Original Article

Validation of Becton Dickinson Barricor™ tubes for use with Abbott Alinity™ and Siemens Atellica® highly sensitive cardiac troponin I measuring systems

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Keywords

Abstract

BACKGROUND: BD Barricor™ tubes have been proposed to decrease laboratory turnaround time (TAT). We analytically validated and then clinically verified these tubes for use with Abbott Alinity™ and Siemens Atellica® highly sensitive cardiac troponin I (hs-cTnI) assays.

METHODS: hs-cTnI measurements were undertaken in paired Barricor™ and in-use PSTII™ tubes on both systems. 359 matched samples with hs-cTnI levels between 3 and 15,000 ng/L (Atellica® values) were used to assess the hemolysis rate and make method comparisons. 599 paired patient samples were collected on emergency department (ED) admission to compare the performance of the rapid acute myocardial infarction (AMI) rule-out strategy based on hs-cTnI concentrations lower than recommended thresholds (<4 ng/L Alinity™; <5 ng/L Atellica®) when different tubes and systems were employed.

RESULTS: No between-tube differences in hemolysis rate were seen when free hemoglobin concentrations in plasma samples were ≥0.25 g/L, even if PSTII™ showed a significant increase of hemolysis rate vs. Barricor™ (31% vs. 22%, p=0.007) when a lower cut-off for hemolysis (≥0.11 g/L) was employed on the Atellica® detection system. The alternate use of these tubes did not influence the hs-cTnI results obtained from either of the two assays, which remained markedly biased (~40%) irrespective of the tube used. The expected optimal ability of very low hs-cTnI values on ED admission for ruling out AMI was confirmed by using both systems regardless of the tube type.

CONCLUSIONS: Barricor™ and PSTII™ tubes can provide analytically equivalent hs-cTnI results when used on either Alinity™ or Atellica® hs-cTnI assays.

Introduction

According to current clinical recommendations [1,2], cardiac troponins I or T are the preferred biomarkers for the detection of myocardial injury and key diagnostic elements in diagnosing acute myocardial infarction (AMI), especially in non-ST-segment elevation myocardial infarction (NSTEMI) [2]. As such, an accurate quantification of these biomarkers, through a detailed knowledge of the preanalytical, analytical, and clinical performance of available assays, is crucial in avoiding erroneous results, potentially leading to wrong diagnosis and inappropriate management of patients with suspected AMI [3].

For measuring cardiac troponin I, several highly sensitive immunoassays are now marketed, which are available on fully automated, high-throughput platforms. Among others, the Alinity™ i STAT High Sensitive Troponin-I and the Atellica® IM High-Sensitivity Troponin I assays have an analytical turnaround time (TAT) <15 min. TAT is an important indicator of laboratory service performance [3,4] and a lag ≤60 min from the time of receipt of blood tubes in the central laboratory to troponin result reporting to clinical wards has been recommended. Meeting appropriate TAT to ensure timeliness in reporting troponin results is a prerequisite for the implementation of fast-track algorithms recommended in clinical guidelines [2]. Furthermore, a rapid TAT for troponin testing can facilitate early diagnosis, timely initiation of treatment, and improved patient outcomes [5,7]. The use of plasma allows for faster blood sample processing compared to serum as the clotting time is eliminated. Therefore, plasma providing tubes are widely used for troponin measurements in emergency departments (ED) [5]. In 2016, Becton Dickinson (BD) introduced a novel type of lithium heparin tube which contains a mechanical separator of blood cells (Barricor™ Lithium Heparin Plasma Blood Collection Tubes) as opposed to classical gel separation, e.g., in Plasma Separator Tubes II (PSTII™). Importantly, Barricor™ tubes require a shorter centrifugation time than PSTII™ tubes (3 minutes as opposed to 10 minutes), potentially offering a further effective reduction of TAT when used in acute clinical setting [6,7]. Although Barricor™ tubes are available since a few years, data evaluating the Barricor™ tube as an alternate sample type for cardiac troponin I measurements are still limited [8,10]. Consequently, in this study we validated the use of Barricor™ tubes on the Alinity™ and Atellica® highly sensitive cardiac troponin I (hs-cTnI) measuring systems, by comparing them with in-use PSTII™ tubes.

Our study sought to evaluate: a) the frequency of hemolysis in using these two tubes, as quantified by the hemolysis index (HI) on both platforms; b) the impact, if any, of the tubes on the assay comparison; and c) the influence of the tubes, if any, on rapid AMI rule-out strategy employing recommended cut offs of hs-cTnI assays.

Materials and Methods

Analytical study design

Sample collection and processing

359 Barricor™-PSTII™ lithium heparin paired samples were collected from hospitalized patients with routinely ordered hs-cTnI testing. All study participants provided informed, written consent prior to adding the Barricor™ tube to PSTII™ tube needed for hs-cTnI measurements. No exclusion criteria, other than insufficient samples (blood volume <3 mL) or with troponin concentrations <3 ng/L, i.e., the lower limit of measurement range, were applied. Blood from each individual was collected into a 3 mL Barricor™ tube (ref. 365044) and then in a 3 mL PSTII™ tube (ref. 367374). Following blood drawing, tubes were gently inverted 4-5 times before immediate transfer to the laboratory, where they were centrifuged according to vendor recommendations, i.e., at 4000g for 3 min for Barricor™, using a dedicated swing bucket DASH Apex 6 Compact STAT centrifuge (Drucker Diagnostics), and at 2000g for 10 min for PSTII™ using a swing bucket Eppendorf centrifuge 5702, respectively. HI measurements and hs-cTnI testing of plasma samples were performed within 30 min following centrifugation. The study was conducted from June 2019 to March 2021.

Characteristics of hs-cTnI assays and HI measurement systems

The hs-cTnI measuring interval was 3 to 50,000 ng/L for Alinity™ i and 3 to 25,000 ng/L for Atellica® IM, respectively. According to the IFCC recommendations to use whole numbers (no decimals) for hs-cTnI reporting in clinical practice [11], all values were rounded up or down to the nearest whole number. The lower end of the measuring interval was defined by the limit of quantitation for Atellica®, rounded to the smallest integer common on both systems. The overall 99th percentile URLs were 26 ng/L and 45 ng/L for Alinity™ and Atellica®, respectively. The respective HI were measured on the Alinity™ c and Atellica® CH. The performance of these photometric determinations has been previously described in detail [12-13]. It should be noted that Alinity™ c permits a quantitatively accurate estimate of free hemoglobin (fHb) concentrations in plasma, while in the Atellica® CH the quantitative results are bucketed into index intervals to report in qualitative terms. Based on previous experiences establishing 0.25 and 1.00 g/L of fHb as the clinically most important thresholds for hemolysis interference [14], we used for Alinity™ the corresponding HI of 25 and 100, and for Atellica® the index ranges of 1 (0.11-1.30 g/L fHb) and 2 (1.31-2.49 g/L fHb) to establish the hemolysis rates by using the two evaluated tubes.

