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Research Article

# Evaluation of high-sensitivity cardiac troponin measurement procedure performance in serum: the vidas 3 high sensitivity cardiac troponin I

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## Abstract

**Objectives:** International recommendations suggest that when laboratories begin to use a new measurement procedure, the analytical performance of the new procedure should be evaluated. In this study, we evaluated the analytical performance of the Vidas 3 high sensitivity cardiac troponin I assay.

**Methods:** The precision, linearity, and measurement procedure comparison study were performed according to international standardized protocols (EP15-A3, EP06-A and EP09c). Serum samples were analyzed 20 times and at a frequency of three runs/day on five consecutive days for the calculation the CV% of repeatability and within-laboratory precision. The highest concentration hs-cTnI calibrator was diluted for the preparation of five pools with a concentration range of 9.6–16450 ng/L. The Beckman Dxl 800 analyzer was chosen as the comparative measurement procedure.

**Results:** For low- and high-level concentrations, CV% of repeatability was calculated as 3.12 and 4.74; CV% of within-laboratory precision was calculated as 3.04 and 2.07, respectively. The linear regression analysis showed a line with a good correlation coefficient (r>0.99) over the entire range tested. PassingBablok regression was described with the equation y=0.2995+1.006 x. There was no significant deviation from linearity (p=0.800). The 95% confidence interval of the intercept value and slope value was calculated as -1.3734 to 1.0214 and 0.9973 to 1.022, respectively. The mean absolute difference was -12.1ng/L (95% CI=-24.2479 to 0.0979).

**Conclusion:** Vidas 3 hs-cTnI measurement procedure is a precise and linear method for the determination of hs-cTnI. There is no significant difference between Vidas 3 and the Beckman Dxl 800 measurement procedure.

**Keywords:** Analytical performance, BioMérieux Vidas 3, Beckman Dxl 800, high sensitivity cardiac troponin

The definition of myocardial infarction (MI) was first made based on electrocardiography by the World Health Organization working groups [1]. With the introduction of more sensitive heart biomarkers, MI is redefined by The European Society of Cardiology (ESC) and the American College of Cardiology (ACC) using a biochemical and clinical approach [2]. It is reported that myocardial injury detected by abnormal biomarkers in the setting of acute myocardial ischemia should be named as MI.

High sensitivity cardiac troponin (hs-cTn) tests provide an early diagnosis in patients with suspected acute coronary syndrome [3]. The ESC guideline recommends the 0/3 or 0/1 hour hs-cTn protocol in patients with suspected non-ST segment elevation myocardial infarction (NSTEMI) [4].

Current generations of troponin assays have analytical sensitivities up to 100 times greater than the previous generation of troponin assays (1 ng/L vs. 100 ng/L) [5]. Therefore, the difference between consecutive levels of hs-cTn (even if both

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measurements are below the 99th percentile upper reference limit) is considered a rapid and sensitive indicator of acute coronary syndrome [3].

The International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers propose that the coefficient of variation (CV) in the 99 percent URL should be less than 10% for an assay to be defined as high-sensitivity [6]. According to The Consensus Document published by ESC and ACC, the CV% of the hs-cTn measurement procedure should be <10 for the URL concentration of the healthy population (99 percentile) [2]. Manufacturers carry out extensive studies to evaluate the performance of the measurement procedures and share their results with users. However, many issues, such as calibrators, instrument components, shipment and storage, local climate control conditions, quality of water, the stability of electric power and the skills of the analyst, may affect the results in different laboratories. Therefore, laboratories should demonstrate that the measurement procedure work under their actual conditions of use. Clinicians should request information from laboratory experts about the performance of their local assay [3, 7, 8]. ISO 15189 (Medical Laboratories-Requirements for Quality and Competence) recommends that medical laboratories use validated measurement procedures, and these procedures are verified by the laboratory before routine use [9].

## Materials and Methods

This study was performed in Izmir Kemalpasa State Hospital laboratory during November-December 2018.

