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A critical evaluation of the Beckman Coulter *Access hsTnI*: Analytical performance, reference interval and concordance

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ABSTRACT

Introduction: We investigated the analytical performance, outlier rate, carryover and reference interval of the Beckman Coulter *Access hsTnI* in detail and compared it with historical and other commercial assays.
Materials and methods: We compared the imprecision, detection capability, analytical sensitivity, outlier rate and carryover against two previous Access AccuTnI assay versions. We established the reference interval with stored samples from a previous study and compared the concordances and variances with the *Access AccuTnI + 3* as well as with two commercial assays.
Results: The *Access hsTnI* had excellent analytical sensitivity with the calibration slope 5.6 times steeper than the *Access AccuTnI + 3*. The detection capability was markedly improved with the SD of the blank 0.18–0.20 ng/L, LoB 0.29–0.33 ng/L and LoD 0.58–0.69 ng/L. All the reference interval samples had a result above the LoB value. At a mean concentration of 2.83 ng/L the SD was 0.28 ng/L (CV 9.8%). Carryover (0.005%) and outlier (0.046%) rates were similar to the Access *AccuTnI + 3*. The combined male and female 99th percentile reference interval was 18.2 ng/L (90% CI 13.2–21.1 ng/L). Concordance amongst the assays was poor with only 16.7%, 19.6% and 15.2% of samples identified by all 4 assays as above the 99th, 97.5th and 95th percentiles. Analytical imprecision was a minor contributor to the observed variances between assays.
Conclusion: The Beckman Coulter *Access hsTnI* assay has excellent analytical sensitivity and precision characteristics close to zero. This allows cTnI measurement in all healthy individuals and the capability to identify numerically small differences between serial samples as statistically significant. Concordance in healthy individuals remains poor amongst assays.

1. Introduction

Cardiac troponins I and T (cTnI, cTnT) are well established as the first choice biomarkers to detect myocardial insults. Over the past decade there has been a rapid improvement of the detection capability that has resulted in improved analytical precision at very low concentrations. The purported advantages of these “high sensitivity” (hs) assays include detection of cTnI and cTnT in the majority of cardiac-healthy individuals with better risk stratification and improved diagnostic sensitivity that may lead to better clinical outcomes. The Beckman Coulter *Access hsTnI* is a recent addition to this category of hs assays and brief evaluations of the method have been published [1,2]. We investigated the analytical performance characteristics, outlier rate and carryover of this assay in detail and compared it with two historical versions of cTnI assays from the same manufacturer. We also determined the reference intervals on stored samples of a previous study [3] and compared the concordances with the *Access AccuTnI + 3* and two commercial hs assays.

Abbreviations: cTnI, cardiac troponin I; cTnT, cardiac troponin T; hs, high sensitive; RLU, relative light units; LoB, limit of the blank; SD_R, short term repeatability standard deviation; SD_{Blank}, standard deviation of the blank; LoD, limit of detection; $SD_{Differences}$, standard deviation of differences between 2 measurements; SD_{WL}, within laboratory intermediate term standard deviation; $LoQ_{10\%}$, limit of quantitation where a 10% CV is achieved

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2. Materials and methods

2.1. Samples and analytical methodology

We used serum samples obtained from venous blood collected into serum separator tubes (Becton Dickinson) and centrifuged at 3000*g* for 10 min after clotting. Pooled sera with target cTnI concentrations were used in the precision and carryover experiments. Aliquots were frozen at -80 °C and were centrifuged for 10 min at 3000*g* after thawing and analysed within 4 h. The *Access hsTnI* reference intervals were determined from aliquots collected for a previous study and stored at −80 °C [3]. All the frozen samples were thawed once only before analysis. The protocol was approved by the local ethical review board.

The *Access hsTnI* assay was performed on a single Beckman Coulter DxI600 analyser (Beckman Coulter, Brea, CA) and the same lot of reagent, calibrators and controls were used throughout the study. We used archived *Access AccuTnI and AccuTnI+3* data from previous studies to compare analytical sensitivity and short term repeatability [3,4]. The results from a previously published reference interval study measured with the *Access AccuTnI+3*, Abbott Architect STAT High Sensitive Troponin-I (Architect hs-TnI) and the Cobas Elecsys TroponinT high sensitive (Elecsys TnT-hs) assays were used to assess concordance of troponin results in a healthy cohort. The claimed limits of the blanks (LoB) for these assays were: 4 ng/L, 0.7–1.3 ng/L, and 3 ng/L respectively [3].

