Analytical evaluation of a new point of care system for measuring cardiac Troponin I

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ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form 31 October 2016
Accepted 9 November 2016
Available online xxx

Keywords:
Emergency medicine
Chest pain
Acute myocardial infarction
Cardiac Troponin-I
Point-of-care

ABSTRACT

Objectives: Point-of-care cardiac troponin testing with adequate analytical performances has the potential to improve chest pain patients flow in the emergency department. We present the analytical evaluation of the newly developed Philips Minicare cTnI point-of-care immunoassay.

Design & methods: Li-heparin whole blood and plasma were used to perform analytical studies. The sample type comparison study was performed at 4 different hospitals. The 99th percentile upper reference limit (URL) study was performed using Li-heparin plasma, Li-heparin whole blood and capillary blood samples from 750 healthy adults, aging from 18 to 86 years.

Results: Limit of the blank, limit of detection and limit of quantitation at 20% coefficient of variation (CV) were determined to be 8.5 ng/L, 18 ng/L and 38 ng/L respectively without significant differences between whole blood and plasma for LoQ. Cross-reactivity and interferences were minimal and no high-dose hook was observed. Total CV was found to be from 7.3% to 12% for cTnI concentrations between 109.6 and 6135.4 ng/L. CV at the 99th percentile URL was 18.6%. The sample type comparison study between capillary blood, Li-heparin whole blood and Li-heparin plasma samples demonstrated correlation coefficients between 0.99 and 1.00 with slopes between 1.03 and 1.08. The method comparison between Minicare cTnI and Beckman Coulter Access, AccuTnI+3 demonstrated a correlation coefficient of 0.973 with a slope of 1.09. The 99th percentile URL of a healthy population was calculated to be 43 ng/L with no significant difference between genders or sample types.

Conclusions: The Minicare cTnI assay is a sensitive and precise, clinical usable test for determination of cTnI concentration that can be used in a near-patient setting as an aid in the diagnosis of acute myocardial infarction. © 2016 The Authors. The Canadian Society of Clinical Chemists. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Measurement of cardiac Troponin-I or Troponin-T (cTnI, cTnT) concentration in blood is required for assessment of patients suspected of Non ST-segment elevation acute myocardial infarction (NSTEMI) to support or exclude a diagnosis of acute myocardial infarction (AMI) [1,2,3]. Measuring cTnI at the point of care (next to the patient) with a short turnaround time (TAT) has the potential to improve patients flow in the emergency department (ED), enabling rapid clinical decision making. A patient blood sample can be withdrawn and directly tested for a diagnosis or exclusion of AMI. In the ED, the cTnI sample can be obtained by a nurse or a paramedic and be sent to the laboratory for cTnI measurement. A point-of-care (POC) cTnI assay may allow the institution to meet the 1-h TAT criteria for the measurement of cTnI from the
guidelines [2,6]. Ideally, the analytical performance of a POC device for cTn testing should not differ from that provided by the central laboratory system [6]. We here present the results of the analytical evaluation of the novel Minicare cTnI POC assay from Philips Electronics.

2. Materials and methods

2.1. Instrumentation

The Philips Minicare cTnI consists of a handheld instrument and plastic disposable cartridge. The system makes use of the Philips Magnotech technology, which is based on the precisely controlled motion of magnetic particles (beads) in a small sample volume (typically 30 μL). The same magnetic particles also serve as labels that are detected using frustrated total internal reflection (FTIR) imaging [7,8].

2.2. Design of the minicare cTnI assay

The Minicare cTnI assay is a homogeneous sandwich immunoassay. The traditional liquid manipulation steps of an immunoassay have been replaced by magnetically controlled movements of magnetic nanoparticles within a stationary liquid (see Fig. 1). The magnetic beads carry antibodies directed against cTnI whereas the other side of the sandwich is formed by antibodies printed on the bottom of the cartridge (the sensor surface).