### Measurement procedure

hs-cTn I test was performed using ELFA measurement procedure in Vidas 3 analyzer (BioMérieux, Marcy l'Etoile, France). Vidas troponin I is a quantitative test using a one-step immuno-enzymatic sandwich method. The serum sample is transferred to wells containing anti-hs-cTn I antibodies (conjugate) labelled with alkaline phosphatase. This allows the formation of the sandwich structure with troponin I, immunoglobulins and conjugate bound to the solid phase inner wall. In the final stage, the substrate is hydrolysed by the conjugate enzyme to a fluorescent product (4-Methyl-umbelliferone). The intensity of the fluorescence is proportional to the antigen concentration. The volume of serum necessary for the analysis is 150 µl. The time of the analysis is below 17 minutes.

### Precision study

The URL of Vidas hs-cTn I assay (99 percentile) is 19 ng/L. In the Vidas hs-cTn I package insert, a "two-hour algorithm" is recommended to rule in or rule out MI. In this algorithm, >100 ng/L hs-cTn I concentrations at the time of patient admission was determined as "rule in". In this context, the concentrations of serum samples (low and high levels) used in our study were determined close to these values (clinical decision points). The serum pool was prepared using the remaining serum samples (low and high level) from routine samples without lipemia, icterus and hemolysis. Samples were aliquoted and frozen within four hours at –20°C until use.

Precision study was performed according to the clinical & laboratory standards institute (CLSI) Guideline EP15-A3 (User verification of precision and estimation of bias, 3rd edition) [10]. Pooled low- and high-level venous serum samples were assayed 20 times consecutively for repeatability study. Separated low- and high-level venous serum samples were tested at a frequency of three runs/day on five consecutive days (15 determinations per level) and CV% of within-laboratory precision were calculated. One reagent lot was used during this study. Normal quality control procedures were conducted during the precision evaluation experiment. In the manufacturer's package insert, CV% of within-laboratory precision at 19 ng/L is reported as 7%. The repeatability and within-laboratory CV% values declared by the manufacturer are reported as <10% for samples with a concentration between 19 and 40000 ng/L. The results obtained were evaluated according to the values declared by the manufacturer and the CV% value recommended for hs-cTn in the Consensus document published by ESC and ASC. The acceptable CV% value for imprecision was determined as ≤10 [2, 6].

### Linearity study

The linearity assessment of the test was conducted in compliance with the description in the CLSI guideline EP06-A (Evaluation of the linearity of quantitative measurement procedures: a statistical approach, 1st edition) [11]. The highest standard of hs-cTn I was diluted for the preparation of five different serum pools. Five pools with a concentration range of 9.6–16450 ng/L were obtained. Serum pools were analyzed twice in one single run.

### Comparison study

The comparison between the measurement procedures was performed in compliance with the description in the EP09c (Measurement procedure comparison and bias estimation using patient samples, 3rd edition) [12]. Operators have spent sufficient time on learning the operation and maintenance procedures of the device. Proper quality control protocol was used throughout the evaluation period of both the test and the comparative measurement procedures. Forty serum samples, covering most of the concentration range (2-5603 ng/L), were selected from routine serum samples requested for troponin analysis of patients admitted to the Emergency Department with chest pain. Samples with lipemia, icterus and hemolysis were excluded from this study. The duration and conditions of storage were determined accord-

ing to the recommendations of the manufacturer. Samples were analyzed within the same run in duplicate on Vidas 3 (BioMérieux, Marcy l'Etoile, France) and Beckman Dxl 800 (Beckman Coulter, Miami, USA) analyzers. The time span for analysis did not exceed two hours.

### Statistical analysis

Statistical analyses were performed using the SPSS software package (version 20.0; SPSS, Inc., Chicago, IL, USA), Microsoft Excel program and MedCalc (MedCalc Software, Inc., Mariakerke, Belgium). The normal distribution of data was tested with Kolmogorov-Smirnov and Shapiro-Wilk test. CV% was calculated using the following equation: CV (%) = (standard deviation × 100)/laboratory mean. The XY plot was visually examined to guide the subsequent assessment of linearity. Polynomial regression analysis was performed. The measurement procedure comparisons were performed by Passing-Bablok regression equation. The distribution of the differences between the measurement procedures was evaluated on the Bland-Altman graph.

