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Evaluation of analytical performance of the Pathfast® cardiac troponin I

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Abstract

Background: Cardiac troponins are considered the cornestore for risk stratification and diagnosis of patients whit acute coronary syndrome (ACS). Following Clinical Laboratory Standards Institute (CLSI) guidelines, we assessed the analytical performances of the Pathfast® (Mitsubishi, Japan) cTnI method.
Methods: We evaluated different sample types. Control materials and lithium heparin plasma pools were used to determine: limit of blank (LoB), limit of detection (LoD), imprecision and linearity. The effects of potential endogenous interfering substances and the possibility of falsely increased cardiac troponin I (cTnI) concentrations attributable to the presence of heterophilic antibodies (HA), rheumatoid factor (RF) and human anti-mouse antibodies (HAMA) in high concentrations were evaluated. The 99th percentile limit of the cTnI value distribution was determined from 320 Caucasian reference individuals.
Results: No significant differences were found when cTnI concentrations of 40 lithium-heparin plasma samples were compared with the matched values of K_2-EDTA plasma, whole blood and serum samples. The LoB and the LoD of the cTnI method were 0.0048 and 0.0066 μg/L, respectively. cTnI mean values from 0.66 to 6.0 μg/L showed a total CV% from 6.0 to 6.4. cTnI at a concentration of 0.02 μg/L was associated with a total CV of 9.6%. The method gave a linear response for cTnI concentrations within the measurement range. In six of 12 samples containing HA, a positive interference was demonstrated. The 99th percentile limit of the cTnI distribution in the reference population was 0.013 μg/L.
Conclusions: The data indicate that the cTnI Pathfast method may be suitable for helping clinicians in the management of patients with ACS.
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Introduction

Coronary heart disease is the leading cause of mortality in developed countries (1). Timely evaluation of patients with chest pain in the emergency/cardiology department is mandatory in order to provide early treatment to reduce morbidity and mortality in those with acute coronary syndrome (ACS). In patients with no ST elevation recorded by ECG, the diagnosis of acute myocardial infarction (AMI) depends almost entirely on serial measurements of biochemical markers of necrosis. In 2000, the European Society of Cardiology (ESC) and the American College of Cardiologists (ACC) recognized the pivotal role of biomarkers, making their increase in the bloodstream the ''cornerstone'' for diagnosis of AMI (2). In particular, cardiac troponins have emerged as a powerful tool for diagnosis of AMI and for risk assessment of cardiac events, including death and recurrent ischemia (3). In 2007, the joint ESC/ACCF/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the redefinition of myocardial infarction (4) confirmed the role of troponin. Thus, in a laboratory medicine context, a high-quality analytical method is mandatory (5, 6). In accordance with Clinical Laboratory Standards Institute (CLSI) guidelines, we evaluated the analytical performance of cardiac troponin I (cTnI) on the Pathfast immunoassay analyzer (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). We also defined the 99th percentile limit of the cTnI value distribution in our reference population.

Materials and methods

Assay method

The Pathfast system is a bench-top enzyme immunoassay analyzer, processing up to six samples at a time and providing simultaneous quantitative determination of creatine kinase-MB mass, myoglobin, cTnI, and NT-proBNP using whole blood or plasma (sodium or lithium-heparin) samples (7).

The chemiluminescence enzyme immunoassay (CLEIA) principle has been enhanced by employing an efficient bound/free separation method whereby magnetic particles are washed and separated at the inner wall of a pipette tip according to a patented Magtration® technology (Precision System Science/PSS USA Inc., Livermore, CA, USA). The term ''Magtration'' is derived by abbreviating ''Magnetic Filtration'': in order to remove excessive reagents or residual materials, the technology performs bound/free separation in pipette tips using magnetic particles. Briefly, the sample (25 μL) containing the target analyte is incubated with

