=-28.4972 [SE 154.74]+0.7698 [SE 0.011] X$, $R=0.9761$, $n=257$).

In Supplemental Figure 1, regression analysis between log-transformed cTnI-values, measured with Access hs-cTnI and AccuTnI + 3 methods, was reported to evaluate in more detail all the working range of cTnI concentrations measured with these two methods. These data demonstrate that cTnI values of the 31 QC samples (black circles) showed the same analytical behavior of 257 samples of healthy subjects and patients (gray circles) because the values of QC samples were always within the 95% prediction interval of calculated regression (indicated by gray zone around linear regression).

Although a close regression was found between the cTnI values measured with the two Access methods (Figure 3 and Supplemental Figure 1), the data of

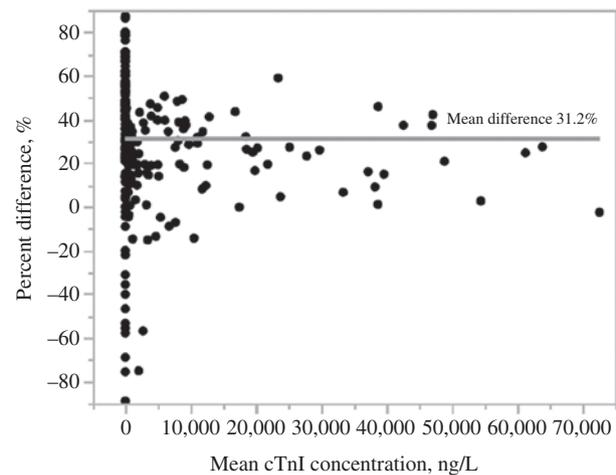


Figure 4: Bland-Altman plot analysis of the linear regression between cTnI concentrations, measured by Access AccuTnI + 3 and hs-cTnI methods.

On the x-axis, the mean cTnI concentrations ($[(\text{AccuTnI} + 3 + \text{hs-cTnI})/2]$) measured by the two methods was reported, whereas the percent difference between the original (not-transformed) values measured cTnI values ($\{[(\text{AccuTnI} + 3 - \text{hs-cTnI})/\text{AccuTnI} + 3] \times 100\}$) were reported on the y-axis. The calculated mean percent difference is also reported in figure.

Bland-Altman plot, considering the original (non-transformed) data (Figure 4), indicate that on average the AccuTnI+3 method showed significantly higher cTnI values than the hs-cTnI method (mean percent difference value: 31.2%, SD: 29.9%, $p < 0.0001$).

To better evaluate the relationship between the cTnI concentrations measured with these two methods in samples with very low cTnI concentration, the linear regression between cTnI values measured in 138 plasma samples with concentration < 150 ng/L is reported in Figure 5. Considering this low range value, the percent difference value between by the cTnI values measured by two Access methods was 43.9% (SD 26.6%, $p < 0.0001$).

In particular, when only the results concerning the 78 healthy subjects are considered, the distribution parameters of cTnI values measured by Access hs-cTnI method were as follows: median 1.9 ng/L, 25th percentile 1.6 ng/L and 75th percentile 2.5 ng/L; on the contrary, the distribution parameters of cTnI values measured by AccuTnI+3 method were as follows: median 3.0 ng/L, 25th percentile 2.0 ng/L and 75th percentile 5.0 ng/L. It is important to note that with Access cTnI method, only nine samples (11.5%, two men and seven women) showed cTnI concentrations below the LoD value (i.e. 1.3 ng/L). On the contrary, the major part (66.7%) of samples showed cTnI value $< \text{LoD}$ value (i.e. 4.5 ng/L) when measured with AccuTnI+3 method. There was a significant difference considering the number of healthy subjects with cTnI $< \text{LoD}$ value (i.e. 1.3 ng/L) between the two Access methods ($p < 0.0001$ by χ^2 -test).