2.2. Study design and statistical procedures

2.2.1. Analytical sensitivity and detection capability

The calibration slopes of three *Access* generations (*hsTnI, AccuTnI +3 and AccuTnI*) were constructed with the relative light unit (RLU) raw data signals. We previously performed this procedure with the *AccuTnI and AccuTnI+3* assays to obtain uncensored results [3,4]. The *Access hsTnI* data were obtained from the precision and reference interval experiments described below. The detection capability parameters - standard deviation of the blank (SD_{Blank}), LoB and limit of detection (LoD) - were determined with two complementary approaches [7]. We repeatedly analysed a blank sample (reagent diluent) and estimated the parameters with an abbreviated protocol (single lot number and calibrator). The LoB and LoD were estimated with a parametric approach with $\alpha = 0.05$ ($z = 1.645$). We also estimated the parameters by extrapolation of the imprecision profile data obtained from pooled patient samples with low cTnI concentrations [7].

2.2.2. Analytical imprecision

We performed a nested ANOVA model experiment with three levels of commercial quality control material (Biorad) and with 9 serum pools [5]. The serum pools were constructed to extend the coverage at low concentrations. The EP05-A3 protocol was modified to 40 runs over 11 days instead of 20 days due to time constraints. In addition we also estimated the short term repeatability (SD_R) for the *Access hsTnI* from the duplicate results obtained from the reference interval data set in 5 ng/L incremental bins to gain a better appreciation of the imprecision at low concentrations than could be obtained with a single QC material. We compared this with data generated for previous studies with the *Access AccuTnI and AccuTnI+3* assays [3,6].

2.2.3. Carryover

We examined carryover contamination by repeatedly analysing a pooled serum sample with a low cTnI concentration immediately before and after a challenge sample as previously described [8]. The sample with the extremely high cTnI concentration (± 750,000 ng/L) was submitted for routine cTnI analysis post cardiac surgery.

2.2.4. Outlier rate

We detected outliers with duplicate analysis of samples as previously described and used a probability of 0.0001 ($z = 3.5$) [6].

2.2.5. Comparison between the Access hsTnI and the AccuTnI+3

We analysed 100 routine serum samples with both the *Access hsTnI* and *AccuTnI+3 assays*. The samples were selected based on the AccuTnI+3 result to cover the expected range of routine cTnI results.

2.2.6. Reference interval

We estimated the *Access hsTnI* reference interval by analysing 1832 stored frozen samples in duplicate (647 females and 1185 males). We excluded 172 of the original study participants due to insufficient sample volume. We used only the first result to calculate the non-parametric 99th percentile reference intervals with 90% confidence intervals (Analyse-it version 2.30). The reference intervals of the *Access AccuTnI+3*, Architect hs-TnI and the Elecsys TnT-hs were also re-calculated after exclusion of participants with missing *Access hsTnI* data.

2.2.7. Concordances amongst assays in the reference population

We sequentially assessed the concordances of the *Access AccuTnI +3*, Architect hs-TnI and Elecsys TnT-hs relative to the *Access hsTnI* at specific percentiles as previously described [3]. The excess variance components, not attributable to imprecision of the assays, were estimated from the standard deviation of the differences ($SD_{Differences}$) between methods and their respective within-laboratory imprecisions (SD_{WL}).

3. Results

3.1. Analytical sensitivity and detection capability

The calibration curves slopes of three Access assay generations in Fig. 1 graphically illustrate the progressive improvement in analytical sensitivity that manifested as improved detection capability and imprecision at low concentrations. The slopes of the *Access AccuTnI+3* and the *Access hsTnI* increased by factors of 1.6 and 8.6 respectively, compared to the original *Access AccuTnI* assay. The directly determined SD_{Blank} was 0.18 ng/L with an estimated LoB of 0.29 ng/L and a LoD of 0.58 ng/L. We corroborated this with the complimentary precision profile approach by plotting the SD_{WL} obtained with pooled sera followed by extrapolation: SD_{Blank} 0.20 ng/L, LoB 0.33 ng/L, LoD 0.69 ng/L and $LoQ_{10\%}$ 2.75 ng/L. All the *Access hsTnI* results in the reference interval study were higher than the theoretical LoB and therefore significantly different from zero. The lowest result obtained was 0.4 ng/L in a female subject with the next lowest result of 0.6 ng/L in a male subject. The rest of the results were 0.8 ng/L and above.

