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Short Communication

Analytical characterization of the Siemens Dimension EXL high-sensitivity cardiac troponin I assay

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ABSTRACT

Background: Siemens Healthcare Diagnostics has four commercially available assays on different analytical platforms using different methodologies to generate signal. We assessed the analytical performance of the Dimension EXL hs-cTnI assay (LOCI method) across different matrices and compared it to two different acridinium ester-based hs-cTnI assays (ADVIA Centaur and Abbott ARCHITECT).

Methods: The analytical sensitivity and precision below the 99th-percentile was determined for the Dimension EXL hs-cTnI assay. Method comparisons were performed between the Dimension EXL contemporary cTnI and the hs-cTnI assays, between different matrices for the EXL hs-cTnI assay (serum, lithium heparin and EDTA plasma), and between different hs-cTnI assays (EXL versus ADVIA Centaur or Abbott ARCHITECT) using non-parametric analyses.

Results: The limit of blank and detection were 0.9 ng/L and 1.7 ng/L, respectively, with imprecision of 5.8% at 8.6 ng/L and 3.2% at 47.5 ng/L. Comparison between the EXL contemporary cTnI and hs-cTnI assay (range: 2.6–4214 ng/L) yielded proportional lower concentrations for the hs-cTnI assay (slope = 0.86; 95%CI: 0.81 to 0.96, n = 40); however, there was no difference in concentrations below 100 ng/L between the assays (median difference = −2.7 ng/L; 95%CI: −9.8 to 9.3). Passing-Bablok regression analysis with EDTA plasma yielded proportionally higher concentrations with the EXL hs-cTnI versus Abbott hs-cTnI (slope = 1.45; 95%CI: 1.02–1.86, n = 40) with proportionally lower concentrations with EDTA versus lithium heparin plasma with the EXL hs-cTnI assay alone (slope = 0.93; 95%CI: 0.90 to 0.99, n = 40). Comparison with Abbott hs-cTnI concentrations below 100 ng/L in the three matrices, indicated that the EXL hs-cTnI assay yielded higher concentrations (median difference range: 3.4–9.4 ng/L), with differences also evident when comparing the EXL hs-cTnI assay to the ADVIA Centaur hs-cTnI assay.

Conclusion: The Siemens EXL hs-cTnI assay meets the analytical criteria for a high-sensitivity assay, with assay specific cutoffs important to maximize clinical performance.

1. Introduction

Clinical guidelines have firmly endorsed high-sensitivity cardiac troponin (hs-cTn) as the preferred test in evaluating patients with possible myocardial infarction [1,2]. Recent laboratory recommendations have also provided important information on the analytical attributes of hs-cTn assays [3]. However, recommendations regarding sample type/matrix and cardiac troponin assays have not changed from previous laboratory recommendations with a continued emphasis of not interchanging the measurements with different sample types; and equally important, that the selection of the sample type should be based on evidence [3]. To that end, it is important to provide evidence for both the analytical and clinical performance in the sample type selected for hs-cTn testing in the institution transitioning to a hs-cTnI assay [4,5].

Evaluating the effect of different matrices on hs-cTn concentrations is important as different hs-cTn assays yield different results when assessed in serum versus lithium heparin versus EDTA plasma [6–8]. Also, for reassurance that indeed the assay being evaluated is a hs-cTn assay, quality control (QC) should be measured in the “normal range” and near the 99-percentile [3,9]. Siemens has four different analytical platforms with hs-cTnI assays, with the ATELICA and ADVIA Centaur instruments using acridinium ester-based technology whereas the Dimension VISTA and Dimension EXL instruments employ LOCI