magnetic particles coated with the corresponding capture antibodies and alkaline phosphatase (ALP) conjugated antibodies. The resulting immunocomplexes are separated, washed and transferred by Magtration technology to a luminescence measurement well containing a CDP-Star® reagent (1,2-dioxetane compound Phototope® ALP) chemiluminescent substrate. The generated luminescence is measured and the analyte concentration calculated using a standard curve relating these two parameters. The cTnI method is a one-step, two site immunoassay using a mouse monoclonal antibody (MoAb A) immobilized on magnetic particles (capture: G00811) recognizing cTnI (residues 41–49), and two monoclonal antibodies (MoAb B and C) bound to ALP (tag: G00821 and G00831) recognizing the cTnI mid (residues 71–116) and C-terminal portion (residues 163–210), respectively. The human cardiac troponin complex (CTI), supplied by Hytest Ltd. (Turku, Finland), was used as calibration material in the Pathfast assay. Upon receiving a new lot of reagents, the specifications of the lot are recorded in the instrument through the master lot entry sheet, included in each package; the calibration adjustment is made using two calibrators supplied with the kit. The working range of the master curve is 0.02–50 μg/L and calibration stability lasts 28 days. All reagents needed for the assay are incorporated into a single cartridge. Analysis time is 15 min.

Sample types

Lithium-heparin plasma samples (polyethylene terephthalate/PET tubes, 68 IU lithium-heparin in 4 mL blood; code: 367374, Becton Dickinson, Franklin Lakes, NJ, USA) were used as reference for the Pathfast cTnI analytical evaluation throughout the entire study. In order to evaluate the potential impact of anticoagulants on cTnI concentrations, EDTA plasma, whole blood (K_2-EDTA: 1 mg/mL blood, in plastic tubes; code: 368861, Becton Dickinson) and serum (PET tubes without a gel barrier; code: 368815; Becton Dickinson) samples were used. Forty paired samples with detectable cTnI concentrations were obtained from AMI patients admitted to the Cardiology Department of the University Hospital of Bari. cTnI was measured in all sample types within 1 h following collection. cTnI results in whole blood were obtained after software correction for individual hematocrit values, according to the following formula: corrected cTnI = measured cTnI/[(1-(Hct/100)].

Estimation of limit of blank (LoB) and limit of detection (LoD), imprecision studies, linearity on dilution

The LoB and the LoD were calculated according to CLSI EP 17-A (8). The LoB, which is defined as the highest measurement result that is likely to be observed (with a stated probability = 5%) for a blank sample, was estimated from 60 repeated measurements of the cTnI Pathfast zero calibrator; the analytical signal was expressed in arbitrary units. A sample with a cTnI concentration of 25.3 μg/L was diluted until an expected concentration equal to zero was obtained. The parameters of the linear regression (arbitrary units on the y-axis and expected cTnI concentrations on the x-axis) were used to transform the mean analytical signal of LoB into cTnI concentrations. To determine the LoD, which is defined as the lowest amount of analyte in a sample that can be detected with a stated probability (usually, 95%), a pooled estimate of standard deviations (SDs) was obtained from 12 repeated measurements of five pools with low cTnI concentrations (cTnI concentrations from LoB to ~3×LoB). The imprecision of the cTnI Pathfast method was determined in accordance with CLSI EP5-A guidelines (9): a lithium-heparin plasma pool and BioRad Liquicheck™ Cardiac Markers control LT (level 2, 3) (BioRad Laboratories, Headquarters, Hercules, CA, USA) were analyzed daily, in duplicate, for 20 consecutive days. Two aliquots of test material, for each concentration, were tested within each run (80 measurement per sample). To define the method imprecision in the low concentration range, a plasma pool containing a known troponin concentration (0.11 μg/L) was serially diluted with a lithium-heparin plasma sample showing undetectable concentrations of cTnI. Eleven pools containing troponin concentrations ranging from 0.1 to 0.01 μg/L were aliquoted and stored frozen at –20°C until analysis. For each of the 11 pools, a new aliquot was thawed, centrifuged and analysed in duplicate every day for 10 consecutive days; two different reagent lots and two different calibration curves were used. The linearity of the method was determined according to CLSI guidelines EP6-P (10): a lithium-heparin plasma pool (pool A; TnI concentration = 42 μg/L) was diluted with a lithium-heparin plasma pool showing undetectable concentrations of cTnI (pool E), at a ratio of 3/1, 2/2 and 1/3. Using this protocol, three different samples (pool B, C and D) with theoretical cTnI concentrations of 31.5, 21.0, 10.5 μg/L, respectively, were obtained. Each of the five pools were tested in quadruplicate in a single run.