## Results

### Precision study

Data of the precision study are shown in Table 1. For low- and high-level concentrations, CV% of repeatability was calculated as 3.12% and 4.74%; CV% of within-laboratory was calculated as 3.04% and 2.07%, respectively (Table 1).

### Linearity Study

The linear regression analysis showed a line with a good correlation coefficient (r>0.99) over the entire range tested. Polynomial regression analyses are presented in Figure1.

### Comparison study

The passing-Bablok regression equation was calculated as y=0.2995+1.0057 x. The 95% confidence interval of the intercept value and slope value was calculated as -1.3734 to 1.0214 and 0.9973 to 1.022, respectively (Fig. 2). There was no significant deviation from linearity (p=0.800). The mean absolute difference was calculated as -12.1ng/L (95% CI=-24.2479 to 0.0979) (Fig. 3).

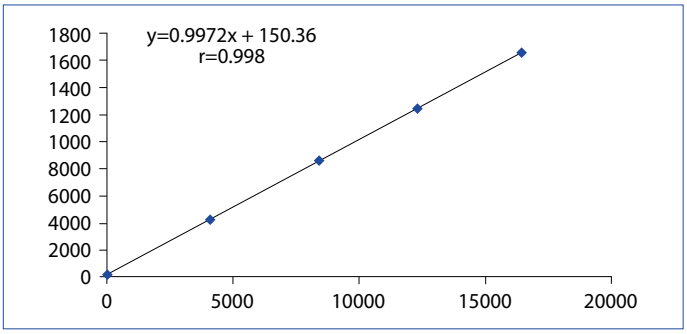


**Figure 1.** Linear regression analysis for the serial dilutions of the hs-cTnI.

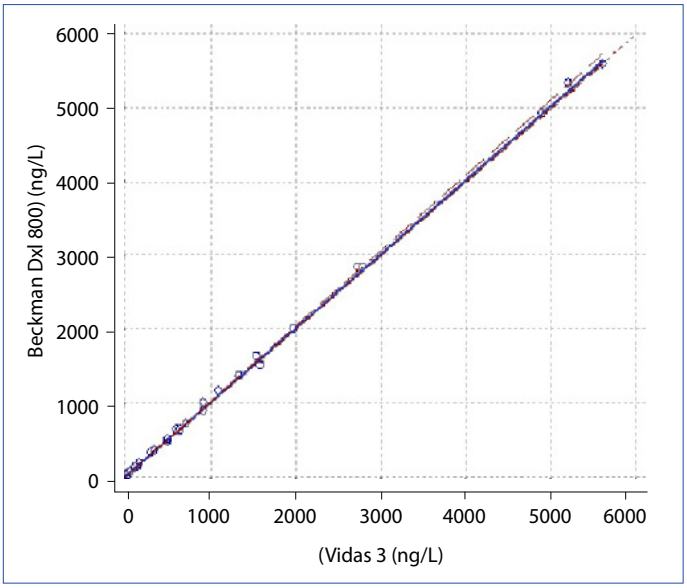


**Figure 2.** Passing-Bablok regression plot.

## Discussion

Our findings showed that the within-laboratory precision and repeatability of the hs-cTn I measurement procedure were "guideline acceptable" performance for low and high concentrations of hs-cTnI [2, 3, 13, 14]. The hs-cTnI measurement procedure was of acceptable linearity between 9 and 16450 ng/L concentrations to rule in and rule out acute MI. In comparison procedure study, Vidas 3 and Beckman Dxi 800 hs-cTnI measurement procedures were shown to be agreement.

The hs-cTn tests are significant in the diagnosis and management of patients with the suspected acute MI. Therefore,

**Table 1. Mean, SD and CV% values of repeatability and within-laboratory precision for low and high-level samples**

| | Repeatability | | | Within-laboratory precision | | |
|---|---|---|---|---|---|---|
| **hs-cTn I Level** | **Mean(ng/L)** | **SD (ng/L)** | **CV (%)** | **Mean(ng/L)** | **SD (ng/L)** | **CV (%)** |
| Low | 20.2 | 0.63 | 3.12 | 19.05 | 0.58 | 3.04 |
| High | 97.7 | 4.63 | 4.74 | 94.3 | 1.95 | 2.07 |

SD: Standard deviation; CV: Coefficient of variation.