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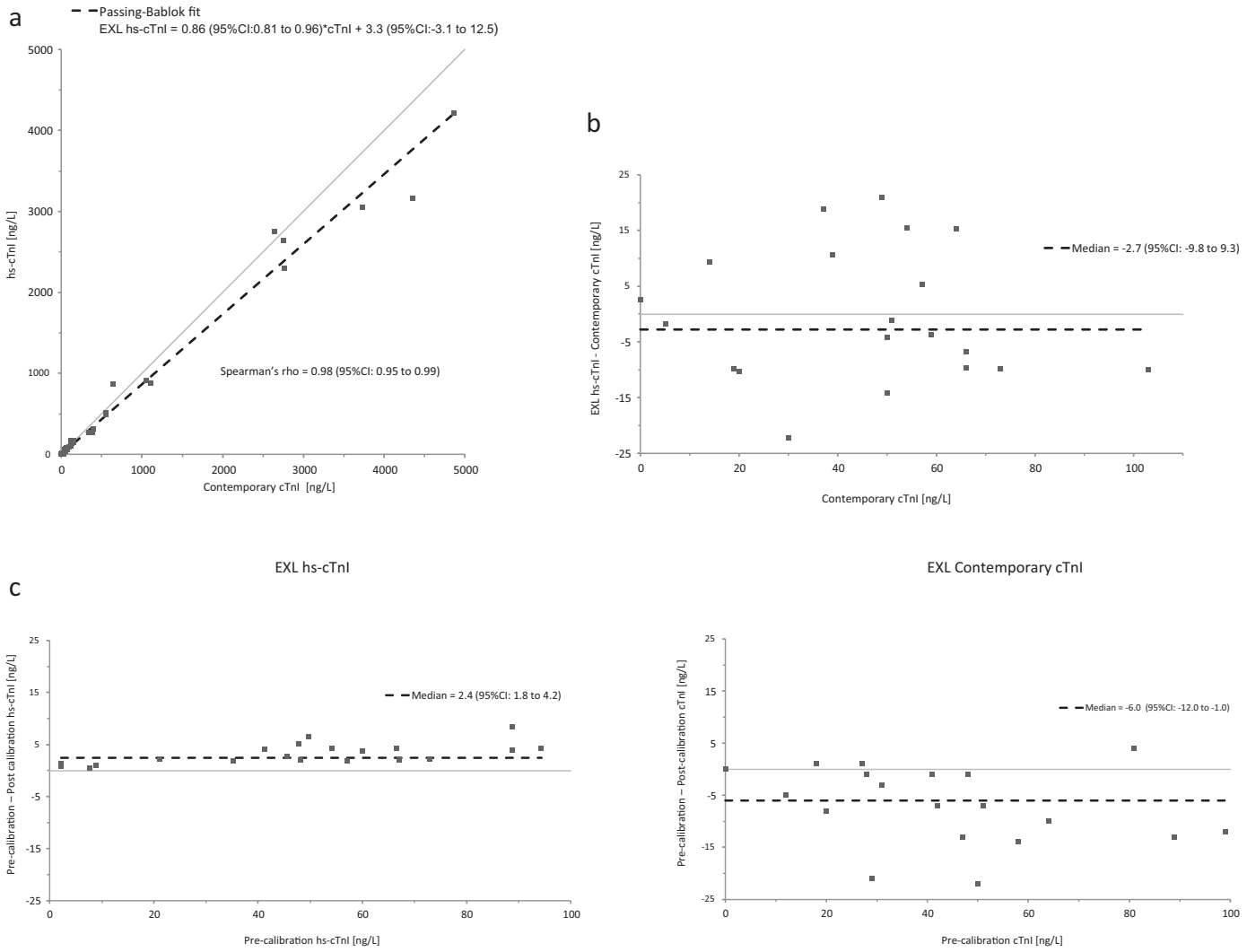

Fig. 1. Dimension EXL comparison between the contemporary cTnI assay versus high-sensitivity cTnI assay via Passing-Bablok (hs-cTnI range: 2.6–4214 ng/L, n = 40) (a) and difference plot below 100 ng/L between cTnI and hs-cTnI (hs-cTnI range: 2.6–93 ng/L, n = 20) (b); and after recalibration for hs-cTnI (post-calibration hs-cTnI range: 3.1–97 ng/L) and for cTnI (post-calibration cTnI range: 0–87 ng/L) (c).

technology to generate the signal [8,10]. Differences extend beyond signal generation and despite the same three cTnI epitopes being targeted by three antibodies in all four assays, differences exist between which antibodies are used in capture versus detection, in the analytical testing time, in the prevalence of measurable hs-cTnI concentrations in the healthy population as well as the 99th-percentiles [11]. To that end it is incumbent to validate each assay [5,8], with the objective of the present study to analytically characterize the Siemens EXL hs-cTnI assay.