Effect of potential interfering substances

Lithium-heparin plasma pools (P) containing high concentrations of potentially interfering substance (total bilirubin, 771 μmol/L; triglycerides, 8.8 mmol/L; hemoglobin, 8.2 g/L) and TnI concentrations lower than the detection limit were prepared. These were added in fixed ratios (0+10, 1+9, 2+8, 3+7, 4+6, 5+5, 6+4, 7+3, 8+2, 9+1, 10+0) to two lithium-heparin plasma pools containing elevated TnI concentrations (S1: 1.13 μg/L; S2: 2.72 μg/L) and physiological concentrations of each of the potential interfering substances. In addition, diluting both the S1 and S2 pools (in the same fixed ratios mentioned above) with a lithium-heparin plasma pool with undetectable concentrations of TnI allowed us to produce a set of control dilutions (control-1, C1; control-2, C2).

To evaluate the potential interference due to heterophilic antibodies (HA), human anti-mouse antibodies (HAMA) and rheumatoid factor (RF), 12 lithium heparin plasma samples known to cause false positive cTnI results when tested with the Dimension RxL (Siemens Healthcare Diagnostics, Deerfield, IL, USA), and seven commercially available (Scantibodies Inc, Santee, CA, USA) samples containing high HAMA (concentration range: 100–782 ng/mL) and RF titers (concentration range: 162–1080 UI/mL) were tested using the Pathfast method. All these samples were treated with heterophile blocking tubes (Scantibodies Inc).

Comparison study

The Pathfast cTnI method was compared to the Stratus CS cTnI method (Siemens Healthcare Diagnostics) in accordance with CLSI guidelines (11). A total of 115 paired lithium-heparin plasma samples were collected and cTnI concentrations measured with the Stratus CS and Pathfast. In the comparison study, only samples with cTnI concentrations >0.015 μg/L (LoD for Stratus CS) and lower than 50 μg/L (linearity limit for Pathfast) were used.

Upper reference limit

In order to define the cTnI upper reference limit, lithium-heparin plasma samples were collected from 320 healthy Caucasian subjects: (160 women and 160 men aged 26–89 years; mean ± SD = 56.7 ± 15.6 years). Exclusion criteria included subjects with a suspected history of ACS, biochemical abnormalities in serum creatinine, glucose, urea, alanine aminotransferase (ALT), aspartata aminotransferase (AST), C-reactive protein, creatine kinase-MB, leukocyte blood count, or hemoglobin and hyperpyrexia. According to the ESC/ACCF/AHA/WHF (2, 4), the upper reference limit was calculated at the 99th percentile limit of the cTnI value distribution in the reference group; the 95% confidence interval (CI) for the percentile was determined assuming a non-Gaussian distribution of the data.

Statistical analysis

Statistical comparison among different sample types was assessed with the Wilcoxon rank sum test for paired samples; Deming regression and Bland-Altman analysis (12) were also performed.

For 60 measured values of the sample blank, a Gaussian distribution was not assumed (Anderson-Darling-test: $p < 0.01$) and the 95th percentile of the LoB was calculated as [60(95/100) + 0.5] ordered observations following logarithmic transformation of the original data set. The LoD was defined by the relation $LoD = LoB + c_\beta \times SDs$. SDs is the pooled estimate of the SD of pools with low cTnI concentrations, and $c_\beta = z_{1-\beta}/(1-1/(4\times f))$ where the $z_{1-\beta}$ is the standard normal deviation (SD = 1.645), and f is the number of degrees of freedom for estimation of SDs (13). ANOVA was used to assess the total imprecision (CV%) for the cTnI method at different concentrations of analyte. Dilution linearity was determined by least-squares linear regression analysis with the lack-of-fit-test (10). Bland-Altman analysis was used to evaluate the effect of potentially interfering endogenous substances. We considered the significance of the bias on the basis of the 95% CI. Taking into account the analytical imprecision of the method, a difference > 10% among results obtained in pools with and without added substances was considered to be clinically significant. Correlation between Pathfast and Stratus CS cTnI methods were assessed using Passing and Bablok regression (14) and Bland-Altman analysis.

Statistical analysis was performed using Analyse-it Software for Microsoft Excel version 2.11 (Analyse-it Software, Leeds, UK) and MedCalc version 9.5 (Mariakerke, Belgium).