3.2. Analytical imprecision

The *Access hsTnI* imprecision data are presented in Fig. 1 and in Supplementary Table 1. The lowest serum pool, with a mean cTnI concentration of 2.83 ng/L, had an SD_{WL} of 0.28 ng/L and a 9.9%

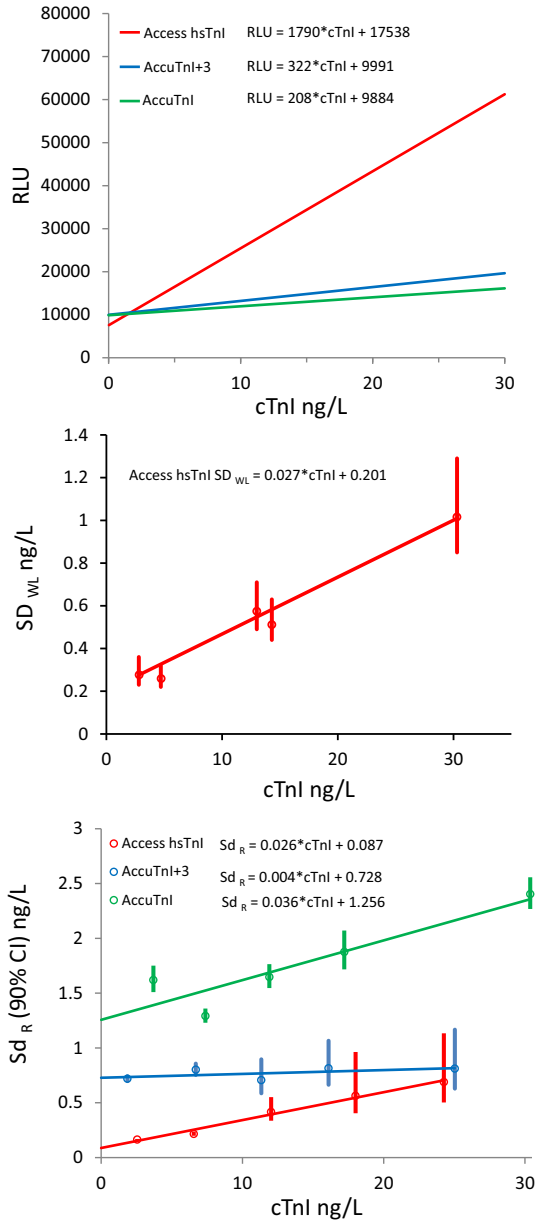

Fig. 1. Analytical sensitivity and detection capability of the *Access hsTnI* assay.
Top panel: Calibration curves of successive assay generations. The instrument raw data in relative light units (RLU) are plotted against the concentration cTnI.
Middle panel: The estimation of the blank SD by extrapolation of the within laboratory precision (SD_{WL}).
Bottom panel: Repeatability (SD_{WL}) of three assay generations calculated from duplicate results in 5 ng/L bins.
The 95% confidence intervals are indicated by the error bars.

coefficient of variation (CV). This compared favourably with our historically determined SD_{WL} of 1.76 ng/L at a mean concentration of 2.61 ng/L (CV 67.5%) for the *Access AccuTnI+3*. The short term imprecision (SD_R) of three of Access cTnI generations illustrate the progressive improvement in imprecision at low concentrations (Fig. 1). In the lowest bin (< 5 ng/L) the SD_R of the *Access hsTnI* assay (0.16 ng/L) was 4.5 fold lower than those of the *Access AccuTnI+3* (0.72 ng/L) and approximately 10 times better than the *AccuTnI* (1.62 ng/L).

3.3. Carryover

The carryover experiment demonstrated clinically significant carryover similar to those previously reported [8] with previous generations of Access assays (Supplementary Table 2). Although the carryover fraction is small (0.005%), a result of 44.1 ng/L was observed immediately after the challenge sample compared to the expected mean of 2.72 ng/L. The increased day 2 mean cTnI determined on fresh aliquots indicated that contamination of the reagent pack occurred.

