

Letter to the Editor

Masotti Silvia, Musetti Veronica, Prontera Concetta, Simona Storti, Claudio Passino, Giancarlo Zucchelli and Aldo Clerico*

Evaluation of analytical performance of a chemiluminescence enzyme immunoassay (CLEIA) for cTnI using the automated AIA-CL2400 platform

<https://doi.org/10.1515/cclm-2017-1101>

Received November 25, 2017; accepted December 15, 2017

Keywords: analytical sensitivity; cardiac biomarkers; cTnI; cTnT; myocardial infarction.

To the Editor,

In the last 10 years, a new generation of methods for the measurement of cardiac troponin I (cTnI) has been set-up in order to improve the analytical performance in accordance with the quality specifications recommended by international guidelines [1–3].

We evaluated, from September to November 2017, the analytical performance parameters and the clinical results of a new chemiluminescence enzyme immunoassay (CLEIA) for cTnI measurement (CL AIA-PACK cTnI TEST) using the automated AIA-CL2400 platform (TOSOH BIOSCIENCE, Tessenderlo, Belgium). This method is a two-step CLEIA method using a combination of two monoclonal antibodies. The assay of plasma concentrations of cTnI was performed according to the recommendations suggested by the manufacturer. Two different lots of reagents and calibrators were used. The calibration curve is standardized against the human cardiac troponin complex SRM 2921 material, certified by the National Institute of Standards and Technology (NIST). The cTnI value

of the 99th percentile for the reference Caucasian population, suggested by the manufacturer, is 31 ng/L (bulletin A0008871001-116B Rev. 11/16, TOSOH Europe N.V., Tessenderlo, Belgium).

Blood samples were collected in polypropylene tubes with lithium heparin. After blood collection, samples were centrifuged at 3000 × g for 10 min and plasma was then analyzed, as soon as possible. If it was not possible to perform the assay within 1 h, the samples were stored at –80 °C.

Patients with cardiac diseases were admitted to the clinical wards and intensive coronary unit of the Fondazione CNR Regione Toscana G. Monasterio (Pisa and Massa, Italy). Healthy people were recruited from laboratory staff, blood donors or voluntary subjects, included in screening programs for preventive medicine, as previously described in detail [4]. The presence of cardiac or systemic acute or chronic diseases was excluded in healthy subjects by history, accurate clinical examination, ECG, cardiac imaging and laboratory tests, including BNP or NT-proBNP assay [4]. Furthermore, all healthy volunteers denied the use of drugs for at least 2 weeks before the sample collection. The informed consent was obtained by all subjects and patients enrolled in the study, and the screening programs were approved by the local Ethical Committee [4].

The limits of blank (LoB) and detection (LoD) were determined according to the CLSI EP17-A protocol [5]. The sample diluent solution of the cTnI assay, considered as the blank of the method (BoM), was measured using three different calibration curves during 60 working days; the rate of chemiluminescence signal detection (RLU) was recorded for each measurements of BoM (mean 51.7 RLU, SD 11.4 RLU, n = 150). To calculate the LoB value (expressed as RLU), the following formula was used:

$$\begin{aligned}\text{LoB (RLU)} &= \text{mean BoM (RLU)} + \text{SD (RLU)} \times 1.645 \\ &= 70.5 \text{ RLU.}\end{aligned}$$

The LoB value (i.e. cTnI = 1.1 ng/L) (Table 1) was then calculated by interpolation considering the regression,

*Corresponding author: Prof. Aldo Clerico, MD, Department of Laboratory Medicine, Fondazione CNR-Regione Toscana G. Monasterio, Pisa, Italy; and Scuola Superiore Sant'Anna, Via Trieste 41, 56126 Pisa, Italy, Fax: +(39)-0585-493601, Phone: +(39)-0585-493569, E-mail: clerico@ifc.cnr.it

Masotti Silvia, Musetti Veronica and Claudio Passino: Fondazione CNR Regione Toscana G. Monasterio, Pisa, Italy; and Scuola Superiore Sant'Anna, Pisa, Italy

Prontera Concetta and Simona Storti: Fondazione CNR Regione Toscana G. Monasterio, Pisa, Italy

Giancarlo Zucchelli: QualiMedLab, CNR Institute of Clinical Physiology, Pisa, Italy

Table 1: Comparison of analytical sensitivity parameters of the FLUOR and CLEIA methods for cTnI immunoassays using the AIA platform, as evaluated in the present study and suggested by the manufacturer.