Method comparison studies

The between-assay comparisons using the same tube and the between-tube intra-assay comparisons were carried out using the same 359 matched samples having hs-cTnI concentrations covering the range between 3 and 15,000 ng/L (Atellica® values). To highlight correlation results in the most important clinical range, comparisons were also done on a subgroup of 300 paired samples with hs-cTnI ranging from 3 to 300 ng/L, a value previously identified as threshold for immediate rule-in at patient admission when using Abbott Architect™ platform [15]. All hs-cTnI measurements were performed in duplicate and the mean value was calculated. Method comparison studies were undertaken in compliance with CLSI EP09-A3 standards [16].

Clinical study design

Study population and blood sampling

We prospectively enrolled 599 unselected patients admitted to the ED with chest pain of possible cardiac origin and suspected AMI and with pain onset within the last 6 h. Paired Barricor™ and PSTII™ samples were collected at patient presentation and processed promptly. Testing of paired plasma samples from each patient was performed firstly on the routinely employed Atellica® and shortly after on the Alinity™ measuring system.

AMI rule-out strategy on ED admission and study endpoint

An hs-cTnI-based AMI rule-out strategy using previously recommended thresholds (<4 ng/L for Alinity™ [2], and <5 ng/L for Atellica® [17], was employed using single sample results obtained from these two types of primary tubes. The primary endpoint was to compare the performance of the aforementioned strategy to rule-out AMI using Barricor™ and PSTII™ tubes.

AMI adjudication

After review of relevant clinical information and the standards of the University Hospital No.1 in Bydgoszcz, cases were adjudicated for AMI (including type 1 and 2) following the Fourth Universal Definition of AMI consensus recommendations [1]. The adjudicators (cardiologists EL and MJ) were blinded to the investigational Alinity™ and Atellica® hs-cTnI. The hs-cTnI Atellica® PSTII™ results were available to the adjudicators during the hospitalization period of the patient.

Compliance with ethical standards

In compliance with the ethical principles for medical research involving human subjects the study protocol was approved by the Bioethics Committee of the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland in agreement with the Helsinki declaration on ethical standards [No. 402/2019]. All patients provided written informed consent for enrollment in the study.

Statistical analysis

Categorical variables were compared using the chi-square test and their percentage share in the entire group of results was determined. The compliance with the normal distribution was checked using the Shapiro-Wilk W test. Normally distributed values were presented as mean and standard deviation (SD). Values whose distribution deviated from normal were presented as medians with the 25th and 75th percentiles.

Depending on whether a given distribution met the criteria of a normal distribution, the obtained values were compared using the Student's t-test or, if these conditions were not met, the U-Mann-Whitney test. Comparison of hs-cTnI concentrations in BD Barricor™ and BD PSTII™ tubes on Alinity i and Atellica IM analyzers was made using Deming regression analysis with the determination of the Pearson linear correlation coefficient. Scatter plots of hs-cTnI concentrations measured by the Alinity™ and Atellica® measuring systems were generated, slopes and intercepts [with corresponding 95% confidence intervals (CI) were estimated, and between-assay percentage differences calculated. Diagnostic sensitivities and negative predictive values (NPV), with corresponding CIs, were calculated to examine the diagnostic performance of AMI rule-out strategy for both the hs-cTnI Alinity™ and Atellica® assays and using the two different tubes. Differences in the proportion of results obtained with both tubes were compared. A p value <0.05 was considered statistically significant. All statistical analyzes were performed using MedCalc v.20.023 software (MedCalc Software, Ostend, Belgium).

Results

Comparisons of hemolysis rates using Barricor™ and PSTII™ tubes, as automatically detected by the Alinity™ c and Atellica® CH systems, are shown in Table 1. No between-tube differences were seen in the hemolysis rate on either platform when a medium degree of hemolysis, defined as fHb ≥1.00 g/L, was detected. The impact of different thresholds in detecting low degree hemolysis on these two platforms, i.e., Alinity™ ≥0.25 g/L and Atellica® ≥0.11 g/L, may explain the significant increase in the percentage of hemolyzed samples detected by Atellica® compared to Alinity™ when using PSTII™ tubes (31% vs. 22%, p=0.011). It is indeed possible that some part of samples reported with HI ≥1 on Atellica® were not detected as hemolyzed on Alinity™, as an fHb range between 0.11 and 0.25 g/L approached the threshold for a low degree of hemolysis on Alinity™. The same observation may explain significant increases in low-degree hemolysis rate (31% vs. 22%, p=0.007) on Atellica® when PSTII™ tubes were compared with Barricor™ tubes, indicating that PSTII™ may increase the number of samples displaying relatively low fHb (between 0.11 to 0.25 g/L). A direct relationship of hemolysis indices of the two blood collection tubes and the two analytical systems is presented in Supplementary Figure 1.

Table 1: Hemolysis rates in 359 paired Barricor™ and PSTIITM lithium heparin plasma samples as detected by automatic hemolysis index on the two measuring systems. Chi-square test was used for comparisons.

	Low-degree hemolysis*	P value between tubes	P value between platforms
Alinity™ Barricor™	19%	0.229	0.307† — 0.011§
Alinity™ PSTII™	22%		
Atellica® Barricor™	22%	0.007	
Atellica® PSTII™	31%		
	Medium-degree hemolysis†		
Alinity™ Barricor™	5%	0.613	0.247† — 0.494§
Alinity™ PSTII™	6%		
Atellica® Barricor™	3%	0.327	
Atellica® PSTII™	5%		

* H-index ≥0.25 g/L for Alinity™ and ≥1 g/L (quantitative values in the range 0.11-1.30 g/L) for Atellica®.

† H-index ≥1 g/L for Alinity™ and ≥2 g/L (quantitative values in the range 1.31-2.49 g/L) for Atellica®.