3.4. Outlier rate

We observed 2 outliers in 4336 possible events with an outlier rate of 0.046% (95% CI 0.01–0.17%) which is significantly lower than the 0.44% (95%CI 0.25–0.63%) reported for the *AccuTnI* [6] and similar to the 0.025% (95% CI 0.00–0.14%) for the *AccuTnI+3* assay [3].

3.5. Comparison against the AccuTnI+3

A comparison of a 100 samples ranging up to approximately 30,000 ng/L was performed and the slope of the *Access hsTnI* relative to the *Access AccuTnI+3* assay was 0.85 (95% CI 0.83 to 0.89) (Supplementary Fig. 1). Trimming the range to below 100 ng/L did not materially affect the slope (0.80; 0.73 to 0.89).

3.6. Reference interval

The non-parametric 99th percentile reference intervals with 90% confidence intervals are summarised in Table 1. The median age of 647 female participants was 33.6 years and ranged from 18.0 to 80.4 years. The median age of 1185 males was 43.9 years with a range between 18.0 and 79.1 years. The 99th percentiles of males (20.88 ng/L 90%CI 16.32–25.77) and females (9.57 ng/L 90%CI 7.34–16.26) differed significantly with no overlap of their confidence intervals. The regression of cTnI against age revealed small and gradual increases in the median cTnI of 0.22 ng/L/decade (95%CI 0.18–0.25 ng/L) in females and 0.35 ng/L/decade (95%CI 0.30–0.40 ng/L) in male participants (Fig. 2).

The 99th percentiles determined in this study compared well with those previously determined despite the exclusion of 114 male and 58 female participants [9]. Although the numeric differences of the female 99th percentiles for the *Access AccuTnI+3* and Architect hs-TnI assays appeared numerically large, the 90% confidence intervals overlapped. Using the Architect hs-TnI cut-off value from this study (12.7 ng/L) on the original cohort we identified 9 subjects compared to the 6 above the original 20.2 ng/L cut-off. The proportions were not significantly different (9 vs. 6 in 705 females, p 0.439). The results for the *Access AccuTnI+3* were similar with 2 additional individuals in the original cohort identified by the lower cut-off from this study (p 0.645).

3.7. Concordances amongst assays in the reference population

The concordances of the *Access AccuTnI+3*, Architect hs-TnI and Elecsys hs-TnT assays relative to the *Access hsTnI* were below 60% at the 99th and 95th percentiles (Supplementary Table 3). The poor concordance between the *Access hsTnI* and *AccuTnI+3* was particularly notable and was approximately 50% even at the 75th percentile. The concordance between the duplicate *Access hsTnI* results served as a

Table 1
Cardiac troponin non-parametric 99th percentile reference intervals (ng/L).

	Access hsTnI	AccuTnI + 3	Architect hs-TnI	Elecsys TnT-hs
Female (n = 647)				
Median	1.91	1.58	1.80	1.49
99th percentile	9.57	18.45	12.72	9.58
90% CI	7.34 to 16.26	9.53 to 33.00	9.40 to 26.00	8.47 to 10.57
Male (n = 1185)				
Median	2.95	3.06	2.90	3.51
99th percentile	20.88	33.08	37.22	17.83
90% CI	16.32 to 25.77	25.00 to 57.50	26.71 to 44.04	15.65 to 19.37
Combined (n = 1832)				
Median	2.54	2.53	2.50	2.68
99th percentile	18.19	30.65	28.64	15.80
90% CI	13.18 to 21.07	25.17 to 36.98	22.90 to 44.10	14.55 to 18.11