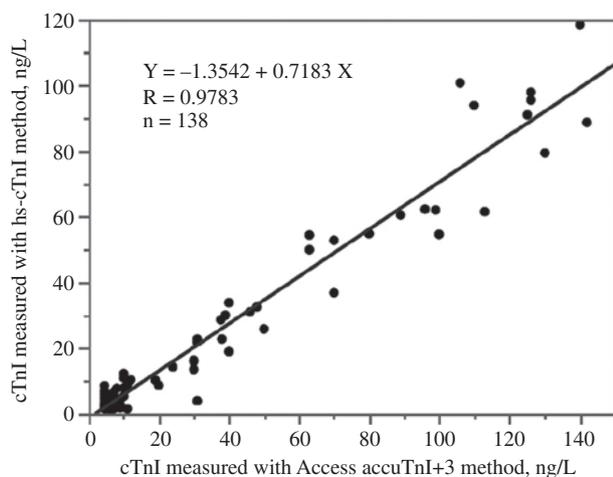


Figure 5: Linear regression between cTnI concentrations, measured by Access AccuTnI+3 (x-axis) and hs-cTnI (y-axis) methods considering the range of cTnI values < 150 ng/L.

For the regression analysis, 138 heparinized plasma samples of healthy subjects and patients with cardiac disease were evaluated. The calculated linear equation is also reported in figure.

Comparison between Access cTnI and ARCHITECT cTnI methods

The linear regression between cTnI concentrations measured with Access hs-cTnI and ARCHITECT hs-cTnI methods in 318 samples (also including 31 QC samples) is reported in Figure 6. In the Supplemental Figure 2, the regression analysis between log-transformed cTnI-values, measured with the Access hs-cTnI method and the ARCHITECT hs-cTnI method, is reported to better evaluate all the working range of cTnI concentrations measured with the two methods. These data also demonstrate that cTnI values of the 31 QC samples (black circles) showed the same analytical behavior of 287 samples of healthy subjects and patients (gray circles) because the values of QC samples were included within the 95% prediction interval (gray zone around the linear regression).

Although a close correlation was found between the results of these methods, a significant percent difference ($[\text{ARCHITECT method} - \text{Access method}]/\text{mean concentration}$) was found between cTnI values measured by these two methods, which tends to increase with mean concentration values, as demonstrated by Bland-Altman plot analysis reported in Figure 7 (mean percent difference 3.9%, SD 38.3%, $p < 0.0001$).

To better evaluate the relationship between the Access hs-cTnI and ARCHITECT hs-cTnI methods in samples with very low cTnI concentrations, the linear regression between cTnI values measured in 177 plasma samples

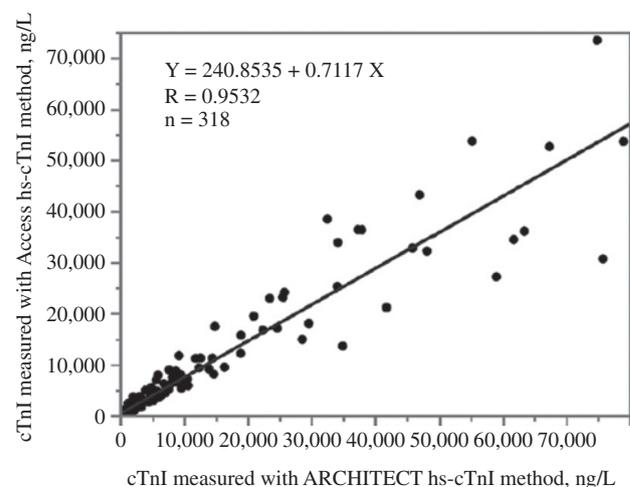


Figure 6: Linear regression between cTnI concentrations, measured by ARCHITECT hs-cTnI (x-axis) and Access hs-cTnI (y-axis).

For this regression analysis, 287 heparinized plasma samples of healthy subjects and patients with cardiac disease (including some patients with AMI) and 31 QC samples were evaluated. The calculated linear equation is reported in figure.