2. Methods

2.1. High-sensitivity cardiac troponin I assays

The Siemens Dimension EXL hs-cTnI assay uses three antibodies (one mouse and two sheep monoclonal), in a homogenous format using LOCI technology to generate signal (i.e., illumination generates oxygen from the Sensibeads which diffuse into the Chemibeads and triggers a chemiluminescent signal) with the sample volume being 10 μL (package insert). The amino acid (aa) epitopes on cTnI targeted by the antibodies for detection are 41-50aa and 171-190aa and for capture is 29-34aa [11]. Serum and lithium heparin plasma are the manufacturer supported sample types [11]. The two other hs-cTnI assays used in this study were the Siemens ADVIA Centaur hs-cTnI assay and the Abbott ARCHITECT hs-cTnI assay with their analytical performance in different matrices previously reported [4–8].

2.2. Analytical sensitivity, precision, stability, and linearity

The limit of blank (LoB) was assessed by 10 measurements with water, saline, diluent, and zero calibrator before formally selecting one matrix to determine the LoB. The LoB was determined over 3 days of testing and 80 measurements with the following equation: $LoB = mean_{blank} + (1.645*(SD_{blank}))$ [7]. A low-patient pool QC was measured over 3 weeks (n = 25) to determine the SD_{low} to calculate the limit of detection (LoD):$LoD = LoB + (1.645*(SD_{low}))$ [7]. Another QC patient pool near the 99th-percentile concentration was also measured over this timeframe [7,8]. Freeze thaw stability testing was performed by pooling different lithium heparin samples to construct one pool below 10 ng/L and another pool near the 99th-percentile concentration. The pools were tested, frozen at −20 °C, thawed and tested again for a total of 5 freeze/thaw cycles. Stability was deemed acceptable after the freeze/thaw cycles if the low pool (below 10 ng/L) concentrations were within ± 1.6 ng/L from baseline and ± 20% from baseline for the higher pool [5,8]. Linearity (analytical measurement range: 4.0–25,000 ng/L per the manufacturer) was assessed by performing a 7-