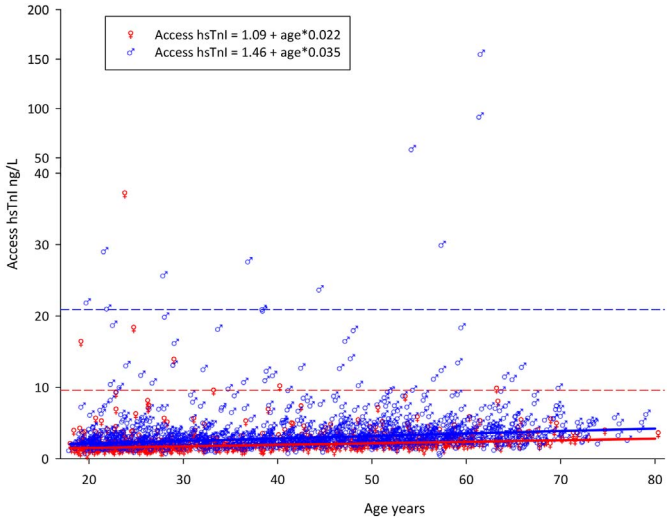

Fig. 2. *Access hsTnI* results as a function of age in male and female volunteers.
The median cTnI results show a proportional increase with age in male and female subjects (solid lines). The non-parametrically determined 99th percentiles are indicated by the dotted lines. Please note the non-linear cTnI scale on the Y-axis.

reference mark for the isolated impact of analytical imprecision and was approximately 95% at all the percentiles tested. Of the potential maximum 18 individuals above the 99th percentile only 3 (16.7%) were identified by all four assays. At the 97.5th and 95th percentiles only 19.6% and 15.2% were identified. This did not materially improve if restricted to the *Access hsTnI*, Architect hs-TnI and Elecsys TnT–hs assays: 22.2%, 23.9% and 20.7%. The poor concordances are graphically illustrated in the plots of ranks in Fig. 3. This non-parametric representation is scale invariant and allows easy inspection of the concordance across the measuring ranges. The ranks values were distributed in a near random pattern due to discordant results at all levels and not only at the higher percentiles. A result at the upper reference interval with one method could have a paired result with another method anywhere, even at the lower end of the spectrum. This observation was particularly notable in the comparison with the *Access AccuTnI + 3*, but was valid for all combinations.

The almost random distribution of results was also evident from scatter and difference plots in Fig. 4. Above 10 ng/L the results were widely scattered with no clustering around the regression lines and with R^2 between 0.16 and 0.25. Variance component analysis of the observed and predicted differences demonstrated that imprecision of the assays were not major contributors to this phenomenon with the estimated sample-method specific variance component the dominant contributor (Table 2). The plots of the *Access hsTnI* duplicate data in Figs. 3 and 4 serve as a reference for the isolated effect of imprecision on concordance and the distribution of results.

4. Discussion

The *Access hsTnI* assay demonstrates an impressive advance in analytical sensitivity compared to the *Access AccuTnI + 3*. The calibration slope increased approximately six fold with excellent detection capability and imprecision at low concentrations. Our LoB estimates of 0.29 to 0.33 ng/L were lower than the 0.8 to 1.7 ng/L of Budd [1] and may be explained by our modified protocol of a single analyser and one lot of reagents and calibrators. Lippi reported an even lower LoB of 0.14 ng/L [2] and differences in methodology may again be responsible. This variability amongst the studies however illustrates the flaw of using analytical performance characteristics, such as LoB or LoD, as clinical decision levels.

The *Access hsTnI* regression slope (0.80 to 0.85) may be due to different antibodies and/or a change in calibration material. The proportional bias could not fully explain the 99th percentiles that were 52 to 63% those of the *AccuTnI + 3*. Antibodies that react with different epitopes may contribute to the poor correlation and wide scatter at low concentrations. The scale of the redesign was highlighted by the marked discordance between two assay versions from the same manufacturer which was comparable with the discordance with other manufacturers. The robustness, as assessed by carryover (Supplementary Table 1) and outlier rates were similar to our previous estimates with the AccuTnI + 3 and probably reflect the mechanical characteristics of the analysers and not the assay formulations. Laboratories and clinicians must therefore continue to be alert about the existence of these potential sources of error and should implement procedures to minimize their clinical impact.