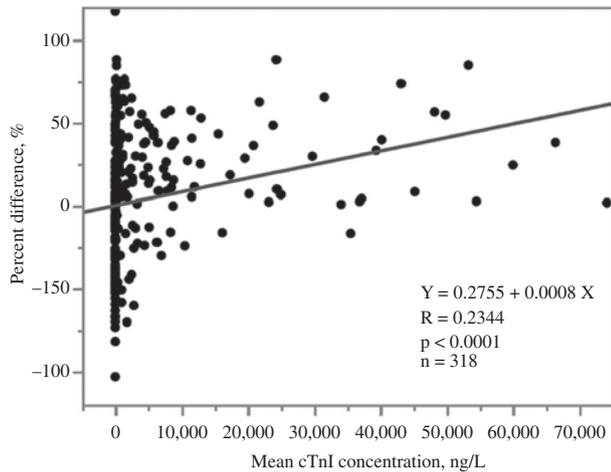


Figure 7: Bland-Altman plot analysis of linear regression between the cTnI concentrations, measured by ARCHITECT hs-cTnI and Access hs-cTnI methods (Figure 6).

On the x-axis, the mean cTnI concentrations ($[\text{ARCHITECT hs-cTnI} + \text{Access hs-cTnI}]/2$) measured by the two methods is reported, whereas the percent difference values between the measured cTnI values $\{([\text{ARCHITECT hs-cTnI} - \text{Access hs-cTnI}]/\text{mean concentration}) \times 100\}$ are reported on the y-axis. The linear regression between mean cTnI concentration and percent difference values is also reported in the figure.

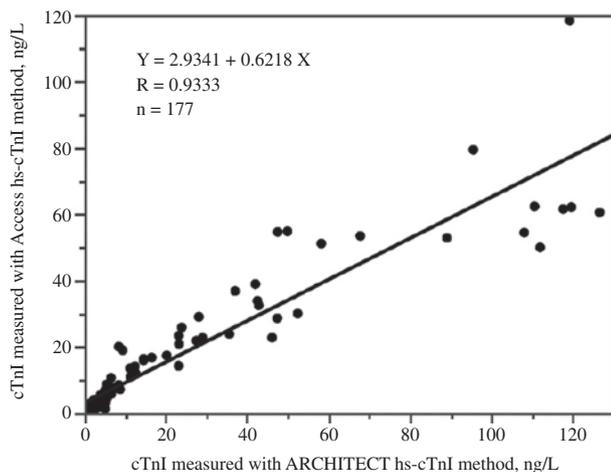


Figure 8: Linear regression between cTnI concentrations, measured by ARCHITECT hs-cTnI (x-axis) and Access hs-cTnI (y-axis) methods considering only the range of cTnI values <120 ng/L.

For this regression analysis, 177 heparinized plasma samples of healthy subjects and patients with cardiac disease are evaluated. The calculated linear equation is also reported in the figure.

with concentration <120 ng/L is reported in Figure 8. Considering this low range value, a significant difference was found between cTnI values measured by these two methods ($p=0.0052$): Access hs-cTnI method, mean 11.5 ng/L, SD 17.1 ng/L, median 3.5 ng/L, 25th percentile

1.9 ng/L and 75th percentile 14.1 ng/L; ARCHITECT hs-cTnI method mean 13.9 ng/L, SD 25.8 ng/L, median 3.7 ng/L, 25th percentile 1.4 ng/L and 75th percentile 11.7 ng/L.

In particular, when only the results concerning the 78 healthy subjects are considered, distribution parameters of cTnI values measured by ARCHITECT hs-cTnI method were as follows: median 1.8 ng/L, 25th percentile 1.0 ng/L and 75th percentile 2.1 ng/L. Furthermore, 31 samples (39.7%) showed cTnI value <LoD value (i.e. 1.3 ng/L) with the ARCHITECT hs-cTnI method. There was a significant difference considering the number of healthy subjects with cTnI < LoD value (i.e. 1.3 ng/L) between Access hs-cTnI and ARCHITECT hs-cTnI methods ($p < 0.0001$ by χ^2 -test).

