Letter to the Editor

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Analytical assessment of the Beckman Coulter Unicel DxI AccuTnI+3 immunoassay

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To the Editor,

The diagnostics of myocardial injury and, especially, of ischemic heart disease strongly rely on laboratory data [1]. In particular, the assessment of cardiospecific troponin(s) is now almost unavoidable in clinical practice, as for current guidelines and recommendations [2]. It is also noteworthy, however, that specific criteria have been defined for characterising and classifying the different troponin immunoassays that are currently available in the market. These specifically include the calculation of the limit of blank (LOB; i.e., the highest value expectable in replicates of samples containing no analyte), the limit of detection (LOD; i.e., the lowest amount of analyte that can be detected in a sample) [3], the value characterized by optimal imprecision (i.e., coefficient of variation [CV] of 10% or lower), along with the 99th percentile of the upper reference limit (URL) of a reference population of presumably healthy subjects [4, 5].

In a previous article, Zaninotto et al. assessed the analytical performance of the Beckman Coulter AccuTnI (Beckman Coulter, Brea, CA, USA) immunoassay on both Beckman Coulter Unicel DxI and Beckman Coulter Access-2 [6]. In two following investigations, it has then been reported that this method [7, 8], but not its prototype high-sensitivity evolution which is not commercially available so far [9], may suffer from problems of carryover that may ultimately bias test results. To overcome this drawback, the manufacturer has recently released an evolution of this assay that would make it more resistant against carry-over. The processing of reagent material has also been improved, thus resulting in better to lot-to-lot consistency of AccuTnI+3 assay reagents. Therefore, the aim of this study (performed between December the 2nd and the 12th, 2013) was the analytical evaluation of the novel AccuTnI+3 on Unicel DxI, along with evaluation of carry-over.

The detailed characteristics of this immunoassay have been earlier described by Zaninotto et al. in a previous publication [6]. In brief, Unicel DxI AccuTnI is a two-site chemiluminescent immunoassay employing mouse monoclonal anti-human troponin I (TnI) antibody-alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti-human TnI antibody. The main differences of this novel version of the immunoassay consist in modifications to the calibrator value assignment to harmonize data of Unicel DxI with those of Access-2, implementation of an algorithm to adjust results for changes in room temperature, along with modifications to the assay protocol file for improvement of mixing and probe wash process.

As regards the analytical evaluation of AccuTnI+3, the value associated with optimal imprecision was calculated by preparation of serial dilutions (i.e., 1:2; 1:4; 1:8 and 1:16) of an EDTA plasma sample with a TnI concentration of 120 ng/L and an EDTA plasma sample with undetectable TnI, 10 replicated measurements of each dilution, calculation of the CV for each dilution and creation of a model fit to estimate the TnI value with 10% CV. The LOB was calculated as the value corresponding to the sum of the mean and 1.645* standard deviation (SD) of 20 replicates of saline tested in one single run, as for Clinical and Laboratory Standards Institute (CLSI) recommendations [3]. The LOD was calculated as the sum of the LOB and 1.645*SD of 20 replicates of an EDTA plasma sample with the lowest measurable value of TnI, as estimated from the study for calculation of the 10% CV value [3]. The 99th percentile was calculated using 125 EDTA plasma samples obtained.
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from healthy Caucasian blood donors (77 males and 48 females, age range 21–54 years), according to the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the CLSI [10]. No subjects had current or past history of ischemic heart disease, cardiac-related medical conditions or non-cardiac-related diseases as established by a health questionnaire. The carryover was calculated testing the sequence A1-A2-B1-B2-B3 and using Broughton's formula (carryover, % = 100×(B1–B3)/(A2–B3)), where A was an EDTA plasma specimen with a very high TnI concentration (i.e., 484,176 ng/L, final value obtained after 1:50 dilution with a TnI negative sample), and B was an EDTA plasma sample with a TnI value close to the diagnostic cut-off (i.e., 60 ng/L) [11]. The study was based on pre-existing EDTA plasma samples obtained after routine analysis was completed and no informed consent or Ethics Committee approval were hence necessary. The investigation was however performed in accord with the Declaration of Helsinki and under the terms of all relevant local legislation. The statistical analysis was performed with Analyse-it (Analyse-it Software Ltd, Leeds, UK).

According to the study protocol described earlier, the value corresponding to 10% CV was 37 ng/L, and thereby lower than that previously reported for the former assay on the same analytical platform (i.e., 58 ng/L) (Figure 1). We also obtained a value of 9 ng/L for the LOB and a value of 13 ng/L for the LOD for the new AccuTnI+3, respectively. The LOD was hence globally comparable to that earlier found for the former method (i.e., 12 ng/mL). The 99th percentile URL calculated from our local population of healthy blood donors was 30 ng/L (95% CI, 26–35 ng/L), and thus virtually identical to that estimated with the former Unicel DxI AccuTnI commercial assay (i.e., 34 ng/L; 95% CI, 30–41 ng/mL). Nevertheless, the number of measurable values (i.e., ≥9 ng/L) obtained in our presumably healthy population with AccuTnI+3 was lower (i.e., 13%) than that reported for the former assay (i.e., 42%). It is noteworthy, however, that 125 individuals may be not sufficient for a reliable determination of the 99th percentile URL, and we can hence conclude that we have performed a rough validation of this threshold with some degrees of uncertainty. Finally, the carry-over was both analytically and clinically negligible with AccuTnI+3, as attested by the very low value (i.e., 0.004%) and the modest percentage bias, which remained lower than the 50% variation that is currently recommended for diagnosing ischemic heart disease during serial testing (80 ng/L in aliquot B1 vs. 60 ng/L in aliquot B3, i.e., 33% increase).

In conclusions, the results of this study attest that the Unicel DxI Accu-TnI+3 immunoassay display an analytical performance that is globally comparable to that of the earlier method, except for an approx. 40% lower TnI value associated with 10% CV. It is also noteworthy that the problem of carry-over that seemed to afflict the former method was apparently eliminated after development of this evolution of the assay.
Conflict of interest statement

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