‡ Differences between Alinity™ Barricor™ and Atellica® Barricor™

§ Differences between Alinity™ PSTII™ and Atellica® PSTII™

Comparisons between plasma samples obtained from the two types of tubes run on either the Alinity™ or the Atellica® hs-cTnI measuring systems are shown in Supplemental Figure 2. The regression equations revealed near equivalence between tubes, showing that the alternate use of the two types of tubes did not influence hs-cTnI results obtained by each of the two measuring systems. Supplemental Figure 3 shows the between-assay comparisons using the same tube. Regression analyses remained the same regardless of tube type employed for obtaining plasma. As expected, the two hs-cTnI systems showed non-comparable results, with Alinity™ giving hs-cTnI results markedly lower than Atellica®. Slopes and intercepts for both comparisons indicated both constant and proportional difference. Difference plots confirmed the substantial between-assay bias, in average ranging from 38% to 40%, that was however unaffected by the employed type of tube (Figure 1). We also compared distributions of Barricor™ and PSTII™ paired samples according to the specific categories on either system: <4ng/L Alinity™, <5ng/L Atellica®, between these low values and the assay-specific 99th percentiles, and >99th percentiles which showed no statistically significant differences between tubes regardless of the system used (Supplemental Table 1). A rapid AMI rule-out strategy using the two hs-cTnI measuring systems and the two types of tubes was applied to 599 patients admitted to ED with suspected AMI. Baseline characteristics of these patients and corresponding hs-cTnI concentrations measured using both systems and tubes are shown in Table 2. The average age in patients with suspected AMI was 68.7 years and the majority of them were men. Patients finally diagnosed with AMI were of similar age to patients with AMI excluded. However, AMI was diagnosed significantly more frequently in men compared to women. The average age of women was 71.9 ± 11.7 years, while that of men was 66.8 ± 11.8 years and the observed difference was statistically significant ($P < 0.001$). The median hs-cTnI concentrations in the study group did not differ statistically significantly between both tubes on the same analyzer. Statistically significant differences were observed between median hs-cTnI concentrations obtained in the same tubes using two measurement systems. As expected, median hs-cTnI concentrations were statistically significantly higher in patients with confirmed AMI compared to patients with AMI excluded.

Table 2: Baseline characteristics of patients with suspected AMI and corresponding hs-cTn concentrations.

	All patients (n=599)	Non-AMI patients (n-530)	AMI patients (n=69)	p value
Age [years]	68.7 ± 12.0	68.7 ± 12.0	68.5 ± 11.9	0.988
Sex [female]	227 (38%)	209 (39%)	18 (26%)	<0.001
Alinity I BD Barricor™ hs-cTnI [ng/L]	10 (5-33)	9 (5-26)	41 (13-428)	<0.001
Alinity i BD PSTII™ hs-cTnI [ng/L]	10 (5-33)	9 (5-26)	39 (11-406)	<0.001
Atellica IM BD Barricor™ hs-cTnI [ng/L]	15 (8-52)	14 (8-41)	86 (24-747)	<0.001
Atellica IM BD PSTII™ hs-cTnI [ng/L]	15 (8-52)	13 (7-40)	88 (23-751)	<0.001

Using this approach, excellent sensitivities and NPV were obtained, irrespective of the tube type employed (Table 3).

Table 3: Diagnostic performance of acute myocardial infarction rule-out strategy using recommended cut-offs for hs-cTnI on Alinity™ (<4 ng/L) and Atellica® (<5 ng/L) measuring systems with Barricor™ and PSTII™ tubes. 95% confidence intervals in parentheses.

	Alinity™ Barricor™	Alinity™ PSTII™	Atellica® Barricor™	Atellica® PSTII™
Sensitivity	100% (95-100)	100% (95-100)	99% (92-100)	99% (92-100)
Negative Predictive Value	100% (94-100)	100% (93-100)	98% (90-100)	98% (90-100)

Sixty-nine (12%) patients were finally diagnosed with AMI. In reporting hs-cTnI values lower than the recommended cut-offs for the AMI rule-out procedure, we found overall agreement between both hs-cTnI measuring systems and both tube types employed (Supplemental Table 2). However, it should be noted that whilst Alinity™ did not show any false negative results (i.e., AMI patients with hs-cTnI <4 ng/L on ED admission), Atellica® displayed two false negative results in two different AMI patients, with both showing a hs-cTnI value of 4 ng/L, one when using the Barricor™ tube, the second the PSTII™.

Discussion

In this study, we successfully performed an analytical validation and clinical verification of BD Barricor™ tubes for use on both the Alinity™ and the Atellica® hs-cTnI measuring systems in comparison with the in-use PSTII™ tubes. Our results clearly demonstrate that BD Barricor™ tubes displayed an acceptable analytical and clinical performance on both hs-cTnI measuring systems and that they are fit for purpose in an emergency setting for patients presenting with chest pain. Initially, we assessed the effect of Barricor™ tubes on the incidence of hemolysis. In vitro hemolysis is an undesirable, though relatively common

problem, which may adversely affect patient management. Our data revealed that the incidence of a medium degree of hemolysis, as defined by a fHb concentration of ≥1.00 g/L, detected in our setting in approximately 5% of plasma samples, was independent of the blood collection tubes used. On the other hand, PSTII™ showed a significant increase in rate of hemolysis when compared with Barricor™ tubes if a lower cut-off for hemolysis (≥0.11 g/L) was employed on the Atellica® CH detection system, demonstrating a slightly better quality of plasma being obtained from Barricor™ tubes and indicating that PSTII™ may increase the number of samples showing a very low hemolysis degree, which were however still within the physiological fHb range [18]. Other authors also noted a significantly lower frequency of hemolysis and a better quality of plasma in Barricor™ when compared to PSTII™ tubes, even if different centrifugation protocols for Barricor™ tubes were employed in the various studies [6, 7, 19, 20]. Our results showed that significant between-tube differences occurred where HI was relatively low. Increases in hemolysis severity cancelled out these differences. Nevertheless, interference thresholds for HI differed slightly in employed analyzers, further highlighting the need for establishing standardized and universally accepted