Discussion

According to Apple and Collinson [30], two basic criteria are needed to define an hs troponin assay: (1) the total imprecision (expressed as CV%) at the 99th percentile value should be <10%; (2) measurable concentrations below the 99th percentile should be attainable with an assay at a concentration value above the assay's limit of detection for at least 50% (and ideally 95%) of healthy individuals.

The results of present study demonstrate that the Access hs-cTnI method using DxI platform shows analytical performance significantly better than that of Access AccuTnI+3 method (Table 2). Moreover, the analytical performances of Access hs-cTnI method are similar to those previously found in our laboratory for ARCHITECT hs-cTnI method using the same standardized protocols (Table 2) [10].

The manufacturer of the Access hs-cTnI method suggests a value of 17.5 ng/L (CI 12.6–20.7 ng/L) as the 99th reference limit for the overall reference population (combined population including adult men and women). According to the imprecision profile of the Access hs-cTnI reported in Figure 2, cTnI values between 12 and 21 ng/L are measured with an analytical error <10%, which is the analytical imprecision recommended by international guidelines for evaluation of the 99th URL values [14–20]. In particular, the cutoff value of 17.5, suggested by the manufacturer, should be measured with a CV value of 5.3% (Figure 2). Therefore, the Access hs-cTnI method actually satisfies the first of the two criteria to be defined an hs method [30].

Preliminary results obtained in the present study, considering 78 healthy subjects (including 51 women, 65%, with very low cTnI concentrations), suggest that the Access hs-cTnI is able to measure cTnI values > LoD (i.e. 1.3 ng/L) in the major part of healthy individuals (in

the present study in 88.5% of enrolled healthy subjects), as requested by international guidelines and consensus documents for hs cTnI methods [30]. However, further studies, based on large reference populations (divided for age, gender and ethnic origin) are needed to demonstrate that Access hs-cTnI is able to measure cTnI concentrations above the LoD value (i.e. 1.3 ng/L) in the majority of healthy subjects, according to the second criteria required for definition of an hs method for cTn assay [19, 30].

Acknowledgments: All the immunoassay kits used in this study were kindly supported by the manufacturers, namely, Beckman Coulter S.r.l. Italy (Cassina de' Pecchi, Milan, Italy) and Abbott Diagnostics Italia (Rome, Italy).