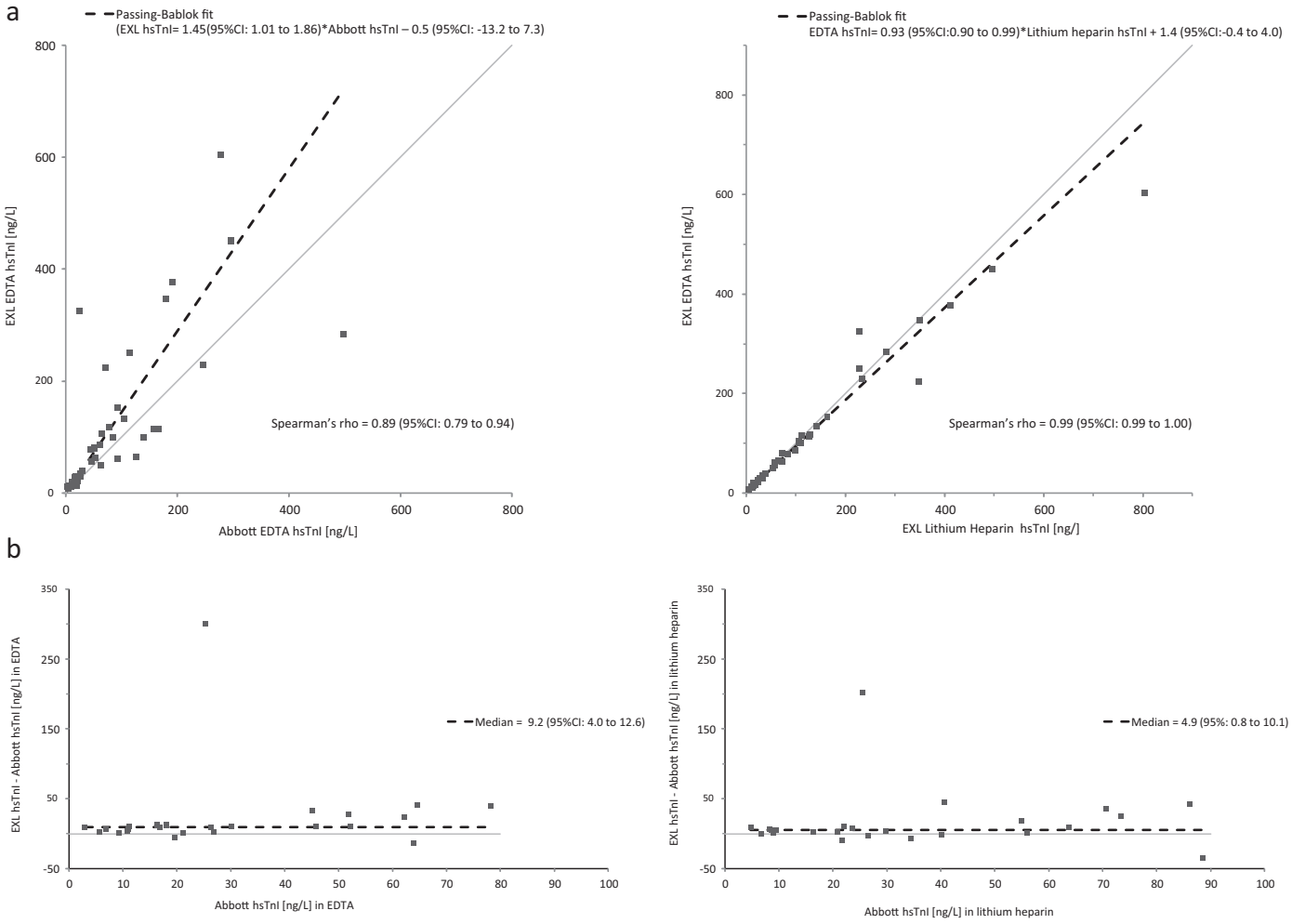

Fig. 2. Dimension EXL hs-cTnI versus Abbott hs-cTnI in EDTA plasma and Dimension EXL in EDTA plasma versus lithium heparin plasma (n = 40) (a) and difference plot below 100 ng/L (n = 24) between EXL hs-cTnI versus Abbott hs-cTnI in EDTA plasma and in lithium heparin plasma (b).

point mix-sample dilution study (testing in triplicate) with a high concentration lithium heparin pool (H = 19,516 ng/L) and a low lithium heparin pool (L = 3.5 ng/L) via the following protocol: low pool or (L), 0.833(L) + 0.167(H), 0.667(L) + 0.333(H), 0.5(L) + 0.5(H), 0.667(H) + 0.333(L), 0.833(H) + 0.167(L), high pool or (H), with analyses performed in Analyse-it.

2.3. Analytical comparison between different hs-cTnI assays and different matrices

Non-parametric analyses were performed for all comparison studies via Analyse-it software (Passing-Bablok regression, Spearman correlation (rho), and median difference). First, comparison between the Dimension EXL contemporary cTnI assay (concentrations converted to ng/L from μg/L for this analysis) versus the hs-cTnI assay was performed on 40 fresh lithium heparin samples where cTnI was clinically reported. Secondary analyses were performed on concentrations below 100 ng/L and after recalibration for both assays (n = 20). Second, comparison between the EXL hs-cTnI assay and the Abbott hs-cTnI assay was performed with EDTA plasma (n = 40, with EDTA plasma being a validated sample type for the Abbott hs-cTnI assay) [4] and between EDTA plasma and lithium heparin plasma for the EXL hs-cTnI assay. Both the EDTA and lithium heparin plasma aliquots were frozen at -20 °C, thawed for approximately 1 h at room temperature, mixed 10 times via hand inversion and then centrifuged for 10 min@2300 *g* before testing on the respective platforms. Secondary analyses included comparison between the two assays in lithium heparin plasma and EDTA plasma for concentrations below 100 ng/L (n = 24 matched sample pairs). Third, comparisons below 100 ng/L were performed in lithium heparin plasma and serum between the EXL hs-cTnI, the Abbott hs-cTnI, and the ADVIA Centaur hs-cTnI assays. The results from the Abbott hs-cTnI and the ADVIA Centaur hs-cTnI assays have been previously reported [8], with testing performed with the EXL hs-cTnI assay on the second thaw from samples with sufficient volume for testing and stored below −70 °C (n = 40 paired samples that were thawed, mixed and centrifuged as detailed above).