A droplet of sample (30 μL) of whole blood or plasma is applied to the cartridge and the reaction chamber fills by capillary action. Red blood cells are retained by an integrated separation membrane to prevent impact on the assay. About 2 μL of plasma will be extracted and the microfluidic design ensures that precisely 0.25 μL is metered into the reaction chamber. In the first phase of the assay, beads coated with antibody capture cTnI molecules in the sample. Subsequently, magnetic fields gradients are engaged to transport the particles rapidly to the sensor surface towards immobilized antibodies able to capture the troponin-bearing nanobeads. Thereafter, a sequence of finely tuned magnetic pulses is applied to facilitate optimal binding and mixing of the beads containing cTnI molecules at the antibody-functionalized surface. After the beads react with the sensor surface, un-bound and non-specifically bound beads are rapidly removed with a magnetic wash by applying a magnetic field gradient oriented away from the detection surface [7,8].

2.3. Selection of antibodies

Antibodies are applied to the magnetic beads and onto the plastic surface of the cartridge to form both parts of the sandwich, as described above. The choice of antibodies is crucial for assay performance. Mouse-monoclonal antibodies were selected for the Philips Minicare cTnI assay. The primary anti-cTnI mouse-monoclonal antibody is directed against the stable region of the cTn molecule (amino acid (AA) 41–49) and has been covalently bound to the magnetic beads. A mixture of three secondary antibodies have been attached to the sensor surface by physisorption, which consists of two anti-cTn antibodies with epitopes in the range AA 20–100 and a single anti-cTnC antibody, to optimize measurement of total cTnI including cTnI-TnC complexes.

2.4. Standardization

Due to the lack of a suitable commutable primary calibrator for cTnI immunoassay standardization, new cTnI standards have been developed from pooled native human samples. These standards are dose-assigned on the Beckman Coulter Access 2, AccuTnI + 3 as a reference method. Primary calibrators are prepared, based on the standard reference material of the National Institute of Standards and Technology (NIST SRM) 2921 Human Cardiac Troponin Complex, and are dose-assigned on the native standards. Additionally, secondary calibrators are prepared from NIST SRM 2921 and dose assigned using the primary calibrators. Calibration parameters were obtained from dose-response curves and programmed in the radio-frequency identification (RFID) of each cartridge. For the method comparison and sample type comparison studies, three sample types (capillary whole blood from finger stick, Li-heparin whole blood and Li-heparin plasma) were collected for each patient at four European hospitals (Medizinische Universität Innsbruck, Austria, Klinikum Nürnberg Germany, Catharina Ziekenhuis Eindhoven, The Netherlands and Hôpital de la Pitié-Salpêtrière Paris, France) participating to the Lab2Go project. Lab2Go is a European Union funded multicenter Research and Development project involving several hospitals in the European Union. Patients were selected to represent the range of cTn concentrations likely to be encountered in clinical practice, covering the measurement range of the Minicare cTnI. Samples were analysed on Minicare cTn by Minicare-trained users (nurses, research assistants) within 2 h after blood drawn. Li-heparin plasma samples were centrifuged a second time and transferred to a new container before freezing at ≤– 55 °C. Frozen samples were sent to the core lab at Philips on dry ice for parallel testing on the Beckman Coulter AccuTnI + 3 assay and

Fig. 1. Depiction of the reaction chamber and actuation magnets showing the assay processes: analyte binding by beads bearing anti-cTnI antibodies (top and bottom magnets off), bead binding to the sensor surface (bottom magnet on) and magnetic removal of free and weakly bound beads (top magnet off).

Please cite this article as: D.W.M. Kemper, et al., Analytical evaluation of a new point of care system for measuring cardiac Troponin I, Clin Biochem (2016), http://dx.doi.org/10.1016/j.clinbiochem.2016.11.011
the Minicare cTnI for the method comparison study. This study has been approved by all local ethical committees and informed consent was obtained from patients prior to their participation in the study.

For the 99th percentile upper reference limit (URL) study Li-heparin whole blood, capillary whole blood and Li-heparin plasma samples were obtained from healthy volunteers at PRA Health Sciences Zuidlaren, The Netherlands. Informed consent was obtained from all volunteers prior to their participation in the study.

2.6. Precision

To assess the Minicare cTnI imprecision according to CLSI EP05-A3 recommendations [9] three Li-heparin plasma pools with different levels of cTnl distributed over the measuring range were tested in two runs per day on twenty different days, using two different lots of Minicare cTnI cartridges.