criteria for detecting and reporting HI among manufacturers as well as defining significant assay interference thresholds according to both the analytical criteria and clinical relevance. Our study is the first to demonstrate that hs-cTnI results obtained in both BD lithium heparin tubes fully agreed across the measuring range within the same system (Alinity™ or Atellica®). Dupuy et al. previously compared highly sensitive cardiac troponin T (hs-cTnT) measurements in both lithium heparin tubes, revealing only a negligible difference between the tubes [21]. Although these data were limited by the small sample size (samples were collected from only 9 patients and 5 healthy individuals) and the narrow range of evaluated hs-cTnT values (3-159 ng/L), those authors concluded that the use of Barricor™ tubes with a shorter centrifugation time did not affect hs-cTnT measurements, suggesting that both Barricor™ and PSTII™ tubes can be used interchangeably [21]. Our results confirmed that both tubes can also provide analytically equivalent results when used on either of the evaluated hs-cTnI measuring systems. As demonstrated in previous studies [22], marked differences in hs-cTnI concentrations between the two systems were observed even when using the same sample tube. The lack of both a commutable reference material and the different antibody configuration of assays may explain these differences. While Atellica® hs-cTnI is traceable to an internal standard manufactured using human heart homogenate, Alinity™ hs-cTnI is believed traceable to National Institute of Standards and Technology SRM 2921 through an alignment to the Architect™ assay, even though specific information on traceability implementation and the assessment of SRM 2921 commutability is not available [23]. It is however noteworthy, that when between-assay comparisons were focused on the 3 to 300 ng/L range, i.e., the hs-cTnI values having the most clinically important role in classifying patients with suspected AMI and in which assay harmonization is most desirable, the intercept, indicating the existence of a constant bias due to the different selectivity of antibody sandwiches in the two assays, was reduced to 5 ng/L. This supports the concept that differences between hs-cTnI assays could be markedly reduced by the availability of a commutable reference material utilized as a common calibrator in commercial systems [24]. Finally, in employing an AMI rapid single-measurement rule-out strategy using hs-cTnI with assay-specific cut-offs, we have clinically validated Barricor™ tubes [2,17]. With this approach, we showed that the rule-out ability for both evaluated hs-cTnI systems was excellent, with high NPV irrespective of the employed tube type. Sensitivities and NPVs found in this study corresponded with those found in previous reports using hs-cTnI assays, in which the safety and clinical efficacy of early AMI rule-out strategies using marker concentrations near to the assay limit of detection were evaluated [25, 27]. As recently highlighted in guidelines released by the UK National Institute for Health and Care Excellence (NICE) [28], no specific diagnostic accuracy evidence has been published to date for Alinity™ hs-cTnI.

In selecting the Alinity™ cut-off for the optimal ruling out of AMI in this study, we followed the NICE suggestion in using the recommended Architect™ cut-off, as Alinity™ uses the same method principle and reagents as an alternative version of the test, the only marked difference being that they are run on different analyzers [28]. With accord to this approach, several studies previously evaluated the diagnostic performance of very low Architect™ hs-cTnI concentrations on ED admission, with their results being consistent with our Alinity™ data [29,31]. Only three studies evaluating AMI rule-out power at patient ED admission have been published [17,22,32]. Our study obtained the same sensitivity figure (99%) as Sandoval et al. [17], and that approached (98%) by Chapman et al. [32], with both studies using the same 5 ng/L cut-off. Our NPV was slightly lower possibly influenced by differences in cohort patient numbers and recruitment protocols. It has been shown that the NPV may be higher in enrolled populations that have a higher prevalence of non-ischemic myocardial injury [33]. Although AMI rule-out performance may vary in principle among measuring systems [34], our study showed that the rule-out strategy based on a single sample with very low hs-cTnI concentrations measured at ED admission did not alter its outcome whichever of the two tubes were employed. Nevertheless, we observed two false negative hs-cTnI results with using Atellica® measuring system. Two hypothesis may explain this undesirable situation. Importantly, is the possible variability of hs-cTnI measurements due to the imprecision of the assay at these very low concentrations. Similarly, rounding results to the smallest integer may introduce a bias of an estimator. There are several limitations which should be acknowledged. Firstly, we did not provide a detailed characteristics of patients with suspected AMI as we focused specifically on the rapid rule-out strategy in non-selected ED patients with suspected AMI which may limit the generalizability of our findings. Secondly, our study is limited by the lack of information on a 30-day risk of major adverse cardiovascular events (MACE). Furthermore, the quality of plasma obtained using BD Barricor tubes has not been evaluated by plasma residual cells. In conclusion, we demonstrated that Barricor™ tubes performed equally well, both analytically and clinically, when compared with PSTII™ tubes. Assuming a potential reduction in laboratory TAT, without impairment of the quality of laboratory service, Barricor™ tubes may provide an advantage which is of particular interest in hs-cTnI testing where a more expeditious availability of results has a central clinical role.

Figure 1: Difference plots for hs-cTnI results: (A) in PSTII™ tubes on the Alinity™ and Atellica® systems for hs-cTnI in the range 3 to 15,000 ng/L; (B) in Barricor™ tubes on the Alinity™ and Atellica® systems for hs-cTnI in the range 3 to 15,000 ng/L; (C) in PSTII™ tubes on the Alinity™ and Atellica systems for hs-cTnI in the range <300 ng/L; (D) in Barricor™ tubes on the Alinity™ and Atellica® systems for hs-cTnI in the range <300 ng/L.
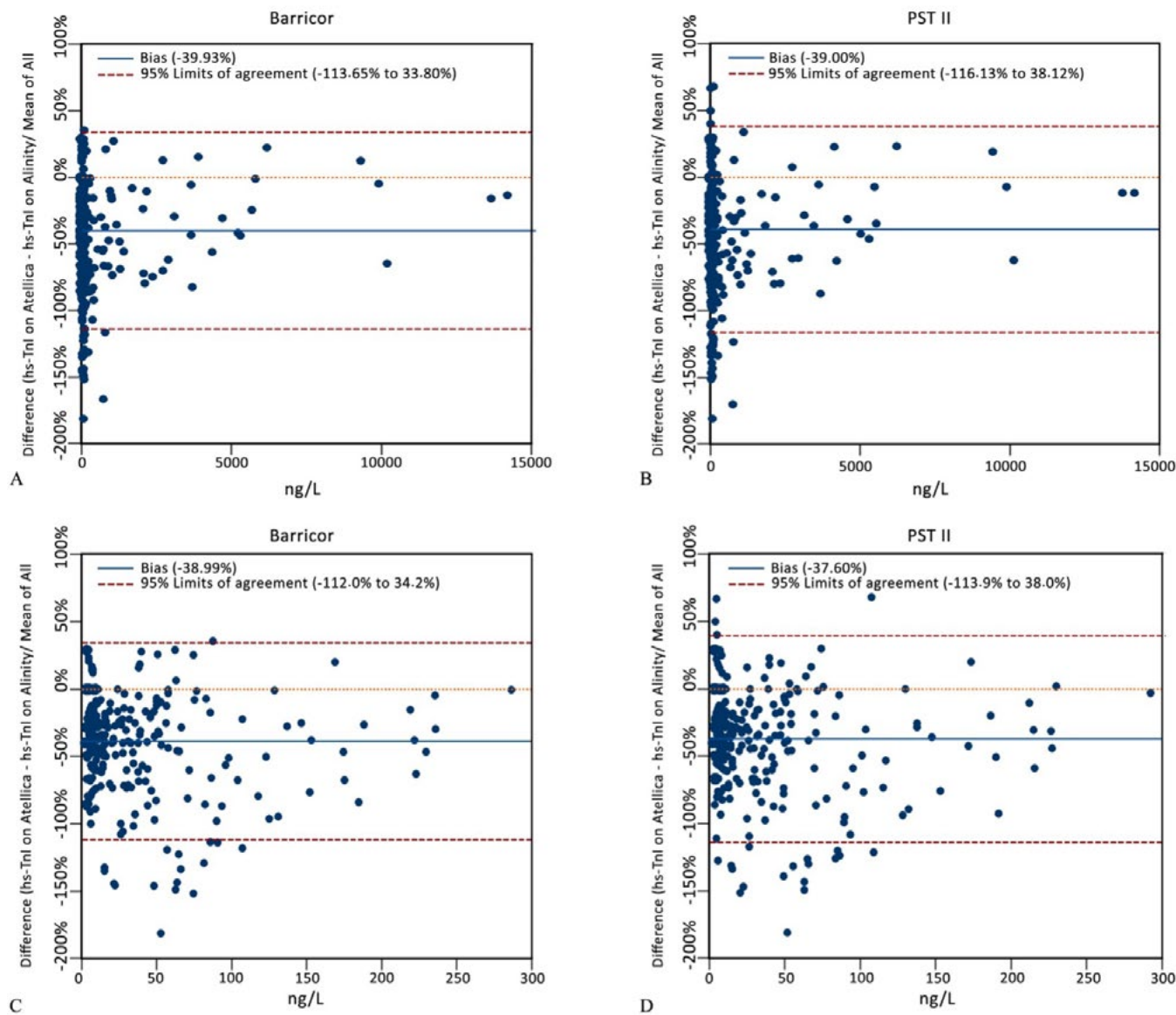
Supplemental Table 1: Distributions of Barricor and PSTII paired samples according to the categories on either system: <4ng/L Alinity, <5ng/L Atellica, between these low values and the assay-specific 99th percentiles and > 99th percentiles. Chi-square test was used for comparisons.