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

- Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined: a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol* 2000;36:959–69.
- Clerico A, Fortunato A, Ripoli A, Prontera C, Zucchelli GC, Emdin M. Distribution of plasma cardiac troponin I values in healthy subjects: pathophysiological considerations. *Clin Chem Lab Med* 2008;46:804–8.
- Giannoni A, Giovannini S, Clerico A. Measurement of circulating concentrations of cardiac troponin I and T in healthy subjects: a tool for monitoring myocardial tissue renewal? *Clin Chem Lab Med* 2009;47:1167–77.
- Clerico A, Zaninotto M, Ripoli A, Masotti S, Prontera C, Passino C, et al. The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology and clinical implications. *Clin Chem Lab Med* 2017;55:1634–51.
- Marjot J, Kaier TE, Martin ED, Reji SS, Copeland O, Iqbal M, et al. Quantifying the release of biomarkers of myocardial necrosis from cardiac myocytes and intact myocardium. *Clin Chem* 2017;63:990–6.
- Prontera C, Fortunato A, Storti S, Mercuri A, Longombardo G, Zucchelli GC, et al. Evaluation of analytical performance of the Siemens ADVIA TnI ultra immunoassay. *Clin Chem* 2007;53:1722–3.
- Saenger AK, Beyrau R, Braun S, Cooray R, Dolci A, Freidank H, et al. Multicenter analytical evaluation of a high-sensitivity troponin T assay. *Clin Chim Acta* 2011;412:748–54.
- Venge P, James S, Jansson L, Lindahl B. Clinical performance of two highly sensitive cardiac troponin I assays. *Clin Chem* 2009;55:109–16.
- Krintus M, Kozinsk M, Boudry P, Capell NE, Köller U, Lackner K, et al. European multicenter analytical evaluation of the Abbott ARCHITECT STAT high sensitive troponin I immunoassay. *Clin Chem Lab Med* 2016;52:1657–65.
- Caselli C, Cangemi G, Masotti S, Ragusa R, Gennai I, Del Ry S, et al. Plasma cardiac troponin I concentrations in healthy neonates, children and adolescents measured with a high sensitive immunoassay method: high sensitive troponin I in pediatric age. *Clin Chim Acta* 2016;458:68–71.
- Morrow DA, Cannon CP, Jesse RL, Newby LK, Ravkilde J, Storrow AB, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: clinical characteristics and utilization of biochemical markers in acute coronary syndromes. *Clin Chem* 2007;53:552–74.
- Apple FS, Jesse RL, Newby LK, Wu AH, Christenson RH, Cannon CP, et al. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: analytical issues for biochemical markers of acute coronary syndromes. *Clin Chem* 2007;53:547–51.
- Apple FS, Ler R, Murakami MM. Determination of 19 cardiac troponin I and T assay 99th percentile values from a common presumably healthy population. *Clin Chem* 2012;58:1574–81.
- Thygesen K, Alpert JS, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. *J Am Coll Cardiol* 2007;50:2173–95.
- Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third Universal Definition of myocardial infarction. *Eur Heart J* 2012;33:2551–67.
- Apple FS, Collinson PO; IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. *Clin Chem* 2012;58:54–61.
- Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J* 2012;33:2252–7.
- Casagrande I, Cavazza M, Clerico A, Galvani M, Ottani F, Zaninotto M, et al. Proposal for the use in emergency departments of cardiac troponins measured with the latest generation methods in patients with suspected acute coronary syndrome without persistent ST-segment elevation. *Clin Chem Lab Med* 2013;51:1727–37.
- Sandoval Y, Apple FS. The global need to define normality: the 99th percentile value of cardiac troponin. *Clin Chem* 2014;60:455–62.
- Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2016;37:267–315.
- Apple FS, Saenger AK. The state of cardiac troponin assay: looking bright and moving in the tight direction. *Clin Chem* 2013;59:1014–6.

22. CLSI EP17-A protocol. Protocols for determination of limits of detection and limits of quantitation; approved guidelines. Wayne, Pennsylvania, 2004;24.
23. CLSI EP5-A2 protocol. Evaluation of precision performance of quantitative measurement methods; Approved guideline – Second edition, Wayne, Pennsylvania, 2004;24.
24. Storti S, Masotti S, Prontera C, Franzini M, Buzzi P, Casagrande I, et al. Evaluation of analytical performance and comparison of clinical results of the new generation method AccuTnI + 3 for the measurement of cardiac troponin I using both patients and quality control plasma samples. *Clin Chim Acta* 2015;451:129–34.
25. Franzini M, Prontera C, Masotti S, Zucchelli GC, Storti S, Passino C, et al. Evaluation of analytical performance of a novel immunoenzymometric assay for cTnI. *Clin Chim Acta* 2013;416:48–9.
26. Clerico A, Ripoli A, Masotti S, Prontera C, Storti S, Fortunato A, et al. Pilot study on harmonization of cardiac troponin I immunoassays using patients and quality control plasma samples. On behalf of the Italian Section of the European Ligand Assay Society (ELAS) and of the Study Group on Cardiovascular Biomarkers of the Società Italiana di Biochimica Clinica (SIBioC). *Clin Chem Acta* 2016;456:42–8.
27. Franzini M, Lorenzoni V, Masotti S, Prontera C, Chiappino D, Latta DD, et al. The calculation of the cardiac troponin T 99th percentile of the reference population is affected by age, gender, and population selection: a multicenter study in Italy. *Clin Chim Acta* 2015;438:376–81.
28. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, et al. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2013;62:e147–239.
29. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016;18:891–975.
30. Apple FS, Collinson PO. Analytical characteristics of high-sensitivity cardiac troponin assay. *Clin Chem* 2012;58:54–61.

Supplemental Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/cclm-2017-0387>).