2.7. Detection capability

Limit of the blank (LoB) and limit of detection (LoD) in Li-heparin plasma and limit of quantitation (LoQ) in Li-heparin whole blood and Li-heparin plasma for the Minicare cTnI were established in accordance with CLSI document EP17-A2 [10]. A total of four blank Li-heparin plasma pools for LoB, four Li-heparin plasma pools with cTnl concentrations of 15, 20, 25 and 30 ng/L for LoD and four Li-heparin plasma pools with a cTnl concentration between 45 and 55 ng/L for LoQ were measured on three different days, in five-fold per day, on two cartridge lots giving 120 measurements for LoQ, LoD and LoQ in Li-heparin plasma. Furthermore, for measuring LoQ in whole blood, 16 Li-heparin whole blood samples (concentration between 10 and 200 ng/L) were measured in 20-fold on ten analyzers in parallel, on one cartridge lot and on multiple days to determine LoQ in Li-heparin whole blood.

2.8. Linearity on dilution

According to CLSI EP06-A recommendation [11], 11 Li-heparin plasma pools with different levels of cTnl were prepared from a high cTnl Li-heparin plasma pool and a negative Li-heparin plasma pool with steps of 10% dilution. The pool with the highest cTnl level and the pool with the lowest cTnl level were measured in five-fold. All the other pools were measured in three-fold. All tests were performed on one Minicare cTnI cartridge lot.

2.9. High-dose hook effect

11 dilutions blends prepared from the NIST standard of human cTnl (SRM 2921) and a negative Li-heparin plasma pool were tested. All high-dose hook samples were measured in duplicate on one Minicare cTnI cartridge lot.

2.10. Cross-reactivity and interferences

Various blood components (hemoglobin, bilirubin, triglycerides, lecithin, human anti-mouse antibodies (HAMA)) and drugs (alloporin, acetaminophen, ampicillin, ascorbic acid, acetylsalicylic acid, atenolol, caffeine, captopril, digoxin, dopamine-HCL, erythromycin, furosemide, methyldopa, naphedipine, phenytoin, theophylline, verapamill) were tested for interference and human skeletal troponin I, human cardiac troponin T, human cardiac troponin C and human skeletal troponin T (Hytest 8T25, 8T13, 8T57 and 8T24 respectively) for cross-reactivity according to CLSI EP07-A2 recommendations [12] in a Li-heparin plasma pool with a cTnl concentration of 105 ng/L. As a reference, only the dilution matrix of the substances was tested in the Li-heparin plasma pool. Results for samples enriched with these possible interferences that were within 10% of the results for the control samples were considered acceptable. Four endogenous interfering substances (human albumin, globulin, rheumatoid factor (RF) and total protein) were tested on interference in samples from a normal population (without spiking) on Minicare cTnI and a comparative method (Beckman Coulter Access 2, AccuTnI + 3).

2.11. Method comparison

The Minicare cTnI was compared with the Beckman Coulter Access 2, AccuTnI + 3 by testing and comparing 119 Li-Heparin plasma samples in accordance with CLSI document EP09-A3 [13].

2.12. Sample type comparison

The sample type comparison study was conducted on 138 pairent samples from 4 hospitals, all part of the Lab2Go consortium, in accordance with CLSI EP09-A3 recommendations [13]. From each patient capillary whole blood from finger stick, Li-heparin whole blood and Li-heparin plasma from venous puncture were assayed in duplicate on Minicare cTnI.

2.13. 99th percentile upper reference limit (URL)

A single site study was performed at PRA Health Sciences, Stationsweg 163, 9471 GP, Zuidlaren, The Netherlands. The health condition of 848 apparently healthy adults was screened based on a questionnaire, on the measurement of a cardiac marker (NTproBNP on the Siemens Immulite 2000) and a marker for kidney function (creatinine to estimate Glomerular Filtration Rate).

Volunteers with a personal history of AMI or other cardiac vascular diseases, COPD, immunological disease, diabetes, hypertension, renal disease, drug-of-abuse or cancer in the last 5 years were excluded from the study. The cutoff values used to exclude patients based on NTproBNP test were taken from the package insert of the Siemens Immulite 2000 NT-proBNP and were >125 pg/mL for subjects <75 years old and >450 pg/mL for subjects 75 years old or older. Acceptance criteria for eGFR was above 60 mL/min/1.73 m2. In total 750 healthy volunteers (373 males and 377 females; age range from 18 to 86 years) were qualified as final study population for the 99th percentile URL study. Males and females were equally distributed over five age groups (18–30, 31–40, 41–50, 51–60 and >60). The 99th percentile URL for the Minicare cTnI was determined by testing capillary whole blood, Li-Heparin whole blood and Li-Heparin plasma samples and has been calculated per sample type and per gender.