	Barricor™ No. of samples	PSTII™ No. of samples	P value for comparisons between tubes
Alinity™ <4 ng/L	37	29	0.301
Atellica® <5 ng/L	37	37	1.000
Alinity™ 4 ng/L – 26 ng/L	181	185	0.765
Atellica® 5 ng/L – 45 ng/L	179	179	1.000
Alinity™ >26 ng/L	141	145	0.760
Atellica® >45 ng/L	143	143	1.000

Supplemental Table 2: Paired comparisons between both measuring systems and the two tube types regarding the number and proportion of hs-cTnI results <4 ng/L for Alinity and <5 ng/L for Atellica. Chi-square test was used for comparisons.

| System and tube type	System and tube type	P value
Alinity™ Barricor™ vs. Alinity™ PSTII™	29	0.301
Alinity™ Barricor™ vs. Atellica® Barricor™	37	1.000
Alinity™ Barricor™ vs. Atellica® PSTII™	185	0.765
Alinity™ PSTII™ vs. Atellica® Barricor™	179	1.000
Alinity™ PSTII™ – Atellica® PSTII™	145	0.760
Atellica™ Barricor™ vs. Atellica® PSTII™	143	1.000

Supplemental Figure 1: Comparisons of hemolysis indices in the Barricor™ vs. PSTII™ tubes employing each of the two evaluated measuring systems:

(A) Comparison between Barricor™ tubes and PSTII™ tubes on the Alinity™; rho = -0.11; CI: -0.18, -0.01; p = 0.032

(B) Comparison between Barricor™ tubes and PSTII™ tubes on the Atellica®; rho = 0.38; CI: 0.29, 0.48; p < 0.001

(C) Comparison between AlinityTM and Atellica® systems in BarricorTM tubes; rho = 0.82; CI: 0.73, 0.87; p < 0.001

