

Letter to the Editor

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Evaluation of the analytical performance of a new ADVIA immunoassay using the Centaur XPT platform system for the measurement of cardiac troponin I

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To the Editor,

At the present time, there are few commercially available methods able to completely satisfy the quality specifications recommended by international guidelines for cardiac troponin I (cTnI) assay [1, 2]. An accurate evaluation of analytical performance is needed to demonstrate that a new immunoassay method for cTnI assay actually fulfills all quality specifications required by international guidelines [1, 2]. This study is aimed at using standardized protocols [3, 4] to evaluate the analytical performances of the new immunoassay method, named ADVIA Centaur High-Sensitivity Troponin I (TNIH) (Ref. 10994774-5), distributed in Italy by Siemens Healthineers (Milano, Italy). We compared the results from the new immunoassay with those of the ADVIA ultra-cTnI method, using the same Centaur platform [5, 6], and those of the STAT ARCHITECT cTnI, using the ARCHITECT platform [7].

The ADVIA Centaur TNIH is a three-site sandwich immunoassay using the direct chemiluminometric technology and the fully automated Centaur XPT platform.

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This method uses magnetic latex particles combined with streptavidin through two bound biotinylated capture monoclonal antibodies (as solid phase reagent) and a recombinant anti-human cTnI sheep Fab antibody covalently attached to bovine serum albumin for chemiluminescent detection. This assay requires a 100- μ L sample for a single determination. The ADVIA Centaur TNIH assay measures cTnI concentrations from 2.50 to 25,000.00 ng/L. The first result is obtained within 20 min after the assay starts.

The study was performed in the laboratory of the Fondazione CNR Regione Toscana G. Monasterio (Pisa, Italy) from September 1 to December 20, 2017. Heparinized blood samples were collected from 134 patients (30.4% women, ages 40–88 years) with different cardiac diseases (including acute coronary syndromes, heart failure, myocarditis and pericarditis) and 105 healthy subjects (65% women, ages 20–76 years). Plasma was obtained shortly after venipuncture by centrifugation for 10 min at room temperature (about 22 °C). Samples, if not immediately assayed, were frozen and stored at –20 °C in 0.5-mL aliquots in polypropylene tubes until assayed with ADVIA Centaur platform some weeks later.

Limits of blank (LoB) and detection (LoD) were calculated following the CLSI EP17-A protocol [3], using Centaur XPT platform. The “0 calibrator” of the ADVIA Centaur TNIH method was considered the blank of the method because it contains no cTnI. This calibrator was measured in 72 different runs, using two batches of reagents throughout 60 working days (mean = 2299.0 RLU, standard deviation [SD] = 174.6 RLU, n = 72). To calculate the LoB value [expressed as Relative Luminescence Units (RLU)], the following formula was used: $\text{LoB (RLU)} = 0 \text{ calibrator (2299.0 RLU)} + \text{SD of 0 calibrator (174.62 RLU)} \times 1.645 = 2585.6 \text{ RLU}$. LoB value (1.0 ng/L) was then calculated by interpolation considering the linear regression estimated between RLU values (y-axis) and the respective cTnI concentrations measured with the ADVIA Centaur TNIH (x-axis) in 603 cTnI values within the concentration range from 0 to 60 ng/L ($y = 2298.83 + 286.141 x$, $R = 0.9960$, $n = 603$). LoD value (i.e. 2.2 ng/L) was then calculated

according to the following formula [3]: $\text{LoD (ng/L)} = \text{LoB (1.0 ng/L)} + 1.645 \times \text{SD (0.70 ng/L)} = 1.0 + 1.15 = 2.15 \text{ ng/L}$, where SD was estimated by the distribution of cTnI values measured in a sample with low cTnI concentration (mean 0.51 ng/L, SD 0.70 ng/L, $n = 45$).