2.14. Statistical analysis

The LoB was calculated using the nonparametric method with formula: Rank position = 0.5 + B * 0.95 (where B is the number of replicates.). The LoD was calculated using the formula: LoD = LoB + cSD1, where c is a multiplier to give the 95th percentile of a normal distribution (equal to 1.653) and SD1 represents the standard deviation (SD) of all the results of the pooled samples. For LoQ a coefficient of variation (CV) profile of 16 Li-heparin venous blood samples with concentrations between 10 and 200 ng/L was calculated to determine the LoQ at a total CV of 20%. The calculations were modelled using the equation CV = a + b * c to model the CV's with concentration, where a, b and c are constants to fit and, in addition, background CV c was added. This nonlinear model was solved with Gauss-Newton. This model was furthermore used to calculate the CV at 99th percentile URL. The linearity was checked using IVDfit as statistical analysis tools. A 2nd polynomial fit was calculated with a weight factor (1/SD^2) as described in CLSI guideline EP06-A section 5.3.3 [11]. Method comparison regression analyses were performed using the Deming regression method. The sample type comparison analysis was performed according to the Passing and Bablock linear regression procedure [14]. The agreement between the two sample types was assessed according to

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the method as described by Bland and Altman [15]. For these analyses methods and additional basic statistics Analyze-it for Microsoft Excel was used as software tool. Since no common statistical distribution described the data set properly at percentiles over 98–99, the 99th percentile was determined using the non-parametric method (PROC UNIVARIATE with interpolation option (1) in SAS® statistical software, i.e. linear interpolation between the data points closest to the 99th percentile) as described in CLSI standard EP28-A3c [16]. Furthermore, as described in this guideline, 90% CIs were calculated based on binomial probabilities. The lower and upper ranks corresponding to the confidence limits are symmetric in ranks and chosen so that their values are as close to the 99th percentile as possible while satisfying the coverage requirement.

3. Results

3.1. Precision

For the 3 Li-heparin plasma pools 80 replicates were measured. The total imprecision of the Li-heparin plasma pools with concentrations between 109.6 and 6135.4 ng/L and across two lots was found to be between 7.3% and 12.0%. At low cTnI level the total imprecision for lot 1 was 11.6% and for lot 2 it was 12.0%. In the medium range of cTnI level, total imprecision for lot 1 was 9.6% and for lot 2 was 8.4%. For the high cTnI level sample, the total imprecision for lot 1 was 7.3% and lot 2 it was 7.7%.

3.2. Detection capability

For the four samples for LoB, a total of 60 replicates were measured and ranked on increasing concentration. The LoB for lot 1 and lot 2 was found to be 6.5 ng/L and 8.5 ng/L respectively. The highest value of LoB was used to calculate the LoD. The LoD was determined to be 18 ng/L and 17 ng/L for lot 1 and lot 2 respectively. For Li-heparin whole blood a CV profile was calculated in order to determine the LoQ at 20%CV (Fig. 2). The LoQ for Li-heparin whole blood was found to be 38 ng/L [95% CI 28.3–47.7 ng/L]. The LoQ at 20%CV for Li-heparin plasma was determined to be 37 and 29 ng/L for lot 1 and lot 2 respectively.

3.3. Linearity

The Minicare cTnI demonstrated linearity with a maximum deviation between a linear and non-linear fit of ≤15% throughout the measured range up to and including 8126 ng/L.

The observed percentage of cross-reactivity of the Minicare cTnI to troponin C and human skeletal troponin T was 0.00045%, 0.00664%, 0.00256% and 0.00016% respectively.

3.4. High-dose Hook effect

No High-dose hook effect was found for samples up to and including a cTnI concentration of 2,000,000 ng/L.