(D) Comparison between AlinityTM and Atellica® systems in PSTIITM tubes; rho = -0.15; CI: -0.22, -0.05; p = 0.004
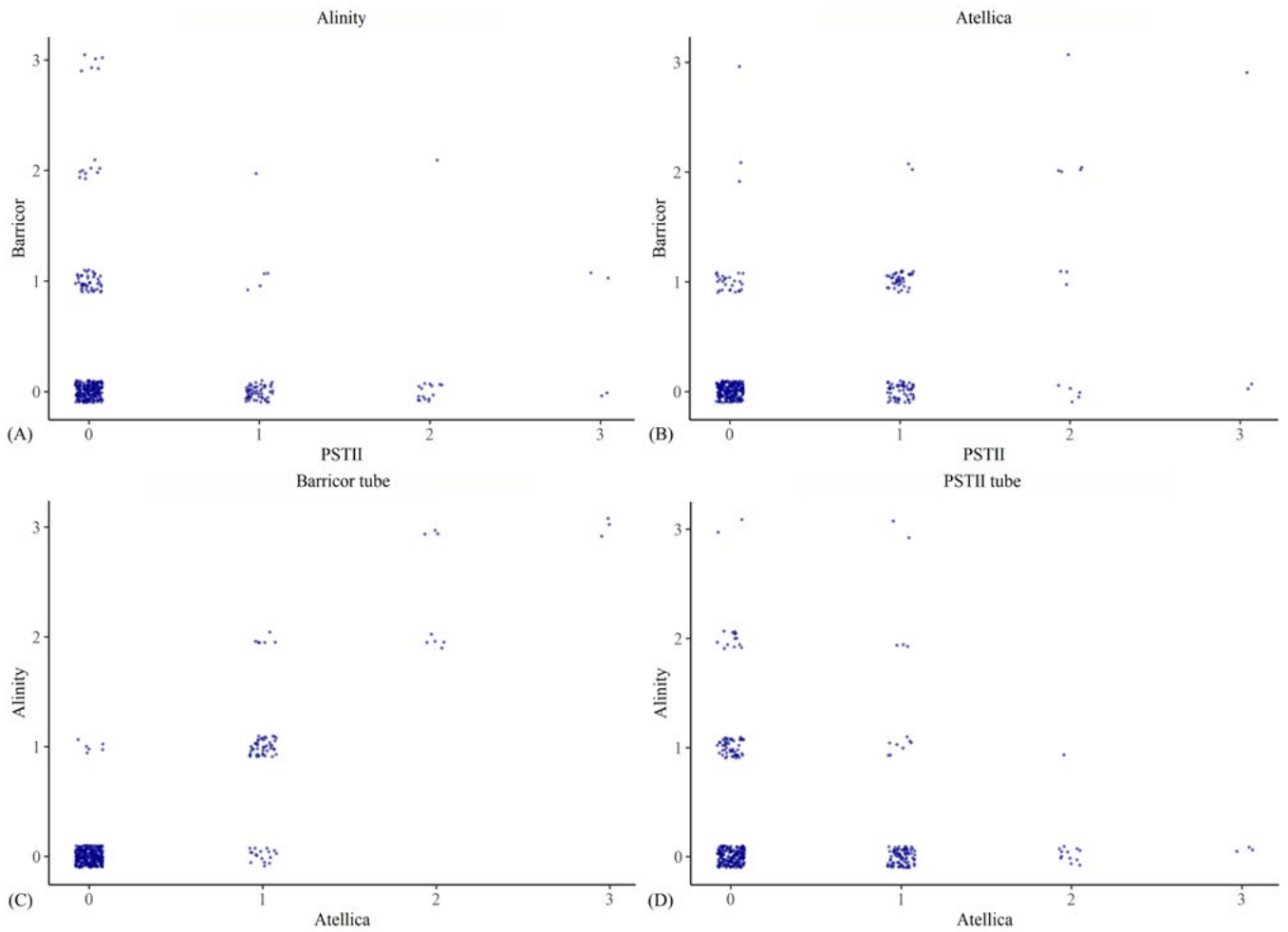
Supplemental Figure 2: Regression analyses of comparisons of hs-cTnI measurements in Barricor™ vs. PSTII™ tubes employing each of the two evaluated measuring systems:
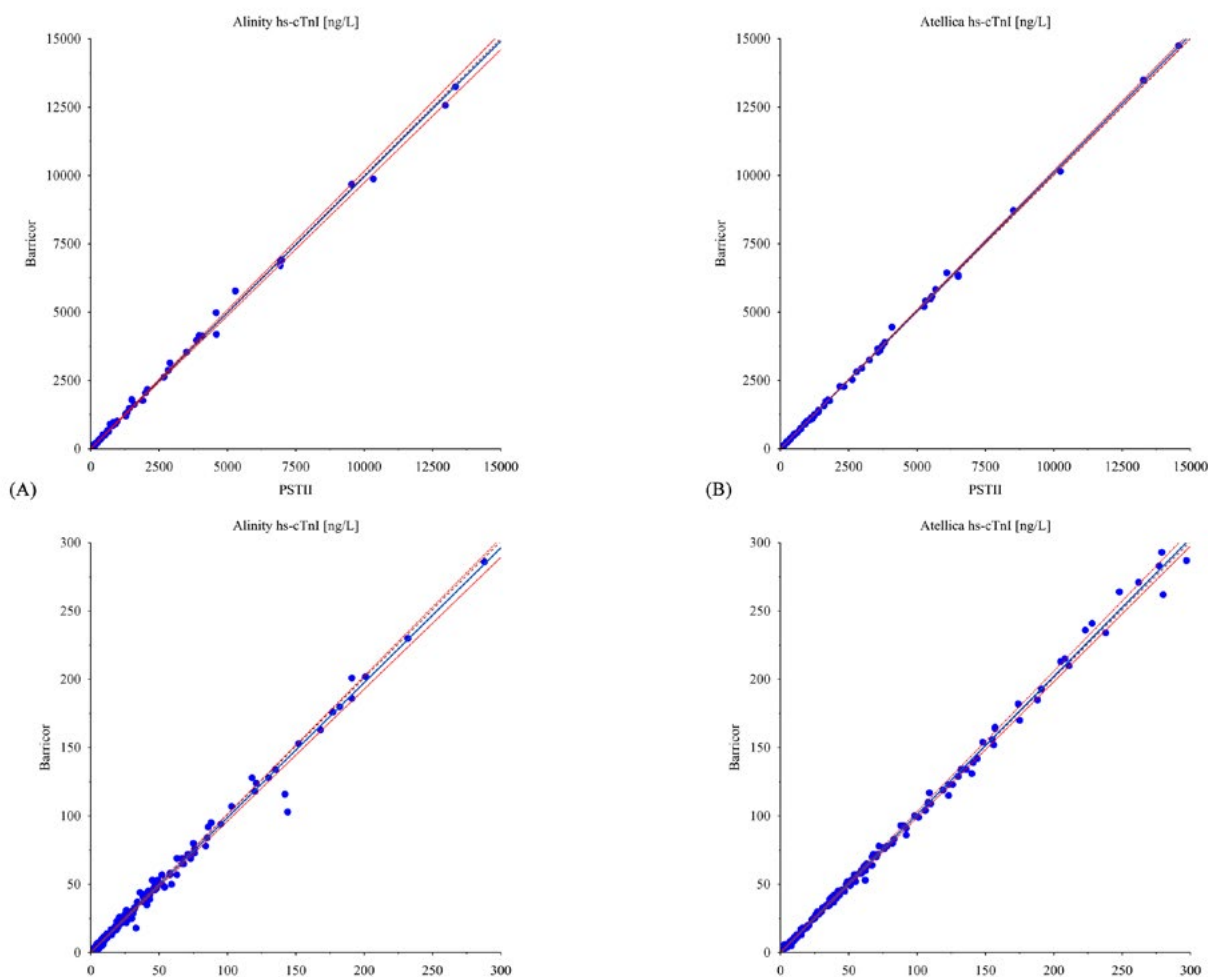
(A) Comparison on the Alinity™ on all 359 samples; regression equation: Barricor™ = 0.99 (CI: 0.97-1.01) PSTII™ + 6 (2-10) ng/L; r=0.99.

(B) Comparison on the Atellica® on all 359 samples; regression equation: Barricor™ = 1.01 (CI: 1.00-1.02) PSTII™ − 1 (−3 to 1) ng/L; r=0.99.

(C) Comparison on the Alinity™ on 300 samples with hs-cTnI <300 ng/L; regression equation: Barricor™ = 0.99 (CI: 0.96-1.01) PSTII™ + 0.2 (−0.04 to 0.5) ng/L; r=0.99.

(D) Comparison on the Atellica® on 300 samples with hs-cTnI <300 ng/L; regression equation: Barricor™ = 1.01 (CI: 0.99-1.03) PSTII™ + 0.1 (−0.3 to 0.4) ng/L. r=0.99.

Dashed line corresponds to the identity line. Continuous red lines correspond to the 95% confidence intervals of the regression line.