The imprecision profile was estimated by measuring 11 heparinized plasma pools collected from healthy subjects as well as patients with cardiovascular diseases, with mean cTnI concentrations ranging from 0.5 to 64.0 ng/L. These pools were repeatedly measured in 45 different runs with three different calibration curves using two batches of reagents throughout 60 working days. To calculate the limit of quantification (LoQ) at 10% CV (Coefficient of Variation) and 20% CV, the relationship between the error of the measurement (expressed as CV values, y-axis) and cTnI concentrations (x-axis) was interpolated by means of a nonlinear reciprocal regression curve (Figure 1). LoB,

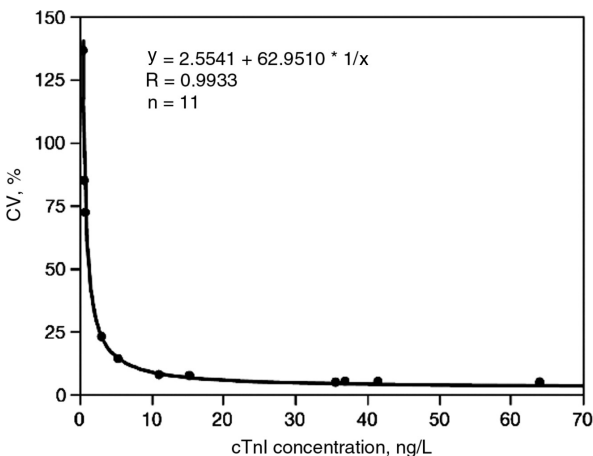


Figure 1: Imprecision profile of the ADVIA Centaur High-Sensitivity Troponin I (TNIH) method.

The imprecision profile was estimated by measuring 11 heparinized plasma pools collected from healthy subjects and patients with cardiovascular diseases with mean cTnI concentrations from 0.5 to 64.0 ng/L (45 repeated measurements). The non-linear relationship calculated between the error of measurement (CV%, y-axis) and the cTnI concentration (ng/L, x-axis) is reported.

LoD and LoQ values, evaluated in the present study, were compared to those previously evaluated in our laboratory for ADVIA ultra Centaur [5, 6] and ARCHITECT hs-cTnI [7] methods and reported in Table 1.

A close linear regression was found between cTnI concentrations measured with ADVIA ultra Centaur (x-axis) and ADVIA Centaur TNIH (y-axis) methods using 222 heparinized plasma samples collected from 91 healthy subjects and 131 patients with cardiac diseases, including some with acute myocardial infarction ($y = 393.1793 + 0.6579 x$, $R = 0.9875$, $n = 222$). A significant systematic difference was found between these two methods with a mean under-estimation of -27.4% of the ADVIA Centaur TNIH compared to the ADVIA ultra Centaur with the largest differences observed at low cTnI concentrations. Indeed, considering only the 125 plasma samples with cTnI concentrations $\leq 1000 \text{ ng/L}$, the relationship between TnI values measured was different to that found in all experimental plasma samples (Figure 2).

A close linear regression was also found between cTnI concentrations measured with ADVIA Centaur TNIH (y-axis) and ARCHITECT hs-cTnI (x-axis) methods using 239 plasma samples collected from 105 healthy subjects and 134 patients with cardiac diseases ($y = -153.258 + 1.1304 x$, $R = 0.9730$, $n = 239$). However, as expected due to the different standard materials used by these two methods, a mean systematic difference of 28.8% (SD 41.9% , $p < 0.0001$) ($[\text{ADVIA-ARCHITECT}]/[\text{mean cTnI concentration}] \times 100$), negatively related to mean cTnI concentrations ($p < 0.0366$), was observed between the cTnI values measured by the two immunoassay methods (ARCHITECT: mean 6288.71 ng/L , SD 20455.40 ng/L , median 130.5 ng/L , 25th percentile 2.3 ng/L , 75th percentile 2479.0 ng/L ; ADVIA: mean 6955.25 ng/L , SD: 23760.61 ng/L , median: 174.35 ng/L , 25th percentile 2.37 ng/L , 75th percentile 3685.29 ng/L).

The results of the present study (Table 1), taken as a whole, indicate that the ADVIA Centaur TNIH method has significantly better analytical performance than ADVIA

Table 1: Comparison of analytical sensitivity parameters of immunoassay methods for cTnI tested in the study.