3. Results

The Siemens Dimension EXL hs-cTnI assay when measured with water (n = 10) yielded a mean (SD) concentration of 7.7 ng/L (0.7), with saline (n = 10) a mean concentration of 5.6 ng/L (0.2) and with diluent (n = 10) a mean concentration of 4.5 ng/L (0.2). Measurement with the zero calibrator (n = 80) yielded a mean (SD) concentration of 0.1 ng/L (0.5), and LoB = 0.9 ng/L, with the highest measured concentration of the zero calibrator over 80 measurements being 4.2 ng/L. Measurement of two QC patient pools (n = 25) yielded the following estimates: low-pool mean|SD|CV = 8.6 ng/L|0.5|5.8% and high-pool mean|SD|CV = 47.5 ng/L|1.5|3.2%. Using the SD from the low-pool coupled with the LoB estimate when using the zero calibrator yielded an LoD = 1.7 ng/L. Stability after 5 freeze/thaw cycles yielded acceptable recovery in lithium heparin plasma pools of 6.3 ng/L and 50.0 ng/

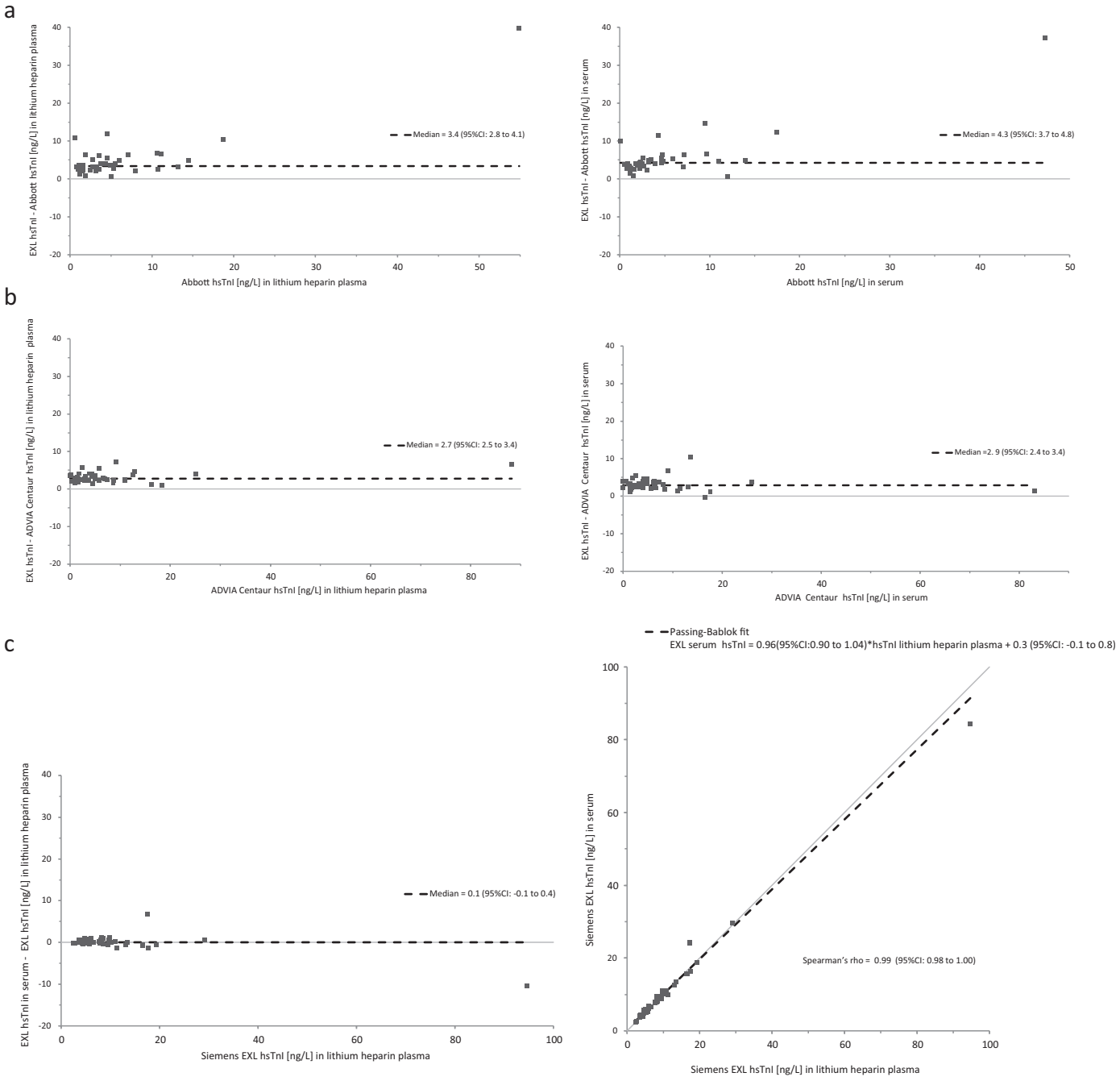

Fig. 3. Difference plots (n = 40) between Dimension EXL hs-cTnI versus Abbott hs-cTnI in lithium heparin and serum (a) and Dimension EXL hs-cTnI versus ADVIA Centaur hs-cTnI in lithium heparin and serum (b) with difference plot and Passing-Bablok regression between serum and lithium heparin plasma for the EXL hs-cTnI assay (c).

L, with the lowest concentration measured being 5.5 ng/L (or 0.8 ng/L lower from baseline) and 48.2 ng/L (or 3% lower from baseline), respectively. The 7-point linearity study demonstrated acceptable linearity from 20,000 to 2500 ng/L (nonlinearity% range: 4.4% to −9.9%), however below there was significant deviation, with overall a polynomial curve a better fit for the data.