3.5. Cross-reactivity and interference

None of the tested blood components and/or drugs showed interference beyond 90% and 110%. The interference of four endogenous interfering substances albumin, globulin, total protein and RF were determined in natural plasma samples via a method comparison study, showing that there is no predictive relationship between the interfering factor content and specific bias of the cTnI results up to a concentration of 4.95 g/dL, 1.75 g/dL, 8.09 g/dL and 45.3 IU/mL respectively. The observed percentage of cross-reactivity of the Minicare cTnI to human skeletal troponin I, human cardiac troponin T, human cardiac troponin C and human skeletal troponin T was 0.00045%, 0.00664%, 0.00256% and 0.00016% respectively.

3.6. Method comparison

In Fig. 3, the Deming regression is shown for the comparison between Beckman Coulter Access, AccuTnI+3 and Minicare cTnI performed on Li-heparin plasma samples from 119 patients. The Pearson correlation was good: over all measurements the correlation coefficient was 0.973 [95% CI 0.961–0.981]. The slope was 1.09 [95% CI 0.97–1.20] with an intercept at 75.18 ng/L [95% CI 33.7–116.7]. No outlier was discarded from the data set.

3.7. Sample type comparison

Sample types were prepared from blood samples from 122 patients with cTnI concentrations that spanned the full measurement range of the Minicare cTnI. In Fig. 4 the Passing-Bablock fit and Bland-Altman figures are shown for the comparison between the three sample types. The sample type correlations are reported in Table 1. Samples with a concentration below LoD (18 ng/L) were excluded from the data set in the Passing-Bablock and correlation plot and samples with a concentration below LoQ whole blood (38 ng/L) were excluded in the Bland-Altman plot. The correlation between the sample types was excellent. Over all measurements, with cTnI values covering 18–7000 ng/L, the sample type comparison for Li-heparin venous whole blood versus capillary whole blood, Li-heparin venous whole blood versus Li-heparin whole blood was excellent.
plasma and Li-heparin plasma versus capillary whole blood showed R values of 0.995, 0.996 and 0.990 and slopes of 1.08, 1.03 and 1.05 respectively. The bias measured for Li-heparin venous whole blood versus capillary whole blood, Li-heparin venous whole blood versus Li-heparin plasma and Li-heparin plasma versus capillary whole blood were 7.7%, 2.2% and 5.5%, respectively.

<table>
<thead>
<tr>
<th>Sample types</th>
<th>R</th>
<th>Slope (95% CI)</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>lower LoA</th>
<th>upper LoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li-heparin whole blood vs. Li-heparin plasma</td>
<td>1.00</td>
<td>1.03 (1.01–1.05)</td>
<td>2.2%</td>
<td>–0.2%–4.6%</td>
<td>–22.0</td>
<td>26.5</td>
</tr>
<tr>
<td>Li-heparin plasma vs. capillary whole blood</td>
<td>0.99</td>
<td>1.05 (1.02–1.08)</td>
<td>5.5%</td>
<td>2.9%–8.0%</td>
<td>–20.7</td>
<td>31.6</td>
</tr>
<tr>
<td>Li-heparin whole blood vs. capillary whole blood</td>
<td>1.00</td>
<td>1.08 (1.07–1.10)</td>
<td>7.7%</td>
<td>5.5%–9.9%</td>
<td>–14.5</td>
<td>29.9</td>
</tr>
</tbody>
</table>

Table 1
Correlation coefficients (R) and slopes with 95% CI for measurements of cTnI in different sample types using the Minicare I-20 analyzer. Data based on 122 patient samples in a range of 18–7000 ng/L and Bland–Altman data (mean difference with 95% CI together with lower and upper limits of agreement (LoA)) using Analyse-it for analysis with 104 samples.

Fig. 4. Correlation between cTnI concentrations as obtained with the Minicare cTnI analyzer in Li-heparin venous whole blood and Li-heparin venous plasma samples (A), Li-heparin venous plasma and capillary whole blood samples (C) and Li-heparin venous whole blood and capillary whole blood samples (E) (122 samples for each pair). Bland–Altman graphs for venous whole blood vs plasma samples (B), plasma vs capillary whole blood samples (D) and venous whole blood vs capillary whole blood samples (F) (104 samples for each pair).

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3.8. 99th percentile URL

In Fig. 5 the distribution of the Minicare cTnI levels of the healthy individuals resulting from the 99th percentile URL study is shown. Measurements on capillary blood are shown as an example. Other sample types show similar distributions. The presence of outliers was verified carefully [17]. As the dataset was clearly non-normal and transformation to a normal distribution was not possible using standard transformations, Tukey’s rule [16] was not applicable. Application of Dixon’s test [16] revealed that the highest data point for all data sets was a potential outlier. However, since for this particular subject all three separate measurements were higher and the subject was qualified as a healthy person, i.e. belonged to the healthy population, these data points were not discarded.