The poor agreement of cardiac troponin results obtained in healthy individuals was a prominent feature of this study. This was characterised by suboptimal correlation coefficients, the dominant contribution of sample-method specific bias to the observed between method variances and in the discordant classification at clinically

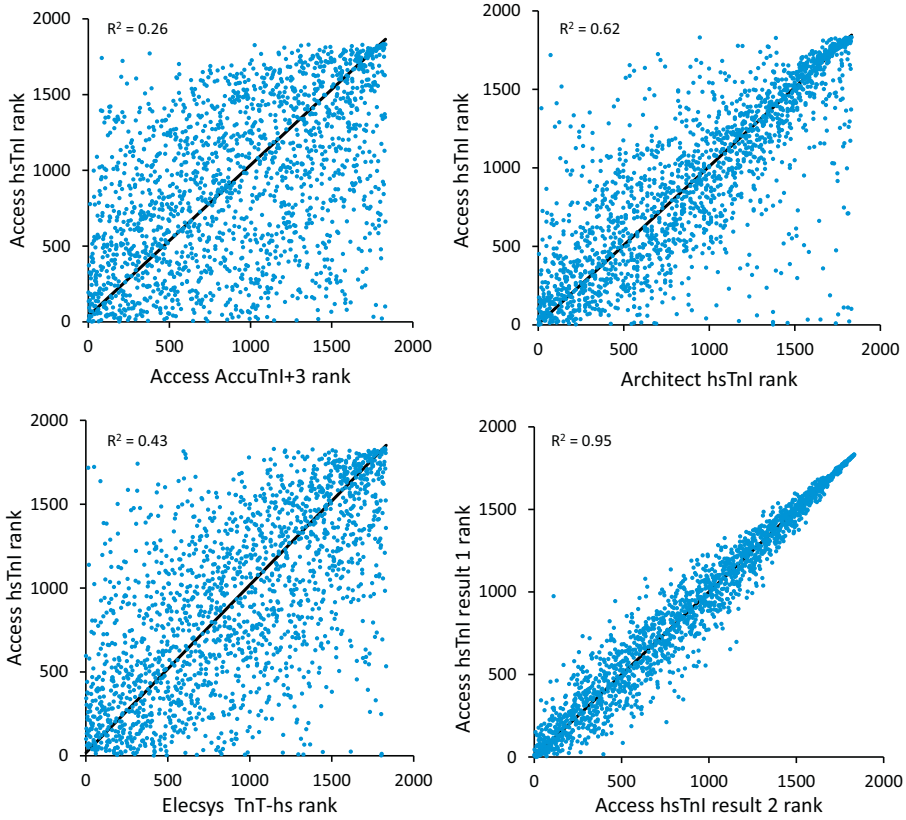

Fig. 3. Distributions of the rank values obtained with the *Access hsTnI* compared with those from the *Access AccuTnI +3*, Architect hs-TnI and Elecsys TnT-hs assays.
The results the reference interval samples as determined with each assay were ranked and those of the three comparative assays were plotted against the Access hsTnI. The plot of the duplicate *Access hsTnI* ranks serve as a reference for the effect of analytical imprecision on the distribution of the ranks in this sample set. The lines of identity are indicated in black. Pearson correlation coefficients were calculated from the rank values.

important percentiles. This raised a number of aspects with regard to the clinical use of cardiac troponin assays and reference intervals. Firstly it reinforces the critical importance of serial measurements and dynamic change in suspected acute coronary syndrome. The improved analytical precision of the *Access hsTnI* assay will allow identification of small numerical differences with a high degree of confidence. At a mean cTnI of 5.0 ng/L a delta > 0.8 ng/L will exceed the 95% confidence level for analytical significance (p 0.05). The biological variation of cTnI measured with the *Access hsTnI* has not been determined, but on the assumption of a 1.5 ng/L physiological variation in an individual, the reference change value would be approximately 4.2 ng/L at a mean cTnI of 5.0 ng/L (p 0.05) [10].

The high degree of discordance also argues against the notion that subclinical cardiac disease should be excluded with extensive (and expensive) screening of reference interval subjects. In our opinion it is possible that the majority of results in the upper range of the population distribution with any of the current assays do not reflect “true” concentrations and we hypothesize that the sample-method specific bias component may in part explain the severely right skewed distributions. With approximately 20% of results concordant above the 99th percentile, it is in our opinion a tenuous argument to infer structural cardiac disease in most participants from our cohort above this threshold.

The 99th percentile upper reference limits of male and female participants with their non-overlapping confidence intervals raises the role of sex specific reference intervals in clinical practice. The small, but statistically significant, difference between male and female age related increments may also be invoked to further support this argument. The relatively small increase in median cTnI with age does not however correspond with the numerically larger jumps in age related 99th percentiles that we [3] and others have previously reported. Although the 99th percentile limits of males and females may differ numerically with a degree of statistical significance, the low density of data points in the vicinity of the 99th percentiles (Fig. 2), and the analytical uncertainty that surrounds the majority of these points may in part explain the questionable benefits derived from sex specific reference intervals [9].