Supplemental Figure 3: Regression analyses of comparisons of hs-cTnI results obtained by the two evaluated measuring systems in the same type of tube:

(A) Comparison between Alinity™ and Atellica® systems in Barricor™ tubes on all 359 samples; regression equation: Alinity™ = 0.83 (CI: 0.72-0.95) Atellica® − 48 (−76 to −19) ng/L; r=0.96.

(B) Comparison between Alinity™ and Atellica® systems in PSTII™ tubes on all 359 samples; regression equation: Alinity™ = 0.85 (CI: 0.73-0.97) Atellica® − 56 (−87 to −24) ng/L; r=0.96.

(C) Comparison between Alinity™ and Atellica® systems in Barricor™ tubes on 300 samples with hs-cTnI <300 ng/L; regression equation: Alinity™= 0.74 (CI: 0.64-0.84) Atellica® − 5 (−8 to −1) ng/L; r=0.89.

(D) Comparison between Alinity™ and Atellica® systems in PSTII™ tubes on 300 samples with hs-cTnI <300 ng/L; regression equation: Alinity™ = 0.76 (CI: 0.66-0.87) Atellica® − 5 (−8 to −2) ng/L; r=0.88.

Dashed line corresponds to the identity line. Continuous red lines correspond to the 95% confidence intervals of the regression line.
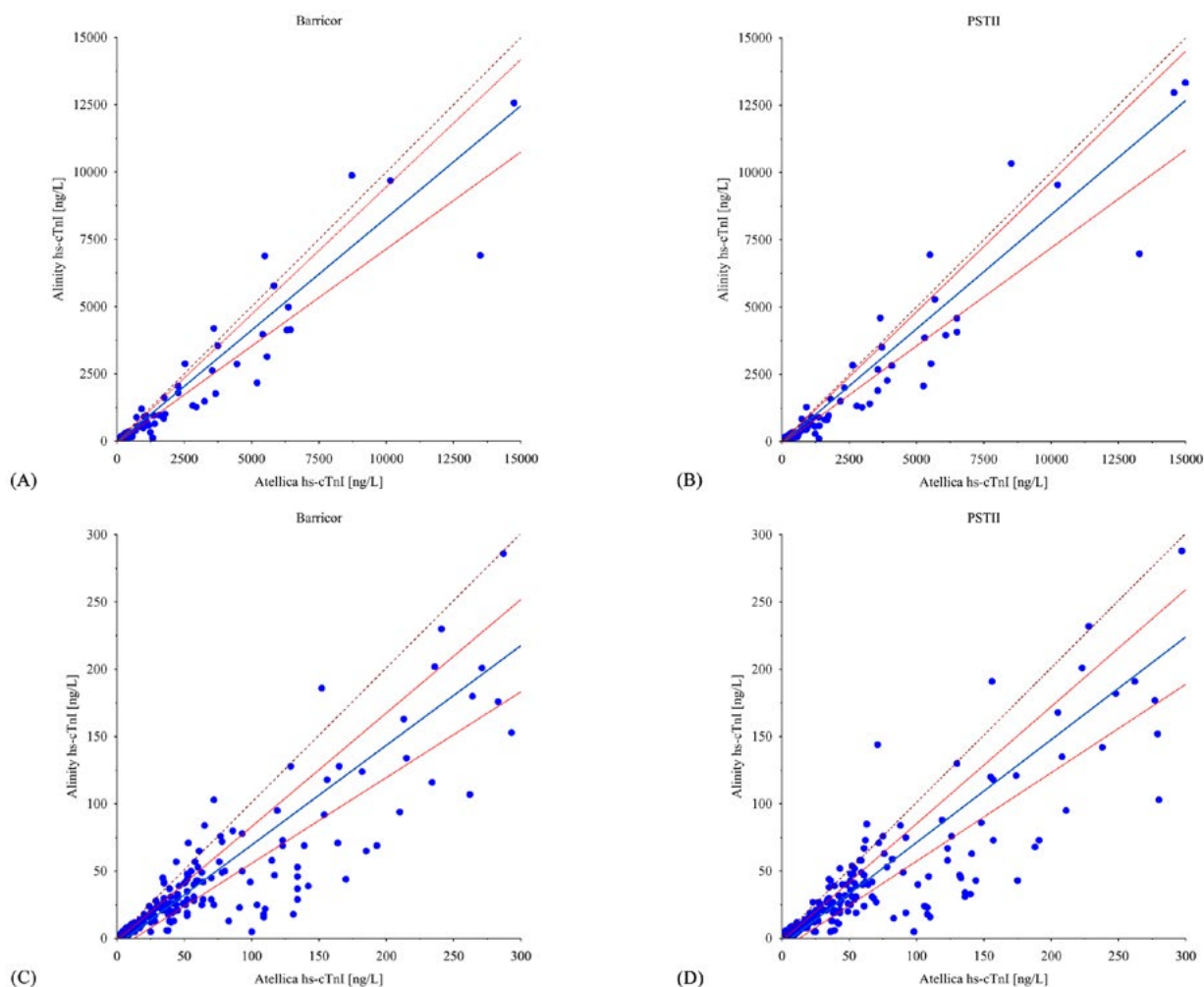
References

1. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). Circulation 2018;138:e618–51.

2. Collet J-P, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J 2021;42:1289–367.

3. Krintus M, Panteghini M. Laboratory-related issues in the measurement of cardiac troponins with highly sensitive assays. Clin Chem Lab Med 2020;58:1773–83.

4. Ferraro S, Dolci A, Panteghini M. Fast track protocols using highly sensitive troponin assays for ruling out and ruling in non-ST elevation acute coronary syndrome. Clin Chem Lab Med 2017;55:1683–9.

5. Backus BE, Body R, Weinstock MB. Troponin Testing in the Emergency Department-When 2 Become 1. JAMA Netw Open. 2021; 1;4:e210329.

6. Ramakers C. BD Vacutainer® Barricor tube in the emergency department: reduced hemolysis rates using partial draw tubes with reduced vacuum. Clin Chem Lab Med 2018;56:e31–2.

7. Badiou S, Vuillot O, Bargnoux AS, Kuster N, Lefebvre S, Sebbane M, et al. Improved quality of samples and laboratory turnaround time using 3.5 mL low vacuum BD Vacutainer® Barricor tubes in the emergency department. Pract Lab Med 2019;16:e00128

8. Füzéry AK, Raizman JE, Goudreau BL, Moses K, Niemann K, Park J, Cembrowski GS. The BD Barricor blood collection tube is an acceptable and robust alternative to the PST for use with the Beckman AccuTnI+3 assay. Clin Biochem 2017;50:851-7.