The 99th percentile URL of Minicare cTnI for 750 healthy volunteers (373 males and 377 females, age range from 18 to 86 years) was calculated at 59 ng/L using capillary blood, 39 ng/L using Li-heparin whole blood and 41 ng/L using Li-heparin plasma (see Table 2). No significant influence of one of the three tested sample types on the overall 99th percentile cut-off values was observed so that the same 99th percentile URL could be used for three sample types. The combined 99th percentile URL value for all sample types was 43 ng/L (90% CI: 35–61 ng/L). There were also no significant differences between cTnI values of male and female subjects for any of the sample types, allowing combining male and female cTnI URL values for the determination of the Minicare cTnI 99th percentile URL. Testing for equality of the 99th percentile URL of male and female subjects for any of the sample types, allowing combining male and female cTnI values for the determination of the Minicare cTnI 99th percentile cut-off values was observed so that the same 99th percentile URL could be used for the three sample types. The combined 99th percentile URL value for all sample types was 43 ng/L (90% CI: 35–61 ng/L). There were also no significant differences between cTnI values of male and female subjects for any of the sample types, allowing combining male and female cTnI URL values for the determination of the Minicare cTnI99th percentile URL. Testing for equality of the 99th percentile URL of all six groups (three blood types combined with two genders) was done by using Pearson’s chi-square [18] and showed no significant difference (Pearson chi2(5) = 4.8570; Pr = 0.434). The 99th percentile URL for all sample types and genders was established at 43 ng/L. From the CV profile of Li-heparin whole blood (Fig. 2), the CV at 99th percentile URL was calculated to be 18.6%.

4. Discussion

The study results show that the Minicare cTnI is a clinically usable cTnI POC test which can accurately and rapidly measure cTnI near the patient with a turn-around time of ~10 min, which is somewhat faster than currently available POC cTnI assays which usually need 10–20 min test time [19], making the Minicare cTnI one of the fastest POC devices for cTnI testing. Unlike other cTn assays which need at least 90 μL of sample, the Minicare cTnI only requires a single droplet of blood and is the first device on the market that has been evaluated carefully for capillary sample testing. This allows for minimally invasive blood collection via finger prick, giving the assay a good flexibility in use. The Minicare cTnI needs no sample preparation on foreground, which is needed for some other cTn POC assays, thereby reducing TAT and the possibility of user errors [19]. Results will be available to the treating physician at bedside together with the ECG after history taking which potentially accelerates the ACS patient’s pathway and reduces ED crowding [20,21]. Further reduction in TAT could be achieved by performing the first measurement in the ambulance; the ruggedness of the device seems suitable for this use environment and studies have been initiated to investigate this workflow.

For LoQ at 20%CV no significant difference between Li-heparin whole blood and Li-heparin plasma was observed. The sample type comparison study between capillary whole blood, Li-heparin whole blood and Li-heparin plasma samples demonstrated an excellent correlation and indicates that the three sample types are substantially equivalent. Thus, sample types can be used interchangeable which is especially beneficial in case of serial testing. Capillary sampling could also ease cTnI testing in other medical fields, like the general practitioner’s office, ambulance cars and at the triage nurse in the ED. Method comparison between Minicare cTnI and the reference cTnI assay (Beckman Coulter Access, AccuTnI + 3) demonstrated a very close correlation. Cross-reactivity and interferences

![Histogram of healthy population](image-url)

Fig. 5. Distribution of Minicare cTnI values of capillary blood from healthy individuals resulting from the 99th percentile URL study.