The impressive improvements in the analytical sensitivity, detection capability and analytical imprecision of hs assays has not resolved the sample-method specific bias as is evident from the discordances and poor correlations. The underlying mechanism of this variance component is unclear and should be the focus of future assay development as both accurate and precise quantitation may contribute to better risk stratification.

5. Conclusion

The new Beckman Coulter *Access hsTnI* assay has excellent analytical sensitivity and imprecision close to zero. This allows cTnI detection in all healthy individuals and the capability to identify numerically small differences between serial samples as statistically significant. Despite these improvements the concordance and correlation amongst assays in healthy individuals remains poor.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2018.03.003.

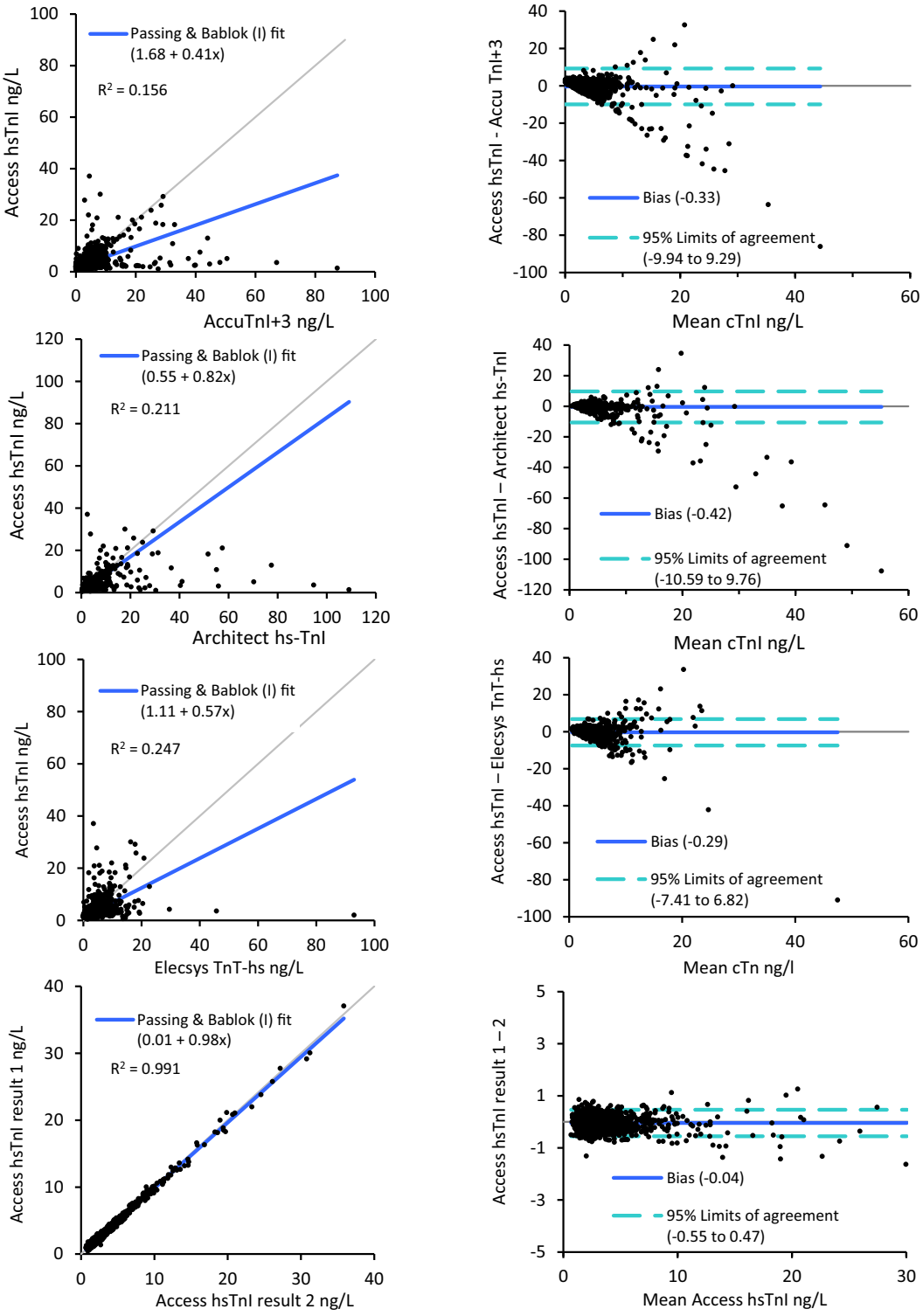

Fig. 4. Comparisons of the *Access hsTnI* with the *AccuTnI+3*, Architect hs-TnI and Elecsys TnT-hs assays in the reference sample set.
The data from 1832 samples analysed in the reference interval study are presented. The plots of the duplicate *Access hsTnI* results serve as a reference for the effect of analytical imprecision. Pearson correlation coefficients were calculated from the numerical results.

Table 2
Variances of difference components determined for the *Access hsTnI* vs. the AccuTnI + 3, Architect hs-TnI and Elecsys TnT-hs assays.

	AccuTnI + 3	Architect hs-TnI	Elecsys TnT-hs
Predicted $SD_{differences}$	1.89	0.79	1.42
Observed $SD_{differences}$	4.90	5.19	2.95
Sample-method bias SD	4.52	5.13	2.59

All the parameters are in ng/L.
The predicted variance for each method pair was calculated from their respective analytical imprecisions at 10 to 15 ng/L. The SD's used in the estimations were: *Access hsTnI* 0.6 ng/L, AccuTnI + 3 1.8 ng/L, Architect hs-TnI 0.55 ng/L and Elecsys TnT-hs 1.3 ng/L. The data for the Architect hs-TnI and the Elecsys TnT-hs assays were obtained from their package inserts.