9. Raizman JE, Tsui AKY, Goudreau BL, Füzéry AK, Estey M, Sadrzadeh H, Higgins T. Multi-platform analytical evaluation of the Beckman Coulter Access high-sensitivity troponin I assay across different laboratory sites using Barricor plasma. Clin Biochem. 2020;78:25-31.

10. Cembrowski G, Qiu Y, Sherazi A, Shea JL. Retrospective analysis of intra-patient laborato-ry variation demonstrates that the BD Vacutainer® Barricor™ blood collection tube reduces troponin variation. Clin Biochem 2023;114:24-29.

11. Barth JH, Panteghini M, Bunk DM, Christenson RH, Katrukha A, Noble JE, et al.; IFCC Working Group on Standardization of Cardiac Troponin I. Recommendation to harmonize the units for reporting cardiac troponin results. Clin Chim Acta 2014;432:166.

12. Capoferri A, Aloisio E, Pasqualetti S, Panteghini M. More about the random uncertainty of photometric determination of hemolysis index on the Abbott AlinityTM c platform. Clin Biochem 2022;105-106:94-5.

13. Snyder JT, Garcia E, Davis J, Cheek J. Performance evaluation of the Atellica CH analyzer HIL alert feature. 72nd AACC Annual Scientific Meeting Abstracts, Abstract B-239, 2020:S144.

14. Pasqualetti S, Szőke D, Panteghini M. Heparinate but not serum tubes are susceptible to hemolysis by pneumatic tube transportation. Clin Chem Lab Med 2016;54:785–9.

15. Boeddinghaus J, Nestelberger T, Badertscher P, Twerenbold R, Fitze B, Wussler D, et al.; APACE Investigators. Predicting acute myocardial infarction with a single blood draw. Clin Chem 2019;65:437-50.

16. CLSI Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline–Third Edition. CLSI document EP09-A3. Wayne, PA. Clinical and Laboratory Standards Institute; 2013

17. Sandoval Y, Nowak R, deFilippi CR, Christenson RH, Peacock WF, McCord J, et al. Myocardial infarction risk stratification with a single measurement of high-sensitivity troponin I. J Am Coll Cardiol 2019;74:271-82.

18. Gils C, Sandberg MB, Nybo M. Verification of the hemolysis index measurement: imprecision, accuracy, measuring range, reference interval and impact of implementing analytically and clinically derived sample rejection criteria. Scand J Clin Lab Invest 2020;80:580-9.

19. Gawria G, Tillmar L, Landberg E. A comparison of stability of chemical analytes in plasma from the BD Vacutainer® Barricor™ tube with mechanical separator versus tubes containing gel separator. J Clin Lab Anal 2020;34:e23060.

20. Cadamuro J, Mrazek C, Leichtle AB, Kipman U, Felder TK, Wiedemann H, et al. Influence of centrifugation conditions on the results of 77 routine clinical chemistry analytes using standard vacuum blood collection tubes and the new BD-Barricor tubes. Biochem Med (Zagreb) 2018;28:010704.

21. Dupuy AM, Badiou S, Daubin D, Bargnoux AS, Magnan C, Klouche K, Cristol JP. Comparison of Barricor™ vs. lithium heparin tubes for selected routine biochemical analytes and evaluation of post centrifugation stability. Biochem Med (Zagreb) 2018;28:020902.

22. Sörensen NA, Goßling A, Neumann JT, Hartikainen TS, Haller PM, Scharlemann L, et al. Diagnostic validation of a high-sensitivity cardiac troponin I assay. Clin Chem 2021;67:1230-9.

23. Sharrod-Cole H, Ford C. Multicenter evaluation of a high-sensitivity troponin I assay and verification of an early rule-out algorithm. J Appl Lab Med 2019;4:95-100.

24. Tate JR, Bunk DM, Christenson RH, Barth JH, Katrukha A, Noble JE, et al; IFCC Working Group on Standardization of Cardiac Troponin I. Evaluation of standardization capability of current cardiac troponin I assays by a correlation study: results of an IFCC pilot project. Clin Chem Lab Med 2015;53:677-90.

25. Carlton EW, Ingram J, Taylor H, Glynn J, Kandiyali R, Campbell S, et al. Limit of detection of troponin discharge strategy versus usual care: randomised controlled trial. Heart 2020;106:1586–94.

26. Anand A, Lee KK, Chapman AR, Ferry AV, Adamson PD, Strachan FE, et al. High-sensitivity cardiac troponin on presentation to rule out myocardial infarction: A stepped-wedge cluster randomized controlled trial. Circulation 2021;143:2214–24.

27. Chiang CH, Chiang CH, Lee GH, Gi WT, Wu YK, Huang SS, et al. Safety and efficacy of the European Society of Cardiology 0/1-hour algorithm for diagnosis of myocardial infarction: systematic review and meta-analysis. Heart 2020;106:985-91.

28. National Institute for Health and Care Excellence. High-sensitivity troponin tests for the early rule out of NSTEMI. Diagnostics guidance [DG40]. Published: 26 August 2020.

29. Boeddinghaus J, Nestelberger T, Twerenbold R, Wildi K, Badertscher P, Cupa J, et al. Direct comparison of 4 very early rule-out strategies for acute myocardial infarction using high sensitivity cardiac troponin I. Circulation 2017;135:1597–611.

30. Sandoval Y, Smith SW, Love SA, Sexter A, Schulz K, Apple FS. Single high-sensitivity cardiac troponin I to rule out acute myocardial infarction. Am J Med 2017;130:1076–83. e1.

31. Shortt C, Ma J, Clayton N, Sherbino J, Whitlock R, Pare G, et al. Rule-in and rule-out of myocardial infarction using cardiac troponin and glycemic biomarkers in patients with symptoms suggestive of acute coronary syndrome. Clin Chem 2017;63:403–14.

32. Chapman AR, Fujisawa T, Lee KK, Andrews JP, Anand A, Sandeman D, et al. Novel high-sensitivity cardiac troponin I assay in patients with suspected acute coronary syndrome. Heart 2019;105:616–22.

33. Lowry MTH, Anand A, Mills NL. Implementing an early rule-out pathway for acute myocardial infarction in clinical practice. Heart 2021;107:1912-9.

34. Karády J, Mayrhofer T, Ferencik M, Nagurney JT, Udelson JE, Kammerlander AA, et al. Discordance of high-sensitivity troponin assays in patients with suspected acute coronary syndromes. J Am Coll Cardiol 2021;77:1487-99.