Method	LoB, ng/L	LoD, ng/L	LoQ 20% CV, ng/L	LoQ 10% CV, ng/L	Ratio	References
ADVIA ultra	3.2	6.0	30	57	1	[5, 6]
ADVIA TNIH	1.0	2.2	3.6	8.5	5.6	Present study
ARCHITECT	0.7	1.3	1.8	4.7	5.6 ^a	[7]

ADVIA ultra, ADVIA ultra-cTnI method; ADVIA TNIH, ADVIA Centaur High-Sensitivity Troponin I method; ARCHITECT, STAT Architect cTnI method using the ARCHITECT platform. Ratio: ratio between the 99th percentile of the reference population distribution of cTnI, suggested by the manufacturer for general population, and the LoQ 10% CV value evaluated in the laboratory. ^aThe 99th URL percentile value for general population of the ARCHITECT method is 26.2 ng/L (data from Ref. 3p25 G5-9236/R01, B3P2U4, April 2015, Abbott Ireland, Diagnostic Division Lisnamuck, Longford Co., Longford, Ireland).

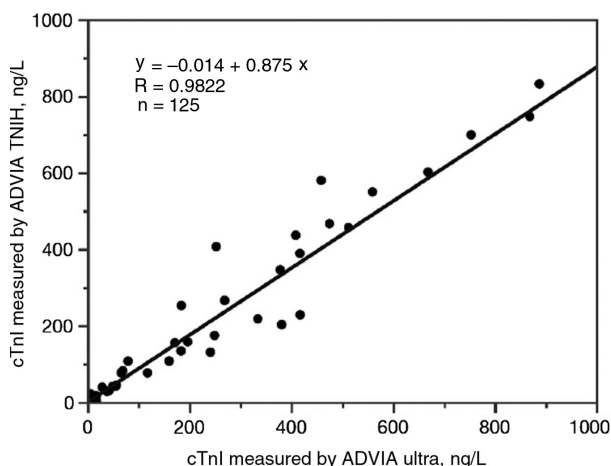


Figure 2: Linear regression between cTnI concentrations, measured by ADVIA ultra (x-axis) and ADVIA Centaur TNIH (y-axis) methods. One hundred and twenty-five plasma samples collected from healthy subjects and patients with cardiac diseases with cTnI values ≤ 1000 ng/L were evaluated in this regression analysis. The calculated linear equation is reported.

ultra Centaur method [5, 6] but performs similarly to the cTnI method using the ARCHITECT platform [7]. According to Apple and Collinson [1], two criteria are needed to define a high-sensitivity assay for cardiac troponin: (1) the total imprecision (expressed as CV %) at the 99th percentile value should be $\leq 10\%$; (2) measurable concentrations below the 99th percentile should be attainable with an assay at a concentration value above the assay LoD for at least 50% (and ideally $\geq 95\%$) of healthy individuals. Unfortunately, an accurate evaluation of the 99th URL of cTnI levels is challenging to analyze due to low biomarker concentrations in healthy subjects [2, 8–10].

The manufacturer determined the 99th URL value of the ADVIA Centaur TNIH method in a combined population of 2010 apparently healthy individuals (1012 women and 998 men, ages 22–91 years); the 99th percentile URL value was 47.30 ng/L (90% confidence interval: 36.39–64.27 ng/L), and the 63% of tested individuals had values greater than the LoD value, suggested by the manufacturer (i.e. 1.19 ng/L). Accordingly, the 99th URL value, suggested by manufacturer for general population (i.e. 47.30 ng/L), is 5.6-fold higher than the LoQ value at 10% CV (i.e. 8.5 ng/L) (Table 1). These data suggest that the ADVIA Centaur TNIH method is able to satisfy the first of the two criteria recommended for a cTnI assay with high-sensitivity performance [1]. Indeed, to demonstrate that the ADVIA Centaur TNIH method is a high-sensitivity assay for plasma cTnI, further evidence from large reference populations, stratified for age, gender and ethnic origin, is required [1, 2].

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