Results and discussion

cTnI concentrations in EDTA plasma (range: 0.004–29.8 μg/L; median, 0.56 μg/L), whole blood (range: 0.006–28.3 μg/L; median, 0.58 μg/L), and serum (range: 0.003–28.8 μg/L; median, 0.48 μg/L) were compared against the cTnI concentrations measured in lithium-heparin plasma samples (range: 0.004–27.4 μg/L; median, 0.52 μg/L).

No significant differences were found between cTnI concentrations measured in 40 lithium-heparin plasma samples and concentrations measured in matched K_2-EDTA plasma samples (2-tailed p = 0.76), whole blood (2-tailed p = 0.56) and serum samples (2-tailed p = 0.48).

Regression analysis (Deming) showed the following results:

K_2-EDTA plasma vs. Li-heparin: slope = 0.96 (± 0.025); intercept = –0.10 (± 0.19) μg/L;
K_2-EDTA whole blood vs. Li-heparin: slope = 0.96 (± 0.025); intercept = –0.09 (± 0.19) μg/L;
Serum vs. Li-heparin: slope = 1.04 (± 0.00); intercept = –0.07 (± 0.07) μg/L.

No significant bias was detected by Bland and Altman analysis:

K_2-EDTA plasma vs. Li-heparin: average absolute bias = –0.2 μg/L (95% CI, from –0.6 to 0.09 μg/L); average percentage bias = 0.1% (95% CI, from –3.8% to 4%).
K_2-EDTA whole blood vs. Li-heparin: average absolute bias = –0.2 μg/L (95% CI, from –0.6 to 0.07 μg/L); average percentage bias = 6% (95% CI, from –0.3% to 12.7%).
Serum vs. Li-heparin: average absolute bias = 0.09 μg/L (95% CI, from –0.05 to 0.2 μg/L); average percentage bias = 0.3% (95% CI, from –2.4% to 3.2%).

The analytical signal corresponding to 60 measurements of Pathfast zero calibrator ranged from 257 to 590 arbitrary units, and the 95th percentile of value distribution was 344 arbitrary units. Following logarithmic transformation, regression analysis performed using the sample with a cTnI concentration of 25.3 μg/L ($y = 0.9093x + 4.6476$; $r^2 = 0.94$) revealed the Pathfast LoB to be 0.0048 μg/L. To evaluate the LoD, 12 measurements for each of the five pools (cTnI concentration from 0.0061 to 0.0156 μg/L) were performed; this provided 55 degrees of freedom ($f = 5\times(12\text{-}1)$). The pooled within-sample SD of the measurements, calculated as the weighted average of the variances (squared SDs), was 0.0011 μg/L. The LoD was calculated as $LoD = LoB + z_{1-\beta}/(1-1/(4\times f))\times SDs = 0.0048 + 1.654/(1-1/220)\times 0.0011 = 0.0066$ μg/L. Imprecision studies gave the following: control material level 2: mean, 0.66 μg/L; within run CV, 5.8%; total CV, 6.3%; control material level 3: mean, 3.1 μg/L; within-run CV, 5.1%; total CV, 6.0%; lithium-heparin plasma pool: mean, 6.0 μg/L; within-run CV, 4.5%; total CV, 6.4%. Figure 1A shows the imprecision profile at low cTnI concentrations. A cTnI concentration of 0.02 μg/L was associated with a total imprecision < 10% (CV = 9.6%). Linearity data showed that the linearity hypothesis was accepted ($F = 2.33$; $p = 0.11$), and a linear response obtained ($r^2 = 0.995$). No significant interferences were observed with icteric (bilirubin up to 704 μmol/L) or hemolyzed (hemoglobin up to 7.2 g/L) samples. Triglyceride concentrations at 7.6 mmol/L appeared to interfere with Pathfast cTnI measurements in one of the two samples tested [S2, absolute mean difference 0.09 μg/L: (95% CI, 0.04–0.15)]. However, given that this difference is well within the imprecision of the assay, it could be considered not clinically relevant.