Method	LoB, ng/L	LoD, ng/L	LoQ 20%, ng/L	LoQ 10%, ng/L	References
FLUOR	–	8	35	100	Manufacturer ^a
FLUOR	3.5	8.7	30	100	Franzini et al. [4]
CLEIA	–	1.6	2.3	5.6	Manufacturer ^b
CLEIA	1.1	2.1	15	30.9	Present study

FLUOR: ST ALA-PACK cTnI 3rd-Gen method; CLEIA: CL AIA-PACK cTnI TEST method. ^aData reported in the bulletin 1008871001-111D, Rev. 11/11, TOSOH EUROPE N.V. (Tessenderlo, Belgium). ^bData reported in the bulletin A008871001-1168, Rev. 11/16, TOSOH EUROPE N.V. (Tessenderlo, Belgium).

which represents the mean calibration curve, fitted considering all the measurements performed for method evaluation. In particular, 707 measured values ≤ 60 ng/L (including 557 plasma samples and 150 BoM determinations) were considered for the evaluation of LoB using this calibration curve, which is linear in the range from 0 ng/L to 60 ng/L ($RLU = 55.251 + 14,4135 \text{ cTnI}$, $R = 0.9877$). The LoD value (i.e. 2.1 ng/L) was then calculated according to the formula [6]:

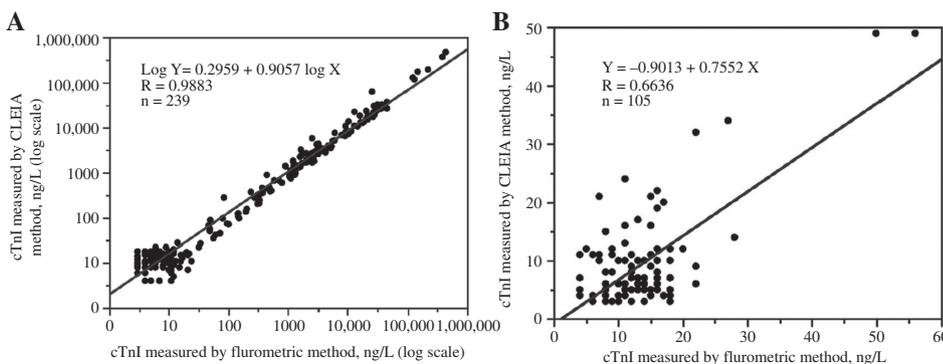
$$\text{LoD (ng/L)} = \text{LoB (1.1 ng/L)} + 1.645 \text{ SD},$$

where SD is the standard deviation of cTnI values of a plasma sample with very low cTnI concentration (mean 0.61 ng/L, SD 0.58 ng/L, $n = 70$).

The limits of quantitation (LoQ) at 20% CV (i.e. 15.5 ng/L) and 10% CV (i.e. 30.9 ng/L) were also calculated using nine heparinized plasma pools with mean cTnI concentrations from 6.5 ng/L to 46.5 ng/L, measured in 40 different runs performed during 60 working days using three different calibration curves. The calculated curvilinear regression between the mean cTnI concentrations

measured in these nine plasma pools (X-axis) and the respective measurement imprecision values (expressed as CV%, Y-axis) was: $Y = 0.570 + 290.959 * 1/X$ ($R = 0.9915$). For comparison, the calculated values for LoB, LoD, and LoQ 20%, and LoQ 10% of the CLEIA method, and also those previously found in our laboratory with the enzymometric fluorescent ST ALA-PACK cTnI 3rd-Gen method, using the same standardized experimental protocols [7], were reported in Table 1. The new CLEIA method actually shows significantly better analytical sensitivity parameters than the fluorescent enzymometric (FLUOR) method.

The cTnI values, measured in healthy subjects and patients with CLEIA method, were compared to those found with ST ALA-PACK cTnI 3rd-Gen method using the AIA-2000LA platform [7]. A very close linear relationship was found between the cTnI values, respectively, measured in 239 plasma samples of healthy subjects ($n = 103$) and patients with cardiac diseases ($n = 136$) with ST ALA-PACK cTnI 3rd-Gen (X-axis) and CLEIA methods (Y-axis) (Figure 1A). Moreover, a mean systematic difference of -3.2% ($[(\text{CLEIA} - \text{FUOR})/\text{FUOR}\%]$; $p < 0.001$ by Wilcoxon