The EXL hs-cTnI assay (range = 2.6–4214 ng/L; n = 40) yielded proportional lower results than the EXL contemporary cTnI assay (slope = 0.86; 95%CI: 0.81 to 0.96/rho = 0.98; 95%CI: 0.95–0.99) (Fig. 1a). However, below 100 ng/L, there was no appreciable difference in concentrations (median difference = −2.7 ng/L; 95%CI: −9.8 to 9.3,n = 20) (Fig. 1b), with recalibration demonstrating closer agreement with hs-cTnI measurements (median difference = 2.4 ng/L; 95%CI: 1.8 to 4.2) as compared to cTnI measurements (median difference = −6.0 ng/L; 95%CI: −12.0 to −1.0) (Fig. 1c).

The EXL hs-cTnI assay yielded higher results as compared to the Abbott hs-cTnI assay in EDTA plasma (slope = 1.45; 95%CI: 1.01 to 1.86, n = 40), with excellent correlation (rho = 0.99; 95%CI: 0.99 to 1.00, n = 40) between the EXL hs-cTnI assay in EDTA plasma versus the EXL hs-cTnI assay in lithium heparin plasma (Fig. 2a). Comparison below 100 ng/L with the EXL hs-cTnI assay in either EDTA or lithium heparin plasma to the Abbott hs-cTnI assay yielded higher results (Fig. 2b). In serum below 100 ng/L, the EXL hs-cTnI assay also produced higher results as compared to the Abbott hs-cTnI assay (median difference = 4.3 ng/L; 95%CI: 3.7 to 4.8) (Fig. 3a) and ADVIA Centaur hs-cTnI assay (median difference = 2.9 ng/L; 95%CI: 2.4 to 3.4)

(Fig. 3b). There was excellent agreement (slope = 0.96; 95%CI: 0.90 to 1.04/intercept = 0.3 ng/L; 95%CI: −0.1 to 0.8) and correlation (rho = 0.99; 95%CI: 0.98 to 1.00) between the EXL hs-cTnI assay measured in serum versus lithium heparin plasma (Fig. 3c).