Six of 12 samples showing elevated cTnI concentrations due to HA when tested on Dimension RxL

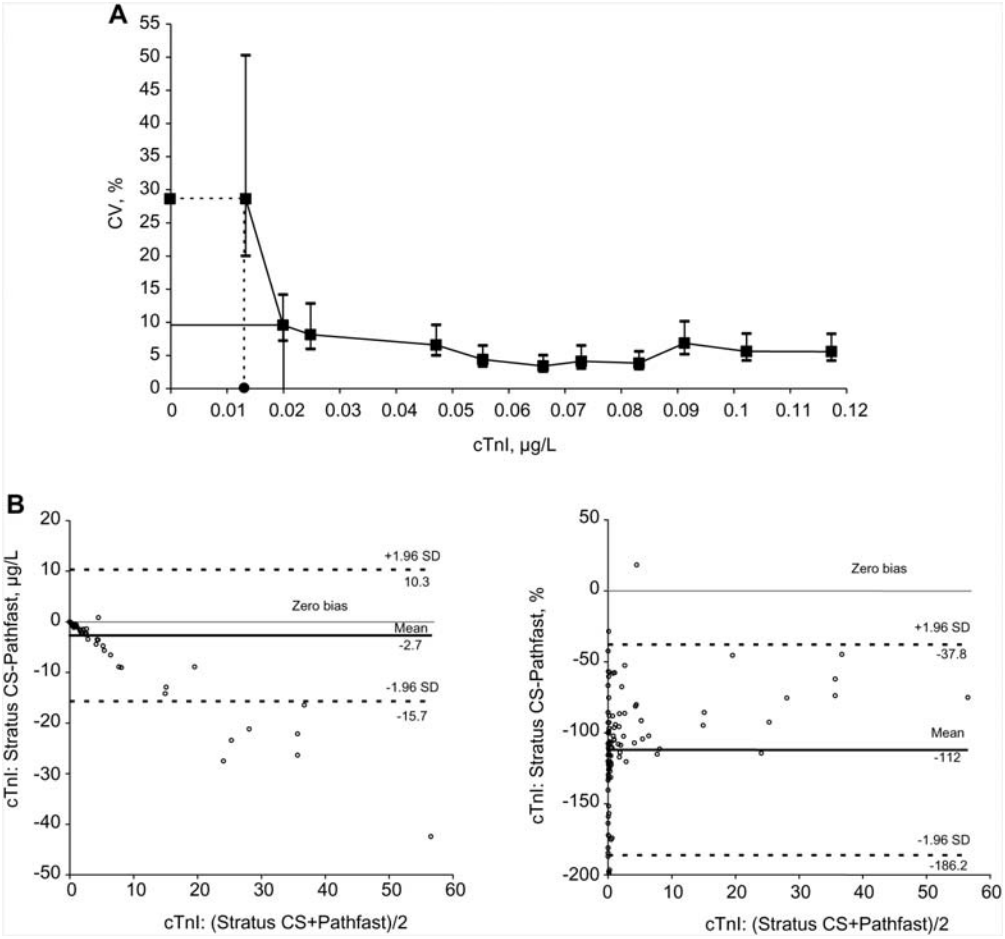

Figure 1 Imprecision profile of Pathfast cTnI assay at low concentrations (A) and Bland-Altman difference plots for the Pathfast vs. Stratus CS method, n = 115 (B).
(A) Plot of cTnI values (x-axis) vs. total imprecision as coefficient of variation (CV%; y axis): cTnI concentration (0.02 µg/L) associated with a 9.6% CV (thick lines), 99th percentile reference limit of cTnI (0.013 µg/L) associated with a 28.7% CV (dashed lines) and imprecision 95% confidence intervals (error bars) at each cTnI concentration level, are shown. (B) Results are absolute (left panel), percentage bias (right panel) and 1.96 SD.

system were obtained from the same patients at different times. The cTnI values measured in these patients are consistently higher than the decision level for AMI (0.15 µg/L). However, there was no typical rise and fall in cTnI values and clinical findings were not consistent with AMI. Treatment with heterophilic blocking tubes provided definitive evidence that all values were false positive; after treatment all values were <0.15 µg/L. When tested on the Pathfast system, these samples showed cTnI concentrations ranging from 0.022 to 0.032 µg/L. These values were higher than the 99th percentile upper reference limit obtained in this study, suggesting interference by HA, as also shown by pre-treatment. cTnI concentrations after treatment with heterophilic blocking tubes were lower than detection limit of the assay. The other samples that showed spuriously high concentrations of troponin when measured with the RxL Dimension, including those with high RF and HAMA concentrations, gave troponin I results below the detection limit of the method when tested on Pathfast.

