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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%