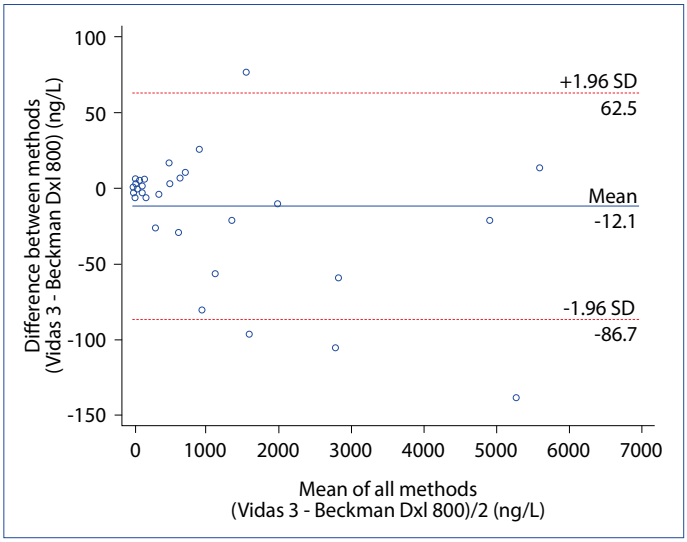


**Figure 3.** Bland-Altman difference plot.

laboratories should select the measurement procedure that manufacturers claim to meet the desired performance characteristics. When the measurement procedure changes, the analytical performance of the new method should be evaluated. The results obtained in the performance analysis can be made according to the manufacturer's declared values or the analytical performance characteristics recommended in the guidelines. Our study results were evaluated according to the values declared in the manufacturer's package insert and analytical performance characteristics suggested in the guidelines.

In our bias estimation study, we determined that four samples showed a small difference with observations beyond the 95% limit of agreement. The hs-cTnI concentrations of these four samples were well above the clinical decision points. No significant difference was seen between the candidate and the comparative measurement procedure. Consistent with our results, the URL concentrations of hs-cTn I measurement procedures reported by the manufacturers of Vidas 3 and Beckman Dxl 800 are close to each other. (99$^{th}$ percentile URL of Vidas 3 and Beckman Dxl 800 hs-cTn I measurement procedures are 19 ng/mL and 18.2 ng/mL, respectively.) The measurement results of low concentration samples were found to be lower in the Vidas 3 hs-cTnI measurement procedure than the Beckman Dxi 800 measurement procedure. This is significant for a laboratory to evaluate cut off-limits and algorithms according to the method they use.

In our study, we did not use a standard or reference measurement procedure for method comparison, which is the limitation of our study. Therefore, we used the term “difference” instead of “bias" [12]. In the comparison study, we selected the Beckman Dxl analyzer, which performs well in analytical performance studies as a comparative measurement procedure [15, 16]. In this study, we used the serum samples. Therefore, if the sample type used in the troponin measurement procedure changes (plasma instead of serum), the performance study should be repeated using the new sample type [12].

The analytical performance studies lead the user to determine the match between the actual performance of the laboratory and the expected performance of the method. In our study, the Vidas 3 hs-cTn I measurement procedure has been shown to provide the performance declared by the manufacturer.

## Conclusion

Good accuracy, linearity, and acceptable precision are crucial criteria for the measurement procedures in the clinical laboratory. The Vidas 3 hs-cTnI measurement procedure showed good analytical performance. Our study may be helpful for laboratories beginning to use a new hs-cTn I measurement method or for the evaluation of hs-cTnI result obtained by different measurement methods.

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**Conflict of Interest:** No conflict of interest was declared by the authors.

**Ethics Committee Approval:** I certify tahat this study involving human subjects is in accordance with the Helsinky declaration of 1975 as revised 2000 and that it has been approved by the relevant institutional Ethical Committee.

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There is no use of any human or animal sample/data in the study. In the study, leftover serums were used, and there was no procedure that involves the collection of additional patient samples from any biological material.

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