Comparison (n = 115) between Pathfast (y) and Stratus CS (x) cTnI gave the following correlation: y = 0.336(SE, 0.009)x–0.005 (SE, 0.12) µg/L; r = 0.98 (n = 115). Figure 1B shows the bias plots. The mean absolute bias was −2.7 µg/L (95% CI, –3.8 to –1.4) and mean percentage bias was –112% (95% CI, –118.9 to –104.9). The intercept was not significant and methods showed a constant bias. The 99th (95% CI) percentile limit of the cTnI values distribution in the reference population was 0.013 (0.0068–0.019) µg/L: measurable cTnI concentrations (i.e., >0.0066 µg/L, assay LoD) were found in only six subjects (2%). Figure 1A shows the imprecision (28.7% CV) obtained with a sample with a cTnI value of 0.013 µg/L. Several assays with relatively high precision for cTnI measurement exist. However, none are able to experimentally achieve a 10% CV at the 99th percentile limit of the reference population (15); this is true also for the cTnI Pathfast method.

In conclusion, this study indicates that the cTnI Pathfast method meets the quality specifications recommended by NACB and the IFCC Committee for the Standardization of Cardiac Damage (5, 6). Therefore, it may be suitable for use in the clinical laboratory to help clinicians in the management of ACS patients. Caution should be taken when comparing the published findings related to *analytical sensitivity* of the

cardiac troponin assays, with the findings of this study. Many LoD values are experimentally calculated as LoB (e.g., as the concentration corresponding to a signal of 3 SD above the mean of N replicates for a sample in which cTnI is absent), so that they can be markedly lower if more statistically rigorous procedures, like those applied in this study, were used.

References

1. American Heart Association. Heart and stroke statistical update. Dallas, TX: American Heart Association; 2001.
2. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined – a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959–69.
3. Braunwald E, Antman EM, Beasley JW, Califf RM, Cheitlin MD, Hochman JS, et al. ACC/AHA 2002 guideline update for the management of patients with unstable angina and non-ST-segment elevation myocardial infarction: executive summary and recommendations. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients with Unstable Angina). Circulation 2000;102:1193–209.
4. Thygesen K, Alpert JS, White HD, Joint ESC/ACCF/AHA/WHF Task Force for the Redefinition of Myocardial Infarction. Universal definition of myocardial infarction. Eur Heart J 2007;28:2525–38.
5. Panteghini M, Gerhardt W, Apple FS, Dati F, Ravkilde J, Wu AH. Quality specifications for cardiac troponin assays. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). IFCC Scientific Division Committee on Standardization of Markers of Cardiac Damage. Clin Chem Lab Med 2001;39:174–8.
6. Apple FS, Jesse RL, Newby LK, Wu AH, Christenson RH. National Academy of Clinical Biochemistry; IFCC Committee for Standardization of Markers of Cardiac Damage. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: analytical issues for biochemical markers of acute coronary syndromes. Circulation 2007;115:e352–5.
7. Kurihara T, Yanagida A, Yokoi H, Koyata A, Matsuya T, Ogawa J, et al. Evaluation of cardiac assays on a benchtop chemiluminescent enzyme immunoassay analyzer, PATHFAST. Anal Biochem 2008;375:144–6.
8. National Committee for Clinical Laboratory Standards. Protocols for determination of limits of detection and limits of quantitation; approved guideline. NCCLS guideline EP17-A. Wayne, PA: NCCLS, October 2004.
9. National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS guideline EP5-A. Wayne, PA: NCCLS; February 1999.
10. National Committee for Clinical Laboratory Standards. Evaluation of the linearity of quantitative measurement procedures: a statistical approach; proposed guideline. NCCLS guideline EP6-A. Wayne, PA: NCCLS; September 1986.
11. National Committee for Clinical Laboratory Standards. Method comparison and bias estimation using patient samples; approved guideline. NCCLS guideline EP9-A. Wayne, PA: NCCLS; December 1995.
12. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;307–10.
13. Linnet K, Kondratovich M. Partly nonparametric approach for determining the limit of detection. Clin Chem 2004;50:732–40.
14. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J Clin Chem Biochem 1983;21:709–20.
15. Panteghini M, Pagani F, Yeo KT, Apple FS, Christenson RH, Dati F, et al. Evaluation of the imprecision at low range concentrations of the assays for cardiac troponin determination. Clin Chem 2004;50:327–32.