**Figure 1:** Linear regression analysis.

(A) Linear regression between cTnI concentrations (log-scale) measured by ST ALA-PACK cTnI 3rd-Gen method (FLUOR, X-axis) and the CLEIA method (Y-axis) in 239 heparinized plasma samples collected from healthy subjects and patients with cardiac diseases, also including some with acute myocardial infarction. The log-transformed values were used in this regression analysis because the use of log-transformed values allows a more accurate evaluation of all the working range from very low to high values. (B) Linear regression between cTnI concentrations measured by ST ALA-PACK cTnI 3rd-Gen method (FLUOR, X-axis) and CLEIA (Y-axis) in 105 heparinized plasma samples collected from healthy subjects.

test) was found between these two methods. Considering only the results found in 105 samples with cTnI concentrations <60 ng/L, a worse agreement was observed between FLUOR (X-axis) and CLEIA (Y-axis) methods (Figure 1B).

It is important to note that cTnI values (mean 8.5 ng/L, median 6.0 ng/L, minimum value 3 ng/L, 25th percentile 5.0 ng/L, 75th percentile 27.2 ng/L, 99th percentile 34.0 ng/L) measured in all the 103 adult healthy subjects (65% women, age range 20–76 years) with the CLEIA method were higher than the LoD value (i.e. 2.1 ng/L). On the contrary 23 of these healthy subjects (corresponding to 22.3% of total number) showed cTnI values \leq LoD value (i.e. 9 ng/L) measured with the FLUOR method.

According to Apple and Collinson [3], two criteria are needed to define a high-sensitivity troponin assay: 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's limit of detection for at least 50% (and ideally 95%) of healthy individuals. Compared to ST ALA-PACK cTnI 3rd-Gen method, previously evaluated in our laboratory [7], the new CLEIA method (CL AIA-PACK cTnI TEST) for cTnI using the AIA-CL2400 platform showed an improved analytical sensitivity and reproducibility, especially at very low cTnI concentrations (Table 1). This improved imprecision at lower cTnI concentrations allows the measurement of the 99th percentile of the reference Caucasian population suggested by the manufacturer (i.e. 31 ng/L) with an imprecision of 10% (bulletin A008871001-1168, Rev. 11/16, TOSOH EUROPE N.V., Tessenderlo, Belgium). These preliminary data suggests that the new CLEIA method for the AIA-CL2400 platform should satisfy the first of the two criteria to be defined a high-sensitivity method [3]. However, these data should be confirmed by multicenter studies using large reference populations, including several healthy individuals, divided for age, gender and ethnic origin (including more than 300 individuals for each group) in order to actually demonstrate that the CLEIA method for the AIA-CL2400 platform is able to measure the 99th URL with a CV value $\geq 10\%$, and with cTnI values > of LoD values in the major part of healthy individuals, as requested by the two criteria recommended by international guidelines [3, 8, 9].

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: TOSOH EUROPE N.V. (Tessenderlo, Belgium) kindly supplied all reagents and calibrators used in the study for the measurement of cTnI.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. *J Am Coll Cardiol* 2012;60:1581–98.
2. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2016;37:267–315.
3. Apple FS, Collinson PO; IFCC Task Force on Clinical Applications of Cardiac Biomarkers. Analytical characteristics of high-sensitivity cardiac troponin assays. *Clin Chem* 2012;58:54–61.
4. Franzini M, Lorenzoni V, Masotti S, Prontera C, Chiappino D, Latta DD, et al. The calculation of the cardiac troponin T 99th percentile of the reference population is affected by age, gender, and population selection: a multicenter study in Italy. *Clin Chim Acta* 2015;438:376–81.
5. CLSI EP17-A protocol. Protocols for determination of limits of detection and limits of quantitation; approved guidelines. 2004, Vol. 24, No. 34. Wayne, Pennsylvania.
6. CLSI EP5-A2 protocol. Evaluation of precision performance of quantitative measurement methods; approved guideline—Second edition. 2004, Vol. 24, No. 25. Wayne, Pennsylvania.
7. Franzini M, Prontera C, Zucchelli GC, Storti S, Passino C, Clerico A. Evaluation of analytical performance of a novel immunoenzymometric assay for cTnI. *Clin Chim Acta* 2013;416:48–9.
8. Sandoval Y, Apple FS. The global need to define normality: the 99th percentile value of cardiac troponin. *Clin Chem* 2014;60:455–62.
9. Clerico A, Zaninotto M, Ripoli A, Masotti S, Prontera C, Passino C, et al. The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology and clinical implications. *Clin Chem Lab Med* 2017;55:1634–51.