Table 2
Summary 99th percentile URL values Minicare cTnI (ng/L).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Overall (n = 750)</th>
<th>Male (n = 373)</th>
<th>Female (n = 377)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary whole blood</td>
<td>59 (90% CI: 40–88)</td>
<td>67 (90% CI: 30–387)</td>
<td>52 (90% CI: 30–88)</td>
</tr>
<tr>
<td>Li-heparin venous whole blood</td>
<td>39 (90% CI: 25–61)</td>
<td>51 (90% CI: 21–329)</td>
<td>33 (90% CI: 23–59)</td>
</tr>
<tr>
<td>Li-heparin plasma</td>
<td>41 (90% CI: 28–93)</td>
<td>68 (90% CI: 23–350)</td>
<td>37 (90% CI: 24–93)</td>
</tr>
<tr>
<td>Capillary whole blood and Li-heparin venous whole blood combined</td>
<td>44 (90% CI: 33–63)</td>
<td>58 (90% CI: 35–165)</td>
<td>40 (90% CI: 25–59)</td>
</tr>
<tr>
<td>Capillary whole blood and Li-heparin plasma combined</td>
<td>49 (90% CI: 36–69)</td>
<td>65 (90% CI: 37–163)</td>
<td>40 (90% CI: 28–64)</td>
</tr>
<tr>
<td>Li-heparin venous whole blood and Li-heparin plasma combined</td>
<td>39 (90% CI: 28–59)</td>
<td>56 (90% CI: 30–101)</td>
<td>33 (90% CI: 24–43)</td>
</tr>
<tr>
<td>Overall</td>
<td>43 (90% CI: 35–61)</td>
<td>60 (90% CI: 37–72)</td>
<td>39 (90% CI: 30–49)</td>
</tr>
</tbody>
</table>

Please cite this article as: D.W.M. Kemper, et al., Analytical evaluation of a new point of care system for measuring cardiac Troponin I, Clin Biochem (2016), http://dx.doi.org/10.1016/j.clinbiochem.2016.11.011
were minimal and no high-dose hook effect was observed. Since the Minicare uses 2 solid phases (with one antibody on the magnetic bead and the complimentary antibodies on the sensor surface) accessibility to the target epitopes could be more challenging compared to systems that only use one solid phase and have the other antibody free in solution. One can hypothesize that the potential negative steric impact from using two solid phases can be partially mitigated by including additional epitopes. This has been the rationale to evaluate configurations with an antibody against cTnC in the spot. On average the signal increased about 25–30% (so 25–30% more beads were bound to the spot for a comparable Tnl concentration) when including the cTnC antibody to the spot. It should be noted that the Minicare cTnl assay is still specific for cTnI (potentially as part of the complex with cTnC) since the antibody on the bead is directed against cTnI and hence a label can only be bound in the presence of cTnI. A potential downside of including a cTnC antibody in the spot could be increased non-specific binding against cTnC for the assay. However, in our studies cross-reactivity with cTnC was very low (<0.005%), which might be explained by the good specificity of the antibody used on the bead. Based on the above it was decided to include a cTnC antibody into our final assay configuration.

The CV at the 99th percentile URL of 43 ng/L, as measured conservatively, on a truly healthy population, was calculated to be 18.6%. According to Jaffe et al. [22], LoQ below or equal to 20%CV at 99th percentile URL leads to a clinically usable system for the diagnosis of AMI. The 99th percentile URL showed no significant difference between genders or sample types in a large healthy population selected according to the most recent recommendations for normal subjects to determine the 99th percentile URL of a cTn assay [23]. These recommendations advise to include at least 300 male and 300 female subjects without clinical history or known cardiovascular disease, no diabetes, age range between 18 to above 70 years old, diversity in race/ethnicity, biomarker negative for NT pro BNP and normal eGFR values.

In conclusion, the Minicare cTnl assay is a sensitive, fast and precise test for determination of cTnI that can be used as an aid in the diagnosis of AMI in a near-patient setting on capillary or Li-heparin venous whole blood sample. This offers for the Minicare cTnl test the potential for good clinical performance in the diagnosis of AMI, which is currently evaluated in a large clinical study.

Acknowledgement

The European Union (project 621035) funded part of the studies by supporting the following sites: Medizinische Universität Innsbruck, Univ. Klinik für Innere Medizin III – Kardiologie und Angiologie (Innsbruck, Austria), Klinik für Notfall- und Internistische Intensivmedizin, Klinikum Nürnberg Nord (Nurnberg, Germany), Hôpital Universitaire La Pitié-Salpêtrière, Service des Urgences du Pr Riou (Paris, France) and Catharina Ziekenhuis Eindhoven, Algemeen Klinisch Laboratorium (Eindhoven, the Netherlands).

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