References

[1] J. Budd, M. Thomas, P. Wynveen, C. Carrolo-Neumann, et al., Characteristics of the Beckman Coulter prototype high sensitivity cardiac troponin assay (hsTnI). EuroMedlab [Abstract], Clin. Chem. Lab. Med. 55 (2017) S1–539.
[2] G. Lippi, A. Ferrari, G. Gandini, M. Gelagi, C. Lo Cascio, G.L. Salvagno, Analytical evaluation of the new Beckman Coulter Access high sensitivity cardiac troponin I immunoassay, Clin. Chem. Lab. Med. (2017), http://dx.doi.org/10.1515/cclm-2017-0350.
[3] J.P.J. Ungerer, J.R. Tate, C.J. Pretorius, Discordance with 3 cardiac troponin I and T assays: implications for the 99th percentile cutoff, Clin. Chem. 62 (2016) 1106–1114.
[4] J.P.J. Ungerer, C.J. Pretorius, A fit-for-purpose approach to analytical sensitivity applied to a cardiac troponin assay: time to escape the 'highly-sensitive' trap, Clin. Chem. Lab. Med. 52 (2014) 553–556.
[5] CLSI, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline —Third Edition. CLSI Document EP05-A3, Clinical and Laboratory Standards Institute, Wayne PA, 2014.
[6] C.J. Pretorius, G. Dimeski, P.K. O'Rourke, L. Marquart, S.A. Tyack, U. Wilgen, J.P.J. Ungerer, Outliers as a cause of false cardiac troponin results: investigating the robustness of 4 contemporary assays, Clin. Chem. 57 (2011) 710–718.
[7] CLSI, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2, Clinical and Laboratory Standards Institute, Wayne PA, 2012.
[8] U. Wilgen, C.J. Pretorius, M.J. Gould, J.P.J. Ungerer, Cardiac Troponin I carryover by very high patient samples still causes false-positive results on the Beckman Coulter AccuTnI + 3, Ann. Clin. Biochem. 53 (2016) 177–179.
[9] K.M. Eggers, B. Lindahl, Impact of sex on cardiac troponin concentrations – a critical appraisal, Clin. Chem. 63 (2017) 1457–1464.
[10] C.J. Pretorius, U. Wilgen, J.P.J. Ungerer, Serial cardiac troponin differences measured on four contempory analyzers: relative differences, actual differences and reference change values compared, Clin. Chim. Acta 413 (2012) 1786–1791.