4. Discussion

The Siemens Dimension EXL hs-cTnI assay has analytical attributes similar to other hs-cTnI assays [4–8]. Importantly, this assay demonstrates excellent precision below 10 ng/L and near the manufacturer's 99th-percentile [11], exceeding recommended target precision goals at these important concentration ranges [3,9]. The fact that the EXL hs-cTnI assay has excellent recovery after several freeze/thaws and only uses 10 μl for the sample volume may be favorable in situations where the amount of sample volume available for testing is limited (e.g., neonate/pediatric) [12,13]. The LoB (0.9 ng/L) and LoD (1.7 ng/L) determined in this study are lower than the manufacturer's estimates [11]. However, the fact that 2 measurements (4.2 and 2.7 ng/L) from the 80 with the zero calibrator exceeded these estimates suggests that using the manufacturer's limit of quantification of 4 ng/L maybe a more robust estimate for the lower limit of reporting. Moreover, the finding that water, saline and diluent, all produced measureable concentrations (average concentrations > 4.0 ng/L) warrants caution and suggests that a protein component is necessary to optimize this assay, similar to the Beckman hs-cTnI assay where measurement of water also yielded detectable concentrations (mean concentration of water being 6.1 ng/L with this assay) [7]. In agreement with this, the zero calibrator (or level 1) for the EXL hs-cTnI assay is stated to be bovine albumin based (manufacturer package insert), with measurement for albumin in this material on the EXL analyzer yielding a concentration > 60 g/L (in house measurement), further supporting the premise that a protein component is needed for accurate measurements.

The EXL hs-cTnI assay can use both serum and lithium heparin plasma for testing, with EDTA plasma yielding slightly lower concentrations, in agreement with other hs-cTnI assays using this matrix [6–8]. Accordingly, it is prudent not to interchange lithium heparin plasma with EDTA plasma as concentration differences may be inappropriately interpreted as a biomarker change when in fact the differences in the concentrations are due to the sample types. Despite there being no published 99th-percentiles for EDTA plasma and the manufacturer excluding EDTA as a sample type, measurement with this matrix may be helpful when assessing discrepant results. Specifically, one patient had an Abbott hs-cTnI concentration of 25 ng/L in both lithium heparin and EDTA plasma with a corresponding EXL hs-cTnI concentration of 228 ng/L in lithium heparin plasma and 326 ng/L in EDTA plasma. Not only does the significantly higher (~10-fold) EXL hs-cTnI concentration raise concerns on possible interferences, the fact that the concentration in the EDTA plasma sample is ~100 ng/L higher than the concentration in the lithium heparin plasma sample further suggests an interferent causing this discrepant result. Accordingly, the ability to measure the EXL hs-cTnI assay in EDTA plasma may be useful when investigating clinically discordant results, which can occur when using hs-cTn assays [7,14].

Finally, the EXL hs-cTnI assay yields higher concentrations than both the Abbott and ADVIA Centaur hs-cTnI assays, especially at lower concentrations. This difference is important for both laboratory professionals and clinicians to appreciate and provides additional analytical evidence that different and assay specific cutoffs will be needed to optimize early decision-making algorithms using hs-cTn assays for patients presenting with possible acute coronary syndrome to the emergency department [15–17]. In addition to these metrics, it is also incumbent that the laboratory community further investigate and assess the analytical attributes and 99th percentiles that have been published, largely from the manufacturers, for hs-cTn assays [11]. In doing so, both laboratory professionals and clinicians may gain confidence in interpreting and understanding hs-cTn concentrations from the various manufacturers.

Disclosures

Dr. Kavsak has received grants/reagents/consultant/advisor/honoraria from Abbott Laboratories, Beckman Coulter, Ortho Clinical Diagnostics, Randox Laboratories, Roche Diagnostics and Siemens Healthcare Diagnostics. McMaster University has filed patents with Dr. Kavsak listed as an inventor in the acute cardiovascular biomarker field.

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