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Letter to the editor

# Evaluation of reproducibility of the cTnT immunoassay using quality control samples

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*To the Editor,*

The Fourth Universal Definition of Myocardial Infarction recommends a decision point for the detection of myocardial injury at the 99th percentile upper reference limit (URL) of the cardiac troponin (cTn) distribution values in a population of healthy subjects [1]. However, reliable quality control (QC) materials with cTn concentrations ≤ of the 99th percentile URL are often not commercially available [2]. As a consequence, a quality gap arises because the users do not know the performance of cTn assay at the 99th percentile URL. The 2018 IFCC guidelines on the application of cardiac biomarkers [2] recommend that laboratories measure at least 3 different concentrations of QC, at least once per day, for the evaluation and monitoring of high-sensitivity (hs) cTn assay: QC 1 with a cTn concentration between the limit of detection (LoD) and the lowest sex-specific 99th percentile URL; QC 2 with a concentration that should be higher than but near (within 20%) to the highest sex-specific 99th percentile URL; QC 3 with a concentration that challenges the upper analytical range of the reportable cTn results.

Since the year 2015, the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Section of the European Ligand Assay Society (ELAS) promoted some studies with the aim to accurately evaluate and compare the analytical performance and the reference values of cTn methods commercially available in Italy, as well as to obtain a harmonization among cTnI methods [3]. In particular, one target of these studies was to prepare and to evaluate some QC materials able to satisfy the recommendations of 2018 IFCC for cTnI and cTnT measurement with high-sensitivity methods [2]. The QC samples were prepared using residuals from heparinized plasma samples collected from healthy volunteers or patients with cardiac diseases [3,4]. A pilot study demonstrated that the prepared QC materials were commutable with the samples of healthy controls and patients [3]. More recently, the results concerning the use of the QC materials to evaluate the analytical performance of some high-sensitivity cTnT [4] and cTnI [5–8] methods were described.

The aim of this paper is to report the results obtained using the QC samples distributed in an external quality assessment (EQA) program. The between-laboratory variability of the Elecsys Troponin T hs Immunoassay (Roche Diagnostics) was assessed by means of QC samples distributed in collaboration with the Italian spin-off QualiMedLab of CNR (Centro Nazionale delle Ricerche) during the EQA 2015–2018 cycles. The Elecsys Troponin T hs method is an electrochemiluminescence immunoassay (ECLA) for measurement of cTnT.

The data reported in this study are related to the results of several Italian laboratories (on average 24 for each EQA cycle), which measured the cTnT concentrations in QC samples with Cobas e411, e801 and e601 platforms during the EQA 2015–2018 cycles. The control samples were prepared using residuals from heparinized plasma samples collected from healthy volunteers or patients with cardiac diseases admitted to the Heart Hospital of the Fondazione CNR Regione Toscana G. Monasterio (Massa, Italy), as previously described in details [3,4]. All healthy subjects and patients gave the informed consensus for the use of their residual blood samples in the study. For every annual EQA cycle, 12 control samples with different cTnT concentrations were prepared according to the ILAC G13 guidelines and sent to clinical laboratories, as previously described in detail [3,4]. Briefly, several heparinized plasma specimens, containing different cTnI and cTnT concentrations, were pooled together (about 30–50 patients for each pool) to obtain plasma pools with a final volume of about 100 m.L. After the preparation, the pools were immediately stored at −20 °C. QC samples were sent by mail as lyophilized materials. Lyophilization procedure was performed within two weeks after preparation of sample pools, as previously reported [3]. The mean recovery of cTnT after lyophilization procedure was 103.8% (CV 19.1%). The lyophilized materials were reconstituted with 0.5 mL of distilled water by participant laboratories before the assay. To test the within-laboratory variability, some of QC samples were repeatedly sent to the clinical laboratories participating to the EQA (about two times every year).

Total variability was estimated by averaging the CVs computed from the results of each study sample. Considering the 2015–2018 EQA cycles, 48 QC samples with cTnT mean concentration ranging from 7.8 ng/L to 324.5 ng/L were prepared and then sent to clinical laboratories. The clinical laboratories measured these QC samples in the annual EQA cycles and produced a total of 1054 results regarding cTnT values. The mean ( ± SD) total variability of cTnT assay was 4.96 ± 2.20% (minimum value 2.2%, maximum value 12.8%). The non-linear relationship (i.e., the imprecision profile) between cTnT concentrations (X-axis) and imprecision values (expressed as CV. %, Y-

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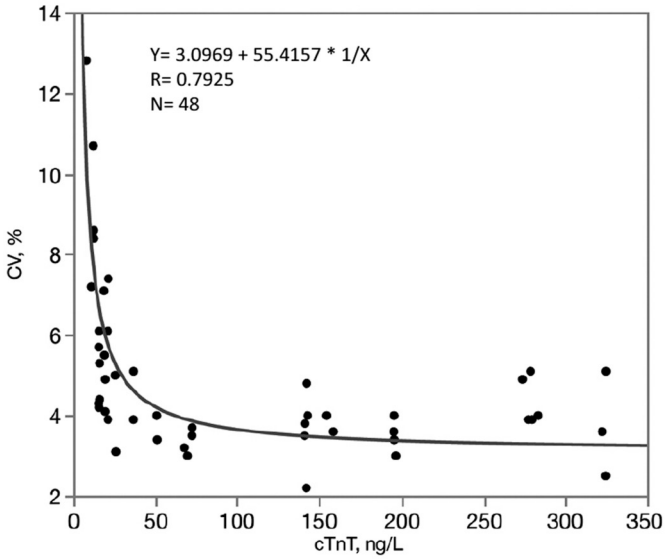


**Fig. 1.** Imprecision profile of the ECLIA hs-cTnT method using Cobas platform. For the calculation of this imprecision profile, 48 QC samples were assayed. The interpolated reciprocal curve was reported in Figure.

axis) is reported in Fig. 1. According to the results of imprecision profile, the 99th percentile URL of cTnT distribution values suggested by the manufacturer (i.e., 14 ng/L) is measured with a mean imprecision of 7.1%, while the cTnT value measured with an imprecision of 10% is 8.0 ng/L (Fig. 1). Moreover, cTnT values > 15 ng/L are measured with a mean imprecision of 4.3% (minimum value: 2.2%, maximum value: 7.4%). Two QC materials were sent to clinical laboratories for 3 consecutive years and the results obtained were: QC 1, mean cTnT concentration 12.2 ng/L with a CV of 1.7%; QC 2, mean cTnT concentration 278.6 ng/L with a CV of 0.3%, (the detailed data are reported in Supplementary Table 1). These data indicate that the QC materials are stable for at least 3 years.

The results of the EQA study show, in general, that it is feasible to prepare QC materials with cTnT concentrations within the normal range according to quality specifications required by international guidelines [2]. Moreover, the results of this study indicate that the Elecsys ECLIA cTnT method automatized on Cobas platforms shows a good analytical performance being able to measure the 99th percentile URL, suggested by the manufacturer (i.e., 14 ng/L), with a mean imprecision of 7.1%. while cTnT concentration > 50 ng/L are on average measured with an imprecision of 3%.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.04.068.

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